INVESTIGATION OF ANTIFUNGAL ACTIVITY OF DIFFERENCE PARTS OF *Cerbera odollam* AND EFFECT OF EXTRACTS ON SOME PROPERTIES OF PARTICLEBOARDS

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UNIVERSITI SAINS MALAYSIA

2009

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by

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Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

OCT 2009

ACKNOWLEGDEMENT

I would like to acknowledge Ministry of Science, Technology and Innovation (MOSTI) for the Science Fund Grant 03-01-05SF017. I would like to thank Universiti Sains Malaysia for awarding me the fellowship. I would like to acknowledge Associate Professor Rokiah Hashim for supervising this project, Associate Professor Othman Sulaiman and Dr Fumio Kawamura for bundle of constructive ideas and advices, Professor Lee Chow Yang in assisting the termite study. Thanks also to Hexion Butterworth Penang, Malaysia for providing the resins. Appreciation to lab mates for warmth helps and supports. Last but not least, to my family and girlfriend for understanding and cares.

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LIST OF ABBREVIATIONS

LM	=leaf methanol extracts
FRM	= fruit methanol extracts
WM	= wood methanol extracts
BM	= bark methanol extracts
FLM	= flower methanol extracts
SM	= seed methanol extracts
UF	= urea formaldehyde
PF	= phenol formaldehyde

KAJIAN KEGIATAN ANTI KULAT PELBAGAI BAHAGIAN *Cerbera odollam* DAN KESAN EKSTRAK TERHADAP SESETENGAH SIFAT PAPAN SERPAI

ABSTRAK

Kajian potensi ekstraktif daripada daun, buah, kayu, kulit, bunga and biji Pong Pong (*Cerbera odollam*) sebagai pengawet kayu dalam menentang kulat dan anai anai telah dijalankan. Penilaian sifat anti kulat ekstraktif pada tahap awal yang diekstrak dengan heksana, etil asetat, etanol dan metanol telah dikaji melalui kaedah anti kulat kertas turas. Kandungan bahan kimia terkandung dalam ekstrak yang aktif semasa anti kulat telah dianalisis melalui komatografi gas spektrometer jisim. Kadar kematian anai anai terhadap ekstraktif yang diekstrak dengan metanol telah dinilaikan. Kesan ekstraktif terhadap kayu padu getah termasuk pereputan disebabkan oleh kulat pereputan, anai anai dan kajian tanam dalam tanah telah dikaji. Kesan ekstraktif terhadap papan serpai termasuk pereputan disebabkan oleh kulat, pengembangan ketebalan, kekuatan ikatan dalaman dan pembebasan formadehid papan serpai, pereputan disebabkan oleh anai anai dan kajian tanam dalam tanah telah dikaji. Ekstrak kayu diekstrak dengan metanol menunjukkan nilai kandungan perencatan yang paling rendah menentang *Trametes versicolor, Pycnoporus sanguineus, Schizophyllum commune* melalui kaedah anti kulat kertas turas. Kandungan fenolik dan asid organik ditemui dalam ekstraktif yang aktif dalam anti kulat. Ekstraktif kayu diekstrak dengan metanol menunjukkan keputusan yang paling bagus dalam meningkatkan ketahanan kayupadu getah terhadap *Schizophyllum commune*, *Trametas versicolor*, *Pycnoporus sanguineus*, *Fusarium palustris*,

Gloeophyllum trabeum, dan setanding dengan pengawet pasaran dalam menentang *Schizophyllum commune* and *Gloeophyllum trabeum*. Ekstraktif daripada bahagian bunga yang diekstrak dengan metanol menunjukkan keputusan terbaik dalam menentang *Coptotermes gestroi* melalui kajian kadar kematian anai anai dan kajian pereputan anai anai. Keputusan dalam kajian pereputan anai anai setanding dengan pengawet pasaran. Secara keseluruhan, ekstraktif *Cerbera odollam* tidak menjejaskan pengembangan ketebalan, kekuatan ikatan dalaman dan pembebasan formadehid. Mengikut piawai EN, papan serpai dengan matrik urea formadehid and papan serpai dengan matrik fenol formadehid yang dikaji telah memenuhi syarat pengembangan ketebalan 24 jam papan serpai jenis P3 dan P4. Papan serpai ini juga memenuhi syarat kekuatan ikatan dalaman untuk papan serpai jenis P2, P3 dan P4, dan pembebasan formadehid untuk papan serpai ini dikelaskan sebagai E1.

INVESTIGATION OFANTIFUNGAL ACTIVITY OF DIFFERENCE PARTS OF *Cerbera odollam* AND EFFECT OF EXTRACTS ON SOME PROPERTIES OF PARTICLEBOARDS

ABSTRACT

This research investigated the potential of extracts from leaf, fruit, wood, bark, flower and seed of Pong Pong (*Cerbera odollam*) as wood preservatives against fungal and termite. Preliminary evaluation of antifungal properties of *n*-hexane, ethyl acetate, ethanol and methanol extracts was evaluated via paper disc antifungal assay. Chemical compounds of antifungal active extracts were analysed via gas chromatography mass spectrometer. Termite mortality of methanol extracts was evaluated. The effect of extracts to solid Rubberwood including fungal decay, termite decay, and soil burial were evaluated. The effect of extracts to particleboard including thickness swelling, internal bond strength, formaldehyde release of particleboard, termite decay, and soil burial were also evaluated. Methanol wood extracts showed the lowest minimum inhibitory amount against *Trametes versicolor, Pycnoporus sanguineus, Schizophyllum commune* via paper disc antifungal assay. Majority of phenolic compounds and organic acids were detected in the antifungal active extracts. Methanol wood extracts showed the best result in increasing durability of solid Rubberwood against *Schizophyllum commune*, *Trametas versicolor*, *Pycnoporus sanguineus*, *Fusarium palustris*, *Gloeophyllum trabeum,* and compatible with commercial preservative against *Schizophyllum commune* and *Gloeophyllum trabeum*. Flower methanol extracts showed the best result against *Coptotermes gestroi* in termite mortality and termite decay. The result of termite

decay was compatible with commercial preservatives. Overall, the extracts of *Cerbera odollam* did not affect the properties of thickness swelling, internal bond and formaldehyde emission of particleboard. According to EN standard, the evaluated urea formaldehyde matrix particleboards and phenol formaldehyde matrix particleboards fulfilled the requirement of 24h thickness swelling for particleboard type P3 and type P4 respectively. The particleboards also fulfilled the requirement of internal bond strength for particleboard type P2, P3 and P4, and the formaldehyde emission of these particleboards were classified as E1.

1. Introduction

Wood is a remarkable material. Wood product has great value and importance in the world economy. However, wood is biodegradable, and essentially need to be treated against biological attack. This is because wood and wood based panels are normally manufactured from non-durable species. Wood and wood based panel without treatment are easily attacked by fungal, termite, bacteria and borer.

Biodegradation of wood results the devaluation of structural and aesthetic standpoints of wood. According to Savluchinske-Feio et al. (2007), wood decay by fungal can lead to economic losses for wood and wood product manufacturers. Wood decay by termites is also one of the serious biodegradation problems for wood utilization. The lost of wooden product and cellulosic product by termites decay exceed $3 billion annually worldwide (Cheng et al., 2007).

Previously, artificial chemicals are used to preserve wood and wood based panels. However, the concern of safety and environmental effects of artificial chemicals used to preserve wood and wood composites has increased recently (Hashim et al., 1997). The replacement of conventional artificial chemical preservatives which believes lead to environmental impact is necessary. This phenomenon has initiated the research of environmental friendly preservatives from natural constituents. One of the potential alternatives to reduce artificial chemical preservatives consumption belongs to the extracts from durable wood species.

The research of biologically active compounds from tropical species against wood rotten fungal and termite have been done by several researchers. Chang et al. (2001) studied antitermitic activity of essential oil and component from Taiwania. Ganapaty et al. (2004) studied antitermitic activities of quinines from *Diospyros sylvatica*. Kawamura et al. (2005) studied antifungal activities of iridoid glycosides from heartwood of *Gmelina arborea*. Savluchinske-Feio et al. (2007) studied activity of dehydroabietic acid derivatives against wood contaminant fungi. Yen et al. (2007) studied antifungal activities of ethanolic extract and its active compounds from *Calocedrus macrolepis*. Cheng et al. (2008) studied antifungal activities of cinnamaldehyde and eugenol congeners against wood rot fungi. Matan et al. (2008) studied the anise oil, lime oil and tangerine oil in against fungal found on Rubberwood and Temiz et al. (2008) studied the decay resistance of wood treated with mixture of boric acid and tall oil to wood decay fungal. These researchers had success in finding active compounds in their studies specimens. These compounds exhibited good repellent effect on the selected fungal and termite in their researches respectively.

Cerbera odollam belongs to the poisonous Apocynaceae family (Corner, 1952). The trees grow in coastal swamps, creeks and along riverbanks in a lot of Asian countries. The Burmese mixed the oil from the seed of *Cerbera odollam* with other oils as insecticide. In Malaysia, the oil from seed has been applied as an insecticide of hair mites (Gaillard et al., 2004). The fruit and seed of *Cerbera odollam* has been used against mice in many Asian communities.

Based on the literature, the kernels of *Cerbera odollam* contains cerberin which is a potent alkaloid toxin related to digoxin (Gaillard et al., 2004). The oil from the seed of *Cerbera odollam* is not the only part which contains toxic compounds. Other parts of *Cerbera odollam* are also the toxic. The immediate and delayed toxicity on the central nervous system of *Cerbera odollam* leaf extract was studied in mice by Minh Hiˆen et al. (1991). The leaf appeared to be quite devoid of the marked toxicity found in the seeds. The root of *Cerbera odollam* contains some of cardenolides that belongs to steroid (Chang et al., 2000).

With the conventional application and the research studies that has been done on the *Cerbera odollam* previously, we believe *Cerbera odollam* contain bioactive compounds that could potentially be used against wood rotten fungal and termites. It has great potential as new resource to be developed as environmental friendly preservative. However, to my best knowledge, the study of the potential of chemical compounds contain in *Cerbera odollam* against wood rotten fungal and termites has not been done yet. Therefore, this research investigates the potential in incorporating *Cerbera odollam* extracts to increase the durability of the wood and wood based panel against fungal decay and termite decay.

1.1 Objectives

The main objectives of this research were to study and investigate the potential of extracts from *Cerbera odollam* and to increase durability of wood and particleboards from fungal and termite decay. The specific objectives are as follows:

1. To analyse extracts of different parts of biomass component of *Cerbera odollam*
2. To investigate the effectiveness of various biomass components of *Cerbera odollam* against fungal attack and termite attack
3. To investigate the effect of incorporating the extracts on the properties of particleboard

2. Literature review

2.1 Cerbera odollam

Cerbera odollam belongs to the kingdom of Plantae, division of Magnoliophyta, and class Magnoliopsida, with order Gentianales and Genus *Cerbera* (Corner, 1952). *Cerbera odollam,* known as suicide tree, belongs to poisonous Apocynaceae family. *Cerbera odollam* is usually found at coastal swamp, riverbank, creep in a lot Asian countries, it can be seen along north south highway in Malaysia. It is one of ornamental plants in many cities in Malaysia including Penang. Figure 2.1 shows mature trees of *Cerbera odollam* planted as ornamental plant in Penang.

Figure 2.1: *Cerbera odollam*

The average height of mature tree of *Cerbera odollam* is usually around 6-15m. Generally, *Cerbera odollam* have dark green leaf, aroma flower and mango like fruit (Figure 2.2). The seed located in the centre of fruit is covered with protective kernel. The whole tree of *Cerbera odollam* contains milky white latex. Pong Pong is the local name of *Cerbera odollam* given by South Asian (Gaillard et al., 2004).

Figure 2.2: Fruit of *Cerbera odollam*

The toxicity of *Cerbera odollam* has been discovered previously, however, its poison has not been fully understood, and only been utilised conventionally (Gaillard et al., 2004). The Burmese applied the oil of seed for lighting and to kill mites. Malaysian used its seed to kill hair mites and mice.

Cerbera odollam contain Cardenolides compounds, such as cerberin, neriifolin, cerberoside, odollin, cardenolides are sub type of class of steroids, and it is poisonous and cause heart arresting (Gaillard et al., 2004; Laphookhieo et al., 2004). Most of plants that contain cardenolides are used for defence mechanism.

According to Gaillard et al. (2004), *Cerbera odollam* tree is the tree with most high record responsible for human death by suicide. Half of the *Cerbera odollam* kernel is sufficient to kill an adult life within few hours if without any immediate proper rescue. Kerala, a state of India, has the highest record of death attributed to *Cerbera odollam*. This is due to the distribution of the tree in Kerala, ease of availability of the fruit. Furthermore, some of the deaths credited by *Cerbera odollam*, were caused by the child mistaken with the shape of mango like fruit as good fruit and consumed it.

2.2 Rubberwood

Hevea brasiliensis is a tropical tree, initially grew in Amazon rainforest. Rubber tree has been introduced into Malaysia in end of 19 century by British Colonial Council. Referring to Corner (1952), rubber tree belongs to the kingdom of Plantae, and family of Euphorbiaceae with Genus *Hevea,* and species *H. brasiliensis.*

The major economic important of rubber tree is the latex, which is a primary source of natural rubber. The cycle of the rubber tree producing latex is approximately 26-30 years. After it stops producing latex, usually rubber tree wood will be utilized for the next major economic important, usually will be used in manufacture furniture (Matan, 2008).

Rubberwood is one of the common wood resources used in Malaysia for wood furniture product. It is estimated about 80% of the furniture industries in Malaysia are using Rubberwood as raw materials (Sulaiman, 2008). Rubberwood is also used as material for wood panel in Malaysia. For example, according to MTC (2009), rubberwood is one of main source of fibre to produce medium density fibreboard in Malaysia.

2.3 Wood degradation

Natural wood which is unpreserved is easy to biodegrade. The wood and wood products may have change in physical and mechanical properties when biodegrade. Basically, the major changes in wood properties are associated with biodegradation including reduce in strength, lost of weight, more hygroscopic, increased permeability and decrease in dimension (Zabel and Morrell, 1992).

Furthermore, wood decay microbial (microorganism) such as wood rotten fungal, and wood destroying insects (organism) such as termites, are unable to distinguish waste wood and useful wood (Richardson, 1993). Thus, wood and wood products have encountered enormous lost in term of economic and resources worldwide annually.

2.3.1. Wood rotten fungal

Wood rotten fungal is the type of fungal that attack woody plant to obtain the basic of needs and might defect wood properties. Wood rotten fungal have same morphology like other fungal. Fungal belongs to kingdom of fungi. However, there was misunderstood of fungal morphology, where people have been convinced and taught that fungi are plants. Fungi are not plant. The fungal cell is a segment of a tubular hypha, in the mycelia of fungal, branch formation of fungal is equal to cell division in animals and plants (Moore, 1998).

Fungal have certain requirements for living and surviving like other living organism. If it meet the suitable condition, fungal are able to grow rapidly. The major needs of fungal to grow in wood are water, oxygen, favorable temperature range from 15°C – 45°C, digestible substrate to provide energy, favorable pH range from 3 to 6, and other chemical growth factors such as nitrogen compounds, vitamins etc. Some of the fungi require visible light to develop spore bearing structure. For fungal that can be found on plant, including wood rotten fungal, the absence of toxic extracts is one of the important parameter for fungal to live (Zabel and Morrell, 1992).

As mention earlier, nevertheless, fungal like plant and animal, they also need nutrient to metabolise and survive. Generally, there are 3 modes of fungal obtain nutrient, First is called *saprotrophs*, where fungal obtain nutrient from dead organic material not killed by fungal itself, Secondly, *Necrotrophs*, where fungal obtain nutrient from dead organic material invaded, killed by fungal itself, lastly, *biotrophs*, where fungal obtain nutrient from invaded host cells, and the invaded host cells remain alive (Moore, 1998).

Carbon and nitrogen are the major groups of nutrient source, which are important for cell structure and functionality (Raychaudhuri et al., 1975). Wood is one of natural organic material, it can also be source of the nutrient for wood rotten fungal. Wood is bio resource that consists of large amount of cellulose, hemicellulose, and lignin, and also small amount of lipids, protein, nitrogen compound and other organic compound that contribute as source of nutrient and energy for wood rotten fungal.

Basically, wood decay fungal was sorted into white rots, brown rots, and soft rots. White rots break down lignin and followed by cellulose. Brown rots break down cellulose and hemicelluloses. Soft rots are able to break down cellulose, hemicellulose faster than lignin, but soft rots grow slower than brown and white rots. The major nutrient and energy resource for wood rotten fungal belong to sugar either mono saccharides or disaccharides, obtained by breaking down cellulose and hemicellulose (Moore, 1998).

2.3.2. Termites

Termite belongs to the kingdom Animalia, and classified as Insecta, with order of isoptera. Termites are group of hemimetabolous social insect. It is different from hymenopteran social insects like ants, bees and wasps. Termites usually bisexual and have unknown sub-social group. Cellulose is source of food for most of termites, therefore, termites usually decay cellulose contain in dead plant material. The dead plant material can be wood, leaf litter, soil and etc. Termites are self-organized and cooperative, in term of finding food and exploit food source and environments. (Krishna and Weesner, 1969)

Basically, there are four main types of termites: subterranean, drywood termites, dampwood termites and harvest termites (Wilkinson, 1979). A typical colony contains reproductive unit, workers, soldier and nymphs. The reproductive unit can be divided into primary reproductive and the supplementary reproductive. The primary reproductive is consisting of king and queen, and the supplementary reproductive which are short wing or without wing pad compared to primary reproductive. In the mature colonies, the population can hit up to several hundred or several million individuals. A worker termite is in charge of foraging of food, brood, and nest maintenance. Soldiers with long and powerful mandibles take lead on the defence for the community against predators (Krishna and Weesner, 1969).

Not all termites are classified as pest. There are only about 10% of termite species that can cause severe decay to building, crops and plantation. Among group of termite, subterranean termite is a major group of pests in urban of Malaysia. *Coptotermes* termites are one of the common structural pests (Lee, 2002). All species of *Coptotermes* consume wood, and 28 species of *Coptotermes* termites are important structural pests. *Coptotermes gestroi* is one of the species among these 28 species. It is primary pest species of *Coptotermes* come from Indo-Malayan Region (Jenkins et al., 2007).

2.4 Particleboard

The first industrial particleboard production began in early of 1940 during the World War II in Bremen, Germany. Particleboard was intended to replace plywood which were not afford to be manufactured due to lack of lumber during World War II. Nowadays, particleboard shows the rapid growth in the world market, including Malaysia. According to MTIB (2008), in year 2007, the export of particleboards of Malaysia reached RM 365 million, compared to 2004, only RM196 million.

Generally, the process of making particleboard involves few basic steps, including wood chipping, screening, blending, mat forming, hot press and finishing. Chipping is the process of reducing the size of wood source to the required size and shape. Screening removes the particle with size that is larger or smaller than required. Usually, the large particle will be sent to chipping again, and the fine will be utilized in the finishing for smoothing the surface of the board. The process of blending is to mix the selected wood particles with adhesives. In addition, additives such as wax and preservatives will be added. The mixture will be formed into mat of particles according to the required specific weight in specific volume. The mat will be then hot pressed under specified pressure. In this process, the pressure improves the contact of adhesives with particles and maintains the thickness of the board, and the temperature of hot press cures the adhesives. The process of finishing such as sanding, cutting, is the final step of the process of making particleboard (Shawkataly and Hashim, 2004).

There are many factors that can influence the properties of particleboard. These include moisture and relative humidity, type of adhesives, density of the board, and also, the extractives of the wood particle itself. The extractives can provide the positive and also negative effect to the particleboards. For example, the wax and oil containing in the wood can be a problem for adhesion between wood and adhesives. However, in the same time, wax and oil can improve the water resistant ability (Moslemi, 1935).

2.4.1 Wood based panel adhesives

In the wood based panel, the major components consist of wood material and adhesives, and in addition, additives. Adhesive acts as a binder to bond the wood material together. The adhesive on the surface of wood materials joins the wood materials together and resist separation. The strength of adhesion is one of the important elements in wood panel. It is one of the major factors for the strength of the wood panel.

Wood based panel adhesives essentially are solid. However to achieve the intimate contact, it is usually applied in liquid form (Wake, 1976). Generally, adhesives in liquid form are able to penetrate into wood material pits easily, thus, increase the adhesion between surfaces. Other than adsorption, adhesion between wood materials surfaces also depends on many parameters. These parameters include surface of wood material, chemical composition of wood material, contact area, quality of contact and etc (Shawkataly and Hashim, 2004).

There are few common artificial adhesives widely used in wood based panel industries, including urea formaldehyde resin, phenol formaldehyde resin, melamine urea formaldehyde resin, tannin formaldehyde resin, isocyanate resin, thermoplastic adhesives and etc.

2.4.1.1 Phenol formaldehyde

Phenol formaldehyde resin is the earliest commercial synthetic adhesives. Phenol formaldehyde resin is developed in 1909, and established in 1940 (Shawkataly and Hashim, 2004). Phenol formaldehyde resin is one of the most common used adhesives in wood based panel. Theoretically, the functionality of phenol is three and functionality of formaldehyde is two. Which means, phenol can react with formaldehyde at any one of three possible sites of ortho and para, while formaldehyde can react with up to two phenols. The actual functionality found in polymer depends on the phenol: formaldehyde ratio (Pizzi, 1983).

Generally, phenol formaldehyde can be prepared by two different methods. The first type of phenol formaldehyde is resol. Resol is synthesised from benzene with excess formaldehyde with alkali catalyst. The second type of phenol formaldehyde is novalacs. This is involves the reaction of excess phenol with formaldehyde with acid catalysts. In the particleboard formation, the resol type of phenol formaldehyde is commonly used, either in liquid or powder form (Pizzi, 1983).

Phenol formaldehyde resin is usually applied in wood based panel for exterior usage. Phenol formaldehyde has strong adhesion for wood panel and good resistance properties. Phenol formaldehyde has good water resistance in both cold water and hot water. Phenol formaldehyde resin also has good resistant to common organic solvent, weak acid and weak alkali, fungal and insect. Furthermore, phenol formaldehyde has high thermal resistance (Shawkataly and Hashim, 2004).

However, phenol formaldehyde does have some disadvantages. Phenol formaldehyde requires higher curing temperature, approximately 140-160°C. Indirectly, this increases the cost of production, consuming more power and energy to produce higher temperature in hot press. Furthermore, phenol formaldehyde leaving dark colour to the wood product, which the dark colour might not be desirable with the end product in term of aesthetic standpoint. Phenol formaldehyde might have compatibility problem with some of the additives such as wax emulsion (Moslemi, 1935).

2.4.1.2 Urea formaldehyde

Urea formaldehyde is one of the thermoset resins. Urea formaldehyde belongs to aminoresin. Other than urea formaldehyde, examples of other aminoresin include melamine formaldehyde, aniline formaldehyde melamine urea formaldehyde. Urea formaldehyde can be produced with the oxidation of methanol prepared from carbon monoxide and hydrogen, or from petroleum (Pizzi, 1983).

Usually, the ratio of urea: formaldehyde is in between 1:1.4 – 1:2.2. By increasing the portion of formaldehyde, the resins can provide better water resistance properties, high reactivity and good mechanical properties to wood panel. However, the price is expensive due to the price of that formaldehyde is more expensive than urea. Furthermore, high ratio of formaldehyde to urea will increase the formaldehyde release of end product (Shawkataly and Hashim, 2004). For interior usage of particleboard, low ratio of formaldehyde to urea usually is preferable.

Generally, urea formaldehyde needs to cure in acidic condition. Ammonium chloride is one of the common additives as accelerator use to cure urea formaldehyde. Ammonium chloride will react with formaldehyde and form hydrochloric acid. Small amount of ammonium chloride is sufficient to accelerate the curing rate. The consumption of ammonium chloride usually is less than 1.5% of the solid weight of urea formaldehyde resins (Shawkataly and Hashim, 2004).

Urea formaldehyde is widely used in particleboard industries. This is because of its low cost compared to phenol formaldehyde. Secondly, urea formaldehyde has good thermal properties and good in anti flammable. Furthermore, by comparing particleboard bonded with phenol formaldehyde, particleboard bonded with urea formaldehyde showed better appearance properties. Thus, particleboard bonded with urea formaldehyde is usually intended for interior application, due to the fair appearance of the product. Furthermore, the bonding strength of particles with the urea formaldehyde is sufficient for interior application (Moslemi, 1935; Pizzi, 1983).

However, urea formaldehyde has some disadvantages. The adhesions of the urea formaldehyde can be interrupted by water and moisture, due to the hydrolysis of aminomethylenic bond. Urea contain in the urea formaldehyde has high solubility in water. Thus, wood panel bonded with urea formaldehyde will show high thickness swelling rate. Due to the weak water resistance of urea formaldehyde, particleboard bonded with urea formaldehyde is intends for interior usage than for exterior usage (Pizzi, 1983).

2.4.1.3 Formaldehyde release

The common adhesives widely used in wood panel are urea formaldehyde, phenol formaldehyde and melamine formaldehyde. Particleboard is one of the common wood panels used formaldehyde based resins as binder. Formaldehyde emission from formaldehyde resins begins during its manufacture as well as during its usage.

According to Roffael (1993), the formaldehyde release mainly irritated the upper respiratory tract. However, due to strong odour of formaldehyde act as warning, gas poisoning by formaldehyde is seldom occurred, but the hazardous of formaldehyde must be concerned. Based on the investigation of United State Formaldehyde institute mentioned by Roffael (1993), they found that a monkey placed on concentration as low as1ppm formaldehyde concentration with 22 hours daily for 6 months suffered nasal mucous membrane irritation. However, this might varies from human, and different people will have different reaction.

Due to its hazardous, many countries and the world significant material testing and methods organisation such as America Standard Testing and Materials, European Standard, British Standard, has set the regulation of formaldehyde emission of wood based panel bonded with formaldehyde resins (Meyer, 1986).

2.5. Wood preservation

There are many wood species that are durable to biological attack. However, the price of these kinds of wood species is not reasonable to produce low end or moderate wood and wood products. In wood and wood panel industries, low cost wood are preferable. Most of the low cost wood belongs to non durable wood species. These non durable wood species are not resistant to biological attack, such as fungal, termite, and other pests. The wood lumber, wood panel and wood product infected by biological attack might defect on its strength and outlook, and will lead money lost to industrial and end user.

Thus, wood preservation is an important process in wood and wood panel industries, to ensure the long life of the wood and wood panel, especially if the wood and wood panel are manufactured from non durable wood species. The purpose of wood preservation is to increase the durability and resistance of wood and wood products from being destroyed by wood biological attack. Furthermore, the price of wood is keep increasing due to the conservation of wood resource. To avoid unnecessary lost caused by biological degrade, wood preservation is necessary (Richardson, 1993).

Most of the common preservation chemicals use nowadays consist of artificial chemical compounds, such as chromated copper arsenate (CCA), borate compounds (Zabel and Morrell, 1992). These compounds have high toxicity and slow degradation rate. These substances might cause pollution. The treatment run off might pollute the soil and the next, underground water source. These bring serious immediate and long term consequences. Thus, solutions to alternative artificial chemical preservatives are necessary and urgent.

Nowadays, people are more aware about the environment problem. The enforcement of laws and the concern of people about environment, the action to replace the non environmental friendly artificial chemical product with environmental friendly product progresses rapidly. It includes finding new solution to alternative artificial preservatives which are widely available in industries.

2.5.1. Antifungal agent

Artificial antifungal agents is a chemical that apply to inhibit or kill the fungal by damaging the cell components of fungal, either affecting the proteins, membrane or other cell components of fungal. Chemical antifungal agents are used in many sectors, including wood industries, food industries and etc. Usually, chemical antifungal agent is used to preserve the products. Chemical antifungal agents are also applied in medical field for sanitary and sterilization purpose (Black, 2005).

The effectiveness of antifungal mechanism via chemical agent was affected by time, temperature, pH and concentration. Focus on aspect of concentration, high concentration of chemical agents is usually giving effect of killing, whereas low concentration of chemical agents is giving effect of growth inhibiting. (Black, 2005)

Generally, few of the antifungal chemical agents have been discovered. It can be grouped into acid and alkali, heavy metal, halogen, alcohols, phenols and other agents. These agents have different general principles of sterilization and disinfection on fungal. In term of antifungal, alkali are able to destroy microbes whereas acids can lower the pH of material. Selenium, mercury, copper and silver are the common heavy metal usually used in chemical agents to inhibit fungal growth by disturb on the protein of the cell. Halogens such as chlorine and iodine have been well known as antifungal agent in daily life. Alcohols when mixed with water, is able to denature protein of fungal cell. Phenol and its derivatives able to disturb cell membrane, denature protein of fungal (Hedgecock, 1967; Black, 2005).

2.5.2. Insecticide

Insecticide is a pesticide used against insects. It is a toxic chemical applied to resist insect attack. Insecticide is commonly used in agriculture, industry, household and etc. Most of the artificial chemical insecticide alters ecosystem and toxic to humans. It is necessary to balance the needs and with environmental and health issue when using insecticide.

The insecticide is used to preserve wood to resist from insect attack, usually can be waterborne preservatives or oilborne preservatives. The waterborne preservatives included chromated copper arsenate, chromated zinc chloride, ammoniacal copper arsenate and etc. The oilborne preservatives included coal tar creosote, pentachlorophenol, copper naphthenate and etc (Nicholas, 1973).

These preservatives are also able to resist most of the microbial attack. These toxic preservatives consist toxicant to fungal and bacteria. Thus, it is popular to use in industries, due to its ability to provide high efficiency of preservation to the product.

2.5.3. Extracts

Some wood species contain natural bioactive compounds that act as natural preservatives to prevent biodegradation. The natural preservatives occur in wood are related primarily to the presence of the extractives that are toxic to the wood decay microbial or insect. Extractives are one of the components contain in the wood composition and represent in minor fraction, and soluble in polar or non polar solvents (Zabel and Morrell, 1992).

The decay resistance of wood have variation. First, different species have different susceptibility of durability status. Secondly, the position in the wood itself does also have different durability status due to the bioactive compounds not distributed evenly in the wood (Zabel and Morrell, 1992). The phenomenon of different durability status at different position is not only limited in the wood trunk. The different parts of tree such as leaf, fruit, flower also consists of different type of extractives and different amount of yield of extractives, thus, the durability status is also different.

These extracts have the importance to the wood itself and also to our life. For example, to the wood, the extracts such as fats can act as energy resource to wood cell, and also as defence system (Browning, 1963). To our life, the extracts have been utilized for many purposes, including medical, leather tanning, cosmetic, food and beverage, and etc.

Recently, many researches have been carried and revived the potential of the extracts of durable wood species work as preservatives for non durable wood species. The initial of the research in reviving the potential of extracts as wood preservative is because the raise of concern of environmental problems leads by artificial chemical.

From the previous studies done by many researchers, there are many compounds contain in extracts potentially work as preservatives for wood and wood based panel product, for anti wood decay fungal, such as aroma group compound (Yen et al., 2007), essential oil (Temiz et al., 2008), phenolic and terpenoid compound (Kawamura et al., 2004) and etc, and for antitermitic, essential oil compound (Cheng et al., 2007), quinone compound (Ganapaty et al., 2004) and etc.

The essential oil is responsible for the odours characteristic of the fresh wood. They are usually liquid and have boiling point over 100°C. Although essential oil has only consisted of carbon, hydrogen and oxygen element, however, each individual compound of essential oil have different characteristic. The essential oil usually includes other chemical substances belong to the class of hydrocarbon, acid and phenol, alcohol, ester, ether, aldehyde (Wise and Jahn, 1952; Browning, 1963)

Many phenolic compounds are deposited in heartwood but there are also small amount of phenolic compounds present in the sapwood. It is one of the important compounds in the extracts. Most of the wood durability is credited to the phenolic compounds. Other than active in microbial and insect, phenolic compounds are also characterized with the antioxidizing properties and act as antioxidant in wood (Wise and Jahn, 1952).

Usually fat are concentrated in animal tissue, but fat also contain in the wood of some species. In the sapwood, most of the fatty acid appears as triglycerides. In some of the hardwood, the fatty material concentrated in the ray cells (Browning, 1963). Sterols present as minor component in plants fat. Sterols are one of the important chemical compounds in wood science. Sterols are secondary alcohol and usually contain 27 – 29 carbon atoms. One of the sterols found in plant is sitosterol, it is a notable compound in phytochemistry.

There are still many chemical compound contain in wood extractives, including tannin, quinones, flavonoids, nitrogen compounds, sugar, inorganic compound and etc. Each of the compounds has its own characteristic and role in wood.

3. Methodology

The general flow charts of overall methodology are shown in Figure 3.1 and Figure 3.2.

3.1 Sample preparation

Fresh samples of various biomass components including leaf, fruit, wood, bark, flower and seed of *Cerbera odollam* were collected around Penang, Malaysia. Samples of fruit, leaf, flower, bark and seed of *cerbera odollam* were cut into small pieces. The wood sample was grounded into small particles, sieved and passed through a mesh of 2mm aperture size. All samples were freeze dried with setting below -40°C and 12 Pa to remove excessive moisture.

Moisture content of each sample was determined as follows:

About 0.3g of each type of freeze dried samples was put into oven at 103±2°C for 3 days. Samples were taken out from oven and put in desiccators immediately for 15-30 minutes. After samples were cold, samples were taken out and weighed immediately.

Moisture content (%) = $[(m_a - m_b)/m_a] \times 100$

Where m_a is freeze dried weight, and is m_b oven dried weight.

3.2 Extraction

3.2.1 Extracts for antifungal assay

The extraction was conducted according to Kawamura et al (2004) with modification, successive extraction was replaced with series solvent extraction. The freeze dried samples were extracted with *n*-hexane, ethyl acetate, ethanol and methanol respectively. Each extraction consisted 3 cycles of duration. First cycle consisted of 1 h, second cycle consisted of 2 h and third cycle consisted of 3 h of extraction. The extract was collected