PHYSICAL AND MECHANICAL PROPERTIES OF EXTRACTED WOOD OF *TECTONA GRANDIS* AND ANTIFUNGAL TEST OF THE EXTRACTIVE WITH *PHANEROCHAETE CHRYSOSPORIUM*

by

NOR SHIFA SHUIB

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CC	Column chromatography
BIH	Bottom inner heartwood
BOH	Bottom outer heartwood
TIH	Top inner heartwood
TOH	Top outer heartwood
DCM	Dichloromethane
GCMS	Gas chromatography Mass spectrometry
HPLC	High Performance Liquid Chromatography
ID	Internal diameter
ND	Not detected
SD	Standard deviation
Std	Standard
TLC	Thin layer chromatography
ppm	Part per million
v/v	Volume over volume

SIFAT-SIFAT FIZIKAL DAN MEKANIKAL KAYU *TECTONA GRANDIS* TEREKSTRAK DAN UJIAN ANTI KULAT EKSTRAKTIF TERHADAP *PHANEROCHAETE CHRYSOSPORIUM*

ABSTRAK

Kesan terhadap pengekstrakan yang berturutan terhadap sifat mekanikal kayu Jati telah dijalankan. Variasi dari segi sifat mekanikal pada ketinggian dan jarak yang berbeza pada sampel terekstrak telah dijalankan dan dibuat perbandingan dengan sampel belum diekstrak. Walaupun, kayu jati merupakan kayu yang baik, biodegradasi dan ekstrak kayu digunakan sebagai sumber alternatif untuk antimikrobial dan lain-lain kegunaan, kesan daripada pengekstrakan terhadap sifat mekanikal dibincangkan didalam kajian ini.

Ekstrak dari kayu teras diperolehi daripada *Tectona grandis* melalui pengekstrakan secara berturutan menggunakan diklorometana, etanol dan etanol-toluena (1:2 isipadu/isipadu) telah diuji untuk aktiviti biologi terhadap kulat *Phanerochete chrysosporium*. Bahan bioaktif utama yang diperolehi daripada kayu teras termasuklah 2 - Methyl anthraquinone, 1,4 - Naphthoquinone dan Lapachol, yang telah dianalisis menggunakan GCMS dan HPLC. Keputusan yang diperolehi menunjukkan ekstrak diklorometana memberikan aktiviti anti kulat yang tinggi berbanding dengan ekstrak daripada etanol dan etanol-toluena (1:2 isipadu/isipadu).

PHYSICAL AND MECHANICAL PROPERTIES OF EXTRACTED WOOD OF *TECTONA GRANDIS* AND ANTIFUNGAL TEST OF THE EXTRACTIVES WITH *PHANEROCHAETE CHRYSOSPORIUM*

ABSTRACT

Effects of sequential extraction of wood extractives on mechanical properties of teak were evaluated. Variations in mechanical properties of extracted teak samples at the different heights within the stem and at different distances from the pith of the tree were determined and compared with un-extracted samples. Although, teak may be an excellent, biodegradable and renewable alternative source of wood extractives used as antimicrobial and other non-biocidal protection, the effect of its extraction on the mechanical properties of derived wood material is discussed in this study.

Heartwood extracts obtained from *Tectona grandis* by successive extractions with dichloromethane, ethanol and ethanol-toluene (1:2 v/v) were tested for their biological activity against fungus *Phanerochete chrysosporium*. The major bioactive compounds obtained from heartwood include 2 - Methyl anthraquinone, 1,4 - Naphthoquinone and Lapachol, which were analyzed by GCMS and HPLC. The results revealed that dichloromethane extract exhibit higher antifungal activity as compared to ethanol and ethanol-toluene (1:2 v/v) extracts.

CHAPTER 1

INTRODUCTION

1.1 General

People have relied on trees for shelter, building materials, flavors/fragrances, and not least, medicines, since ancient times The special properties of wood, including its aesthetic appearance, low density, low thermal expansion, and desirable mechanical strength, have led to indoor and outdoor applications. Durability is one of the most important considerations for the use of wood in construction. Poor durability has often been recognized as one of wood's 'disadvantages' in certain application. Developing methods to prolong the service life of wood has always been the interest of wood industry researchers. From the environmental perspective, finding naturally existent constituents in highly durable tree species and understanding their mechanisms are the most appropriate approaches to achieve wood protection while preserving the environment (Wang et al., 2005).

The estimation of heartwood content helps to define differences in durability and other wood characteristics, while dry density is considered as an indicator of timber strength (Bhat, 1995). *Tectona grandis* has gained a worldwide reputation due to its attractiveness and durability (Cordero and Kanninen, 2003).

1.2 Teak

Teak has worldwide reputation. Teak *(Tectona grandis* L.) is a species of a small genus *Tectona* belonging to the family Verbenaceae. The genus of Tectona consist of the true teak tree and two species of much less importance, *T. tenuifolia* and *T.*

philippinensis. which were found in Myanmar and northern Philippine Island (Burkill, 1966). Teak has worldwide reputation including as a quality lumber resistant resistance to fungal infection (Tee et al., 1995).

Teak was originated from asia monsoon area such as India, Thailand, Myannmar and Laos. Then, it was introduced to many places such as Papua New Guniea, Indonesia, Philipines and Malaysia. Different parts of the plant are used as medicines (Laskar et al., 1985). It has been a common practice among women in Kerala state in India to crush the tender leaves into a paste and rub it in the palms which get dyed a light reddish shade (Agarwal et al., 1965). In Malaysia it was called Jati (Appanah and Weinland, 1993). The words kayu jati means the true or real wood (Burkill, 1966).

Teak is regarded as all purpose utility timber for its high quality characteristic. Among them, moderate weight, easy to process. Colours yellowish golden. Density about 700 kg/m^3 for dry wood and categorized under light hardwood. Therefore the wood is widely used for furniture, carving and building materials. The presence of oily substance give the teak its shiny appearance. As a log, teak bole is straight, cylindrical well formed except for the fluting on the buttress of the bass. The sapwood has light yellowish colour. Recent technologies makes it possible to produce veneer, strips and parquet (Tee et al., 1995). The wood contains oil that prevent nails from rusting (Appanah and Weinland, 1993).

Due to this natural durability, teak is often used for outdoor purposes, e.g. boat decks, bridge building, and garden furniture, as well as traditional indoor uses, e.g. flooring and furnishings. Such uses require a highly stable wood with regard to physical properties, as well as an aesthetically pleasing colour and adequate resistance to pathogens. Teak has been identified as the most potential species for establishing high quality tropical hardwood plantations under the sustainable forest management (Bhat et al., 2001). Teak is quite often used as a reference species for standardization of wood property evaluation and end use classification of tropical hardwoods (Bhat et al., 2001). Therefore, further information concerning the natural durability of teak wood is indispensable, if such knowledge is to be used in breeding programs, or the wood used to its best advantage by the end-user (Kokutse et al., 2004).

Teak was grown successfully together with agricultural species such as rice, cotton, tapioca, chilli and ginger. In India it was found that teak plantations with peanut and soy bean were very successful and negative effects on the growth of teak were not found. In Venezuela, maize seeded between young teak trees reduced the number of cleanings. In Cuba, the use of this agroforestry system in maize or bean cultures has given excellent harvests. In addition, fields remained clean, without the competition of undesired plants. Later studies proved the allelophatic effects of teak leaves on the germination of peanut and maize (Macias et al., 2008).

1.2.1 Teak in Malaysia

Teak was first planted in 1909 in the Sungai Raya Estate, Langkawi Island by a Dutch rubber planter, who brought the seed from Medan, Sumatera (Appanah and Weinland, 1993). Following the introduction, teak was planted as roadside trees in Kuala Kangsar, Perak. It was only promising in north-west Malaysia (Kedah, Perlis and the Langkawi Islands), which borders Thailand, where a monsoonal climate prevails

(Appanah and Weinland, 1993). In Sabah, teak was originally introduced into Sabah and planted in Kota Marudu in 1926 by the Dutch Tobacco Company (Tee et al., 1995).

In 1957, provenance trials were started in Changlun and Jeniang, Kedah (Appanah and Weinland, 1993). In 1958, teak from the Changlun nursery were planted in compartment 23, Mata Ayer Forest Reserve, Perlis. Seedling from Jeniang were planted in Bukit Enggang Forest reserve, Kedah also on the same year. Other sites in Kedah were Bukit Perak and Gunung Raya. The trial plot comprised an area of 40 hectares (Appanah and Weinland, 1993).

Teak makes an attractive plantation species. The seed are easily available, and the seedlings can be tended in nursery without many problems. Teak can be stump-planted in the open, and the growth rates are high enough to produce very high quality timber in 30-year rotations (Appanah and Weinland, 1993). In Malaysia, efforts have been geared to solve shortage of timber supply through various strategies, including the establishment of large scale forest plantations. Linear planting of timber trees replacing traditional method by restricted to planting of trees in rows at specified spacing and densities. There is potential for linear planting of timber trees for high value and luxury timber production in non-forested areas in Malaysia. Teak are among trees widely used in linear planting along the canals, roadsides and highways (Noor et al., 2005). Good irrigation system allow teak to be grown in various places in Malaysia (Tee et al., 1995).

1.3 Decay

As a natural organic material, wood is degraded by many organisms, principally fungi and insects (Schultz and Nicholas, 2002). The decay fungi are the most important microorganisms affecting wood which are from higher fungi - class basidiomycetes (Findlay, 1975). Decay of wood caused by fungi require favourable moisture (above saturation point), (Wilcox et al., 1991).

Wood is biologically degraded because organisms recognize the polysaccharide polymers in the cell wall and have specific enzyme systems that hydrolyze these polymers into digestible units. The hemicelluloses are probably first to be attacked because they are the most accessible. The accessible portion of cellulose is attacked next. Because high molecular weight cellulose is primarily responsible for strength in wood, its biological degradation through oxidation, hydrolysis and dehydration reactions causes wood to lose strength (Rowell, 1984). Even at early stage, fungal decay already cause wood lose most of its important structural properties without detection (Wilcox et al., 1991). A very low weight loss caused by brown-rot fungi attack already causes a very large loss in the degree of polymerization in the holocellulose fraction (Rowell, 2005).

1.3.1 *Phanerochaete chrysosporium*

The fungus *Phanerochaete chrysosporium*, a white rot fungi has been intensively studied due to its potential in industrial processes: animal feed, biological pre-treatment of wood for pulping, biological bleaching of crude pulp, and treatment of waste effluents generated in pulp and paper production. Its main features includes high optimum temperature (over 40ºC) make it useful for making compost and biotechnology purpose (Agosin et al., 1990).

1.4 Wood Classification

Wood is classified into two main categories of plant known as softwoods and hardwoods (Figure 1.1). The softwood trees are botanically known as gymnosperms (from the Greek: naked seeded) because the seeds develop exposed on the surface of a cone scale. The softwoods also refer to as conifers because many produce seed cones, pollen cones or both. Hardwoods are classified under angiosperms, or hidden – seeded plants which having seed inside the fruits and dicotyledenous (two seed leaves). Hardwood is used to describe timber from the so-called broad-leafed trees (Rowell, 1984).

Timber trees in Peninsular Malaysia are divided into the following: Heavy hardwoods, Medium Hardwoods, Light Hardwoods and Softwoods (conifers). Teak is included into light hardwood under exotic species.

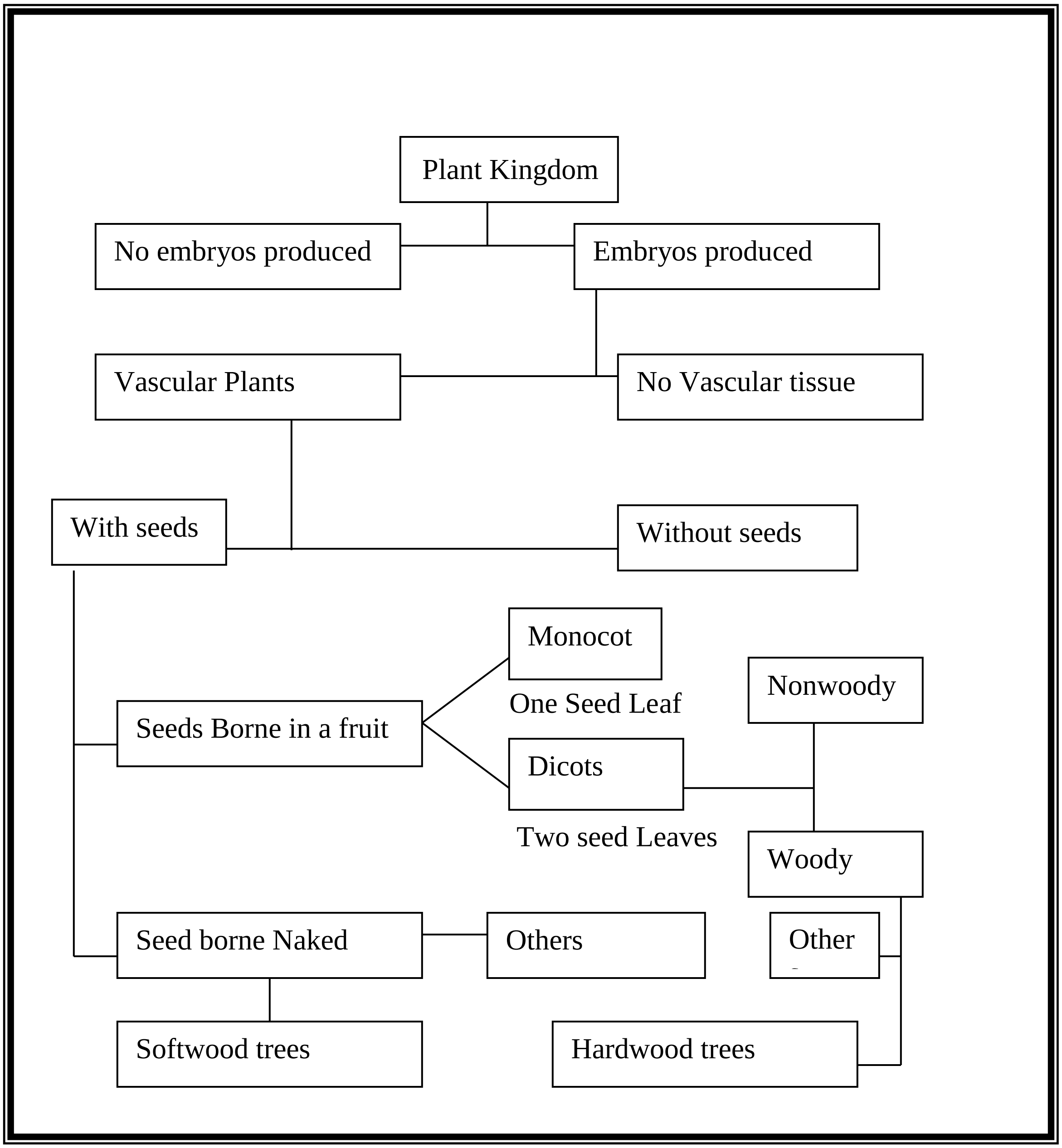

Figure 1.1 Wood classification (Rowell, 1984)

1.4.1 Heavy hardwoods

Wood under this categories possessed density above 880 kg/m^3, moisture content at about 15 % and have natural durability (Menon, 1993).

1.4.2 Medium hardwoods

Wood under this categories having density between 720 - 880 kg/m^3. Moisture content is about 15 % (Menon, 1993).

1.4.3 Light hardwoods

Wood having density below 720 kg/m^3 and moisture content at about 15% are given for this category (Menon, 1993).

1.4.4 Soft woods

Softwood are light and having density at about 385 - 735 kg/m^3 (Menon, 1993). There are called gymnosperm which having exposed seed.

1.5 Wood Anatomy

1.5.1 Tree growth

Wood is defined as the hard fibrous substance basically xylem that makes up the greater part of the stems and branches of trees and shrubs beneath the bark. Woody plants are vascular, possessing conducting tissues consisting of wood (xylem) and bark (phloem); perennial: and exhibit stem thickening, the division of a growing layer (cambium) that annually forms new wood and new bark which are inserted between the older wood and bark (Lewin and Goldstein, 1991).

Wood tissue can be referenced by three major planes or surfaces (Rowel, 1984). Each surface has a unique appearance that stems from the manner in which cells of specific orientations are cut by planes directed either parallel or perpendicular to the tree's longitudinal axis. These trees planes are the cross sectional or transverse plane (A), the tangential plane (B) and the radial plane (C), (Figure 1.2).

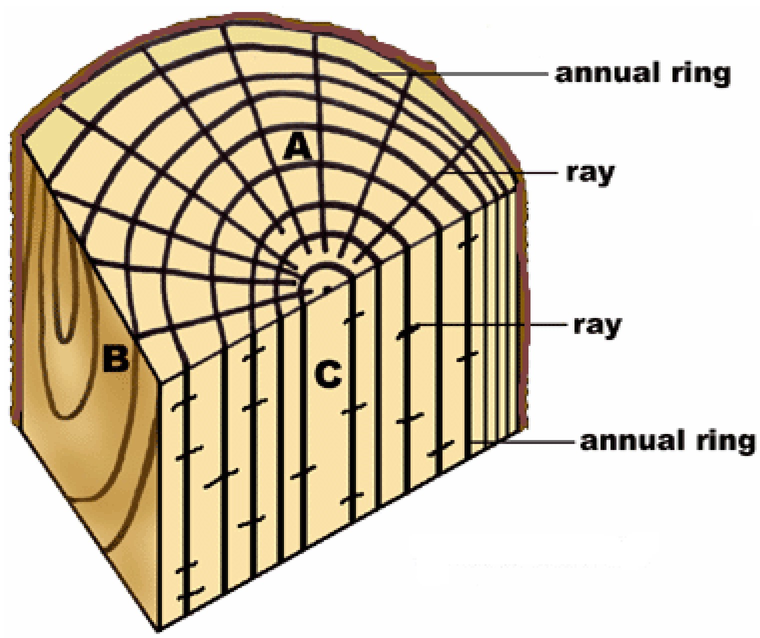

Figure 1.2 Cross section of a tree trunk (Rowell, 1984)

1.6 Moisture Content

Moisture content is the amount of water in a piece of wood It is expressed in the percentage of the dry weight of the wood. Wood is a hygroscopic material and its moisture content will always have a tendency to change until it is in equilibrium with the amount of water vapour in the surrounding atmosphere. Therefore, for any combination of the air temperature and a relative humidity there is a corresponding wood moisture content (Haygreen and Bowyer, 1994).

Moisture content changes wood dimension because the cell wall polymers contain hydroxyl and other oxygen containing groups that attract moisture through hydrogen bonding. The hemicelluloses are the most hygroscopic component in the wood cell wall, but lignin also contributes to the hygroscopicity of wood. Moisture swells the cell wall, and wood expands until the cell wall is saturated with water. Water, beyond this point is free water in the void structure and does not contribute to further expansion. This process is reversible, and the wood shrinks as moisture is lost (Rowell, 2005).

1.7 Wood Durability

Wood is an organic material, so it can suffer from degradation by physical, chemical, and biological factors; among the biological are the microorganisms belonging to the Basidiomycetes. According to Eaton and Hale (1993), the natural durability of the wood depends on its chemical composition and accessibility of its nutrients to organisms. The main chemical factors that influence the natural durability of wood are the polymers of the cell wall and the extractives in the form of terpenes and phenolic compounds, such as quinones, lignan, tannin barks and stilbenes. Teak wood belongs to Durability Class I (Highly Resistant Timbers) of the general classification system (Bakshi et al.,1967).

1.8 Wood Composition

Wood can be defined as three dimensional biopolymer composite containing a solid phase and voids. The solid phase or cell wall is composed of a network of polymers. These polymers-primarily cellulose, several types of hemicelluloses, and lignin are responsible for most of the physical and chemical properties of wood. Degradation of these components reduce wood properties. For example, wood undergoes major changes in properties when cell wall polymers are attacked by microorganisms (rotting), (Hon and Shiraishi, 1990).

Cell walls may comprise 90% of the mass of the woody plants. Main component of woody plant cell walls are cellulose, hemicelluloses and lignin. The basic cellulose component of a cell wall at the molecular level is usually called a microfibril which are about 10-30 nm in diameter. Each consists of bundles of cellulose chains arranged at various angles along the long axis of microfibril. The cell wall is constructed from two, or sometimes three distinct layers: primary wall, the secondary wall and sometimes an innermost tertiary wall (Ridout, 2000).

1.9 Wood extractives

Wood extractives are the extraneous wood components that may be separated from the insoluble cell wall by their solubility in water or organic solvents. They are often genus- or species-specific, and it is possible to use their presence and abundance in taxonomic schemes based on chemical composition. The extracted materials include volatile oils, terpene (turpentines, resin acids, sterols), fatty acids and their esters, waxes, polyhydric alcohols, mono- and polysaccharides, alkaloids and aromatic compounds (acids, aldehydes, alcohols, phenylpropane dimmers, stibenes, flavonoids, tannins and quinines). The content of extractives and their compositions vary with wood species, and within different parts of the same trees. Most of the extractives are located in the ray tracheid and vessel, and phenolic compounds are accumulated in the heartwood (Lewin and Goldstein, 1991). The extractives in the wood can be removed by extracting with a single solvent or combinations of solvents such as ethanol, benzene, dichloromethane, chloroform and water (Maldas and Kamdem, 1999).

Wood extractives are recognised as principal sources of wood decay resistance (Temiz et al., 2008). Teak (*Tectona grandis* L.) has long been known to possess highly durable wood, due largely to the presence of extracts in the heartwood, e.g. anthraquinones and tectoquinones (Yamamoto et al., 1998).

1.10 Extractives Compounds

1.10.1 Quinone derivatives

Major quinone derivatives found in teak wood includes naphtoquinone and anthraquinone. It was reported that 2 - Methyl anthraquinone was identified as a bioactive compound for the inhibition of *Coniophora puteana, a* brown rot fungi (Haupt et al., 2003). This compound also known as techtoquinone (Windeisen et al., 2003). It is consider as major extractives component in teak (Thulasidas and Bhat, 2007).

Naphtoquinone is one of the secondary metabolite groups widespread in nature, where they mostly appear as chromatic pigments. Plant extracts containing naphtoquinones have been used for a long time in traditional medicines of number of nations for cancer and rheumatoid arthritis treatment (Babula et al., 2006).

Lapachol and deoxylapachol are naphtoquinone derivatives which also reported as biologically active compound (Windeisen et al., 2003). This compound and related naphthoquinones are of pharmaceutical interest due to their anticancer and antiviral effect (Lui et al., 1985).

Lapachol is a natural quinone (Figure 1.3) which has been isolated in very good yield from several species of bignoniaceae found in South America (Steinert et al., 1995). It was also found in the heartwood of Asian and South American Bignoniaceae such as species from the genera *Tabebuia*, *Taigu*, and *Tecoma* (e.g., Surinam greenheart), (Mailcar et al., 2002).

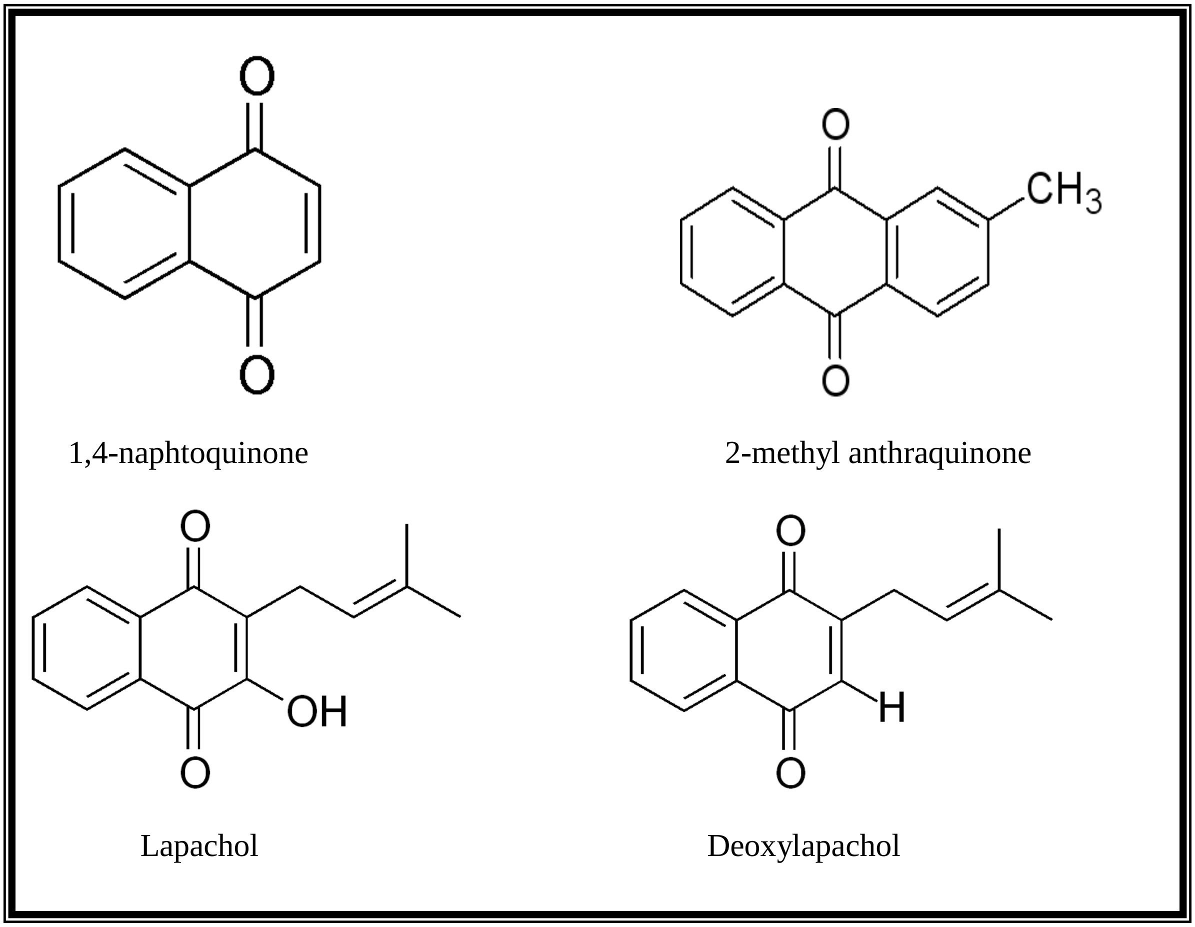

Figure 1.3 Biologically active compound of teak (Windeisen et al., 2003)

1.11 Wood preservatives

The addition of preservative chemicals is necessary because most wood products are belong from non-durable species (Hashim et al., 2009). Current wood preservatives are using hazardous chemical. In the recent years, there has been a rapid replacement of wood preservatives containing arsenic and chromium due to the public concern about health and negative influence on the environment (Temiz et al., 2008).

Lumber and other wood products are extensively used in residential construction, utility poles, railroad ties, decking, etc. As a natural organic material wood is degraded by many organisms, principally fungi and insects. Consequently, in certain applications such as ground-contact or above-ground applications where the wood is frequently wet, wood or wood-based composites should be treated with biocides for protection against wood destroying organisms (Preston, 2000). The three major USA wood preservative systems are the oilborne, organic pentachlorophenol and creosote systems and the water-borne, inorganic ‘‘chromated copper arsenate’’ (CCA-type C) preservative. Most of the treated wood products in the USA, about 80% by wood volume, are treated with CCA (Schultz and Nicholas, 2002) . CCA is highly effective in protecting wood against a wide variety of wood-destroying organisms, besides being inexpensive, water-soluble, and leach-resistant. However, the perceived environmental hazards of the disposal of wood which contain such metals may limit the future use of CCA in the US (Preston, 2000). Indeed, the availability of CCA-treated lumber has been greatly reduced in Hawaii, and CCA use has been restricted in many European countries and Japan. Also, while current US regulations permit disposal of CCA treated wood by landfill burial, discarding CCA-treated products will likely become more expensive and onerous in the future. The State of Florida is in fact considering prohibiting the disposal of treated wood in public landfills, which is already mandated in some countries. Consequently, a need exists for developing alternative, environmentally-benign non-metallic wood preservatives. One possible approach for developing new wood preservatives is to study the heartwood of naturally durable wood species, since an understanding of the cause for natural durability might suggest alternative ways to protect lumber (Schultz and Nicholas, 2002). These natural preservatives include extracts from durable species and natural toxic chemicals from plants that could inhibit growth of biological degrades such as fungi (Temiz et al., 2008). Due to safety and environmental concern, research has been focused on using natural preservatives (Yamamoto et al., 1998). Schultz et al. (1995) reported the role of extractives, particularly stilbenols, which play in the natural durability of heartwood. They suggested that extractives may protect heartwood by having some fungicidal activity and possibly other non-biocidal properties. One possibility is that extractives have both fungicidal and antioxidant properties, which work together to protect the heartwood against fungal colonization and degradation (Schultz and Nicholas, 2000).

1.12 Rubberwood

It is whitish yellow when freshly cut and turns to light brown during drying. The wood is soft to moderately hard with an average weight of 515 kg/m^3 at 12% moisture content. There is insignificant heartwood formation and no transition between sapwood and heartwood, which is confined near the pith only. Growth ring or annual rings are not visible in rubberwood. Defects can occur in rubberwood which includes decay or rot due to attack by fungi.

The importance of plantation wood resources to the furniture manufacturing industry in Malaysia cannot be underestimated. Plantation wood resources, particularly rubberwood (*Hevea brasiliensis*), contributes nearly 85 % of the solid wood needs of the furniture industry, and with a steady reduction in supply from the natural forest, such wood resources will gain further prominence in years to come. Despite its extensive use, plantation wood resources is often accepted to be of 'lower quality' material, which in turns affect the selling price of the finished products made. In order to evaluate this perception of plantation as furniture materials, an extensive survey involving the furniture manufacturing and trade fraternity was carried out at the exhibition site of the Malaysian International Furniture Fair (MIFF) in 2002, 2003 and 2004. A total of 1587 respondents were obtained from this survey, which comprised of manufacturers (32%), traders (17%), and foreign buyers (51%). According to 78% of the respondents, rubberwood exhibits several desirable properties compared to other materials for furniture manufacturing. The results from this survey showed that the market perception of Rubberwood as a furniture material is dominated by customer concerns about non-durability and the environment. Therefore issues of non-durability will have to be addressed if the material is to shed its low cost image, which seem to have pronounced effect on the selling price of the finished good (Ratnasingam et al., 2005).

Investigations related to accelerated laboratory testing using rubberwood have been reported. Okino et al., (2008) reported 50% mass loss after 12 week exposure to *Phanerochaete chrysosporium* which is among the highest decay compared to other fungal attack. The author also reported mass losses obtained after 12 week exposure to *Gloeophyllum trabeum* (29.3 % to 38.5%)..

1.13 Wood physical properties

1.13.1 Shrinkage

Shrinkage is defined as the reduction in size, which occurs when wood is dried from the green condition down to a moisture content of 12%. The amount of shrinkage which occurs not normally be uniform along each dimensional axis of a piece. In longitudinal direction the amount of shrinkage will usually be not significant and can be ignored, but across the grain it can be quite large, particularly in a direction tangential to the growth rings. It is this differential shrinkage which can lead to the development of distortion during drying (Handbook of Some Sarawak Timbers, 1999). Shrinkage is an important property in wood industry due to dimensional changes cause by distortion of wood products (Pometti et al., 2009).

1.13.2 Wood Specific Gravity

Wood specific gravity is often considered as an indicator of decay resistance in both angiosperms and gymnosperms (Panshin and Zeeuw, 1980) The size of cell cavities and the thickness of cell walls affect the density of the wood. Density can vary within a tree and from tree to tree of the same species. The apparent density will also change with moisture content and with the presence of preservative treatment. In the absence of knots and other defects, the denser timber will be the stronger and stiffer timber (Pratt and Turner, 1986).

1.14 Wood mechanical properties

According to ASTM Standard (2005), tensile strength is defined as the ultimate strength of a material subjected to tensile loading. It is the maximum stress developed in a material in a tensile test. Total energy at break is the total energy absorbed per unit volume of the specimens up to the point of rupture (ASTM, 2005). Elongation at break measures the percentage changes in length before fracture (ASTM, 2005). Bending being the common stress appearing in different kinds of wood products (Herajarvi, 2004). The modulus of rupture (MOR) measures the load-carrying capacity in bending until breaking occurs (Handbook of Some Sarawak Timbers, 1999). The tangent modulus of elasticity, often called the 'modulus of elasticity' (MOE) measures the stiffness or flexibility of the timber. The modulus of elasticity is the ratio, within the elastic limit, of stress to corresponding strain. It is calculated by drawing a tangent to the steepest initial straight line portion of the load deflection curve (Handbook of Some Sarawak Timbers, 1999). Impact resistance is resistance to breakage by flexural schock as indicated by the the energy extracted from standardized pendulum type hammers, mounted in standardized machine, in breaking standard specimens with pendulum swing (ASTM, 2005).

1.15 Objectives of study

Based on literature review, there is no document about the fungal effect either on teak extract or on teak after solvent extraction against *Phanerochaete chrysosporium* with regards to radial and height position. Moreover, the effect of teak after extraction has not been carried out elsewhere.

The objectives of the present work are:

1. To study wood decay resistance on rubber wood after vacuum impregnated with teak extract from various solvent and different radial and height location.
2. To determine dimensional stability, physical and mechanical properties of teak after solvent extraction.
3. To study chemical component of extractives which are resistant to white rot *Phanarochaete chrysosporium*.

CHAPTER TWO

MATERIALS AND EXPERIMENTAL PROCEDURE

2.1 Chemicals

A list of chemicals used in this study are as follows:

NAME	BRAND	SPECIFICATION
Dichloromethane	System ChemAR	Analytical grade
Ethanol	System ChemAR	Analytical grade
Toluene	System ChemAR	Analytical grade
Acetonitrile	System ChemAR	HPLC grade
Methanol	System ChemAR	HPLC grade
Water	Water Purifier – Barnstard	Pure water
Hexane	System ChemAR	Analytical grade
Chloroform	System ChemAR	Analytical grade

Acetic acid	System ChemAR	Analytical grade
Hydrochloric acid	System ChemAR	Analytical grade
Tartaric acid	System ChemAR	Analytical grade
1,4 naphtoquinone	Merck	Analytical grade
2-methyl anthraquinone	Merck	Analytical grade
Lapachol	Merck	Analytical grade
Potato Dextrose agar	Merck	
Silica gel 60	Merck	70 – 230 mesh

2.2 Apparatus

The apparatus used in this study are as follows:

NAME	BRAND	SPECIFICATION
Soxhlet	Schott Duran	-
Condensor	Schott Duran	-
Round bottom flask	Schott Duran	Class A
Thimble	Whatmann	Cellulose
Disposable Petri dish		9 cm (Inner diameter)
Glass bottle	-	30 ml
Vial	Agilent	1.5 ml
Cellulose Thin layer chromatography	Merck	-