# DOCTOR OF PHILOSOPHY

## Wood Extractives as Natural Preservatives against Termites and Fungi

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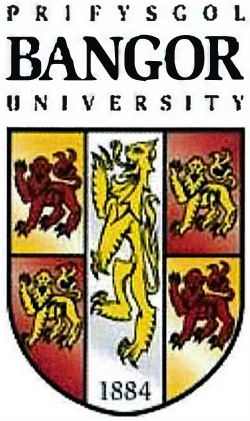
# WOOD EXTRACTIVES AS NATURAL PRESERVATIVES AGAINST TERMITES AND FUNGI

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By

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## ABSTRACT

### Wood Extractives as Natural Preservatives against Termites and Fungi

The importance of factors contributing to the natural resistance of twelve Malaysian wood species was studied.

Twelve Malaysian hardwoods species were selected from three different durability classes. The tests involved physical properties, fungal and termite natural durability, and extractives as antitermitic, antifungals and antioxidants. The wood densities were examined in order to know the variation between and within wood species. Statistical analyses indicated that significant differences existed between and within trees for density. Density was largely influenced by wood species while within tree, it was significantly affected by location of the samples.

For the natural durability of the outer heartwood of these twelve wood species, a series of tests against biological agents were done. Tests with two species of subterranean termites (*Coptotermes curvignathus* and *C. gestroi*) and three species of white-rot fungi (*Pycnoporus sanguineus, Trametes versicolor* and *Lentinus sajor-caju*) showed that *N. heimii* was the most resistant and *H. brasiliensis* the most susceptible wood against all biological agents. However, the order of resistance and susceptibility of the other wood species varied to a minor extent to the challenge by termites and fungi.

Extractives were quantified from all species. Standard extraction with Toluene: IMS (2:1) according to standard method ASTM D1105-96 (2001) was followed by hot water extraction. Results showed that high amounts of wood extractives were found in the durable class timbers and lowest from non-durable class. The highest yields were detected in bark and the lowest in heartwood.

Bioassay studies were done to investigate the toxicity of wood extracts to termites and fungi. Extracts from four selected wood species (*N. heimii, M. utilis, C. lanceolatum* and *S. curtisii*) were impregnated into filter paper discs (termite test) and *H. brasiliensis* blocks (fungus test) in different concentrations. The above termite species (*Coptotermes curvignathus* and *C. gestroi*) were used and the brown-rot fungus (*Coniophora puteana*) was added to fungus test together with *T. versicolor* and *L. sajor-caju*. The study showed some very promising results. Both subterranean termites (*C. curvignathus* and *C. gestroi*) had high mortality with *N. heimii* extracts at the highest dose, 2%, whereas other species gave lower mortality. *S. curtisii* had the least effect with some 30% survival at the highest dose. However higher concentrations were needed to protect the wood from fungal decay.

A study was also undertaken to examine the antioxidant activity of the wood extracts of all twelve wood species. Two tests (total phenolic content and radical scavenging activity – DPPH) were performed for bark and heartwood extracts. The differences in heartwood mass losses were best explained by the antioxidant capacity as well as by the concentration of total phenols determined by the Folin-Ciocalteu method. A significantly higher total phenolic content and stronger antioxidant activity were measured in *N. heimii* bark and heartwood extracts compared to the other extracts examined in this study. However, some of the extracts showed higher values than some well-known antioxidant-rich plant extracts and synthetic antioxidants.

Correlation analyses were run to explore the relationships between the properties studied. This study found that wood density had negative correlation with durability against termites but not with fungi. Wood extractive also has a significant influence on the durability rather than wood density. However, it is observed that higher wood consumption was generally obtained in species with lower wood density and lower extractive contents. The results of this study indicated that in some species there is a strong relationship between densities and extractive contents with durability and that this could be used as a tool to determine the durability of Malaysian hardwoods.

Correlation also exists between the total extractives and total phenolic contents in majority of wood species studied. The total extractive contents had a significant positive correlation with total phenolic content and thus gave a significant correlation between durability and total phenolic content. It was also found that total phenolic content was significantly correlated with antioxidant activity.

In parallel to the termites, fungus, bioassay and antioxidant studies, the extractives of the most durable wood species, *N. heimii,* were characterised by MALDI-TOF. Analysis of methanol extracts afforded two major polyphenols; stilbenes (resveratrols like derivatives as polymers) and flavonoids (kaempferol) which were suspected as defence against both termites and fungi. These two classes of compounds (polyphenols) also could be the reason for higher antioxidant activity in *N. heimii*. A possible way ahead for wood preservation could be to examine in more detail the nature of polymerisation of such compounds inside wood cell walls during heartwood formation.

## GLOSSARY

| | |
|---|---|
| ANOVA | Analysis of variance |
| AS | American Standard |
| AWL | Average weight loss |
| AWPA | American Wood Preservers' Association |
| BHA | Butylated hydroxyanisole |
| BHT | Butylated hydroxytoluene |
| Cc | *Coptotermes curvignathus* |
| Cg | *Coptotermes gestroi* |
| Cp | *Coniophora puteana* |
| DHB | 2,5-dihydroxybenzoic acid |
| DPPH | 2, 2-diphenyl-2-picrylhydrazyl |
| FT-NIR | Fourier Transform Near Infrared |
| GC | Gas Chromatography |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| HPLC | High Performance Liquid Chromatography |
| HR | Highly resistant |
| kg $m^{-3}$ | Kilograms per cubic metre |
| Lsc | *Lentinus sajor-caju* |
| MA | Malt-agar |
| MC | Moisture content |
| mg | Milligram |
| min | minutes |
| ml | millilitre |
| MALDI-TOF | Matrix-Assisted Laser Desorption/Ionization Time-of-Flight |
| MR | Moderately resistant |
| MS | Mean of squares |
| MSD | Mass Selective Detector |
| nm | nanometre |
| NIST | National Institute of Standards and Technology |
| NMR | Nuclear magnetic resonance |
| ppm | parts per million |
| p value | statistics indicator to test null hypothesis |

| | |
|---|---|
| Ps | *Pycnoporus sanguineus* |
| R | Correlation coefficient |
| r | Resistant |
| $r^2$ | Coefficient of determination |
| RH | Relative humidity |
| ROS | Reactive Oxygen Species |
| r value | statistics values to determine the relationship between the factors |
| SD | Standard deviation |
| SS | Sum of squares |
| TBHQ | Tertiary-Butylhydroquinone |
| TIMSW | Toluene: IMS: Hot water |
| TLC | Thin Layer Chromatography |
| Tol:IMS | Toluene: Industrial Methylated Spirit |
| Tv | *Trametes versicolor* |
| *µl* | Microlitre |
| WC | Wood consumption |
| WL | Weight loss |

**Published peer-reviewed articles related to the work in this thesis**

1. Roszaini, K. and Mike D. Hale. 2010. Comparative termite resistance of twelve Malaysian timber species in accelerated laboratory tests. *Holzforschung* (accepted-manuscript review – Ref. No.: HOLZ-D-10-00139R1).
2. Roszaini K., Mike D. Hale and Salmiah, U. Variation in the natural decay resistance of twelve Malaysian broadleaved trees (hardwoods) as a function of wood density and extractives compounds (*Wood Science and Technology* – under review - Ref. No.: WST-11-0114).

**Conferences where work related to this thesis was presented by the author**

1. Roszaini, K. and Mike D. Hale. 2010. Does the activity or amount of extractable compounds account for antitermitic activity in durable Malaysian hardwoods? *PlantMicro Wales Meeting. 12 – 13th July, 2010. University of Aberystwyth, United Kingdom* – First Prize Poster.

2. Roszaini, K. and Mike D. Hale. 2010. Heartwood extractives of different Malaysian broadleaved tree (hardwoods) species and their relationship to termite and fungal decay resistance. *PlantMicro Wales Meeting. 12 – 13th July, 2010. University of Aberystwyth, United Kingdom.*

3. Roszaini K. 2010. Toxicity and anti-termite activities of the wood extractives from tropical heartwood species. *College of Natural Sciences PhD conference day 14th June 2010, Bangor University.*

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# CHAPTER 1: INTRODUCTION

One of the most important processes in nature is the microbiological degradation of lignocellulosic materials (Blanchette, 1995). Decay by fungi, beetles, marine borers and some termite species is important while bacteria are of lesser significance on wood. These organisms cause detrimental effects in wood quality, including unwanted staining and various forms and levels of decay and browsing damage (Istek *et al*, 2005; Goncalves and Oliveira, 2006). Use of either naturally-resistant wood, preservative treatments or engineered wood products with enhanced beetle/termite/decay resistance represents a final line of defence for structural protection. In many structures, proper construction techniques, physical or chemical barriers and baiting systems can be used to prevent termites and fungi from ever entering the structure, however in contact with the ground or immersed in water, its needs some treatment.

Generally, conventional wood preservative techniques rely on biocides containing copper (e.g. CCA, copper azole, copper quat) and boron salts or a variety of organic preservatives, e.g. phenols, and a variety of inorganic salt preservatives (Crawford *et al,* 2000; Peylo and Willeitner, 2001; Humphrey, 2002). Creosote has been used as a preservative since 19$^{th}$ century for treating transmission poles and railway sleepers. For more than 50 years, the waterborne preservative chromated copper arsenate (CCA) was the preferred treatment for the preservation of wood against fungal and insect attack for most applications and environments (Micklewright, 1992). This preservative gave high level of protection against micro-organisms and insects at an economically favourable cost as compared to preservatives based on copper and organic fungicides or those only based on organic fungicides (Edlund *et al*, 2006).

However, this and other traditional wood preservatives, including creosote oil and PCP (pentachlorophenol), have caused various environmental problems due to unintended release at some point during their life cycle at the production, transport or treatment stage (Lesage and Jackson, 1992; Lebow *et al,* 1999; Ejechi, 2001; Lyytikäinen *et al*, 2001; Lebow *et al,* 2003; Schultz *et al*, 2006) or losses in service by leaching, volatilisation or mechanical wear.

From an environmental perspective, finding natural substances in highly durable tree species and understanding their mechanisms is an appropriate approach to achieving wood protection which may have less environmental impact (Chang *et al*, 2000).

A trend towards less hazardous wood preservatives has been apparent for the last 20 years. The use of some traditional wood preservatives is already restricted by legislation. For example, the Environmental Protecting Agency (EPA) in the USA has banned the use of arsenicals in residential structures since 1 January 2004 (US EPA, 2002; FR, 2002) and the European Union also restricted the use of arsenicals from 30 June 2004 (www.bancca.org/CCA_References/EU_CCA_ban_notice). Other developed countries now have totally banned the use of Chrome-copper Arsenic (CCA) as a wood preservative (Berard *et al*, 2006). This type of preservative is only allowed in special purposes which located far from centres of population and in special professional applications (Vähäoja *et al*, 2005) and it is likely that other countries will follow this trend. It has been totally withdrawn and is unavailable throughout Europe. Due to concerns about the safety and environmental impact of preservatives and restrictions on the older established preservatives, less ecotoxic, low efficacy or corrosiveness, alternative products and methods are being developed and used. Pyrethroids have become key candidates for repellent termiticides out of ground contact (Yeoh and Lee, 2007) while Alkaline copper quat (ACQ), Copper boron azole (CBA), Copper dimethyl-dithiocarbamate (CDDC) and Micronized copper quats (MCQ) have been introduced as alternatives for ground contact use and various azole containing fungicide formulations have been introduced for less severe applications (Lebow and Winandy, 2003).

To date, new copper-based preservatives have been introduced as alternatives to arsenic and chromium containing preservatives. These have however been developed more to control fungal decay and lesser problematic insects than termites. Some termite species represent considerable challenges to control (Zhu *et al,* 2007). These preservatives have emerged on the market and had been approved by the Malaysian environmental authorities because they are more environmentally acceptable. However, their performance as wood preservatives against tropical hazards is often less well established.

It is in this context that many highly persistent and environmentally damaging biocides have been retained as termiticides and fungicides. Effective alternatives may be found as natural products and extracted from the bark and heartwood of naturally durable species. Understanding the capability of wood extracts to protect against termites and fungi is one possible approach for developing new termiticides and fungicides (Celimene *et al* 1999; Schultz and Nicholas 2002; Peng *et al*, 2004). However, it is important that the extractive chemicals should be biologically active, cost effective, environmentally

acceptable and have low toxicity to or no impact on non-target organisms (Hon and Shiraishi, 1991).

Wood extractives are naturally formed in trees particularly during heartwood formation and may have little environmental impact. Developing an extractive-based preservative may have less environmental impact because the commercial application of plant extractives has a long history and they are widely used in our everyday life for uses such as medicines, food, cosmetics and perfume (Franzios *et al*, 1997). In the case of termite control, extractives can act as feeding and oviposition deterrents, repellents, toxicants and anti-trail-following compounds. In the case of controlling fungi, they may be toxic, repellent or interfere with their decay mechanisms e. g. blocking the cell wall from hyphal penetration, inhibiting their enzymes, quenching the radicals or blocking the cell wall micropore structure thus reducing access of water and the degradative agents to their sites in the wood cell wall (Schultz and Nicholas, 2000; 2002; Schultz *et al,* 2005).

Since there are only a few naturally occurring compounds known of any single structural type, a comprehensive analysis of data for structure/action relations is difficult. This is more complex because there are different target organism groups e. g. termites and fungi. Although many reports have revealed that several wood species have shown natural resistance for many decades to termite and fungal infestation, surprisingly only a few of timbers in terms of their extractive content have been examined in detail (Wolcott, 1947; Sandermann *et al,* 1958; Mihara *et al,* 2005). Because of the complexity of the constituents and chemical structures, research is still being continued on the chemical properties and potential uses of the wood extractives.

Considering the importance to isolate and identify new materials for the preservative treatment of wood, information concerning extractives that make commercially important timbers resistant to termites and fungi would be of great value. Therefore, this study was undertaken to investigate the anti-termitic efficacy and anti-fungal properties of extractives of selected Malaysian heartwood species and their bark. It latterly developed into a study looking at actions which protected wood by non-fungicidal means, partly because the study failed to show pronounced fungicidal or fungistatic activity.

The following hyphothesis were put forward.

- Wood density is not a single factor for natural durability determination.
- Durable class timbers have higher extractives yield than non-durable timbers.

- Different wood species and different portions of the tree have different amounts of wood extractives.
- Extractives have dual functions. Besides toxicity, they also have antioxidant properties that could affect termites and fungal degradative abilities.

The first objective of the present study was to isolate and identify new materials effective for the preservative treatment of wood to inhibit termite and fungal attack. It also aimed to evaluate the influence of extractive compounds on the biodeterioration of Malaysian timbers.

A second objective of this study was to evaluate the individual efficacy of the major wood and bark extractives components in order to identify the main active compounds in crude wood extracts. Thus wood extractives efficacies were assessed for their ability to protect wood against termites, brown rot and white rot fungi using accelerated termite and decay tests. The crude extracts also were determined for their performance as antioxidants.

All data were subjected to one-way analysis of variance (ANOVA) using Statistical Analysis System (SAS). In this respect wood species, physical properties, natural durability and yield of extractive compounds were compared. All data were indicated as mean $\pm$ SD. The Tukey's Studentized Range Test was employed to compare all the properties studied between each wood species. The differences between means were considered significantly different when the ANOVA's P value test was less than 0.05. The correlation analyses also were performed to determine the correlation between each property.

The thesis is presented in nine chapters. The general introduction, this chapter, is presented in Chapter 1 while Chapter 2 discusses the current state of relevant knowledge about termites, fungal decay and wood extractives. Chapter 3 has quantitatively analysed the wood density. The extractive content from the bark and heartwood of each wood species and were determined quantitatively and qualitatively using standard solvent extraction techniques also in Chapter 3. Several *in vitro* assays including 2,2-diphenyl-2-picryl-hydrazyl radical scavenging assay and total phenolic content with Folin-Ciocalteu's method were conducted for antioxidant activities also in Chapter 3. The natural durability of all timber species tested against termites and fungi (in decay tests) are reported in Chapters 4 and 5, respectively. The activity of extractives from four timbers selected as limiting the decay fungi and increasing the termites' mortality were also determined and

presented in Chapter 6. In Chapter 7, details of the analysis of the methanol extractives of *N. heimii* using MALDI-TOF is reported. Chapter 8 examines the relationship between the main extractive components, density natural durability and antioxidant. The final chapter (Chapter 9) draws the general discussion, final conclusions, criticisms and scope for further study that should be approached.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Biological deterioration and hazard classes

Wood is a biodegradable material that principally consists of cellulose, hemicellulose and lignin. Cellulose has been reported partially resistant to microbial attack and lignin is extremely resistant to some decay fungi (Scheffer and Morrell, 1998) and when combined together in the solid state specialist decay organisms attack it. The ability of these natural agents (insects, termites and fungi) to decay wood and wood products results in large economic losses. It is difficult to quantify this directly but losses over a period of years are estimated to be substantial. However, different timber species exhibit different resistance to attack by these agents. In Malaysia the greatest degree of deterioration are caused by termites and wood-decaying fungi (Wong *et al*, 2000).

The demand for protection of wood differs according to different end-uses (Table 2.1). Normally CEN EN 335-1(2006) is used as a guide for use in different environments. However, it must be used together with e.g. CEN EN 113 (1997) for testing with pure cultures of white- and brown-rot fungi or with CEN EN 599-1 (2009) to evaluate the effectiveness of preservatives.

If the durability of individual logs or sawn timber could be measured rapidly by an indirect method, specific grading of timber would be possible according to its individual service life. Indirect durability measurements could be based on chemical, physiological, or anatomical properties that are associated with resistance to degrading organisms, such as rot fungi, or to abiotic factors, such as weathering. Promising techniques include prediction of the decay resistance by means of FT-NIR spectroscopy (Gierlinger *et al,* 2003) and colour measurements (Gierlinger *et al,* 2004a). It is generally agreed that extractives are ''the principal source of decay resistance'' (Scheffer and Cowling, 1966).

Table 2.1 Occurrence of biological agencies in various use classes (CEN EN 335-1, 2006)

| Hazard classes | General service situation | Description of exposure to wetting in service | Biological agents | |
|---|---|---|---|---|
| 1 | Interior, covered | Dry | Wood boring beetles | If termites also might be present the class is designated **1T** |
| 2 | Interior or covered | Occasionally wet | As above<br>+ Disfiguring fungi<br>+ Decay fungi | If termites also might be present the class is designated **2T** |
| 3 | 3.1 Exterior, above ground, protected | Occasionally wet | As above<br>+ Disfiguring fungi<br>+ Decay fungi | If termites also might be present the class is designated **3.1T**or **3.2T** |
| | 3.2 Exterior, above ground, unprotected | Frequently wet | | |
| 4 | 4.1 Exterior, in ground contact and/or fresh water | Predominantly or permanently wet | As above + Soft rot | If termites also might be present the class is designated **3.1T**or **3.2T** |
| | 4.2 Exterior, in ground contact (severe) and/or fresh water | Permanently wet | | |
| 5 | In salt water | Permanently wet | Decay fungi<br>Soft rot<br>Marine borers | A Teredinids, Limnoria<br>B As in A + creosote tolerant Limnoria<br>C As in B + Pholads |

No single wood species is immune to biodeterioration. In tropical countries, termites and fungi are important for durability classification. Normally, the classification of a timber with regard to its natural durability is often based on the least resistance against one of the above biological agents.

There are five durability classes under European Standards (CEN EN 335 Part 1, 2006) which refers to heartwood. Normally the sapwood will be classified under durability class 5, the least durable class. These classifications are based on either field test or accelerated laboratory test data.

A key point for the long service life is the minimisation of water exposure.

## 2.2 Termites

Termites are one of the world's premier social insects besides ants and bees. They are always reported as the most frequently dominant invertebrates and are mainly more abundant in tropical ecosystems rather than warm temperate climates (Wood and Sands, 1978; Wilson, 1993; Eggleton and Bignell, 1995). The termite contribution in terms of recycling wood and other plant material is significant for most of the world's ecosystems.

Their activities help to aerate soils, improved soil composition and fertility, and contribute significantly to atmospheric gas emissions, including $CO_2$ and $CH_4$ (Zimmerman *et al.* 1982).

The termites are members of the order Isoptera (Ebeling, 1968; Beal *et al,* 1983). The word Isoptera came from Greek work which "*iso*" meaning equal and "*ptera*" meaning wings (Harris, 1957; Thorne and Carpenter, 1992).

There are seven families: Hodotermitidae, Kalotermitidae, Mastotermitidae, Termopsidae, Serritermitidae, Rhinotermitidae and Termitidae which are divided into 14 subfamilies, 270 genera and over 2,800 recognized species. The latest total by Kambhampati and Eggleton (2000) and Ngee *et al* (2004) is more than 282 genera. However only about 6.6 to 8% of these are categorized as wood pests or significantly cause damage to timber in-service (Becker, 1969; Lee and Wood, 1971; Edwards and Mill, 1986; Kambhampati *et al*, 1996; Pearce, 1998; Verma *et al*, 2009). 70% occur in tropical and sub-tropical countries which include Taiwan, China, Malaysia, Japan, Guam, Midway, Hawaii, South Africa, Sri Lanka, north and south Americas. Generally, they are economically significant wherever they occur (Harris, 1958; Tho, 1992). The termites of Peninsular Malaysia comprise a total of about 175 species from 42 genera represented by three families; Kalotermitidae, Rhinotermitidae and Termitidae (Collins, 1988; Thapa, 1980; Tho, 1992).

All termites are social insects, living and working in small to large colonies (groups), a colony in some species containing almost a million or more individuals. The single colony consists of functional reproductives [primary; king and queen, and supplementary; either brachypterous (slightly pigmented with short wing pads) or apterous (very slightly pigmented without wing pad), workers, soldiers and immature individual (Blandford, 1896; Skaife, 1955; Wilson, 1968; Kumar, 1969).

### 2.2.1 Classification of termites

From a practical point of view termites can be divided according to the habitats/place which they are found into one or other of two broad groups; subterranean termites and drywood termites (Beeson, 1941; Findlay 1975; Desch and Dinwoodie, 1994). The major difference among drywood and subterranean groups is the requirement of contact of either soil or moisture by subterranean species. Drywood termites do not require contact with soil or moisture for survival.

#### 2.2.1.1 Subterranean termites

Literature had shown that subterranean termites are the most economically important group of pests in the world (Hickin, 1971; Pearce, 1997; Ahmed, 2000; Su and Scheffrahn, 2000). They attack not only wood but other cellulosic materials (Beal *et al*, 1994). Subterranean termites require more moisture for survival than drywood termites and they always maintain a connection with the soil (ground dwellers), often by the use of soil tubes. Subterranean termites in the genus *Coptotermes* (Isoptera: Rhinotermitidae) are not only responsible for most of the termite damage done to wood structures throughout Malaysia (Ahmad Said *et al* 1982; Lee, 2002a; 2002b) but also throughout the world (Edwards and Mill, 1986). This genus is widespread in lowland forest up to an altitude of approximately 1350 meters (Tho, 1992).

Subterranean termites live in large colonies in the ground, and must retain an unbroken covered earth way from the soil to their feeding grounds. They do not establish themselves in buildings by being carried there in lumber, but enter from ground nests after the building has been constructed. Often there is little indication of their presence but; 1) they construct soil tubes or runways built over the surfaces of foundation walls to reach the wood above, 2) the winged adults swarm at certain times of the year and 3) in the wood itself, the termites make galleries that generally follow the grain, leaving a shell of sound wood to conceal their activities.

#### 2.2.1.2 Drywood termites

Drywood termites which belong to the family Kalotermitidae are common on most continents and do not require contact with moisture or soil and also can tolerate dry conditions for prolonged periods. They get the water from the wood that they feed on and during the digestive process. They live in small colonies which are formed inside sound dead wood rather than in the soil (Gouge *et al*, 2001) and they do not construct soil tubes.

Drywood termites infest a wide variety of structures (including structural lumber as well as dead limbs of native trees, shade and orchard trees, utility poles, posts and lumber in storage) and eat wood rapidly (Cabrera *et al,* 1991). Their presence can be detected by the pellets that they produce and push out from the wood surfaces through small holes when the wood is attacked.

### 2.2.2 Biology

Termites are small (4 to 15 mm long) and variable in colour from white to tan and even black. They are known as social insects which live in colonies. In common with other insects in terms of their body structures, the termite body is divided into head, prothorax (pronotum) and abdomen. Only the reproductive and flying alates have compound eyes. In addition, they have a pair of antennae (packed with olfactory and touch sensors) for smelling and touching simultaneously (Blandford, 1898; Hamilton, 1972).

The colonies of ground dwelling termites (Rhinotermitidae and Termitidae) are always partly in the ground and in connection with it, and is started by a colonizing pair of male and female, which enter the ground or wood in the ground. There are 3 subgroups: subterranean, mound building and carton-nest-building termites. The wood dwelling termites (Kalotermitidae) consist of two subgroups: damp wood and dry wood termites. The colony is confined entirely to wood and is started by a colonizing pair, which enters the wood above ground at the time of swarming.

Termite colonies are comprised of three basic castes: workers (the bulk of the population), soldiers (develop from nymphs, pseudergates or workers) and reproductives (develop either from alates or neotenics). As a rule, a colony has only a pair of primary reproductives: a male (king) and a female (queen), which are generally imprisoned by the workers in a 'royal cell' and their sole function then is to reproduce. Fecundity is high and a physogastric queen in some species of *Odontotermus* sp. are known to lay almost an egg per second throughout a life of some years (Roonwal, 1970; 1975), and can reach up to 30,000 per day (Findlay 1975). The eggs are yellowish-white and take about 50 to 60 days to hatch. The workers and soldiers which are 6 mm long with the pale cream in colour are wingless and usually lack eyes (Myles, 2005).

#### 2.2.2.1 Life cycle

Termites need a reliable source of moisture, which normally they derive from both soil and wood. They have the ability to move their colony up and down in the soil to find the optimal temperature and moisture conditions. When termites feed on wood, which is separated from the nest by an exposed surface, workers build mud-covered shelter tubes or tunnels. Workers need a high humidity to survive and will carry mud up into the wood to maintain at 97% relative humidity. These tunnels function to preserve moisture levels and to protect the vulnerable insects from predators. Details of life cycle vary from species to species but in generally, the pattern below can give some general idea (Figure 2.1).

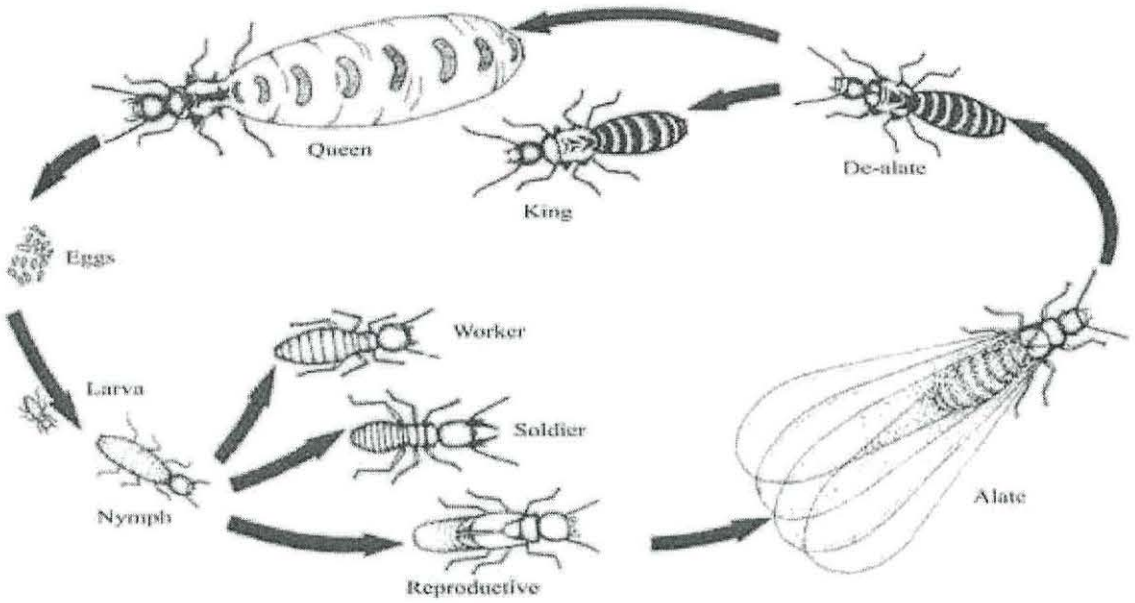



Figure 2.1 The life cycle of termites (modified from Hadlington, 1992)

All termites live a community life (castes, workers, soldiers and the males and females), which no one can survive alone. According to Randall and Doody (1934), termites have an incomplete metamorphosis life cycle. The life cycle of termites begins when the female (queen) lays eggs (paired with a male – king), which both parents look after until the first larvae emerge. After two moults, the young termites reach a stage when they may continue their development in three different directions to end up either as mature winged insects or as workers or as soldiers. The workers perform all the labour works in the colony such as obtaining food, feeding other caste members and larvae, excavating wood and constructing tunnels. Their life span is from three to five years. Another caste, the soldier caste, is responsible for the defence of the colony against its enemies, external and internal; it has a larger head and jaws than the worker and, in some genera is able to squirt out an irritating or sticky fluid. Another caste, termed nymphs, are sexual, becoming male or female and acquiring wings (two pairs) when full-grown; in their young stages they are termed nymphs (Krishna and Wegner, 1970; Verkerk, 1990; Watson and Gay, 1991; Myles, 2005). The winged males and females become mature when the air is humid and leave the shelters, the males and females settle, lose their wings, which break off near the bases and finally the couple pair. A pair finds a suitable place of shelter, they

mate and the female lays eggs and a new colony is started (Beeson, 1941; Harris, 1958). One colony can have between 60,000 to 200,000 workers in one time and take only 4 to 5 years to get to the maximum size (Myles, 2005).

### 2.2.3 Distribution

Termites can be found in a wide range of terrestrial environments and are distributed between 45°N and 45°S up to 3000 m altitude (Wood, 1996) through the tropical, subtropical and temperate regions of the world (Smeathman, 1781; Krishna and Wegner, 1970; Pearce, 1998). Some species extend to the relatively cool zones of temperate regions (Emerson, 1955; Krishna *et al,* 1986; Eggleton, 1999) and some can extend themselves beyond their normal habitat range, especially the family of *Coptotermes* (Takematsu *et al,* 2006). Mostly, they are widely distributed by people who unknowingly transport infested furniture. As a result, many pest termites have very wide distributions.

The first termite distribution of Malaysia was reported by Haviland (1898). Further early surveys were been done by Bugnion (1913), Holmgren (1914), John (1925) and Harris (1957). According to Tho (1992), the distribution of termite fauna of Peninsular Malaysia comprises three broad distinct groups; endemic regional fauna, extra-regional intrusive fauna and tropicopolitan fauna.

### 2.2.4 Economic importance

Subterranean termites are considered as one of the most economically important pests in the world especially the genus of *Coptotermes* in the family of Rhinotermitidae (Edwards and Mill, 1986; Su and Scheffrahn, 1998) and in Malaysia (Harris, 1971; Lee, 2002a; 2007). In Malaysia, the most economically important termite species in relation to timber-in-service are *Coptotermes curvignathus, C. gestroi* and *C. khalsoveni* (Ahmad Said *et al,* 1982). They cause considerable damage to forest plantations, electric poles, fences, stakes and structural timber (Harris, 1961; Dhanarajan 1969; Tho, 1975).

Many efforts have been made to control the termite attack including the usage of wood preservatives, termite barriers etc. It has been estimated that about US$50 billion annual damage caused in worldwide by subterranean termites (Korb, 2007) and about US$22 billion has been spent to solve this problem (preventing and controlling) (Su, 2003). In the early part of this century Japan was spending US$ 800 million/year (Tsunoda, 2003), China RMB 1700-2000 million/year (Zhong and Liu, 2002), U.S.A. $11 billion/year (Wang *et al*, 2007). Economically, termites cause significantly damage

Table 2.2 Crop losses, building damage and economic losses caused by termite worldwide (Verma *et al*, 2009)

| Country | Crop losses (%) | Economic losses/annum (Million US $) |
|---|---|---|
| Australia | – | > 95.24 |
| Brazil | – | – |
| China | – | 248.68 – 292.79 |
| Europe | – | 313 |
| India | 15 – 25 (Maize crop) | 35.12 |
| Japan | – | 800 |
| Malaysia | – | 8 – 10 |
| Southern Africa | 3 – 100 | – |
| Spain | – | – |
| United States | – | >1000 |

especially to wood cellulosic materials, agricultural and forest crops (Table 2.2). Among the two groups, subterranean termites are more aggressive and accounted for about 80% of the economic losses while drywood termites only accounted 20% of the losses (Su and Scheffrahn, 1990). In Malaysia, it runs up to several millions of Malaysian Ringgit per year (US $ 10-12 million) (Lee, 2004).

## 2.3 Fungi

Fungi are eukaryotic organisms devoid of any photosynthetic pigments, have either a hyphal or yeast growth form and commonly are dispersed by spores. Of importance to wood decay and spoilage are fungi from the groups Ascomycota, Basidiomycota and their asexual relatives from the Deuteromycota. Their activities change wood properties either by decay, stain or mould (Zabel and Morrell, 1992). Fungi have been estimated to be large number of species (1.5 million) worldwide but only 5% were thought to have been discovered some 10 years ago (Hawksworth, 1991; 2000).

### 2.3.1 Classification of fungal decay types

Wood decay fungi can be divided into 3 main groups; white-rot, brown-rot and soft-rot. The classification of these 3 groups is related to the way they attack the wood and also to their taxonomic grouping.

#### 2.3.1.1 White-rot

The true white rot fungi together with brown rot fungi predominantly come from the Basidiomycota, although there are some fungi of the Ascomycota which can give a

slow white-rot decay (Shimada *et al*, 1997). A very common species is *Trametes versicolor.* White-rot is named after the bleaching or whitening of the wood which occurs during the later stages of decay as a result of lignin breakdown, and it is the ability to rapidly degrade lignin which defines white-rot decay. White-rot decay fungi are more commonly found in the wood of angiosperm trees (hardwoods) while brown-rot is more commonly found on the wood of coniferous trees (softwoods) (Carlile and Watson, 1994), although this may be an over simplification of a complex interaction of conditions. The hyphae of whiterot grow within the lumens of the cells and penetrate deep into wood; either in living trees, in dead wood within the forest or in service. The hyphae pass from cell to cell within the wood structure either through the pits or bore holes through cell wall pairs (Proctor, 1941; Wilcox, 1965; Bravery, 1968). Ray tissue may also be widely colonised in many non durable species. Some white-rot fungi commence decay by depolymerising the hemicelluloses and fragmenting the lignin and may be termed preferential white-rot fungi while others appear to decay all three structural wood cell wall components at a similar rate and are termed simultaneous white-rot fungi. White-rot fungi have been reported to produce extracellular free radicals including the hydroxyl radical (•OH) (Backa *et al*, 1993; Tanaka *et al*, 1999). In addition, they produce a variety of extracellular enzymes which are involved in lignin degradation: laccases, manganese dependent peroxidases and lignin peroxidases. Carbohydrate depolymerisation is mainly effected by various hemicellulases and a cellulose complex. At advanced stages of decay the wood may appear as a mass of white fibres but these may be yellow, tan or light brown as the pigmented amorphous lignin is removed: weight loss can exceed 95% (Panshin *et al*, 1964; Zabel and Morrell, 1992). To achieve very high weight losses in laboratory tests, white rot may require additional water (Highley and Scheffer, 1970a).

#### 2.3.1.2 Brown-rot

Brown-rot is common; it decays the wood of living trees, dead trees and building timber. The causal fungi most commonly come from within the Basidiomycota. An example species from the Coniophoraceae is *Coniophora puteana* (Eaton and Hale, 1993). As with white-rot, brown-rot also penetrates deep to the wood to produce decay. However, brown-rot only deplete the carbohydrate components of cellulose, hemicelluloses and the associated pentosans but are unable to effectively break down the lignin (Eriksson *et al*, 1990). In contrast to white-rot fungi, most brown-rot fungi have a less comprehensive set of cellulose enzymes. It is thought that they produce free radicals which penetrate deep

into the wood cell wall (Enoki *et al*, 1992; Karem *et al*, 1999; Jensen *et al*, 2001). Because of a rapid depolymerisation of the secondary cell wall, brown-rot causes an early and drastic change in of colour, form and strength of the wood. When brown-rot decayed wood is dried, cubical cracking across and along the grain occurs. Weight losses may go as high as 70% in softwoods (Panshin *et al*, 1964; Zabel and Morrell, 1992). In decay tests, brown-rot fungi require less water than white-rot fungi to achieve maximal wood weight loss (Highley and Scheffer, 1970a).

#### 2.3.1.3 Soft-rot

Soft-rot is a predominantly surface form of decay which arises into prominence since its detailed discovery by Savory (1955). In the 1970's and 1980 there were major concerns about premature failures in CCA treated hardwoods in Australia (Hedley, 1997).

Soft-rot commonly can be found in wood placed in contact with the soil and where the wood is only occasionally wet or is very wet, i.e in waterlogged soil or in aquatic situations. It is difficult to detect the decay in the early stages but the wood becomes generally discoloured later in decay. The infected surface is quite soft when wet and spongy with fine longitudinal and cross cracking when dries (Panshin *et al*, 1964). Soft-rot is predominantly caused by fungi of the Ascomycota and associated Deuteromycota but the microscopic symptoms of their decay (cavity formation in the wood cell wall S2, and lumen erosion) may be similar to some of the Basidiomycota types under special circumstances of conditions (Goodell *et al*, 2008). In soft-rot decay, polysaccharide decay is more pronounced than ligninolytic decay (Eriksson *et al*, 1990) and some have been reported as producing reactive oxygen species (ROS) (Regalado *et al*, 1999; Tanaka *et al*, 2000). Their hyphae colonise the wood cell lumens and, penetrate and grow within the secondary cell walls producing a network of geometric shaped cavities which follow the direction of the S2 wood cell wall layer microfibrils. Decay may also be an erosion around the lumen hyphae. Decomposition is restricted to the immediate neighbourhood of the hyphae (Hudson, 1986) whether within the walls or the lumen, whereas in white rot erosion may be more extensive.

### 2.3.2 Biology

#### 2.3.2.1 The life cycle of fungi

The life cycle of fungi (Figure 2.2) begins with the germination of spores which occurs if factors such as temperature, oxygen, adequate moisture and suitable food occur.

Absence or elimination of any of these requirements prevents or greatly curtails the growth of the fungi (Isenberg, 1963; Panshin *et al*, 1964, Eaton and Hale, 1993). Generally, when spores germinate they swell up, taking in water and become metabolically active. At some point in germination one or more germ tube(s) emerge. Germ tubes extend apically as a tube of cytoplasm surrounded by a cell wall. Technically these are hyphae; living fungal cells. These continue to extend at their tips and then branching occurs, typically sub apically, to produce a network of branched hyphae. At some point this mass of hyphae may be termed the mycelium. Subsequently the mycelium grows. It can grow in different media, for example in soil, dead wood or other living organisms. This hyphal phase is responsible for the main life of fungi and it is in this hyphal phase, most decay of substrates like wood occurs. Fungi live by absorbing molecules which often they have made small enough to absorb through their cell walls and across their cell membranes. At some point however, dissemination and dispersal, sometimes combined with the variation that sex provides occurs. The sexual process will continued with plasmogamy which is the fusion of genetically compatible hyphal structures, followed by karyogamy, the nuclear fusion and then meiosis. Meiosis is the recombination and segregation of genetic characteristics which occur in either Zygomycota, Ascomycota or the Basidiomycota and thereafter, the mycelium will develop fruiting bodies, which produce sexual spores. Then these sexual spores will continue the life cycle (Eaton and Hale, 1993). In the Ascomycota and the Basidiomycota, dikaryons are formed which persist for some time before sexual fruiting occurs and these groups have been referred to as the Dikaryomycota. Asexual spores may also be produced, often in large numbers, but these are effectively produced by mitotic cell division, rather than by meiotic division. Asexual reproduction is often a successful mode of dissemination and survival, and many fungi rarely, if ever reproduce sexually. This presents a problem for taxonomy based on the mode of sexual spore production and the asexual fungi are grouped into the Deuteromycota, although they may be predominantly members of the Ascomycota and less frequently from other groups.

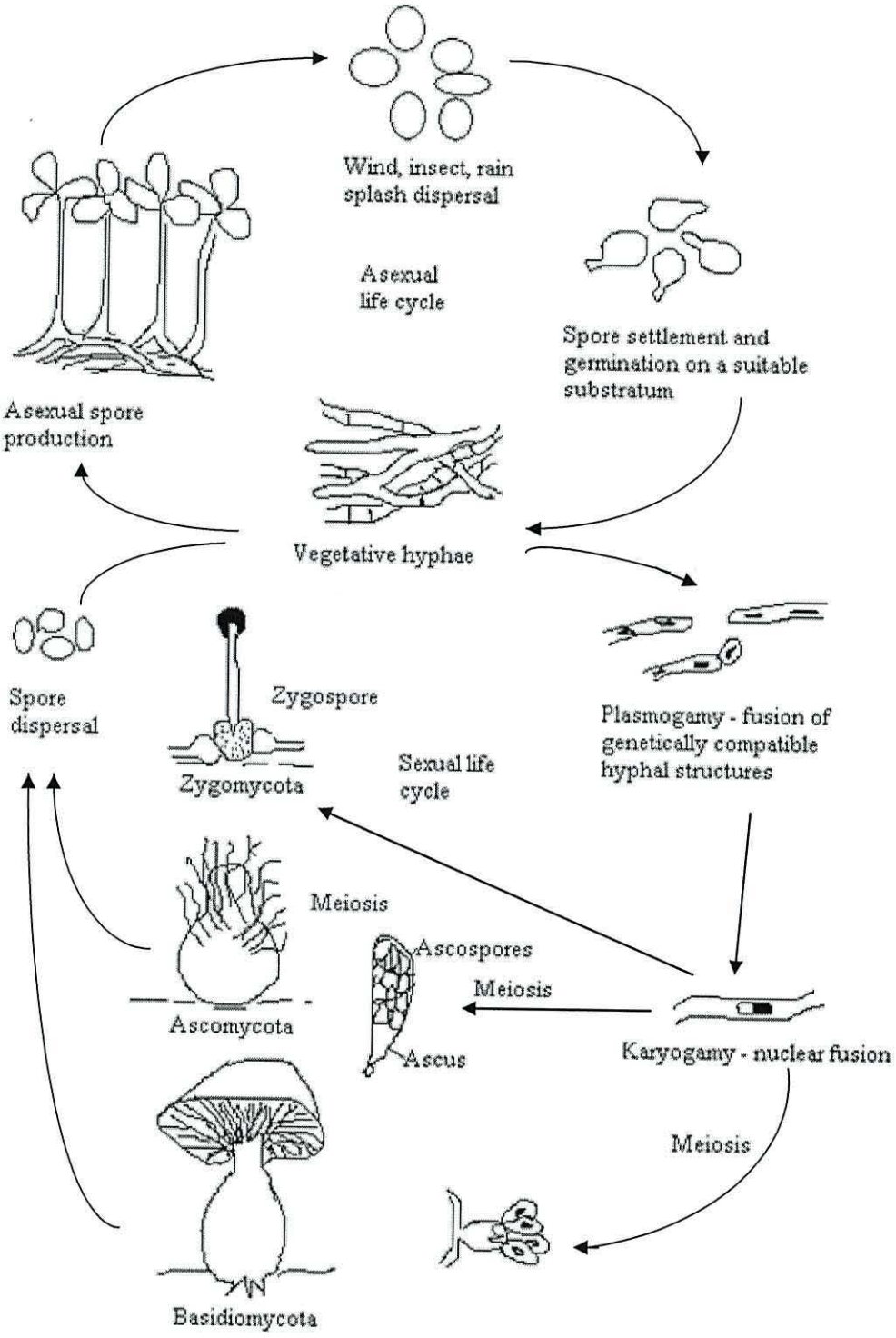



Figure 2.2 The life cycle of fungi (Eaton and Hale, 1993)

**2.3.2.2. Abiotic factors and factors influencing decay rate**

For the wood decay fungi, a number of factors are important for successful decay of the wood. From an environmental point of view moisture content within both high and low limits, the gaseous oxygen environment (which interacts with the moisture content) soluble mineral nutrients, particularly nitrogenous compounds and temperature are important. From the wood features such as density, cell wall lignification and lignin type, refractivity of cellulose and toxic substances (extractives) are important (Hudson, 1986). In addition other features, such are the presence of high quantities of starches may make the wood more susceptible to decay, e.g. *Hevea brasiliensis*.

Moisture is often stated to be the most important factor for wood decay and certainly given adequate oxygen exchange this is true. At low levels of available water, fungi are limited in their ability to grow and little wood cell wall swelling occurs which is necessary for the degradative agents to access the cell wall substrates beyond exposed surfaces. Most of the wood decay fungi from the Basidiomycota require moisture contents between 30% to 80% (Eaton and Hale, 1993; Zabel and Morrell, 1992; Käärik, 1974) and optimum growth can achieved at about 40%. In a special situation, one species, *S. lacrymans,* may require less to initiate decay, because it can transport water by means of strands. It is said to need a minimum moisture content of 20% (Viitanen and Ritschkoff, 1991).

Besides water and oxygen, nitrogen is an important factor in limiting decay rate. Wood is normally very rich in carbon based macromolecules and is around 50% carbon, where as nitrogen is normally very low, giving C:N ratios lower than 250:1 (Merrill and Cowling, 1965). Optimal decay rates usually occur at around 30:1. Herbaceous tissues also contains higher (1.0 to 5.0%) nitrogen than woody tissues (0.03 – 1.0%). Wood decay fungi metabolize large amount of carbohydrates with the presence of small amount of nitrogen. In general, cellulolysis diminishes with the increasing of carbon: nitrogen ratio.

Cell wall lignification is also another important factor in reducing the rate of decay by both white rot and soft rot decay types. Softwoods are more resistant to white rot and soft rot than hardwoods due to the higher degree of lignification and the type of lignin (guaiacyl to syringyl ratio). Lignification may act in a variety of ways: as a physical barrier to cellulases and hemicellulases, and also as a cellulase inhibitor.

Toxic substances which are formed during the heartwood formation contribute to the resistance of wood to decay fungi. Most of these are phenolic compounds and they are discussed in more detail in section 2.6.4.3.

### 2.3.3 Distribution

Many researchers report that it is difficult to determine the geographic distribution of wood-inhabiting fungi (Rayner and Boddy, 1988; Zabel and Morrell, 1992; Ingold and Hudson, 1993). It has been estimated that there may be some 1.5 million fungal species worldwide. However, only an estimated 75,000 species (5% of the total) had been described 10 years ago (Hawksworth, 2001; 2002). Tropical forests are also reported to have high fungal diversity (Hawksworth and Rossman, 1997).

The distribution of fungi is influenced by many factors. Salmiah *et al* (2002) reported that the rainfall, quantities of suitable substrata, damp forests and forest types are among the factors that influence the distribution, species diversity and similarity of fungi in six different forest locations in Peninsular Malaysia. Early works by Corner (1981; 1983; 1984; 1987; 1989a; 1989b; 1991; 1993) recorded about 487 Malaysian lignicolous fungi from Peninsular Malaysia to the Solomon Islands. More recently Hawksworth (2001) reported almost 1,000 species (macromycetes and Ectomycorrhizae) from Malaysia.

### 2.3.4 Economic importance

Though wood decay fungi are economically important for timber in service (Scheffer, 1993), they are also important in the world's ecosystem, within the carbon cycle and to a lesser extent in other nutrient cycles. More recently wood decay fungi have been looked at as sources of food, medicines, biocontrol agents, in animal disease and biodegradation agents for recalcitrant, particularly industrial pollutants, e.g. various polyaromatics and organochlorines.

As decomposers the recycling of fixed carbon to carbon dioxide is indispensible for the continued photosynthesis (Zabel and Morrell, 1992). About 80 billion tons/year of carbon are returned to the atmosphere from these activities (Barron, 2003).

## 2.4 Natural durability

It is well known that the natural durability of wood varies considerably between and within wood species. According to the European standard CEN EN 350-1 (1994) natural durability is "the inherent resistance of wood to attack by wood-destroying organisms". Eaton and Hale (1993) defined natural durability or decay resistance as the ability of the heartwood of any wood species to resist decay. Normally, natural durability is measured by exposing the wood to biological agents such as termites, beetles, fungi and marine borers (e.g., ASTM D2017, 1993; ASTM D3345, 1998). While the natural

durability of heartwood is important in situations either to be used partially or fully exposed to the weather, sapwood is always regarded as having low natural durability (perishable or non-durable) except if treated.

Early work showed that the natural durability of wood is normally derived from the toxicity of extractable compounds, called extractives, in the heartwood (Scheffer and Cowling, 1966; Bamber and Fukuzawa, 1985; Hillis, 1987; Taylor *et al*, 2002), a typical example is β-thujaplicin from western red cedar (*Thuja plicata*). The extractives include certain gums, resins, tannins and possibly other materials (Arthur Koheler, 1924). On the other hand, Öqvist (1988) suggested that the natural durability also can be defined by the ability of the wood to keep below a critical moisture content. A range of structural hardwoods durability ratings is shown in Appendix Table A2.1.

### 2.4.1 Natural durability testing

Although natural durability is the resistance to decay, it is also specific to different groups of organisms and to a certain extent exposure situations. European Standards have classified various ways of determining natural durability. In ground contact (CEN EN 252, 1989), against fungi of the Basidiomycota in the laboratory (CEN EN 113, 1997), above ground conditions (CEN ENV 12037, 1996) and treated wood (CEN EN 330, 1993).

The first series of ground contact natural durability tests of untreated Malaysian timbers were started by Foxworthy in 1918 and results were published in 1930 (Foxworthy and Wooley, 1930). This was continued by Jackson (1957) and Mohd Dahlan and Tam (1985) at a new site of the Forest Research Institute Malaysia (FRIM).

There are many different ways to evaluate the wood performance or decay severity including visual evaluation, microscopic evaluation, image analysis, pick or splinter test, density and mass loss, or various mechanical property (strength) tests (Table 2.3). However, only the first two methods may truly attribute fungi to the change in properties (Råberg *et al,* 2005). Generally, testing with micro-organisms may-encounter difficulties due to unequal aggressive behaviour of all organisms (Van Acker *et al,* 2003).

Table 2.3 An overview of test methods used for evaluating durability (Råberg *et al,* 2005)

| | Subjective | Objective | Fast | Time consuming | Quality | Quantitative | Consider fungi flora |
|---|---|---|---|---|---|---|---|
| Visual evaluation | X | | X | | | X | X |
| Macroscopic evaluation | X | | | X | X | | X |
| Pick or splinter test | X | | X | | | X | |
| Density loss | | X | X | | | X | |
| Weight loss | | X | X | | | X | |
| Strength test | | X | | X | | X | |
| Acoustic test | | X | X | | | X | |

### 2.4.1.1 Field testing

Laboratory test and field test (graveyard/stake tests) are the two main methods used to test the natural durability of wood either against termites or fungi. Although field tests are ultimately more reliable than laboratory tests, laboratory tests are faster and more definable. In addition, different field test sites may give different results, for example a variety of different in ground contact field sites are recognised in Sweden. Even though this may take longer, field test are strongly recommended when dealing with preservative-treated wood (McNamara, 1994). In the past, laboratory tests alone have allowed preservatives to pass but when tested in the field, preservatives failed due to factors such as different organisms to those occurring in the lab tests.

Field tests may be performed by exposing small stakes [19 mm (tangential) x 19 mm (radial) x 500 mm (longitudinal)] partially buried (50% of their length) in the ground and at a 12 inch spacing. The test period takes about 2 years or more to get results, although the test stakes may be evaluated every 6 months. At each assessment, the samples are taken out from the ground and visually rated according to an appropriate standard, e.g. ASTM Standard (ASTM D1758, 2006). Stakes are rated below the ground line and the thickness of each stake is measured at the ground line. The average time taken for these stakes to fail is used to assign a natural durability rating.

### 2.4.1.2 Laboratory termite test

The durability of small test blocks is assessed by wood consumption and visual rating. However, according to ASTM 3345 (1998), the performance of small blocks is assessed by visual rating only.

Laboratory tests are performed in test jars using fine sand as a media for a period of 28 days. The test can be done either using 'no-choice' or 'choice' methods. For 'no-choice', one treated block is placed in a test jar, 400 termites (360 workers and 40 soldiers) are added to each bottle and 30 ml distilled water are added by spraying. For 'choice' methods, one treated block with one untreated block (control) are placed in a test jar and the same amount of termites and water are added. At the end of the period, the blocks are taken out from the test jar and the wood consumption together with termite mortality are determined.

#### 2.4.1.3 Laboratory fungal decay tests with pure cultures

Lab tests and accelerated tests are important tools in the prediction of the natural durability of wood. However, not all wood decay fungi are suitable for tests on decay resistance. Findlay (1935) listed four characteristics for laboratory tests with wood decay fungi: 1) ability to cause rapid decay in the species of wood used; 2) high degree of resistance to antiseptics; 3) preferably of economic importance, and 4) easily cultivated and not unduly sensitive to slight variations in environmental conditions.

Laboratory decay tests are often preferred in early stages of preservative development to field tests because the results are more objective, the tests are quicker and involve exposure to a selection of naturally existing microorganisms. Conversely field test take a longer time and are subject to human assessment variation but they may select decay or detoxifying organisms which would not be present in a laboratory test.

Laboratory tests can be performed either in petri dishes or test jars using pure cultures of decay fungi cultured on a nutrient medium, often malt agar. Standard jar tests with basidiomycotal fungi and a large block size run for16 weeks (standard basidiomycetes test). In a typical test one treated block is placed with one untreated block (control) in an inoculated culture vessel. A range of fungi may be used but these are not mixed within one jar and they are handled to avoid contamination. At the end of the incubation period, the blocks are taken out from the culture vessel, the mass losses are determined and expressed as a percentage mass loss (CEN EN 113, 1996). The performance can be compared by indexing against a reference species.

### 2.4.2 Natural durability classification

Supriana (1988) reported that the natural durability classification is based mainly on records of practical uses. In Europe, timbers are classified into five different classes'

of durability. The five classes are very durable, durable, moderately durable, slightly durable and non durable (CEN EN 335, 2006). However, in Malaysia only four different classes exist for hardwood and softwood Malaysian timbers; very durable (a service life of over 10 years), durable (a service life of 5 to 10 years), moderately durable (a service life of 2 to 5 years) and non durable (a service life of less than 2 years) (Mohd Dahlan and Tam, 1985; Wong *et al,* 2005). Therefore, the durability classes are very dependent on where the wood is exposed. Two major climatic factors are important; temperature and a suitable moisture content, although anthropogenic nitrogen sources may be of increasing importance. In tropical environments the diversity of destructive agents is also greater due to the favourable climatic conditions, therefore some of the results from one climatic region are not applicable all regions (Findlay, 1985; Eaton and Hale, 1993).

It should be noted that in Malaysia, the hazard not only comes from a diverse fungal flora but also termites and other highly destructive insects contribute an additional hazard to wood in service and both are active throughout the year due to the constant warm humid conditions, rather than the fluctuations occurring in temperate zones. Therefore, Mohd Dahlan and Azlan (1994) suggested that factors such as size of timber, type of service conditions and proportion of sapwood to heartwood should be taken into consideration when making the classification.

#### 2.4.2.1 Termite resistance

Different standards classify the extent of termite attack differently. The European Standard divides the durability class into three classes (Table 2.4) whereas the American Standards, whether laboratory (Table 2.5) or field test standard (Table 2.6) use a 0-10 rating system. Even though the classes looked similar in the American Standard, the test periods are different (28 days, lab and 2 years, field). The termite durability of the products can be recommended from these results.

Table 2.4 Classes of natural durability of wood to termite attack (European Standard EN 350, 1994)

| Durability class | Description | Average rating |
|---|---|---|
| D | Durable | 0 – 1 |
| M | Moderately durable | 2 |
| S | Susceptible | 3 - 4 |

Table 2.5 Classes of durability of wood to termite attack based on laboratory test (ASTM D3345, 1998)

| Durability class | Description |
|---|---|
| 10 | Sound, surface nibbles permitted |
| 9 | Light attack |
| 7 | Moderate attack, penetration |
| 4 | Heavy |
| 0 | Failure |

Table 2.6 Classes of durability of wood to termite attack based on field test (ASTM D1758, 1986)

| Durability class | Description of condition |
|---|---|
| 10 | Sound |
| 9 | Trace of attack |
| 7 | Moderate attack |
| 4 | Heavy attack |
| 0 | Failure by termite attack |

### 2.4.2.2 Summary comparison of different fungal durability systems

Eaton and Hale (1993) stated that the abilities of local biodeterioration agents (flora and fauna) may vary the durability rating of the timbers. As termite, wood decay fungi test also can be divided into laboratory tests and field tests, and the classification will be based on the established standards. Based on European Standard (Table 2.7), there are five different classes of durability. However, American Standard (ASTM) classified result for laboratory test to four different classes (Table 2.8) and field test to five classes (Table 2.9).

Table 2.7 Classes of natural durability of wood to fungal attack (European Standard EN 350-1, 1994)

| Durability class | Description | Result of laboratory test (ML, %) | Result of field test (Express as y) |
|---|---|---|---|
| 1 | Very durable | $ML \leq 5$ | $x \leq 0.15$ |
| 2 | Durable | $5 < ML \leq 10$ | $0.15 < x \leq 0.30$ |
| 3 | Moderately durable | $10 < ML \leq 20$ | $0.30 < x \leq 0.60$ |
| 4 | Slightly durable | $20 < ML \leq 30$ | $0.60 < x \leq 0.90$ |
| 5 | Not durable | $ML > 30$ | $x > 0.90$ |

Table 2.8 Classes of durability of wood to fungal attack based on laboratory test (ASTM D2017, 1993)

| Average weight loss (%) | Indicated class of resistance to specific fungi |
|---|---|
| 0 to 10 | Highly resistant |
| 11 to 24 | Resistant |
| 25 to 44 | Moderately resistant |
| 45 or above | Slightly or non resistant |

Table 2.9 Classes of durability of wood based to fungal attack based on field test (ASTM D1758, 1986)

| Durability grade | Description of condition |
|---|---|
| 10 | Sound |
| 9 | Trace of decay |
| 7 | Moderate decay |
| 4 | Heavy decay |
| 0 | Failure due to decay |

### 2.4.3 Wood characteristics affecting durability

The biological and technical properties of wood are mainly determined by the chemical composition of the cell wall. Wood cell walls are made up primarily of cellulose, hemicelluloses (polyoses) and lignin. In addition to the cell wall polymers, the properties of wood are strongly influenced by extractives, accessory compounds extractable by solvents of different polarity (Fengel and Wegener, 1989).

It has been known that wood extractives play an important role towards wood properties (Immamura, 1989). The amount of lignin and other extractives determine natural durability of the wood (Gierlinger *et al*, 2004b). However, other factors such as wood anatomy, other potential nutrients (protein, starch) and growth characteristics (silvicultural factors and second-growth timber) could also be involved in wood service life (Zabel and Morrell, 1992).

Wood density has also been suggested as a factor of durability but it is difficult to correlate with durability. Some wood species of high density are naturally durable against termites (Behr *et al,* 1972; Coulson and Lund, 1973; Bultman *et al,* 1979; Abreu and Silva, 2000; Ahmad Said *et al,* 1982; Ahmad Said and Mohd Hamami, 1983) but some of these species are rapidly degraded by fungi (Jorgensen, 1953; Bultman and Southwell, 1976; Paes *et al*, 2004; Bhat *et al,* 2005).

Many studies have shown that wood density is correlated with the amount of extractives. Higher extractive content may result in higher wood density, thus greater resistance (Arndt, 1968; Jalaluddin and Labosky, 1985; Nagnan and Clement, 1990; Lemaire *et al*, 1990; Scheffrahn, 1991; Grace, 1997; Taylor *et al*, 2006; Roszaini *et al,* 2009).

### 2.4.4 Variation in natural durability

Due to the variation in wood properties (e. g. site growth, age of trees and wood therein, genetics) for each individual species/tree, there is considerable variation in natural durability either between or within wood species (Scheffer and Cowling, 1966).

#### 2.4.4.1 Variation between species

Highly variability of wood durability between wood species in tropical woods has been reported before in many studies (Mannesman, 1973; Reis, 1973; Martawijaya, 1975; Amemiya and Matsuoka, 1979; Wong, 1988; Salmiah and Amburgey, 1992; Torelli and Cufar, 1994; Sukartana and Highley, 1997; Suprapti, 2010). The heartwood resistance of Malaysian commercial timbers have been summarized by Mohd Dahlan and Tam (1985) and Wong *et al* (2005). These are based on field and laboratory evaluation and service records (Table 2.10).

#### 2.4.4.2 Variation within species

The variation of natural durability within a wood species can be divided into two components; the genetic potential of the tree and the environmental conditions of tree growth (Clark and Scheffer, 1983). In the context of genetics Venäläinen *et al* (2001) found that the genetics was a stronger determinant for decay resistance in their study on Siberian larch from northern and central Finland. In the context of growth rate, fertilizer is sometimes used in order to increase tree growth and nitrogen is the most commonly applied fertilizer. However, nitrogen addition as a fertilizer can cause pest problems (Dreistadt and Clark, 2004). The cell wall and fibre characteristics may be changed due to the forest fertilizers and thus affect the decay susceptibility (Yang *et al*, 1988; Mäkinen *et al*, 2002; Heijari *et al*, 2005). On the other hand, the amount of extractives also may be influenced by growth rate (Rudman and DaCosta, 1959; Hillis, 1962). Extractives are formed from carbohydrates translocated or stored near the heartwood-sapwood boundary/zone (Hillis 1971; 1977). This means that the quantity of extractives formed is dependent on the quantity of carbohydrates at that zone (Hillis *et al*, 1962). Higher growth rate as a result of more favourable conditions may produce more carbohydrates thus produce more extractives. In the context of site and condition of growth, the natural durability of wood in one country is not same if used in another country. This is due to differences in environmental conditions (soil, temperature) that affect the condition growth of the trees (Clark and Scheffer, 1983). For examples, Malaysia has different climates

Table 2.10 Variation of natural durability of some hardwoods and softwoods (Mohd Dahlan and Tam, 1985; Wong *et al*, 2005)

| Very durable | Durable | Moderately durable | Non durable |
|---|---|---|---|
| *Neobalanocarpus heimii* | *Koordersiodendron pinnatum* | *Rhizophora* spp., *Bruguiera* spp. / *Cerios* spp. | *Shorea* spp. (Light Red Meranti) |
| *Hopea* spp. | *Aglaia* sect. *Amoora* | *Shorea* spp. (Red balau) | *Calophyllum* spp. |
| *Upuna borneensis* | *Pometia* spp. | *D. kunstleri* | *Mallotus* spp. |
| *Eusideroxylon zwageri* | *Canarium* spp. / *Dacryodes* spp. | *Shorea* spp. (Light Red Meranti) | *Cyathocalys* spp. / *Mezzettia* spp. |
| *Streblus elongatus* | *Hopea* spp. | *Calophyllum* spp. | *Shorea macrophylla* |
| | *Anisophyllea corneri* | *Shorea* spp. (Dark red meranti) | *Blumeodendron* spp. |
| | *Intsia palembanica* | *Strombosia javanica* | *Cratoxylon arborescens* |
| | *Malabera* | *Cantleya corniculata* | *Parashorea* spp. |
| | *Palaquium* spp. | *Parashorea* spp. | *Dyera* spp. |
| | *Mesua ferrea* | *Dryobalanops* spp. | *Dryobalanops* spp. |
| | *Cotylelobium lanceolatum* | *Atuna* spp., *Kostermanthus* spp., *Licania* spp., *Maranthes corymbosa* and *Parinari* spp. | *Shorea* spp. (Yellow Meranti) |
| | *Madhuca utilis* | *Koompassia malaccensis* | *Diospyros spp.* |
| | *Shorea* spp. | *Dacryodes* spp., *Canarium* spp and *Santiria* spp. | *Adinandra, Gordonia* and *Schima* |
| | *Fagrea fragrans* | *Cynometra* spp. | *Artocarpus lanceifolius* |
| | *Dryobalanops* spp. | *Canarium* spp. / *Dacryodes* spp. | *Scaphium* spp. |
| | | Eugenia spp. | *Diallium kunstleri* |
| | | *Scaphium* spp. | Dipterocarpus spp. |
| | | *Pithecellobium* spp. | *Terminalia* spp. |
| | | *Vitex* spp. | *Adinandra* spp. |
| | | *Dipterocarpus grandiflorus* | *Neolamarckia cadamba* |
| | | *Kokoona* spp. | Mangifera spp. |
| | | *Shorea macroptera* | *Macaranga* spp. |
| | | C. scortechinii | *C. scortechinii* |
| | | *Hopea* spp. | *C. scortechinii* limo |
| | | *Quercus* spp. | *Heritiera* spp. |
| | | *Swintonia* spp. | *Kayea* spp. |
| | | *Ctenolophon parvifolius* | *Swintonia* spp. |
| | | *Anisoptera* spp. | *Palaquium* spp. |
| | | *Heritiera* spp. | *Irvingia malayana* |
| | | *Azadiractha excelsa* | *Ficus* spp. |
| | | *Scorodocarpus borneensis* | Podo spp. |
| | | *Acacia* spp. | *Ctenolophon parvifolius* |
| | | *Albizia* spp. | *Agathis borneensis* |
| | | *Palaquium* spp. | *Elateriospermum tapos* |
| | | *Tristaniopsis* spp. | *Parkia* spp |
| | | *Tetramerista glabra* | *Alstonia* spp. |
| | | *Gluta* spp. / *Melanochyla* spp. | *Tetramerista glabra* |
| | | *Koompassia excelsa* | *Koompassia excelsa* |
| | | *Jackiopsis ornata* | *Gonystylus* spp. |
| | | *Dacrydium* spp., *Falcatifolium falciforme* and *Phyllocladus* spp. | *Gymnacranthera* spp. / *Horsfieldia* spp. / *Myristica* spp. |
| | | *Sindora* spp. | *Dillenia* spp. |
| | | *Shorea* spp. (Yellow Meranti) | *Campnosperma* spp. |
| | | *Shorea* spp. (White Meranti) | *H. brasiliensis* |
| | | | *Artocarpus* spp. |
| | | | *Endospermum malaccense* |

Note: The timbers are multispecies, that why it appeared in two different classes.

compared to North America or Northern Europe and also different insect and fungal decomposer species.

Other environmental conditions include soil, tree site and silviculture. 1) *Soil.* Guyette *et al*, (1992) observed that trees (heartwood and sometime also sapwood) grown from a higher mineral content soil had an abundance of mineral elements. Janin *et al* (1990) and Klumpers *et al* (1993) reported that a colour difference (one of the extractive effects in wood) of oaks from different regions was more due to soil properties than genetic control. 2) *Tree site.* Bhat *et al* (2005) studied the natural decay resistance of teak grown in wet, dry and plantation sites. They found that the resistance of teak wood from the wet site was less than the either the dry or the plantation site against brown-rot fungi (*P. palustris* and *G. trabeum*). This was due to a lower quantity of extractives in trees from wet site than the other two sites (dry and plantation).

The last factor for environmental conditions is *silviculture*. A study by Smith (1997) found that thinning (a common silvicultural practice) will increase the resources available to the tree in a stand including water, nutrients and sunlight, and thus the remaining trees to increase their growth rates. In theory more photosynthetic products will give more resources for heartwood extractive production. However, in essence this was not supported by Guilley *et al* (2004), who found that the tallest oak trees (i. e. high log length under crown due to the thinning and pruning) also susceptible to decay in laboratory tests against *Trametes versicolor.* Their findings agreed with Hillis (1971) who stated that silviculture practices (e. g. thinning and pruning) just increase the growth rate but at the same time controlled the formation of heartwood. This practice only contributes to the attainment of wood quality uniformity but not to an increase in extractive formation. Zabel and Morrell (1992) also supported that durability may vary in terms of climatic and geographic regions but not with silviculture. The variations due to these two factors can be more easily found especially in a number of tropical species with very durable heartwood (Scheffer and Duncan, 1947; Scheffer and Cowling, 1966).

### 2.4.4.3 Within tree variation

The wide variation of durability within tree is well reported (Scheffer and Henry, 1949; Scheffer *et al*, 1949; Scheffer and Englerth, 1952; Zabel, 1948; Scheffer and Cowling, 1966). Several factors are involved in within species variation, including size and age of tree, location of the wood in the trunk, characteristics and location of the juvenile wood and its relative proportion to mature wood. Overall the proportion of heartwood increases with the tree age.

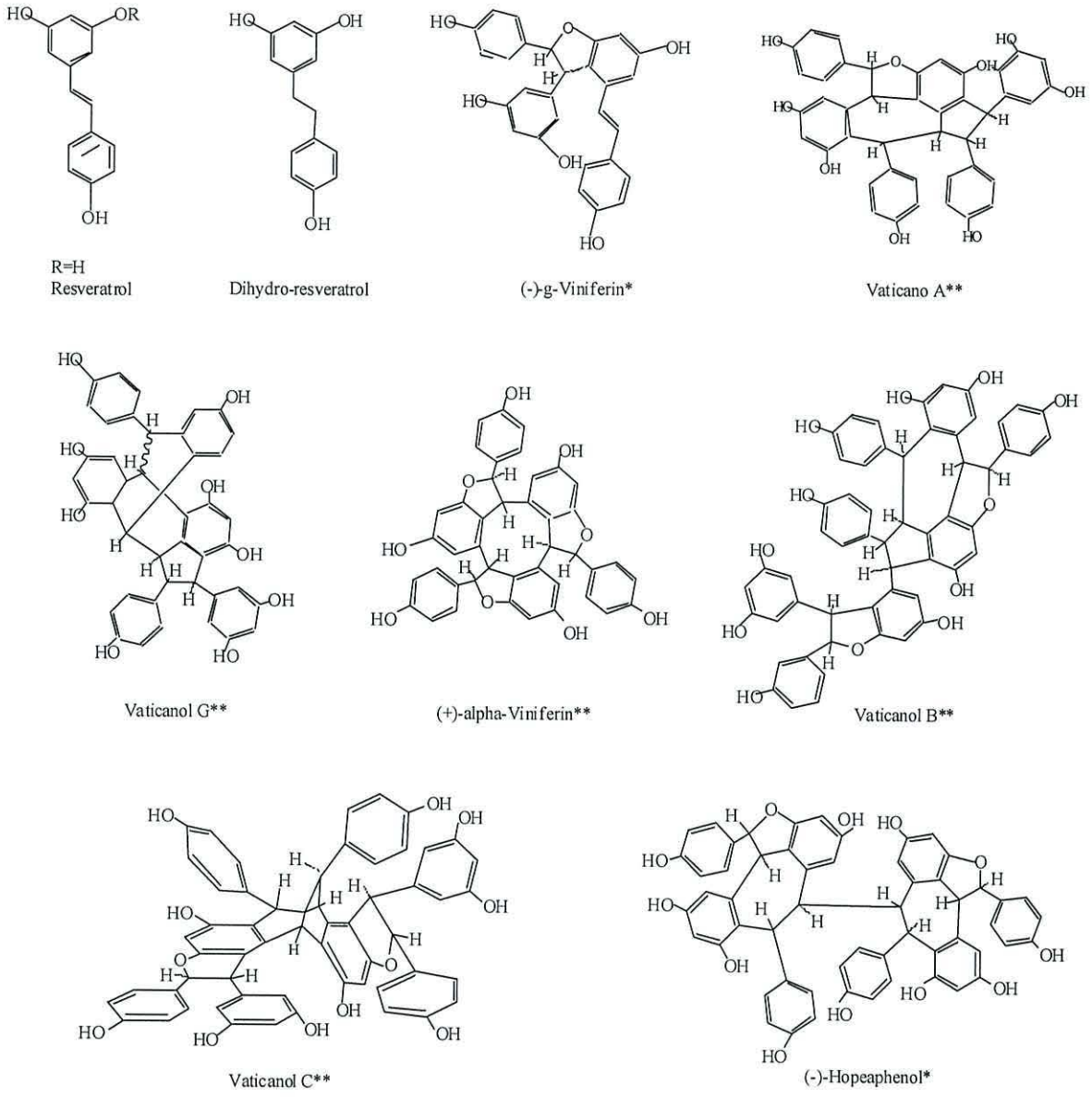



Figure 2.10 Chemical structures of major stilbenoid derivatives from Dipterocarpaceae plants compared to a simple stilbenoid, resveratrol, a monomer similar to that from which the others are derived.
* absolute structure, ** relative structure (Ohguchi *et al*, 2003).

### 2.6.4.4 Tannins

Tannins are soluble in water. They are the complex of polyhydric phenols and occur in hardwoods and many part of plants (wood, bark and leaves) (Burkill, 1935; Umezawa, 2001). The ability to precipitate alkaloids, gelatin and other proteins are the special properties of tannins (Swaim and Bate-Smith, 1962; Haslam, 1989). The use of tannin in adhesives and preservatives has a long history. For example, tannins from forest and agricultural residues have been used since the 1950's as bonding agents (Dalton, 1950;

1953; Plomley *et al*, 1957; Anderson *et al*, 1961) and mangrove tannins as waterproof adhesives in commercial plywood production (Plomley *et al*, 1964; Plomley, 1966). The biological activities of tannins stem from their ability to react with metal ions and other molecules (proteins, polysaccharides and alkaloids) (Haslam, 1996). Tannins also react in antioxidant, radical-scavenging and pharmacological capacities (Bruneton, 1995; Haslam, 1996) (Figure 2.11).

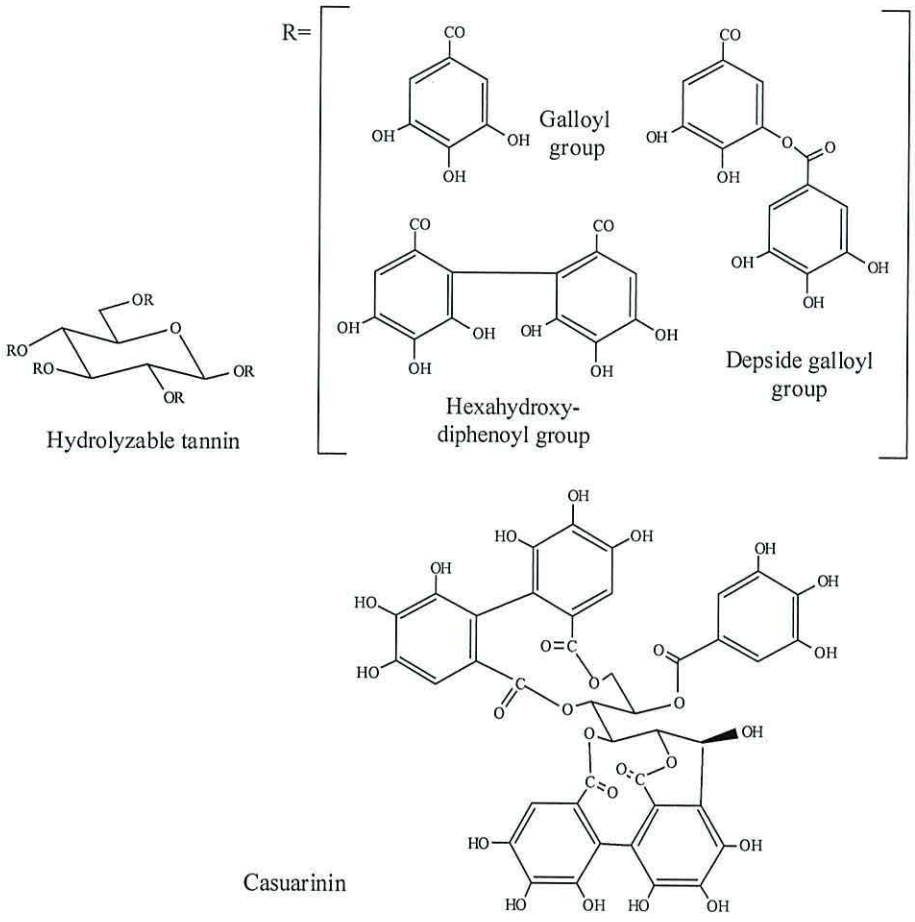



Figure 2.11 Chemical structures of tannins (Hon and Shiraishi, 2001)

### 2.6.4.5 Nitrogenous compounds

Nitrogenous compounds (Figure 2.12) are reported to be low in concentration in wood and that this contributes to decay resistance (Merrill and Cowling, 1965). Soluble, fixed nitrogen is an important nutrient resource for microorganisms (Rana *et al*, 2010) and the nitrogen content of wood is reported to occur at concentration of less than 0.1% in temperate timbers and may be slightly higher in tropical species (Allison *et al*, 1963). In

terms of overall content, nitrogen is more plentiful in sapwood, particularly in living parenchyma, so may be locally distributed in ray tissue. Thus it is more abundant in young sapwood and on a weight for weight basis more plentiful in the thin walled earlywood cells. This also more abundant in the pith, in tissue near the pith, other tissues with higher proportion of parenchyma cells and in root wood of gymnosperms (Merrill and Cowling, 1965; Platt *et al,* 1965; Scheffer and Cowling, 1966). As a result these regions may be more readily colonised and decayed by fungi.

Nitrogenous compounds naturally present in many woods are the remains of proteins and amino acids, and other primary metabolites and, in service wood may acquire a lot of nitrogen from the surrounding environment from soil and rainfall. However, one important class of nitrogenous compounds may have effects on decay resistance, the alkaloids. The alkaloids have been used as drugs probably before the times of Hippocrates. They have served as medicines for a number of diseases such as cancers and, tumour and various antibiotics (Hudson, 1986).

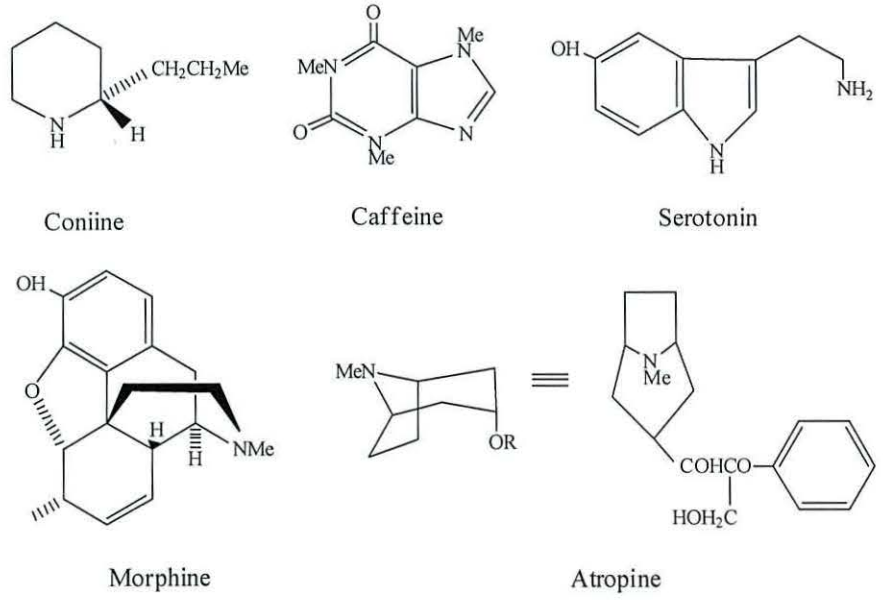



Figure 2.12 Chemical structure of some nitrogen compounds (Mann, 1987)

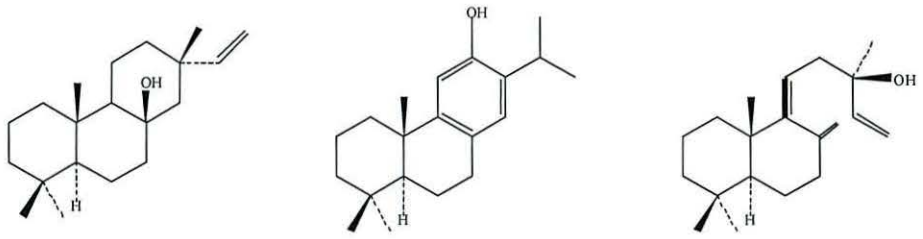
### 2.6.5 Application of wood extractives

Early wood extractives include the fossilized resin (amber), originated from coniferous trees was used by both the Greek and Roman (Sjöström, 1993). Natural extracts from plants or fungi also have been used since Ming Dynasty (AD 1368–1644), as the root of Chinese medicine (Yang, 2009) and crude drugs (Umezawa, 2001).

### 2.6.5.1 Antitermitics

Many studies have demonstrated that wood extractives are a promising source of compounds that inhibit termites and other insect activities (Logan *et al*, 1990; Maistrello *et al*, 2001a; 2001b; Zhu *et al*, 2001a; 2001b; 2003; Lax and Osbrink, 2003; Nix *et al*, 2003; Ibrahim *et al*, 2004). Schultz *et al* (2008) report that many termite-resistant heartwoods have extractive compounds which in addition to being toxic exhibit with higher antioxidant. This combination of two factors makes some heartwood more resistant, rather than being based on a single mode of action, e.g. antitermitic, affecting the termite behaviour.

The activity of a few extractive compounds has been reported in many studies. One of the most studied is the terpenoids. These occur in almost all plants and have been exploited as insect repellents (Obst, 1998). Earlier, Carter *et al* (1978) found that naphthoquinones, 7-methyl-juglone and its dimer isodiospyrin isolated from the wood of *Diospyros virginiana* possess termiticidal activity against *R. flavipes*. More recently Carter and de Camargo (2007) found antitermitic properties in nine Brazilian Amazon wood species (tropical) against *R. flavipes* and *C. formosanus*. Scheffrahn *et al* (1988) detailed that ferruginol and manool in *Taxodium distichum* are the two most active extractive compounds against *C. formosanus*. They also include nezukol even though it is least active but most prevalent (Figure 2.13). Other extractive compounds with antifeedant/antitermitic activities are shown in Appendix Tables A2.11 and A2.12.




Nezukol Ferruginol Manool

Figure 2.13 Major antitermitic compounds from *T. distichum* heartwood (hexane extracts) (Scheffrahn *et al*, 1988)

### 2.6.5.2 Antifungals

Many wood extractive compounds (secondary metabolites) have antifungal activities (Tsoumis, 1991; Quiroga *et al*, 2001; Carpinella *et al*, 2003; Kawamura *et al*,

2004; 2010b; Kawamura and Ohara, 2005; Kusuma *et al*, 2005; Yen *et al*, 2007). Schultz *et al* (1995) and, Schultz and Nicholas (2000) found that stilbenols (hydroxylated stilbenes) protect heartwood by having some fungicidal activity together with antioxidant properties against fungal colonization and degradation.

In addition, isoflavonoids (from group of anthoxanthin flavonoids) have been reported extremely toxic to insects and fungi (Russell *et al*, 1979; Sutherland *et al*, 1980), and can be used as natural biodegradable fungicides to replace the harmful traditional toxic wood preservatives (Carpinella *et al*, 2003). These inhibit fungal spore germination, germ tube elongation and hyphal growth by membrane disruption (Skipp and Bailey, 1977; Higgins, 1978). Some common heartwood extractives known to inhibit fungi are shown in Figure 2.14. Further fungicidal compounds are presented in Appendix Table A2.12.

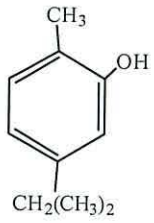
### 2.6.5.3 Antioxidants

The oxidation of lipids or other molecules can be delayed or inhibited by antioxidants (Velioglu *et al*, 1998; Zheng and Wang, 2001). Oxidative damage of cells and biomolecules can be reduced by antioxidants. There has been a lot of interest because antioxidants are thought to be able to defend against cancer and lower the risk of cardiovascular disease, diabetes and dementia, including Alzheimer's disease (Adhami *et al*, 2007; Tahirovic *et al*, 2007). Generally, antioxidants may help our body from damage caused by reactive oxygen nitrogen and chlorine species, abbreviated as ROS, RNS and RCS, respectively (Shahidi, 1997).

Recently, the research interests on antioxidants from plants has aroused more attention from both wood science and pharmacological researchers because some are suspected to cause or promote toxic and carcinogenic effects from the commercial synthetic antioxidant (Figure 2.15); butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone (TBHQ) and gallic acid esters (Denz and Llaurado, 1957; Branen, 1975; Ito *et al*, 1983; Grice, 1986; Barlow, 1990; Kahl and Kappus, 1993; Koleva *et al*, 2002; Tepe *et al*, 2005). A number of studies show that wood extractives have a role as free radical scavengers and antioxidants especially phenolic content (Larson, 1988; Huang *et al*, 1992). Schultz and Nicholas (2000) found that extractives have minimal antitermitic and antifungal activities but were often excellent as antioxidants; this latter property helped to prevent attack by termites and fungi. Antioxidants have therefore been studied as an alternatives wood preservative (Gao *et al*, 2007).

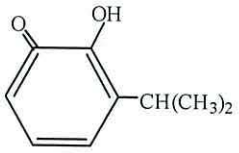
Terpenoids

Carvacrol




ρ-Methoxycarvacrol
ρ -Methoxythymol
Thymoquinone
Sugiol
Totarol
Ferruginol
Chamic acid (nonphenolic)
Chaminic acid (nonphenolic)
1-Citronellic acid (n. p. aliphatic)

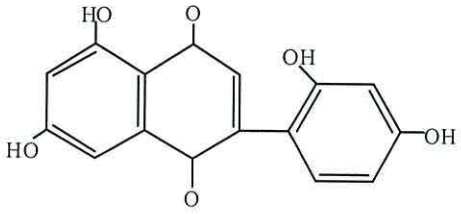
Tropolones




β-Thujaplicin
γ-Thujaplicin
α-Thujaplicinol
β-Thujaplicinol
Pygmaein
β-Dolabrin
Nookatin

Flavonoids

Quercetin



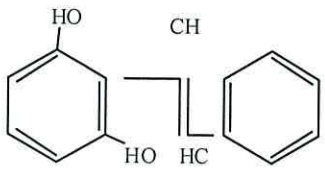

Robinetin
Taxifolin (*trans*-dihydroquercetin)
Dihydrorobinetin (taxifolin isomer)
Homoferreirin
Ougenin[b]

Other Aromatic Compounds

Lapachonon (nonphenolic)
Matairesinol

Stilbenes

Pinosylvin
(3,5-dihydroxy-*trans*-stilbene)



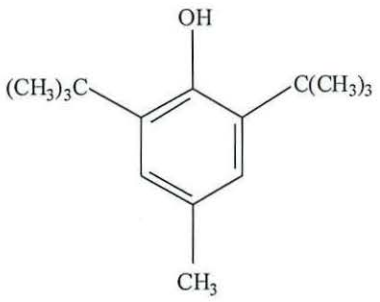

Pinosylvin monomethylether
Pinosylvin dimethylether
3,5,4'-Tetrahydroxystilbene[b]
2,3,5,4'-Tetrahydroxystilbene
3,4,3'5'-Tetrahydroxystilbene[b]
3,4,5,3'5'-Pentahydroxystilbene[b]
4-Hydroxystilbene[b]
Pterostilbene[b]

Figure 2.14 Heartwood extractives known to inhibit decay (Scheffer and Cowling, 1966)

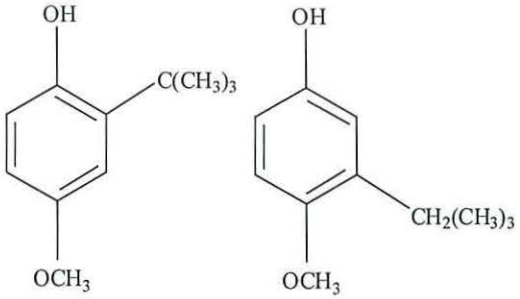
Many plant phenolics are reported as having antioxidant activity against radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994). The potential of extractives as antioxidants depends on the number and arrangement of the hydroxyl groups, the extent of structural conjugation and the presence of substituents in the ring structure (electron donating and electron withdrawing). To function as an antioxidant, hydrogen from phenolic compounds has to donate to highly reactive radicals, thereby preventing further radical formation (Lapornik *et al*, 2005).

An antioxidant can reduce the free radicals and make them stable due to the hydrogen donating ability (initiation or propagation of oxidizing chain reactions) (Gao, 2001). Biological structure in humans (DNA, lipids and proteins) can be damaged because of insufficient entry of antioxidants (Gutteridge and Halliwell, 1994; Papas, 1999).

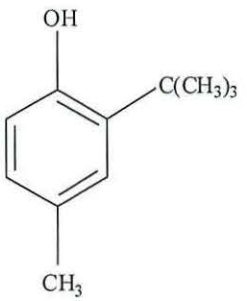
In contrast to the earlier studies, secondary metabolites (extractives) are no longer judged as waste products or as remnants without current function. Due to its complex mixture thus can be exploited in many different ways to get their beneficial role in a diverse array of applications. There are many plant species to be investigated or reinvestigated in the search for new products or other possible uses of wood extractives.



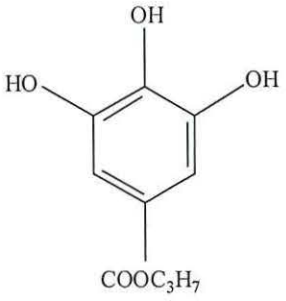

Butylated hydroxytoluene (BHT)




Butylated hydroxyanisole (BHA)




Tertiary-Butylhydroquinone (TBHQ)




Gallic acid esters

Figure 2.15 Chemical structures of food antioxidants of synthetic origin (Shahidi, 2000)

## 2.7 Background of some termite and decay susceptible and resistant trees

In this study a variety of wood species were chosen for study. Instead of just choosing the most durable species for study, the initial selection was made of species across a wide range of durability classes (Table 2.10) and consideration was given to whether adequate quantities of bark would be available for study. In the event verified samples of wood and bark from the log yard at the Forest Research Institute Malaysia were chosen. The range of durabilities was chosen because it was envisaged that performance was not only related to extractive quantities but also extractive characteristics, e.g. toxicity and free radical scavenging activity. Certain species were also chosen because of their known characteristics, including *N. heimii* (very durable and worked on by the supervisor in the past) and *H. brasiliensis* (non durable and probably one of the most susceptible wood species, but also one which was to be used to test the efficacy of extracts in later work, chapter 6).

### 2.7.1 *Alstonia angustifolia* (Apocynaceae)

*Alstonia angustifolia* A. D. C. (pulai) is among the most susceptible woods to termites and fungi (Mohd Dahlan and Tam, 1985; Ani *et al*, 2005). Based on standard graveyard test of untreated specimens of dimension 50 mm x 50 mm x 600 mm, *A. angustifolia* is classified as not durable. All 21 pieces of the specimens tested were destroyed within 6 months (Foxworthy and Woolley, 1930). However, it lasts for about 6 years in indoor application (Ani *et al,* 2005). *A. angustifolia* is usually used as a window frame, skirting, and wood decorative items or for indoor products.

### 2.7.2 *Cotylelobium lanceolatum* (Dipterocarpaceae)

*Cotylelobium lanceolatum* Craib (resak) is often used for all form of heavy constructions, flooring, railway sleepers and wooden containers while the lighter varieties are suitable for cabinet works, high grade joinery, interior fitting and sliced veneer (Choo *et al*, 1999). Heartwood is yellow-brown with an olive tinge, darkening to a dark red-brown. The sapwood is light yellow-brown and well defined.

*C. lanceolatum* has been reported to contain resveratrol oligomers (cotylelophenol C, cotylelosides A and B), *O*-glucosides (vaticasides A, B, C, D) and piceid (Ito *et al*, 2006). This group of stilbenoids showed strong inhibition against termite feeding (Shibutani *et al*, 2004), growth of filamentous fungi (Shibutani *et al*, 1989) and bacteria (Langcake and Pryce, 1976; Hoos and Blaich, 1990).

### 2.7.3 *Cinnamomum scortechinii* (Lauraceae)

*Cinnamomum scortechinii* Gamble (medang) is non-durable, being subject to fungal attacks. Some species of *Cinnamomum* are immune to termite attacks. Due to that, it is only suitable for interior finishing, mouldings, joinery, ornamental items or for interior application as a general. However, the heavier species can be used for covered medium construction (Menon, 1986).

Even though work has not been done yet on *C. scortechinii*, the extractives compounds from the same family; cinnamaldehyde from *C. osmophleum* (Chang and Cheng, 2002) and essential oil from *Cinnamomum* sp. (Lin and Yin, 1995) had antitermitic properties.

### 2.7.4 *Dialium kunstleri* (Leguminosae)

*Dialium kunstleri* var *trifoliolatum* de Wit Rojo (keranji) is classified as moderately durable under the typical Malaysian conditions (Ani and Lim, 1990) with average service life of 4.0 years in graveyard tests (Jackson, 1956) and about 30 to 35 years in above ground contact (Ani *et al*, 2005). The heartwood is golden brown or reddish-brown colour and the sapwood is white to yellowish with fine to moderately coarse texture and interlocked or wavy grains (Choo *et al*, 1999). It is suitable for heavy construction, flooring, handle for striking tools and batons. Generally it is a good general purpose timber. However, the sapwood is susceptible to insect and fungal attack.

### 2.7.5 *Dipterocarpus grandiflorus* (Dipterocarpaceae)

*Dipterocarpus grandiflorus* Blanco (keruing) is a light to dark reddish brown in colour (wood), comparatively coarse to comparatively fine textured, straight grained or very nearly so, strong, hard and heavy. The wood is characterized by the presence of resin ducts which occur in short arcs as seen from end grain surfaces. Although the heartwood is fairly resistant to decay and insect attack, the wood should be treated with preservatives when it is to be used in ground contact (Anon, 1974). The timber (depending on the species) is moderately durable to non-durable under exposed conditions in the tropics.

*D. grandiflorus* heartwood has been reported to contain 0.16% of dipterocarpol (McLean and Watts, 1960), alloaromadendrene, caryophyllene or humulene which have antitermitic properties against *Neotermes* sp. (Messer *et al*, 1990), 3.21% unsaponifiables, 0.27% combined acids, 0.16% fatty acids, 0.05% phenolics, 0.03% resin acids, 0.03%

other acids and 0.06% ethyl ether-insolubles based on the oven-dry weight of heartwood (Salud, 2008).

### 2.7.6 *Fagraea fragrans* (Loganiaceae)

*Fagraea fragrans* Roxb. (tembusu) is a secondary forest species has been classified under strong and durable class (Peters, 1995; Ani *et al*, 2005). It is suitable for heavy construction, marine construction, posts, beams, joists, rafters etc including heavy duty furniture (Menon, 1986).

### 2.7.7 *Khaya ivorensis* (Meliaceae)

*Khaya ivorensis* A. Chev. (khaya), is used principally for furniture, interior finish, boat construction and veneer. Fungi and termites resistance refers to end-uses under temperate climate. The wood are classified under moderately durable (Ani and Lim, 1990). Except for special comments on sapwood, natural durability based on mature wood. Sapwood must always consider as non-durable against wood degrading agents.

Study by Adesida *et al* (1971) shows that *K. ivorensis* contains limonoid (khivorin) and many minor compounds including swietenolide derivatives, and Zhang *et al* (2009) had found another new four limonoid (1-O-deacetyl-6-deoxykhayanolide E, 1-O-deacetyl-2ahydroxykhayanolide E, 3-acetyl-khayalactone, 11a-acetoxy-2a-hydroxy-6-deoxy-destigloylswietenine acetate) besides twelve known limonoids. In addition, study by Severino *et al* (2007) found that limonoids from *Melia azedarch* and *Toona ciliata* (Meliaceae) had higher antifeeding activity against *Heterotermes tenuis*.

### 2.7.8 *Hevea brasiliensis* (Euphorbiaceae)

*Hevea brasiliensis* Willd Muell.-Arg (rubberwood) is a light-coloured hardwood with a density ranging from 435 to 626 $kgm^3$ at 12% moisture content (Rubber Board, 2005). The wood is fine, straight grained and light yellowish to white in colour. It has physical and mechanical properties comparable with timbers like teak, but is very susceptible to attack by organisms due to its high starch content. The sapwood is not differentiated from the heartwood. Blue stain fungi penetrate the ends of logs within a week of felling (Hong *et al*, 1980) and cause an intense stain. The natural resistance of rubber wood to decay and its protection have been studied by Hong *et al* (1982) and Hong and Liew (1989).

*H. brasiliensis* has been classified under non-durable timbers with the average service life of less than 2.0 years under graveyard tests (Jackson, 1956) and only about 6 years in above ground contact (Ani *et al*, 2005).

### 2.7.9 *Madhuca utilis* (Sapotaceae)

*Madhuca utilis* Ridl. H. J. Lam (bitis) is classified as durable under Malaysian classification [average service life of 5.5 years under graveyard tests (Mohd Dahlan and Tam, 1987) more than 56 years in above ground contact (Ani *et al*, 2005)]. It is suitable for all forms of heavy construction including bridges, wharves, piers, piling, posts, railway sleepers, parquet flooring and heavy duty flooring (Lim *et al*, 1998).

A study by Gunasekera *et al* (1977) found a wide range of triterpenoids and steroids in heartwood and bark from members of the Sapotaceae.

### 2.7.10 *Neobalanocarpus heimii* (Dipterocarpaceae)

*Neobalanocarpus heimii* King P. S. Ashton (chengal) was found to be the most resistant to subterranean termites *Cryptotermes cynocephalus* (Ahmad Said and Hamami, 1983), *Coptotermes curvignathus* (Ahmad Said *et al,* 1982), and fungi (Yamamoto and Hong, 1989). *N. heimii* which is synonymous with *Balanocarpus heimii* is a heavy hardwood and classified as naturally durable timber. Termiticidal activity came primarily from hopeaphenol (Coggan *et al*, 1965), Heimiol A, oligostilbenoids, balanocarpol, copalliferol A, and vaticaphenol (Weber *et al,* 2001), and this group of stilbenes also has been reported as having antifungal properties (Robinson, 1980; Hart, 1981).

Untreated specimens can last more than 15.9 years while treated specimens lasted about 19 years in graveyard test conditions (Mohd Dahlan and Azlan, 1994) and more than 50 years in above ground contact (Ani *et al*, 2005). Consequently, it always has been used in heavy construction where strength and durability are essential including railway sleepers, flooring and a favourite timber for boat-building. Besides that, *N. heimii* also a source of fine natural resin (Watson, 1927; Lopez, 1983).

### 2.7.11 *Pommetia pinnata* (Sapindaceae)

*Pometia pinnata* J. R. Forster & J. G. Forster (kasai) has been classified as a moderately durable timber with an average service life of 5 years under natural conditions (Jackson, 1956) and about 34 years in above ground contact (Ani *et al*, 2005). The wood is light red to brown red in colour, had medium texture, straight or interlock grained and

the sapwood is not clearly demarcated. Has been classified under moderate durable class even though it resistant against termite and fungus under temperate climate. Normally use as panelling, flooring, and furniture components as well as exterior joinery with an efficient treatment.

### 2.7.12 *Shorea curtisii* (Dipterocarpaceae)

*Shorea curtisii* Dyer ex King (seraya) is a light hardwood of the dark red meranti type. It has been classified under non durable to moderately durable in ground contact (average service life of 3.1 years under graveyard tests) (Jackson, 1956) which is not resistant against termites. It is not durable under exposed conditions but can last more than 30 years in above ground contact (Ani *et al,* 2005). The timber is one of the more popular dark red merantis, being highly suited for indoor general utility purposes of furniture, highly class interior finishing, flooring, cladding, panelling, fancy doors, mouldings, skirtings and veneers.

# CHAPTER 3: PHYSICAL PROPERTIES AND EXTRACTION

## 3.1 Introduction

Two primary characteristics of wood that are usually taken into account and an influence on other wood properties are moisture content and density. Beside the effects on weight, these also influence the mechanical properties of wood (Kollmann and Krech, 1960; Haygreen and Bowyer, 1982; Armstrong *et al,* 1984; Sandoz, 1993; Zhang, 1994; 1995). Therefore any attempt to utilize a particular wood species should begin by first conducting studies into its physical characteristics.

The best single criterion to determine wood strength is density. Density is the mass contained in a unit volume of wood (Richardson, 1978; Tsoumis, 1991; Desch and Dinwoodie, 1994). It is an important index for wood quality (without defects) (Wangaard, 1950; De Zeew, 1965; Poller, 1967; Zenker, 1967; Landrach, 1986; Tsoumis, 1991) and is much influenced by moisture, structure, extractives and chemical composition (Wangaard, 1950; Kollman and Côté, 1968; Giordano, 1971; Illston *et al*, 1979; Kellogg, 1981; Zobel and Van Buijtenen, 1989; Tsoumis, 1991; Winandy, 1994; Dinwoodie, 2000). A close correlation exists between density and hardness of the wood itself (Hoadley, 1980), drying behaviour, machining characteristics and features such as nail holding properties (Brazier, 1970). Various studies (Einspahr *et al,* 1969; Barefoot *et al,* 1964; 1970; Van Buijtenen *et al,* 1969; Zhang 1992; Zhang *et al,* 1992) have found that density also influences the fibre morphology and paper properties (the most important wood quality characteristics) (Keith and Kellogg, 1986), having major effects on both yield and quality of fibrous and solid wood products (Zobel and Van Buijtenen, 1989; Zhang and Zhong, 1991).

Density also has been reported to correlate with the wood extractives. Density is a ratio of dry weight to the volume of wood which means that how much wood substance is present in a given volume of wood. Low density woods have low wood material per unit volume (Zobel and Van Buijtenen, 1989; Royer *et al*, 2001). Conversely, higher density wood has more cell wall material per unit volume. As wood material may contain extractives in the cell walls and lumens (Panshin *et al*, 1952; Hillis, 1987a; Xu *et al*, 2009), it follows that extractives influence wood density. Esenthers (1977) had studied the natural durability of 21 native and exotic woods and found a positive correlation between specific gravity of different species and decay resistance. Hernandez (2007) in his study found a significant positive correlation between density and wood extractives in tropical hardwoods. Thus wood with higher density had higher extractive contents and were more

toxic to termites than wood with low density. However this is not universally true. Some species like Western red cedar, *T. plicata*, are of low density (ca 330 kg $m^{-3}$ yet show high durability because they contain very toxic extractives.

Consequently, appreciable amounts of information about the timber properties are available and the information gathered is well established. However, studies on tree variation (between and within) of Malaysian wood species on variation of density and the extractives content are not much available. This study has been designed to detail the information about the variation of wood density and determine the extractive contents of twelve Malaysian woods species. The information of it could help in better selection of the wood for specific uses.

Apart from having toxic action, wood extractives from Malaysia timbers have been reported as having free radical scavenging (antioxidant) properties (Pietarinen *et al*, 2006; Gao *et al*, 2007; Kawamura *et al*, 2010). Antioxidants have also been examined as alternatives to toxic preservatives with obvious potential environmental benefits (Gao *et al*, 2007). Small free radicals of various sorts are thought to be released by wood decay fungi because enzymes are too large to move within the wood cell wall matrix (Flournoy *et al*, 1993; Backa *et al*, 1992; 1993) and antioxidants together with fungicides gave more protection against fungal decay (Schultz and Nicholas, 2000; 2002). Bearing in mind the interest in antioxidants a radical scavenging assay on extracts from all 12 species was performed to examine this by the 2, 2-diphenyl-2-picryl-hydrazyl. In addition a total phenolic content assay using the Folin-Ciocalteu method on twelve wood species was made.

Moisture content is also reported here because the wood was shipped partially green from Malaysia and the moisture contents on arrival were not at similar equilibrium moisture contents. Some species had reached a low moisture content while others were still appreciably wetter. This was attributed to their higher densities and extractive contents.

## 3.2 Materials and methods

### 3.2.1 Sampling of the materials

The details of each timber properties are presented in Table 3.1. The sampling procedure for the test samples (Figure 3.1) was the same for each specimen. The heartwood samples came from the outer heartwood. The outer heartwood samples for moisture content and wood density determination were taken from the three different heights of the tree; basal, middle and top. The location of the middle and top levels of the

Table 3.1 Details of 12 Malaysian timber species

| Species | Location | Tree age (years) | Year of felling | Diameter* (cm) |
|---|---|---|---|---|
| *N. heimii* | FRIM (main campus) | 15 | 2004 | 40.3 |
| *C. lanceolatum* | FRIM (Pasoh) | 20 | 2003 | 43.8 |
| *M. utilis* | FRIM (Pasoh) | 18 | 2005 | 52.8 |
| *P. pinnata* | FRIM (Pasoh) | 16 | 2005 | 45.6 |
| *D. grandiflorus* | FRIM (Pasoh) | 15 | 2004 | 42.1 |
| *D. kunstleri* | FRIM (Pasoh) | 18 | 2006 | 50.3 |
| *K. ivorensis* | FRIM (main campus) | 18 | 2006 | 38.4 |
| *F. fragrans* | FRIM (main campus) | 17 | 2006 | 32.3 |
| *S. curtisii* | FRIM (main campus) | 18 | 2007 | 50.1 |
| *A. angustifolia* | FRIM (main campus) | 16 | 2007 | 55.2 |
| *C. scortechinii* | FRIM (main campus) | 17 | 2006 | 48.9 |
| *H. brasiliensis* | RRIM | 18 | 2007 | 48.5 |

Note: FRIM – Forest Research Institute Malaysia area, RRIM – Rubber Research Institute Malaysia
*Diameter at breast height, DBH

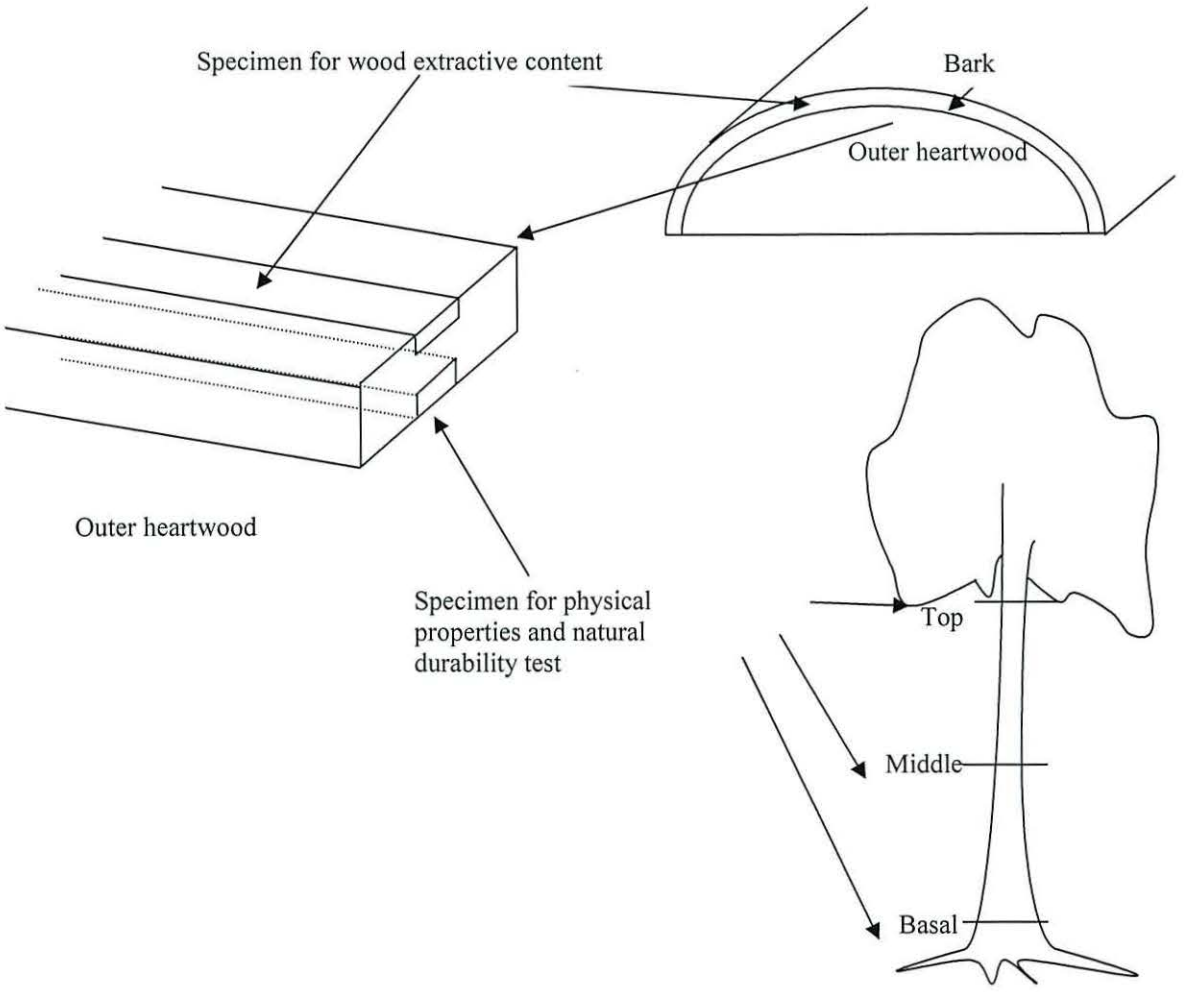



Figure 3.1 Sampling of the evaluation material for physical properties, natural durability test and analysis of wood extractive from dried-planed boards

trees depended on the total height for each tree. Samples from the bark and outer heartwood (basal only) were used to determine the extractive content of each wood species. All the processing materials (cutting and grinding of wood samples) and a part of experiments (durability and bioactivity against termites) were done in Forest Research Institute Malaysia (FRIM). Other experiments (physical properties, extractions, durability and bioactivity against fungus and chemical analysis), were done in Bangor University.

### 3.2.2 Determination of moisture content

Nineteen mm square blocks were cut from the basal, middle and top portion of the outer heartwood of 15 to 20 years old Malaysian trees (with the exception of sapwood from *H. brasiliensis*). They were selected and weighed according to the oven-drying test procedure of ASTM D2016-74n standard (ASTM 1983). Wood species selected in this study were *Neobalanocarpus heimii, Cotylelobium lanceolatum, Madhuca utilis*, *Pometia pinnata, Dipterocarpus grandiflorus, Dialium kunstleri, Khaya ivorensis, Fagraea fragrans, Shorea curtisii, Alstonia angustifolia, Cinnamomum scortechinii* and *Hevea brasiliensis.* The wood species were selected from the three different durability classes; durable, moderately durable and non-durable.

The wood samples were place in a vented, fan-assisted oven at 103±2 °C to dry for 24 hours. Five replicates were prepared for each timber species. The moisture content of the wood samples was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{\text{(Original weight – Oven-dry weight)}}{\text{Oven-dry weight}} \times 100$$

### 3.2.3 Determination of wood density

Wood samples were cut into blocks of 25 x 25 x 9 mm, radial, tangential, longitudinal. The basic density was determined by the Archimedean method (Breese, 1990) using water as a displacement liquid. For each wood species and portion, 15 replicates were taken giving a total of 540 wood blocks.

*Determination of green volume*

All the wood blocks were soaked in deionised water placed in desiccators and vacuum pressure was applied by water pumps. The process was stopped when the wood blocks had sunk. The whole process took about 96 hours (4 days). As the experiment was

conducted at room temperature, density of water was assumed to be 1 (one) and thus the weight of displaced water was equal to the displaced volume. All wood blocks were taken out from desiccators and the volume was measured after 96 hours using digital callipers to 0.01 mm. A displacement method was also used but as there was little variation between the results of the two techniques only the caliper results were used here.

The basic density of wood samples was usually calculated from the oven-dry weight condition of wood as shown below:

$$\text{Basic density (kg m}^{-3}) = \frac{\text{Oven-dry weight of the sample}}{\text{Volume of green wood}}$$

### 3.2.4 Preparation of raw materials for extraction process

The twelve Malaysian wood species were obtained from the lumberyard in FRIM, Malaysia. Each species was divided into two portions; bark and heartwood. The bark and heartwood for this extraction process were taken from the basal portion of each wood species. Both of the portions for each species were chipped and ground in a Wiley Mill, passed through 250 $\mu$m sieve to provide an homogeneous powder for analysis and shipped to Bangor University, UK. All the wood chip samples were kept in a room conditioned at 20 °C and 65% relative humidity before further tests.

### 3.2.5 Extractive content determinations

Extractions with Toluene: Industrial Methylated Spirit (Tol:IMS) (2:1) were done according to the standard method ASTM D1105-96 (2001). Six, 5 g (air-dried at 103±5 °C) portions of sawdust and milled bark of each wood species were weighed to the nearest 0.1 mg then extracted with 240 ml of a solvent mixture of 2:1 Toluene-IMS in a pre-weighed cellulose thimble (porosity 1, height 95 mm) for 5 hours (4 to 6 siphons per hour) in a soxhlet extractor. The Tol:IMS extraction solvent was removed from a pre-weighed round bottom flask by vacuum rotary evaporation at 45 °C and it was re-weighed when the solvent had been removed. The extractive percentages were calculated from the weight of total solids loaded into the extraction thimble and weight of dried extractives recovered from the extracting solvent. A final extraction was then done with 500 ml of deionised water at 100 °C in an autoclave for 3 hours and then filtered in a sintered glass crucible (no. 1), dried in an oven at 103±5 °C, cooled in desiccators and determined the weight loss due to extraction. All the remaining extractives were put in small vials and stored at room

temperature for subsequent analyses. The sum of solvent extracted and water extracted extractives was reported as total extractive contents of the sample. Each wood species were run in 5 replicates.

### 3.2.6 Total phenol assay

The amount of total phenolics in twelve Malaysian wood species was determined according to Folin-Ciocalteu method (Kahkonen *et al.*, 1999). 300 $\mu$l (six replicates) were introduced into test tubes and followed by 1.5 ml Folin-Ciocalteu's reagent (10 x dilutions) and 1.2 ml sodium carbonate (7.5% w/v) was added. The samples were allowed to stand for 30 min and their absorbance was measured at 765 nm using a Shimadzu UV-Vis spectrophotometer. Total phenolic contents were expressed as mg of gallic acid equivalent (GAE $g^{-1}$) of fresh weight. In addition, the total phenols should be scaled to account for the amount of extractive in wood. Unfortunately only the Tol:IMS extractives were quantitatively assayed for total phenols so that the totals from the two extraction procedures cannot be summed. This was because the hot water extract quantities were based on the total weight loss of the wood, minus the Tol:IMS quantity. Thus two sets of data have been produced, that based on the Tol:IMS and that based on the slightly less valid data from the total extractives. The products of these data are calculated as extractive (%) x GAE (%) /100 to give an overall estimations of total extractable phenols.

### 3.2.7 Antioxidant assay

The scavenging activity of Tol:IMS extracts on DPPH radicals was estimated according to the method of Blois (1958). Extracts (4.0 ml of 0.5 mg/ml) were added to 1.0 ml of 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) (1.0 mM in methanol) in 5.0 ml bottle. The mixture was shaken and left for 10 min at room temperature. A control was performed with (+)-catechin alone. The absorbance was measured at 520 nm as above.

The percentage of DPPH scavenging activity was determined by the below formula:

$$A = [(A_o - A_e)/A_o] \times 100$$

Where A = percentage reduction of the DPPH

$A_o$ = initial or blank solution absorbance

$A_e$ = the absorbance value of the sample concentration in the absence of DPPH solution.

All the values of remaining DPPH obtained for each series of dilutions were plotted in a graphic versus the amount of antioxidant/extract and through interpolation the activity of the antioxidant expressed by the parameter $EC_{50}$ (Efficient concentration) was estimated. $EC_{50}$ is the amount of either extract or catechin necessary to decrease a 50 % the initial concentration of DPPH in the steady state. All tests were conducted as six replicates.

To give an estimate of the proportional activity relative to the amount of extractives present the same calculations have been made as for the total phenols.

## 3.3 Results

### 3.3.1 Moisture content

The details and discussions of the 'moisture content as received' in the UK are presented in Appendix 3.

### 3.3.2 Wood density

The average wood densities of the twelve Malaysian wood species (Figure 3.2) varied from basal to the top portions with the highest density at the basal portions. The details of the results are presented in Appendix Table A3.8.

The mean heartwood basic density of the twelve species ranged between 395 kg $m^{-3}$ to 829 kg $m^{-3}$ at basal, 382 kg $m^{-3}$ to 827 kg $m^{-3}$ at middle and 371 kg $m^{-3}$ to 789 kg $m^{-3}$ at top portion. *A. angustifolia* had the lowest basic density at all portions (411 kg $m^{-3}$, 402 kg $m^{-3}$ and 385 kg $m^{-3}$ , respectively) compared to other species . The highest was found in *C. lanceolatum* (803 kg $m^{-3}$) at basal, *N. heimii* (775 kg $m^{-3}$) at middle and *D. grandiflorus/N. heimii* (732 kg $m^{-3}$) at top portion. The basic density is decreased from basal to top portion for every wood species except for *P. pinnata* which are not significant.

Table 3.2 showed that wood species densities were significantly different to each in majority of woods tested. However, there is a trend where durable timber species had higher wood densities than the moderate and non-durable timbers. Table 3.2 showed that majority of wood species were highly significantly different to each other at $P \leq 0.001$ except *H. brasiliensis* and *K. ivorensis* at $P \leq 0.05$. The highest within species variation was found in *N. heimii* (81.8%) and the lowest in *K. ivorensis* (7.2%). The ANOVA table is presented in Appendix Table A3.9.

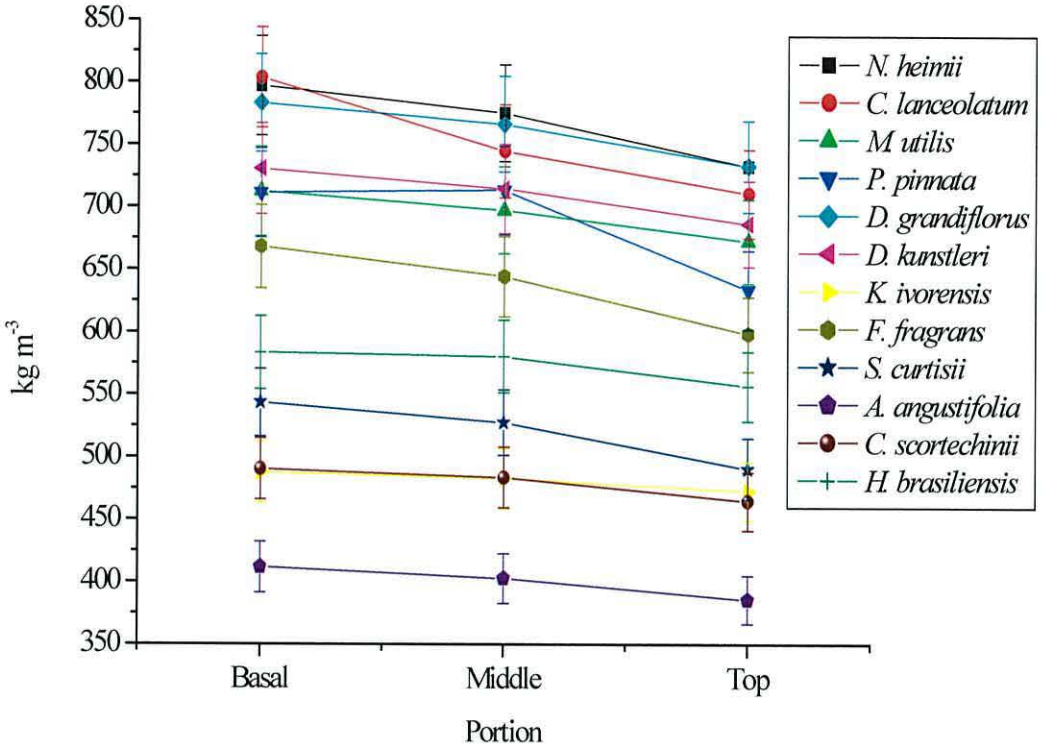



Figure 3.2 Density at different height of twelve Malaysian wood species (means of 15 replicates)

Table 3.2 ANOVA results showing the proportion (%) of total variation ($R^2$) in wood density accounted for the samples within the wood species (general)

| Species | $r^2$ |
|---|---|
| *N. heimii* | 81.8*** |
| *C. lanceolatum* | 65.6*** |
| *M. utilis* | 52.0*** |
| *P. pinnata* | 38.0*** |
| *D. grandiflorus* | 40.0*** |
| *D. kunstleri* | 61.1*** |
| *K. ivorensis* | 7.2* |
| *F. fragrans* | 70.7*** |
| *S. curtisii* | 77.1*** |
| *A. angustifolia* | 46.2*** |
| *C. scortechinii* | 34.6*** |
| *H. brasiliensis* | 12.8* |

* P≤0.05, **P≤0.01, *** P≤0.001, ns – not significant

### 3.3.3 Yields and chemical constituents of wood extractives

A comparison of extractives is more difficult. In previous studies, the results were based separately on either using polar or non-polar solvents. In this study *successive* extractions including both polar and non-polar were performed. Comparing these extractions, therefore involves some approximations.

The crude extracts of bark and heartwood of twelve Malaysian wood species were different in colours. Generally, the bark extracts were darker and gave higher yields (between 3.88% w/w and 34.37% w/w) of the oven dry weight and the heartwood extracts were a little bit lighter in colour with lower yields (between 2.81% w/w and 14.79% w/w). Therefore, a greater quantity of heartwood (440 g) was used to provide enough extractive to evaluate the antitermitic and antifungal activities. The differences in extraction yields between bark and heartwood or heartwood and sapwood are quite consistent in most wood species tested. The quantities of the Tol:IMS and hot water extractives in the bark, heartwood and total amounts in heartwood and bark are presented in Figures 3.3, 3.4 and 3.5. The data relating to this are presented in Appendix Table A3.10.

*N. heimii* exhibited the highest extractive yields both in bark and heartwood (34.37% w/w and 14.79% w/w, respectively). These were significantly higher than those observed for all other species. *H. brasiliensis* exhibited the lowest total extractive yields (3.88% w/w and 2.81% w/w) and these were significantly lower than *M. utilis* (20.86% w/w and 9.02% w/w), *P. pinnata* (17.85% w/w and 9.14% w/w), *D. kunstleri* (10.61% w/w and 5.0 2% w/w), *D. grandiflorus* (6.51% w/w and 4.51% w/w), *K. ivorensis* (13.34% w/w and 8.39%), *C. scortechinii* (5.28% w/w and 4.50% w/w), *A. angustifolia* (4.83% w/w and 4.71% w/w), *C. lanceolatum* (22.07% w/w and 9.34% w/w), *S. curtisii* (19.71% w/w and 7.58% w/w) and *F. fragrans* (9.60% w/w and 8.81% w/w). In general, results show that very durable and durable woods contained higher amounts of wood extractives than moderately and non-durable woods.

The quantities of both the Tol:IMS and hot water extractives were much greater in the bark as is widely reported (Fengel and Wegener, 1989). The results in Figures 3.3, 3.4 and 3.5 also indicate that bark is significantly different from the heartwood in Tol:IMS, hot water and total extractives. In general, more than twice as many total extractives were found in the bark as in the heartwood.

Statistical comparisons of extractive amounts (Figures 3.3 and 3.4) showed that many wood species had significantly different amounts of extractives when extracted with Tol:IMS, except three timber species in bark (*D. kunstleri*, *C. scortechinii* and *F. fragrans*)

and seven for heartwood (*P. pinnata*, *D. kunstleri*, *D. grandiflorus*, *C. scortechinii*, *A. angustifolia*, *S. curtisii* and *F. fragrans*). Only *M. utilis*, *N. heimii*, *P. pinnata*, *D. kunstleri*, *K. ivorensis* and *F. fragrans* showed statistically significant differences in bark extractive contents when extracted with hot water while *M. utilis*, *N. heimii*, *K. ivorensis* and *H. brasiliensis* showed significant differences in heartwood.

However, when all the yields of wood extractives had been totalled up (Figure 3.5), all wood species were highly significantly different at $P \leq 0.001$ for bark while eight (*M. utilis*, *P. pinnata*, *D. kunstleri*, *D. grandiflorus*, *K. ivorensis*, *C. scortechinii*, *A. angustifolia*, and *F. fragrans*) were not significantly different for heartwood at $P \leq 0.001$ and $P \leq 0.05$. The ANOVA tables are presented in the Appendix Tables A3.11, A3.12, A3.13, A3.14, A3.15 and A3.16.

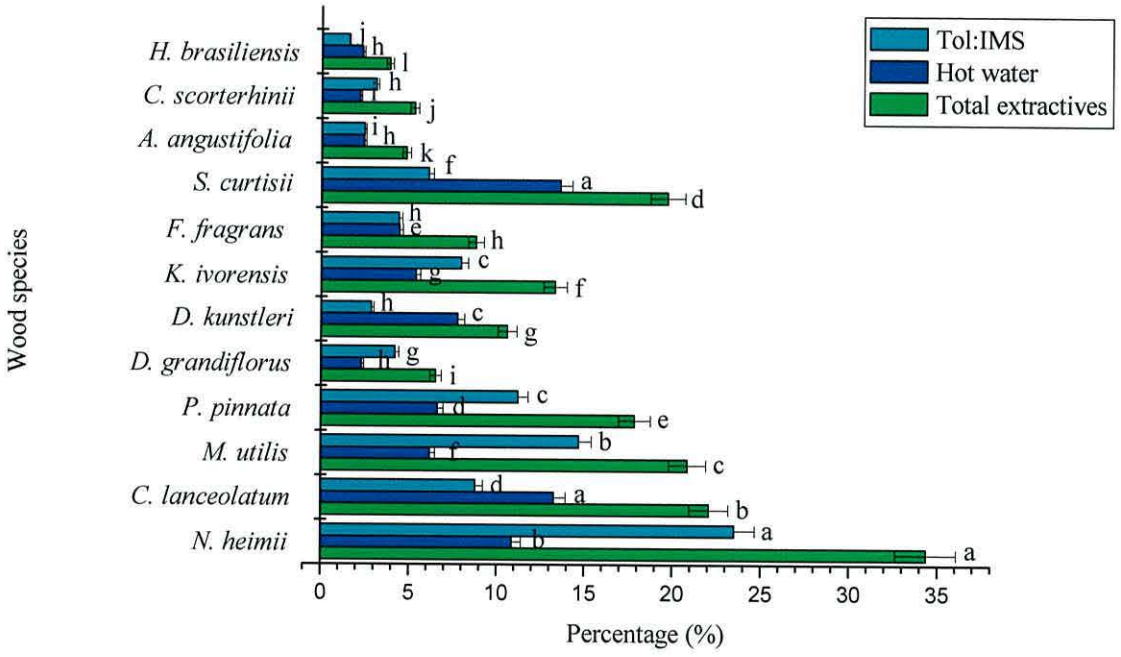



Figure 3.3 Tol:IMS, Hot water and Total extractive contents (%) of bark of twelve Malaysian wood species

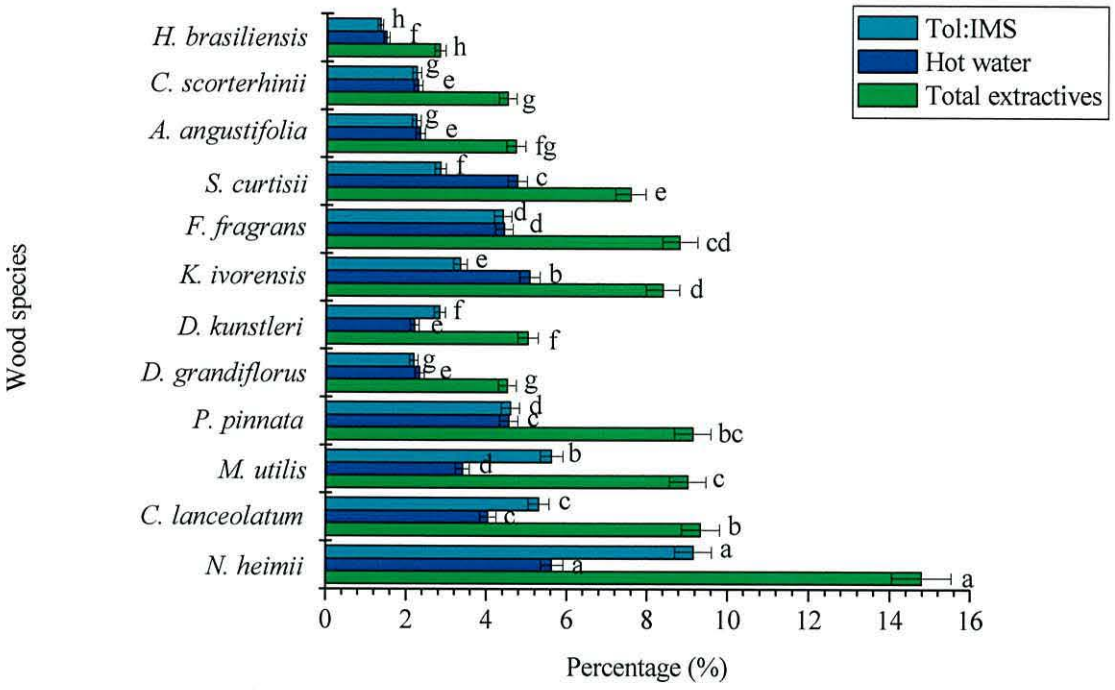



Figure 3.4 Tol:IMS, Hot water and Total extractive contents (%) of heartwood of twelve Malaysian wood species

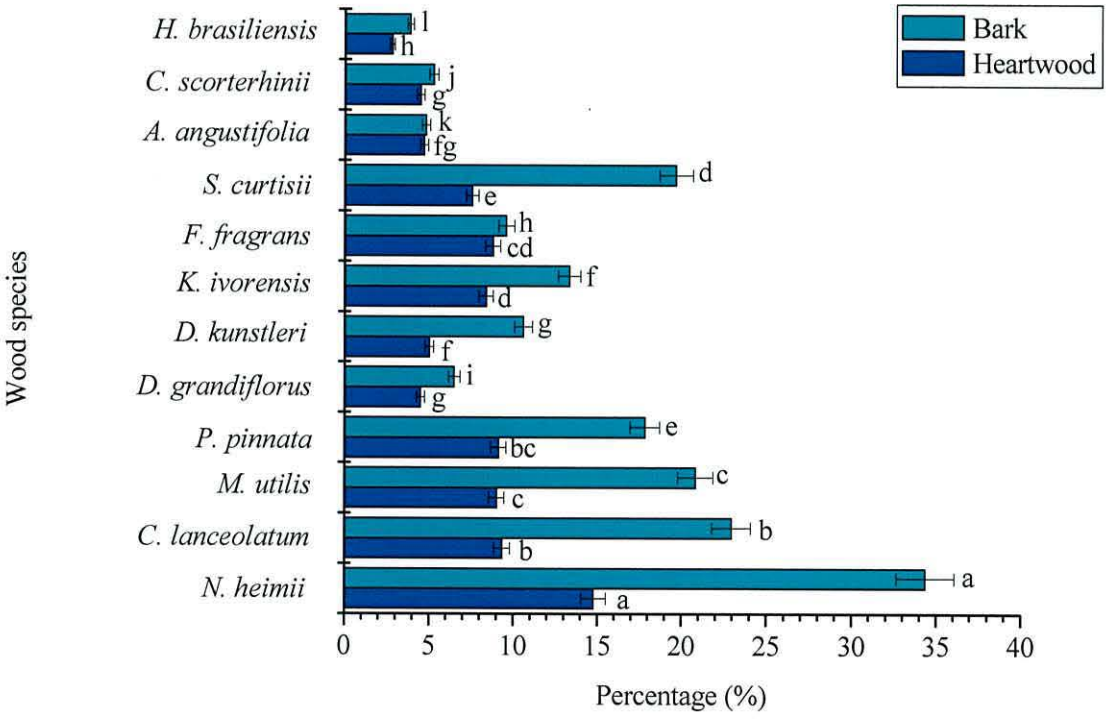



Figure 3.5 A comparison of bark and heartwood total extractive contents

Table 3.3 ANOVA results showing the proportion (%) of total variation ($R^2$) in Tol:IMS, Hot water and Total extractives accounted for samples tested ground into woodflour (heartwood)

| Species | Tol:IMS | Hot water | Total extractives |
|---|---|---|---|
| *N. heimii* | 98.25*** | 99.17*** | 99.10*** |
| *C. lanceolatum* | 99.37*** | 98.70*** | 99.28*** |
| *M. utilis* | 99.86*** | 97.69*** | 99.85*** |
| *P. pinnata* | 96.45*** | 94.70*** | 98.49*** |
| *D. grandiflorus* | 97.82*** | 0.41ns | 91.42*** |
| *D. kunstleri* | 0.15ns | 98.53*** | 95.90*** |
| *K. ivorensis* | 97.98*** | 28.32ns | 97.26*** |
| *F. fragrans* | 56.56* | 96.98*** | 42.74* |
| *S. curtisii* | 93.91*** | 99.69*** | 99.39*** |
| *A. angustifolia* | 28.38ns | 12.82ns | 10.86ns |
| *C. scortechinii* | 86.33*** | 5.93ns | 66.98** |
| *H. brasiliensis* | 48.07* | 87.01*** | 86.39*** |

* P≤0.05, **P≤0.01, *** P≤0.001, ns – not significant.

Table 3.4 General correlation (R) between wood density and extractives yield of twelve Malaysian wood species

| | R |
|---|---|
| Density vs extractive yields | 0.566* |

* P≤0.05, **P≤0.01, *** P≤0.001, ns – not significant.

Table 3.5 Correlation (R) for each replicates of each wood species (within species) between wood density and extractives yield of twelve Malaysian wood species (n = 5)

| Wood species | R | P value |
|---|---|---|
| *N. heimii* | 0.71 | 0.096 |
| *C. lanceolatum* | 0.87 | 0.062 |
| *M. utilis* | 0.94 | 0.027 |
| *P. pinnata* | 0.69 | 0.201 |
| *D. grandiflorus* | 0.91 | 0.034 |
| *D. kunstleri* | 0.64 | 0.259 |
| *K. ivorensis* | 0.66 | 0.231 |
| *F. fragrans* | 0.42 | 0.483 |
| *S. curtisii* | 0.98 | 0.002 |
| *A. angustifolia* | 0.41 | 0.490 |
| *C. scortechinii* | 0.95 | 0.018 |
| *H. brasiliensis* | 0.87 | 0.057 |

Surprisingly, ANOVA (Table 3.3) showed low variation of the Tol:IMS extractive contents in *D. kunstleri* (0.15 %) and of the hot water extracts of *D. grandiflorus*, *K. ivorensis* and *C. scortechinii* (0%). However, highly significant difference (P≤0.001) in variation has been found in majority of woods tested. *A. angustifolia* was the only timber species that showed no significant difference with either solvent extraction system and also in total amount of extractives. The others showed highly significantly differences between

the samples tested. The ANOVA tables are presented in Appendix Tables A3.17, A3.18 and A3.19.

Statistical analysis was done in order to determine the relationships between wood density and extractives contents for each wood species. Analysis shows that in general, wood density was positively correlated with the quantity of wood extractives (Tables 3.4). A strong correlation occurred in three out of twelve wood species ($P<0.01$) (Table 3.5). Only five wood species showed no significant correlation. The other species are significant at $P<0.05$.

### 3.3.4 Antioxidant

#### 3.3.4.1 Total phenolic content

The antioxidant activity of plants often involves phenolic compounds so the total phenol content for the extracts has been determined. The total phenolic content of twelve Malaysian wood species extracts was determined from a regression equation of the calibration curve ($y = 0.2591x + 0.0099$, $R^2 = 0.99$) and was expressed in percentage of gallic acid equivalents (GAE) (Figure 3.6). The data for this are presented in Appendix Table A3.20.

Figure 3.6 shows that there was a large variation in total phenols of the Tol:IMS extracts of the wood species analysed. The values ranged from 4.91 to 29.11% for bark and 4.28 to 24.11% for heartwood. Highest levels of total phenols were obtained from *N. heimii* both for bark and heartwood (29.11% and 24.11%, respectively). *A. angustifolia* gave the lowest total phenol content of both bark and heartwood extracts, at 4.91% and 4.28%, respectively. Other wood species with high total phenols (GAE > 20% dry weight) were *C. lanceolatum* (bark; 23.15% and heartwood; 20.05%) and *M. utilis* (bark; 22.14%).

#### 3.3.4.2 Scavenging activity on 2, 2-diphenyl-2-picrylhydrazyl radical

The DPPH radical scavenging activity of twelve Malaysian wood species extracts was evaluated. Figure 3.7 shows the antioxidant concentration that can scavenge (reduce the concentration by) 50% of the DPPH radical. The details of the result are presented in Appendix Table A3.20.

All twelve Malaysian wood species exhibited concentration-dependent DPPH radical scavenging activity either in bark or heartwood. DPPH radical scavenging activities varied from 22.59 to 93.60% for bark extracts and 7.45 to 83.78% for heartwood extracts. In each case bark extracts were higher than the equivalent weights of heartwood.

Given the protective role of bark this is hardly surprising. Among the extracts isolated, *N. heimii* bark showed the highest anti-oxidant activity for bark and heartwood (93.60% and 83.78%, respectively). The lowest of anti-oxidant activities were found in *A. angustifolia* (22.59% and 7.45%, respectively). Other wood species that gave high values ($EC_{50}$ > 70%, dry weight) were *C. lanceolatum* (bark; 86.28% and heartwood; 81.38%), *F. fragrans* (bark; 71.83%), *H. brasiliensis* (bark; 70.07%), *M. utilis* (bark; 83.61% and heartwood; 75.28%), *P. pinnata* (bark; 72.94%) and *S. curtisii* (bark; 84.11% and heartwood; 76.80%). In general the crude extracts from all wood species (except heartwood of *D. grandiflorus* and, both bark and heartwood of *A. angustifolia*) exhibited stronger antioxidant activity than (+)-catechin and the extracts from *C. scortechinii* and *K. ivorensis* had similar antioxidant potential to (+)-catechin (Figure 3.7).

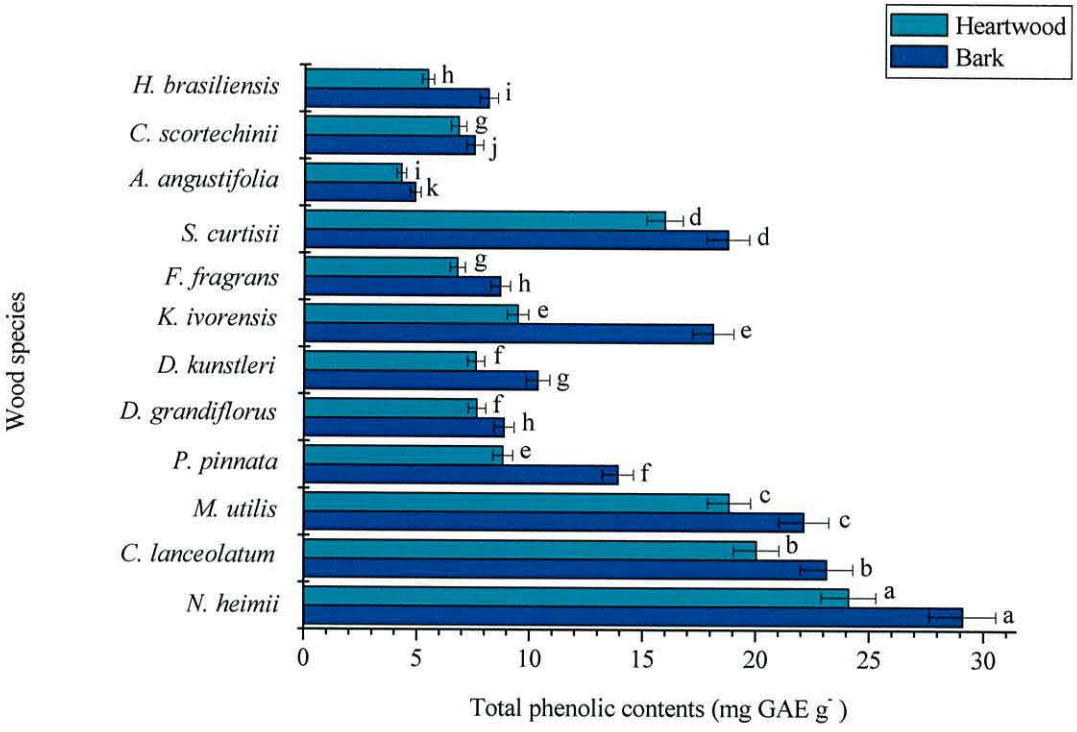



Figure 3.6 Content of total phenolics by gallic acid equivalent (GAE). All the values were calculated on the basis of 0.01 g fresh sample. Results are means of six replicates (n = 6)

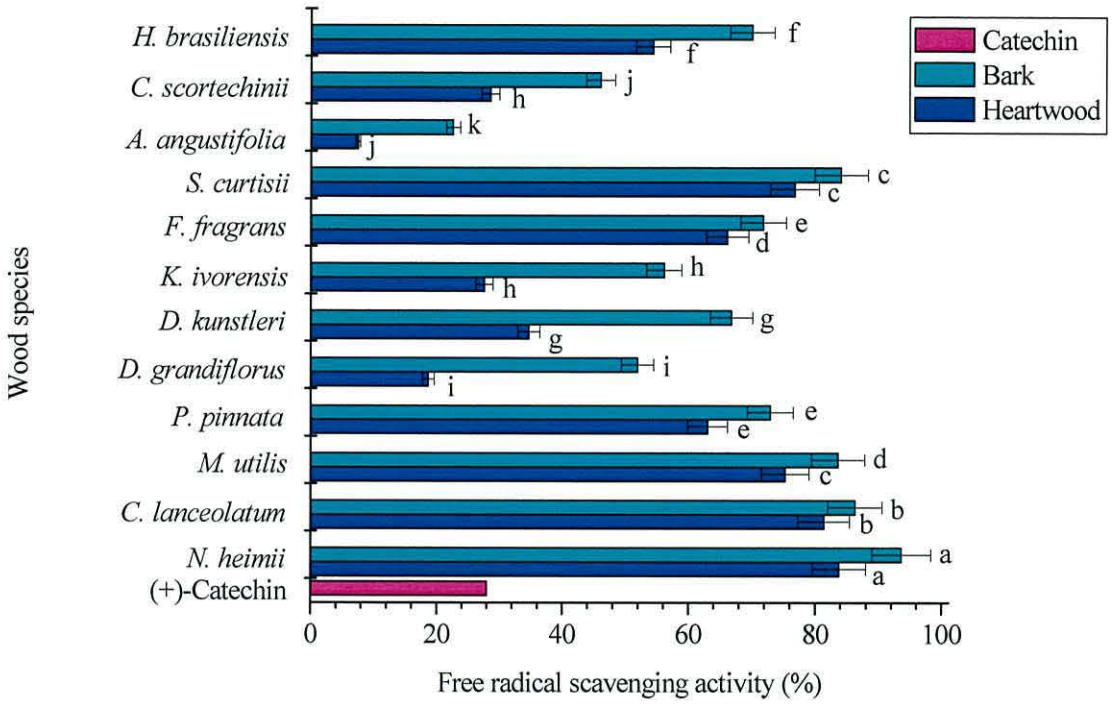



Figure 3.7 Free-radical scavenging activity of twelve Malaysian wood species measured using the DPPH assay. Result are means of six replicates (n = 6).

### 3.3.4.3 Phenolic content and the radical scavenging activity: correlation, total extractable phenols and total anti-oxidant capacity

The total phenolic content (Table 3.6) for each wood species and as general in Figures 3.8 and 3.9 was positively correlated with antioxidant activity for both bark and heartwood but it is clear that the relationship is not best described by a straight line. This will be discussed more fully later. A number of species having higher anti-oxidant activity per phenol unit include the least durable species, *H. brasiliensis*.

Table 3.6 Correlation (R) between total phenolic content and radical scavenging activity (DPPH)

| Wood species | Portion | | | |
|---|---|---|---|---|
| | Bark | | Heartwood | |
| | R | P value | R | P value |
| *N. heimii* | 0.97 | 0.002 | 0.74 | 0.027 |
| *C. lanceolatum* | 0.77 | 0.019 | 0.78 | 0.020 |
| *M. utilis* | 0.64 | 0.167 | 0.86 | 0.012 |
| *P. pinnata* | 0.81 | 0.014 | 0.75 | 0.026 |
| *D. grandiflorus* | 0.56 | 0.250 | 0.61 | 0.197 |
| *D. kunstleri* | 0.96 | 0.013 | 0.84 | 0.016 |
| *K. ivorensis* | 0.58 | 0.228 | 0.73 | 0.101 |
| *F. fragrans* | 0.59 | 0.221 | 0.61 | 0.202 |
| *S. curtisii* | 0.91 | 0.015 | 0.88 | 0.014 |
| *A. angustifolia* | 0.89 | 0.018 | 0.93 | 0.011 |
| *C. scortechinii* | 0.89 | 0.018 | 0.97 | 0.002 |
| *H. brasiliensis* | 0.62 | 0.189 | 0.74 | 0.027 |

When the total extractable phenols are considered (total phenols x total extractive contents, for Tol:IMS (%) or total extractives (%)) more meaningful relationships emerge between durability and total extractable phenols (Table 3.7). The most durable species have high total extractable phenols (>1.2) whereas the less durable species have 0.81 or less.

Consideration of the same transformation for the anti-oxidant capacity, gives a total anti-oxidant capacity (Table 3.8). Although the highly durable timbers clearly have a high anti-oxidant capacity and the very low durability timber have a low capacity, the timbers of intermediate durability (anti-oxidant capacity of 5.8) do not separate out *S. curtisii*, which is more durable than the other two species (*F. fragrans* and *P. pinnata*).

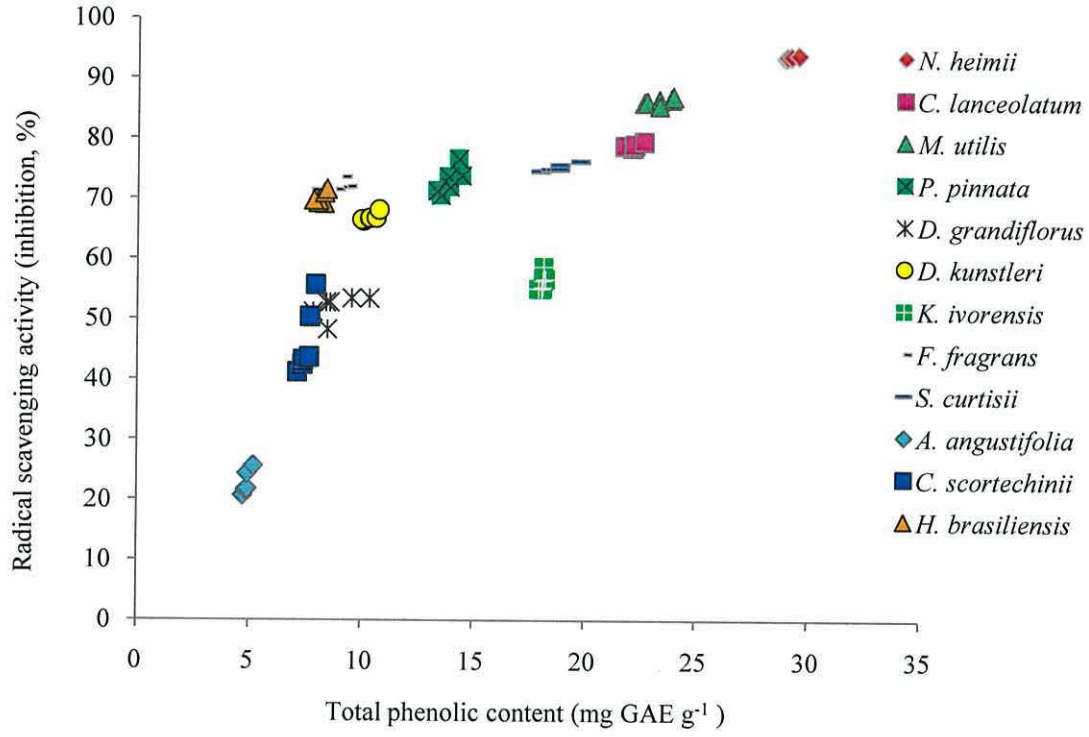



Figure 3.8 Correlation between radical scavenging capacity assays (DPPH) and total phenolic content of twelve Malaysian wood species (bark)

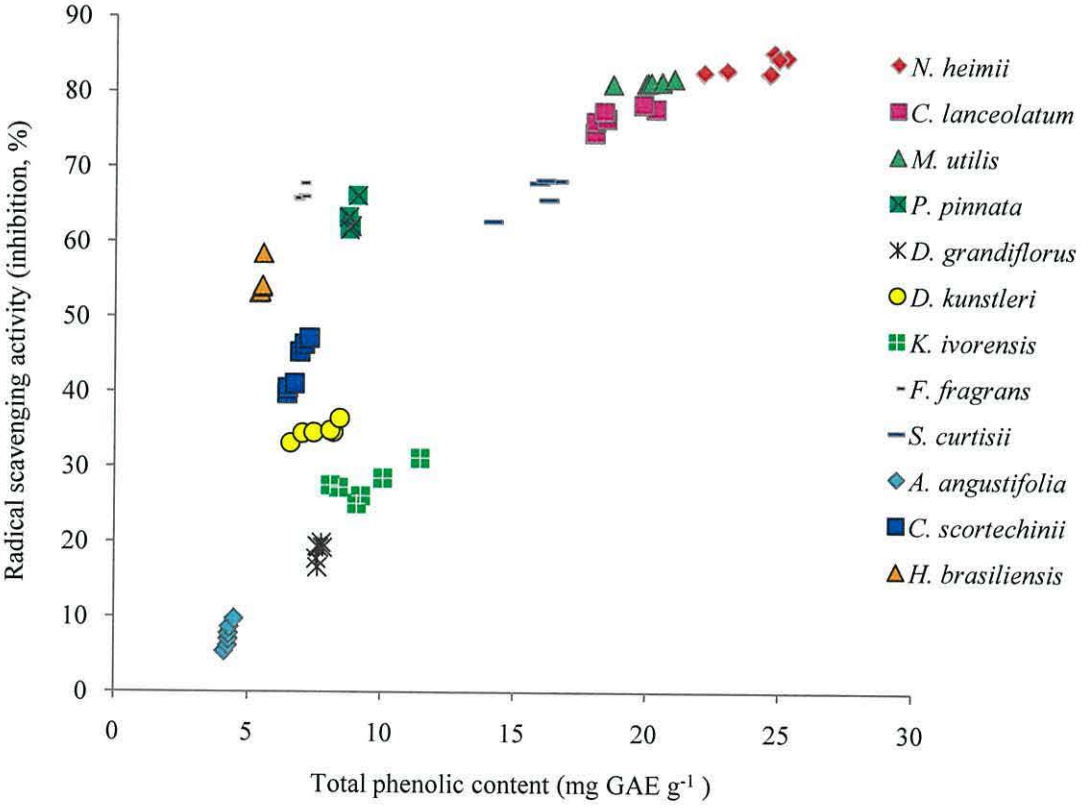



Figure 3.9 Correlation between radical scavenging capacity assays (DPPH) and total phenolic content of twelve Malaysian wood species (heartwood)

Table 3.7 The total extractable phenols as a product of the total phenols x the Tol:IMS and total phenols x total extractives

| Wood species | Tol:IMS (%) | Total extractives (%) | Total phenols (mg GAE $g^{-1}$) | Total extractable phenols Tol:IMS* | Total extractable phenols Total extractives** |
|---|---|---|---|---|---|
| *H. brasiliensis* | 1.33 | 2.8 | 5.5 | 0.07 | 0.15 |
| *D. grandiflorus* | 2.18 | 4.5 | 7.7 | 0.17 | 0.35 |
| *C. scortechinii* | 2.24 | 4.5 | 6.8 | 0.15 | 0.31 |
| *A. angustifolia* | 2.23 | 4.6 | 4.3 | 0.10 | 0.20 |
| *D. kunstleri* | 2.82 | 5.0 | 7.6 | 0.21 | 0.38 |
| *S. curtisii* | 2.83 | 7.6 | 16.0 | 0.45 | 1.21 |
| *K. ivorensis* | 3.33 | 8.4 | 9.5 | 0.32 | 0.79 |
| *F. fragrans* | 4.39 | 8.8 | 6.8 | 0.30 | 0.60 |
| *M. utilis* | 5.62 | 9.0 | 18.8 | 1.06 | 1.70 |
| *P. pinnata* | 4.59 | 9.1 | 8.8 | 0.41 | 0.81 |
| *C. lanceolatum* | 5.3 | 9.3 | 20.1 | 1.06 | 1.87 |
| *N. heimii* | 9.16 | 14.8 | 24.1 | 2.21 | 3.57 |

* This is tol:IMS x total phenols / 100

** This is total extractives x total phenols /100

Table 3.8 The total anti-oxidant capacity as a product of the free radical scavenging capacity x the Tol:IMS and free radical scavenging capacity x total extractives

| Wood species | Tol:IMS (%) | Total extractives (%) | Free radical scavenging activity | Total anti-oxidant capacity Tol:IMS* | Total anti-oxidant capacity Total extractives** |
|---|---|---|---|---|---|
| *H. brasiliensis* | 1.33 | 2.8 | 54.4 | 0.72 | 1.5 |
| *D. grandiflorus* | 2.18 | 4.5 | 18.7 | 0.41 | 0.8 |
| *C. scortechinii* | 2.24 | 4.5 | 28.5 | 0.64 | 1.3 |
| *A. angustifolia* | 2.23 | 4.6 | 7.5 | 0.17 | 0.3 |
| *D. kunstleri* | 2.82 | 5.0 | 34.7 | 0.98 | 1.7 |
| *S. curtisii* | 2.83 | 7.6 | 76.8 | 2.17 | 5.8 |
| *K. ivorensis* | 3.33 | 8.4 | 27.6 | 0.92 | 2.3 |
| *F. fragrans* | 4.39 | 8.8 | 66.2 | 2.91 | 5.8 |
| *M. utilis* | 5.62 | 9.0 | 75.3 | 4.23 | 6.8 |
| *P. pinnata* | 4.59 | 9.1 | 63.1 | 2.89 | 5.8 |
| *C. lanceolatum* | 5.3 | 9.3 | 81.4 | 4.31 | 7.6 |
| *N. heimii* | 9.16 | 14.8 | 83.8 | 7.67 | 12.4 |

* This is tol:IMS x free radical scavenging activity / 100

** This is total extractives x fre radical scavenging activity /100

As a conclusion, the three durable timbers (*N. heimii, C. lanceolatum* and *M. utilis*) extracts had a much higher content of both total extractable phenols and anti-oxidant capacity than the other species examined. Even though *S. curtisii* is not in the durable class, the performance for both properties is close to the durable timbers. It had a lower anti-oxidant capacity than the durable timbers examined but was similar to *F. fragrans* and *P. pinnata*. The total extractable phenols were high and approaching that of *M. utilis*.

Table 3.9 Correlation (R) between wood extractive yield and total phenol content of twelve Malaysian hardwood species (within species) (n = 5)

| Wood species | Portion | | | |
|---|---|---|---|---|
| | Bark | | Heartwood | |
| | R | P value | R | P value |
| *N. heimii* | 0.92 | 0.029 | 0.76 | 0.075 |
| *C. lanceolatum* | 0.88 | 0.051 | 0.94 | 0.018 |
| *M. utilis* | 0.93 | 0.020 | 0.84 | 0.077 |
| *P. pinnata* | 0.53 | 0.578 | 0.96 | 0.008 |
| *D. grandiflorus* | 0.92 | 0.030 | 0.76 | 0.078 |
| *D. kunstleri* | 0.81 | 0.096 | 0.93 | 0.020 |
| *K. ivorensis* | 0.84 | 0.073 | 0.41 | 0.752 |
| *F. fragrans* | 0.86 | 0.062 | 0.84 | 0.078 |
| *S. curtisii* | 0.85 | 0.071 | 0.72 | 0.231 |
| *A. angustifolia* | 0.71 | 0.221 | 0.90 | 0.036 |
| *C. scortechinii* | 0.59 | 0.599 | 0.89 | 0.020 |
| *H. brasiliensis* | 0.62 | 0.315 | 0.60 | 0.326 |

Table 3.9 shows that extractive yield had positive correlation with the total phenol content either for bark or heartwood. Only a few wood species showed no significant correlation.

## 3.4 Discussion

### 3.4.1 Variation of wood density between species

Statistical analysis (Table 3.1) showed that variation in wood density between species were large. Previous studies (Anon, 1962; De Zeew, 1965; Kollmann and Côté, 1968; Panshin and De Zeeuw, 1971; Giordano, 1971; Illston *et al* 1979; Lai *et al,* 1980; Kellogg, 1981; Zhang *et al*, 1996; Dinwoodie, 2000) have shown that the wood structure, extractive content and chemical composition also give significant variation in wood density.

### 3.4.2 Variation of wood density for samples within tree

Variation of outer heartwood density also occurs within tree species (Table 3.2). Half of the wood species tested showed the variation of less than 50% (*A. angustifolia*; 46.2%, *C. scortechinii*; 34.6%, *D. grandiflorus*; 40.0%, *H. brasiliensis*; 12.8%, *K. ivorensis*; 7.2% and *P. pinnata*; 38.0%). The other species showed more than 50% variation. The result from this study seems to be in agreement with Bonnemann (1980), Bowyer *et al* (1982) and Kärkäinenn (1985) that wood density varies significantly within height or a species.

As far as wood density is concerned, factors such as chemical deposits within and between the cell wall can also drastically modify the wood density or specific gravity (Zobel and Van Buijtenen, 1989; Hernandez, 2007). This is true especially for tropical hardwoods which have chemical or crystalline products that deposited in, on or between the cell walls.

### 3.4.3 Extractive contents

Carter *et al* (1975) concluded that there is no single solvent can extract all the non-structural components from wood. Indeed, Fengel and Wegener (1989) reported that the extractive content depends not only on the wood species but also on the solvents used. No single sequence of extractions applies equally to all woods and the degree of milling has an influence too. For 24 Malaysian hardwood species, Yamamoto and Hong (1989) found a range between 0.7 to 12.5% (block) and 1.9 to 23.0% (flour) from hot water extraction and 1.6 to 29.8% (block) and 3.1 to 32.6% (flour) when extracted with methanol.

This study found that the total extractives of bark are higher than heartwood when extracted using standard quantitative extraction methods of Tol:IMS followed by hot water extraction. The bark had a general range between 3.66 to 36.25% while heartwood had 2.47 to 15.17%. Previous studies showed that tropical hardwood timbers have higher extractive contents in the bark than heartwood. Work by Kawamura *et al* (2010) reported a range of between 1.97 to 19.88% for bark, 1.77 to 10.88 for heartwood and 2.11 to 6.99 for sapwood of 15 selected of Malaysian hardwoods with methanol extraction. The higher extractive contents of bark have previously been reported in a chemical study of various softwood and hardwoods tree species (Blankenhorn *et al*, 1985; Jalaluddin and Labosky, 1985; Laks, 1991; Välimaa *et al*, 2007). Indeed, it not only had higher amount of polyphenols but suberin and high-molecular weight tannins which were not commonly found in the wood (Chen and Pan, 1991).

The higher percentage of extractives in the bark was expected, as extractives are active to protect the tree against exterior attack (Ralph *et al*, 2007) due to the large amount of tannins (Schowalter and Morrell, 2002; Yang, 2009). Tannins were reported the main components (Lotz and Hollaway, 1988) and were relatively high in quantity (Nurulhuda *et al*, 1990) in the bark of *Rhizophora mucronata*. They found that tannin extracts are water soluble and difficult to fix in the wood. Other studies also confirmed the present of tannins at very high concentrations in bark of conifers (Aoyama *et al*, 1983), eucalyptus (Cadahia

*et al*, 1997) and leguminous plants (Ohara *et al*, 1994; Yazaki, 1997; Makino *et al*, 2009). Besides that tannin, bark is also richer in other polyphenols (Herrick, 1980; Hillis, 1987a; Kufujita *et al*, 1992; Matthews *et al*, 1997; Karonen *et al*, 2004) and wax-like substances (Blankenhorn *et al*, 1985; Schowalter and Morrell, 2002) than heartwood.

### 3.4.4 Variation of wood extractives between timber species and portions

Figures 3.3 and 3.4 clearly showed that the differences between timber species extractive contents were highly significant, with a few exceptions, irrespective of whether extracted with Tol:IMS or hot water. The total amount of extractive also was significantly different between the timber species and tree portions (bark and heartwood).

### 3.4.5 Phenol content and antioxidants

It is well known that plant phenols (polyphenols) are the highly effective free radical scavengers and anti-oxidants. Extractives from bark and heartwood are reported to have strong anti-oxidant (Chang *et al*, 2001b) and anti-fungal (Chang *et al*, 1999b; Kishino *et al*, 1995) activities mainly due to the number and position of the hydroxyl groups (Rice-Evans *et al*, 1995).

This study found that four (*S. curtisii, M. utilis, C. lanceolatum,* and *N. heimii*) out of twelve wood species extracts had a significantly higher total phenols both in bark (between 18.77% to 29.11%) and heartwood (between 15.97% to 24.11%) (Appendix Table A3.20). The total extractable phenols contents were also high, ranging from 1.21 to 3.57, which matches their durability rating.

Three of the barks from the tree species giving medium durability wood showed similar total phenols contents (*D. grandiflorus*; 8.88%*F. fragrans*; 8.70% and *D. kunstleri*; 10.37% but high contents were found in the other two (*P. pinnata*; 13.92% and *K. ivorensis*; 18.13%). The total phenols from the heartwoods showed less variation (ranging from 6.6 to 9.5%). When the total extractable phenol contents of heartwood are examined a wider relative range is seen (range 0.35 to 0.81). For the low durability class, although *H. brasiliensis* shows a higher total phenol content than the other two wood species (*A. angustifolia and C. scortechinii*) for bark and heartwood, the total extractable phenols content is lower (0.15), some half that of *C. scortechinii* (0.31).

The higher total phenols in the wood of high density hardwoods (*C. lanceolatum, N. heimii and M. utilis)* than medium and light hardwoods (*A. angustifolia*) are expected as reported by Azizol and Rashih (1981) in Malaysian commercial timbers. The variation of

total phenols between the wood species has been reported due to the genetic variation between the wood species (Han *et al*, 2008). Bark extracts showed higher quantities than heartwood extracts.

DPPH radical scavenging activity was reported as the effective concentration of extract fraction needed to decrease the initial DPPH radical by 50% ($EC_{50}$) (Mihara *et al*, 2005) and it is a stable free radical (Blois, 1958; Bondet *et al*, 1997). The test using the reduction of the 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) in the presence of phenolic compounds and has been used for many decades to provide basic information on the reactivity of compounds with regard to their structure (Blois, 1958; Brand-Williams *et al*, 1995; Sanchex-Moreno *et al*, 1998; Silva *et al,* 2000).

Six wood species (*C. lanceolatum, F. fragrans, M. utilis, N. heimii, P. pinnata* and *S. curtisii)* had higher antioxidant activity ($EC_{50} > 60\%$) in the extracts from both bark and heartwood. The higher anti-oxidant activity in the extracts from bark and heartwood of *N. heimii* (94% and 84%, respectively), *C. lanceolatum* (86% and 81%, respectively) and bark of *M. utilis* (84%) and *S. curtisii* (84%) as shown in Figure 3.7, are higher than some well-known anti-oxidant-rich plant extracts (oak – 81% from wood) (Dudonne *et al*, 2009) and higher than 21 of tropical hardwood species from Africa (range between 1.4% to 72%) (Huang *et al*, 2009).

When examined on a total anti-oxidant capacity basis the durable class have high capacities (1.21 to 3.57, group average 2.09) as compared to the moderate durable (0.35 to 0.80, group average 0.62), which is turn is greater than the non-durable class (0.15 to 0.31, group average 0.22). The highest capacity was found in *N. heimii* which was some 24x greater than that of *H. brasiliensis*, with the lowest capacity.

The high activity of *N. heimii* extracts (bark and heartwood) could be due to the availability of stilbenoids (Heimiol A) (Weber *et al*, 2001), flavonoids (Talip *et al*, 2008) and hopeaphenol (Coggan *et al*, 1965). In addition, some studies on Southeast Asia timber had found that plants belonging to Dipterocarpaceae family (in this study; *N. heimii, C. lanceolatum* and *S. curtisii*), Vitaceae, Cyperaceae, Gnetaceae and Leguminosae (Sotheeswaran and Pasupathy, 1993) are rich with resveratrol oligomers (Seo *et al*, 1999; Ito *et al*, 2003). These have been reported to have a variety of biological activities including anti-bacterial, anti-cancer, anti-inflammatory, anti-viral, blood sugar-lowering, cytotoxic effects (Seo and Kinghorn, 2000) and anti-HIV effects (Dai *et al*, 1998). One resveratrol oligomer (3,5,4'-trihydroxystilbene) is one of the best known stilbenes in plants (Cichewicz and Kouzi, 2002).

These studies showed that majority of the wood extracts have good free radical scavenging ability acting possibly as primary antioxidants. It should be noted that only the activity of the Tol:IMS soluble fraction was measured; further activity remains to be explored in the more polar fractions as extractable by water and methanol.

### 3.4.6 Correlation between wood density and extractive contents

Statistical analysis showed that the correlation exists between wood density and extractive yields for all of the species. In general the wood density had a significantly positive correlation with extractive yields ($r = 0.566$, $p = 0.086$) at $P<0.05$ even though it was not strong. However, the correlation is more obvious in individual wood species, i.e. denser samples have higher extractive contents (Table 3.5). *S. curtisii* showed a highly significantly positive correlation ($r = 0.98$). A moderately significantly positive correlation occurred in *N. heimii* ($r = 0.71$), *C. lanceolatum* ($r = 0.87$), *M. utilis* ($r = 0.94$) *D. grandiflorus* ($r = 0.91$), *C. scortechinii* ($r = 0.95$) and *H. brasiliensis* ($r = 0.87$). Other species did not show correlations [*P. pinnata* ($r = 0.69$), *D. kunstleri* ($r = 0.64$), *K. ivorensis* ($r = 0.66$), *F. fragrans* ($r = 0.42$) and *A. angustifolia* ($r = 0.41$)].

### 3.4.7 Correlation between extractives and total phenolic content

In addition to their potential as an antitermitic and antifungal, an important role of plant polyphenols as natural antioxidants has received a lot of attention recently. Interest has been due to the diversity and complexity of the natural mixtures of phenolic compounds and their potential applications (Surveswaran *et al*, 2007).

Table 3.9 shows that total phenolic content had a significantly positive correlation with amount of wood extractives in bark (eight species) and heartwood (nine species). The strong correlation in bark exists in *N. heimii* ($r = 0.92$), *C. lanceolatum* ($r = 0.88$), *M. utilis* ($r = 0.93$), *D. grandiflorus* ($r = 0.92$), *D. kunstleri* ($r = 0.81$), *K. ivorensis* ($r = 0.84$), *F. fragrans* ($r = 0.86$) and *S. curtisii* ($r = 0.85$).

One highly significant correlation has been found in heartwood of *P. pinnata* ($r = 0.96$). The others are moderately significant correlations (*N. heimii*: $r = 0.76$, *C. lanceolatum*: $r = 0.94$, *M. utilis*: $r = 0.84$, *D. grandiflorus*: $r = 0.76$, *D. kunstleri*: $r = 0.93$, *F. fragrans*: $r = 0.84$, *A. angustifolia*: $r = 0.90$, *C. scortechinii*: $r = 0.89$).

The correlation between the above two variables are not significant for bark extracts of *A. angustifolia* ($r = 0.71$), *C. scortechinii* ($r = 0.59$), *H. brasiliensis* ($r = 0.62$)

and *P. pinnata* (r = 0.53). Table 3.9 also showed that the invalid results for heartwood extract (*H. brasiliensis*: r = 0.60, *K. ivorensis*: r = 0.41 and *S. curtisii*: r = 0.72).

The result from this study suggests that the tree portion which had higher extractives content also had higher phenolic content (i.e. bark). A good correlation can be established between the total phenolic content and the Tol:IMS soluble extractive content.

The correlation between the wood extractives and total phenols was also found in Makino *et al* (2009). They found that the amount of total phenols increased parallel with the total extractives irrespective whether water or acetone / water was used as an extraction solvent in four tree species (*Acacia mangium, A. auriculiformis, Rhizophora apiculata* and *Larix leptolepis*). Besides that, the total polyphenolics content also increased when they increase the temperature during extraction process.

Niemz *et al* (2010) also found a highly strong correlation with temperature and extractives ($R^2$ = 0.955) in various hardwood samples extracted with ethanol-toluene solvent. They suggest that this is due to the decomposition of lignin which is break-down by a wider different temperature. The transformation of lignin increased the amounts of phenolic extractives in the wood thus increases the yield of extractives.

### 3.4.8 Correlation between total phenolic content and antioxidant

Several recent studies had shown high correlations between total phenols and anti-oxidant activities in plants (wood, bark, stems, pods, leaves, fruit, roots, flowers, pollen and seeds) (Chanwitheesuk *et al*, 2005). A significant positive correlation occurred between total phenols (Table 3.6) and antioxidant potential for some all wood species. Significant (or better) correlations were found in the bark of seven species (*D. kunstleri, N. heimii, A. angustifolia, C. lanceolatum, C. scortechinii, P. pinnata* and *S. curtisii*) and in the heartwood of nine species (*A. angustifolia, C. scortechinii C. lanceolatum, D. kunstleri, H. brasiliensis, M. utilis, N. heimii, P. pinnata* and *S. curtisii*. The other five wood species showed invalid but reasonable correlations between the total phenols and the anti-oxidant activity from the extracts (*M. utilis*: r = 0.64, *D. grandiflorus*: r = 0.56, *K. ivorensis*: r = 0.58, *F. fragrans*: r = 0.59, and *H. brasiliensis*: r = 0.62) and only three wood species from heartwood extracts (*D. grandiflorus*: r = 0.61, *F. fragrans*: r = 0.61 and *K. ivorensis*: r = 0.73).

This study suggests that majority of Malaysian wood species wood extracts possessed very strong anti-oxidant activities. It is observed that the potential of anti-oxidant activity increases with the total amount of phenols in the wood extracts. In other

words, total phenols content had a positive correlation with anti-oxidant activity, although in some samples this was not significant. The ability of some phenolic substances to act as potential anti-oxidants agreed with previous findings in many tropical timbers (Rice-Evans *et al*, 1995; Kahkonen *et al*, 1999; Pietarinen *et al*, 2006; Rodeiro *et al*, 2006; Rodríguez *et al*, 2006; Banerjee *et al*, 2008; Kawamura *et al*, 2010b). Osawa (1994) explained that total phenols had an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Generally, their potential depends on the redox properties of the phenolic hydroxyl groups which act as reducing agents, hydrogen donating anti-oxidants and oxygen quenchers (Rive-Evans and Miller, 1996; Andlauer and Fürst, 1998).

Some other studies, not involving wood have shown the major contribution or a crucial role of phenolic compounds in oxidative scavenging activity (Ho, 1992; Lien *et al*, 1997; Cooper-Drivers and Bhattacharya, 1998; Larson, 1988; Goldberg *et al*, 1999; Vinson *et al*, 2001; Yang *et al*, 2001; Sroka and Cisowski, 2003; Diouf *et al,* 2006). Cai *et al* (2004) had found a positive linear correlation either for aqueous or methanolic extracts of Chinese medicinal plants and Tawaha *et al* (2007) found similarly in different Jordanian plant species. Tosun *et al* (2009) found a strong positive correlation in eight *Salvia* species from Turkey and Huang *et al* (2009) in 22 tropical hardwoods from Africa. More recently, Kawamura *et al* (2010b) found some positive correlation between total phenols and anti-oxidant activities in thirteen Malaysian hardwoods. The correlation has been reported due to its ability to scavenge free radicals (Kitzberger *et al,* 2007) mainly flavonoids (Pietta, 1998; Chua *et al*, 2008), phenolic acids and phenolic diterpenes (Shahidi *et al*, 1992).

## 3.5 Conclusions

Based on the present study on 12 Malaysian timber species, it is rational to conclude:

1. Density varied significantly from species to species and also with the height within the tree; the highest densities were at the base, then middle then top.
2. The average densities were found between 385 kg $m^{-3}$ to 803 kg $m^{-3}$.
3. *A. angustifolia* had a lowest wood density (basal; 411 kg $m^{-3}$, middle; 402 kg $m^{-3}$ and top; 385 kg $m^{-3}$). The highest density was found in *C. lanceolatum* (basal; 803 kg $m^{-3}$), *D. grandiflorus* (middle; 820 kg $m^{-3}$), and *D. grandiflorus/N. heimii* (topl; 803 kg $m^{-3}$).
4. Bark had a higher total extractive content than heartwood.

5. Wood density was significantly positively correlated with extractive contents. Thus wood density depends not only on the normal wood cell wall material but on the amount of extraneous materials (extractives) in the wood itself.
6. Durable wood contained a greater quantity of phenolic compounds (total phenols and total extractable phenols) than moderate durable and non-durable timbers.
7. Each type of wood species and portion (bark and heartwood) exhibited concentration-dependent DPPH radical scavenging activities. The bark extracts showed the more activity than the heartwood extracts. *N. heimii* extracts showed the strongest anti-oxidant effect. The bark extracts also contained more total phenols than heartwood in all twelve species studied.
8. The anti-oxidant capacity of the durable species was far greater than that of the lower durability classes with the anti-oxidant capacity of the most durable species some 24 x greater than the least durable species.

# CHAPTER 4: LABORATORY TESTING OF NATURAL DURABILITY AGAINST SUBTERRANEAN TERMITES

## 4.1 Introduction

In Malaysia, termites are considered as the most serious insect pest causing extensive damage to timber (Rasadah *et al*, 1987) and other materials including paper, fabrics, and even non-cellulosics such as asphalt, asbestos, bitumen, lead (Greathouse and Wessel, 1954) and metal foils (Bailey, 1954) and thus efforts have been directed towards their control. Their consumption of wood in buildings and structures is a particular economic problem. However their activity is important in many ecosystems because they re-cycle carbon and plant nutrients (Wood and Sands, 1978; Su and La Fage, 1984),

Information on the durability of wood against termites would add an important dimension to the various strategies sought to protect wooden structures in buildings which are prevalent in Malaysia. Termites belonging to the genus *Coptotermes* (family Rhinotermitidae, subfamily *Coptotermitidae*) were selected for laboratory tests since they are widely distributed, cause serious damage to seasoned timber and can tolerate fluctuations in atmospheric conditions (Tho, 1992; Dhanarajan, 1969). Wong *et al* (2001) published an aggressive laboratory method which uses the indigenous *C. curvignathus* and *C. gestroi* to examine resistance of some Malaysian hardwoods to subterranean termites. The laboratory test exposes wood to severe termite attack since large numbers of termites are freshly collected from the field immediately before the test and these are placed in close contact with wood under warm humid conditions that are ideal for termite feeding.

The aim of this study was to investigate the durability of twelve commercial Malaysian wood species against the most aggressive Malaysian subterranean termites; *C. curvignathus* and *C. gestroi.*

## 4.2 Materials and methods

### 4.2.1 Bioassay method

The evaluation of the termites was conducted on no-choice test of twelve Malaysian woods species against the subterranean termites *Coptotermes curvignathus* and *C. gestroi.*

### 4.2.2 Termite field collection and laboratory separation

Subterranean termites, *Coptotermes curvignathus* Holmgren and *C. gestroi* Wasmann (Isoptera: Rhinotermitidae) were collected from an active field colony at the Forest Research Institute Malaysia (FRIM) campus immediately before their use in laboratory assays, using a trapping technique (Kirton *et al,* 1998). The bait containers were stored under standard conditions (25°C and 75% RH). The termites were then separated from the field wood stakes and soil in the container (Figure 4.1).

Figure 4.1 Laboratory separation techniques of termites from field materials

### 4.2.3 Timber specimens

Wood specimen blocks measuring 19 x 19 x 19 mm were cut from the outer heartwood of selected Malaysian woods (with the exception of sapwood from *H. brasiliensis*) and subjected to termite bioassays according to the no-choice test procedure of ASTM D3345-74 (ASTM 1998) standard method.

### 4.2.4 Experimental procedures

The test blocks were oven-dried overnight at 105 °C and weighed. Subsequently, they were conditioned at 22 °C and 75% relative humidity to arrive at constant weight. Twenty replicates were prepared for each timber species with five control samples subjected to the same test conditions but with no termites present.

Screw-top bottles of 8 cm in diameter by 13 cm high were filled with 200 g of sterilized sand and 30 ml distilled water. The bottles were left overnight to equilibrate to laboratory conditions before test initiation. One block from each timber species was placed on the surface of the damp sand and 400 termites (360 workers and 40 soldiers) were added to each bottle. The termite workers used are the older workers, mainly 3$^{rd}$ in-star and above, according to Crosland *et al* (1997). All bottles were stored in an incubator maintained at 26 °C and 95% relative humidity for three weeks (21 days - time check test) and four weeks (28 days – main test). *H. brasiliensis* blocks were used for both tests (time check and main test). The activities of termites were observed daily and within this period, if it was found that all termites appeared dead, the bottle would be taken out and the number of days until 100% mortality would be recorded. At the end of the fourth week the blocks were removed, cleaned, dried overnight (24 hours) at 103 °C. The test blocks were then reweighed and the average percentage wood consumption and moisture contents (dry weight basis) were calculated for each individual timber species and test termite. The remaining live termites were weighed and recorded for each of the bottles. The condition of the test blocks was rated visually according to following scale:

10 - Sound
9 - Light attack
7 - Moderate attack and penetration
4 - Heavy attack
0 - Total failure

Wood consumption of the samples was also determined because it is one of the appropriate measures for termite attack, as termite attack does not alter the specific gravity (basic density) of unconsumed wood components (Essenther, 1977). The wood consumption and termite mortality was calculated according to the following equation:

$$\text{Wood consumption (\%)} = \frac{\text{(Initial oven dry weight – Final oven-dry weight)}}{\text{Initial oven dry weight}} \times 100$$

This is essentially the same as the weight loss used later in the fungal decay tests.

$$\text{Termite mortality (\%)} = \frac{\text{Number of all termites died}}{\text{Total number of termites}} \times 100$$

The X factor was obtained by dividing the wood consumption for any one species by the wood consumption of the most susceptible species. So that the score of both termites could be compared the wood consumption of both species was multiplied to give a product and this was multiplied by 1000 to give results as whole numbers above 1.

## 4.3 Results

### 4.3.1 Incubation time check test

Summary results of wood consumption, visual rating, termite mortality and wood moisture content of the time check test are shown in Figures 4.2 to 4.5. Details of the results are presented in Appendix Tables A4.1 and A4.2.

The study shows that wood consumption increases every week where the highest was noted after 3 weeks. Both termite species (*C. curvignathus* and *C. gestroi*) were not aggressive during the first week of exposure. *H. brasiliensis* samples were slightly attacked (2.1% of wood consumption with 9.3 visual rating and 2.2% of wood consumption with 9.10 visual rating, respectively) against both termites. Meanwhile, about 25.3% of *C. curvignathus* and 23.5% of *C. gestroi* died during the first week of exposure.

*C. curvignathus* consumed about 10.5% of *H. brasiliensis* by weight; ingesting enough nutrients for 42.6% of the termites to survive at the end of third week which is more than *C. gestroi* (only 6.4% of wood consumption and 39.0% of termite survived). With the increment of wood consumption towards the third week, a visual rating decreased to 7.5 for *C. curvignathus* and 8.0 for *C. gestroi*. The moisture content of the wood samples also increased from the first week (49.0% and 58.6%, respectively) until the end of the test (third weeks) (76.2% and 65.2%, respectively) with both termite tests.

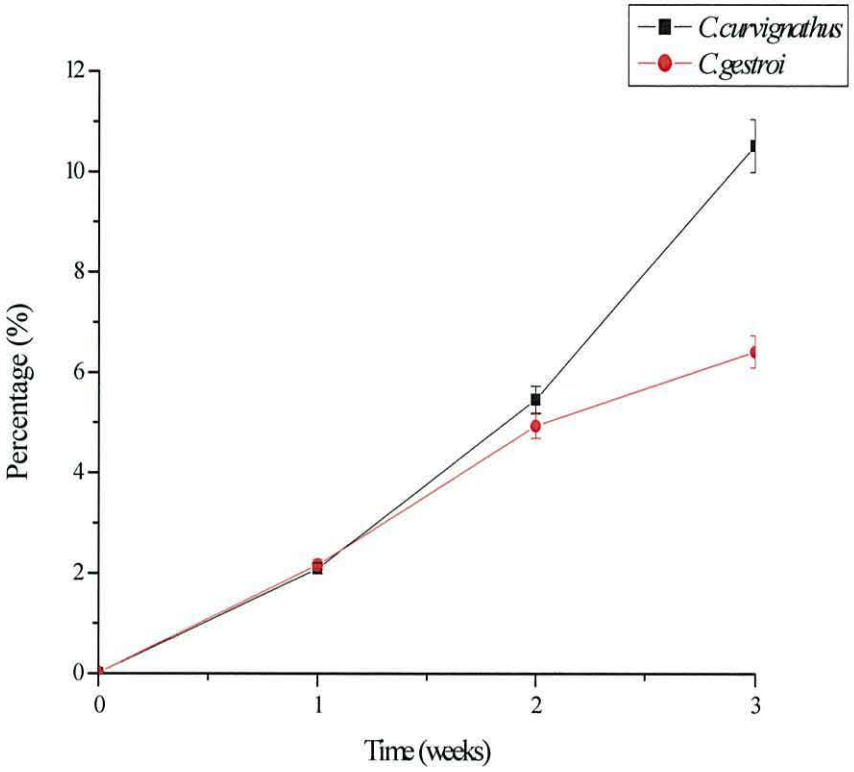



Figure 4.2 Change in wood consumption during incubation (%) of *H. brasiliensis* blocks during exposure to two termite species

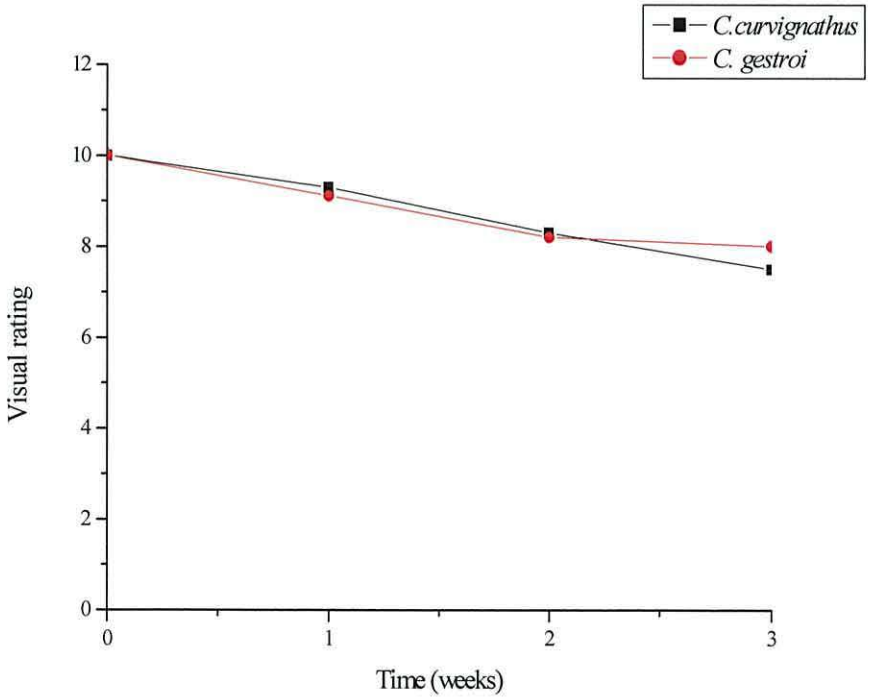



Figure 4.3 Change in visual rating during incubation of *H. brasiliensis* blocks exposed to two termite species

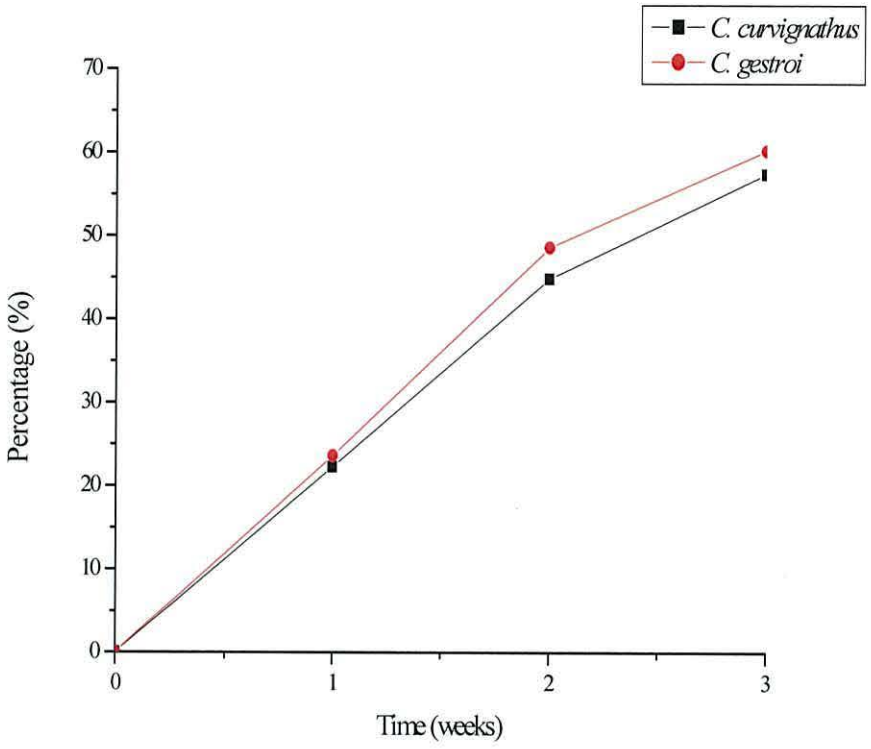



Figure 4.4 Change in termite mortality (%) of two termite species during incubation with *H. brasiliensis* wood blocks

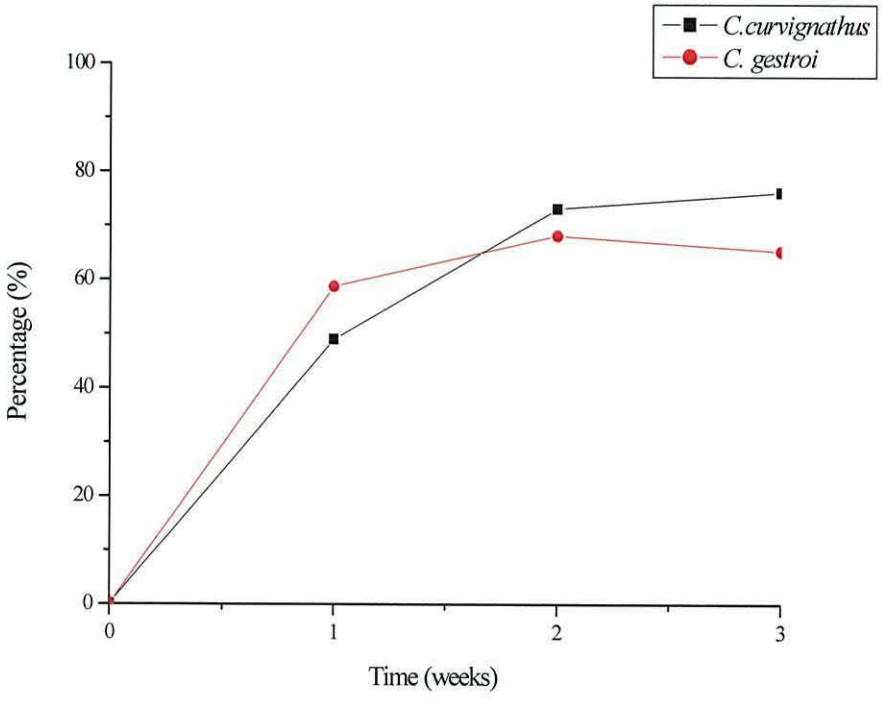



Figure 4.5 Change in wood moisture content (%) of *H. brasiliensis* blocks during incubation

### 4.3.2 Wood consumption and visual rating

The wood consumption of the different wood species as a result of termite feeding is expressed as the percentage of initial dry weight (Figures 4.6 and 4.7). Details of the results are presented in Appendix Tables A4.1 and A4.2.

Both termites were very active on the sand and started digging tunnels downward into all the samples tested. The presence of tunnels indicates the vigorous nest of the termites (Figures 4.8 and 4.9) (ASTM D3345, 1998). After the first week, all wood block showed some surface damage (except for *C. scortechinii*, *A. angustifolia* and *H. brasiliensis*) where the termites had attempted to feed. The *H. brasiliensis* test blocks were severely damaged after four weeks with extensive tunnelling visible on the lower surface of the blocks.

Out of twelve wood species tested, *N. heimii* and *C. lanceolatum* were the most resistant to attack by *Coptotermes curvignathus* with lowest mean of wood consumption and excellent visual rating (0.9% of wood consumption with 9.8 visual rating and 1.0% of wood consumption with 9.6 visual rating, respectively). Similar performance also occurred with *C. gestroi* (0.7% of wood consumption with 9.9 visual rating and 0.8% of wood consumption with 9.67 visual rating, respectively). Two other wood species performed well, *M. utilis* and *S. curtisii.* They showed some attack by *C. curvignathus* (visual rating of 9.7 and 9.5, respectively and wood consumption 1.4% and 1.5% respectively), and by *C. gestroi* (visual ratings for both of 9.5 and wood consumption 1.5% and 1.4% respectively).

Two other wood species were resistant against *C. gestroi*, ending the 28 days exposure period with only a trace of termite damage (rating of more than 9.0) were *P. pinnata* (1.6% of wood consumption with 9.5 visual rating) and *F. fragrans* (1.9% of wood consumption with 9.4 visual rating). At the other end of the performance scale was *H. brasiliensis*. It suffered the highest wood consumption (12.36% and 11.08%, respectively) among the twelve species and lowest visual rating (5.87 and 6.60, respectively) which made it the most susceptible species to *C. curvignathus* and *C. gestroi.* The other timber species were intermediate in their susceptibility to the two termite species.

Based on the X factor (Table 4.1), it is clearly that *N. heimii* and *C. lanceolatum* performed very much better in the termite tests than the other ten wood species.

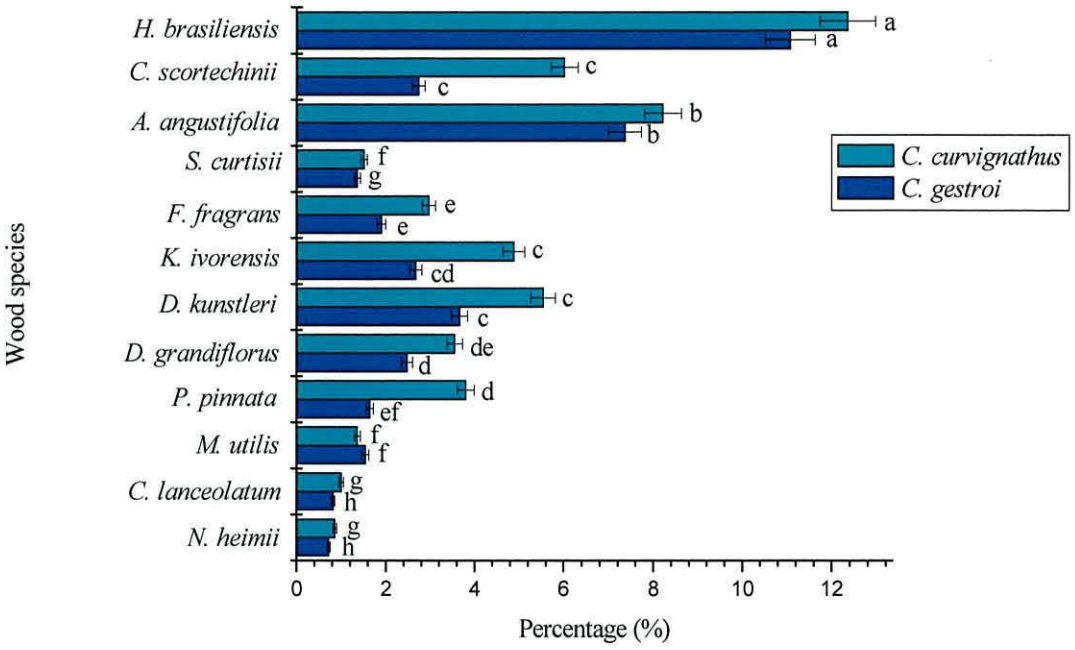



Figure 4.6 Wood consumption (%) after 28 days of twelve Malaysian wood species by two different subterranean termites. Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test in the same category

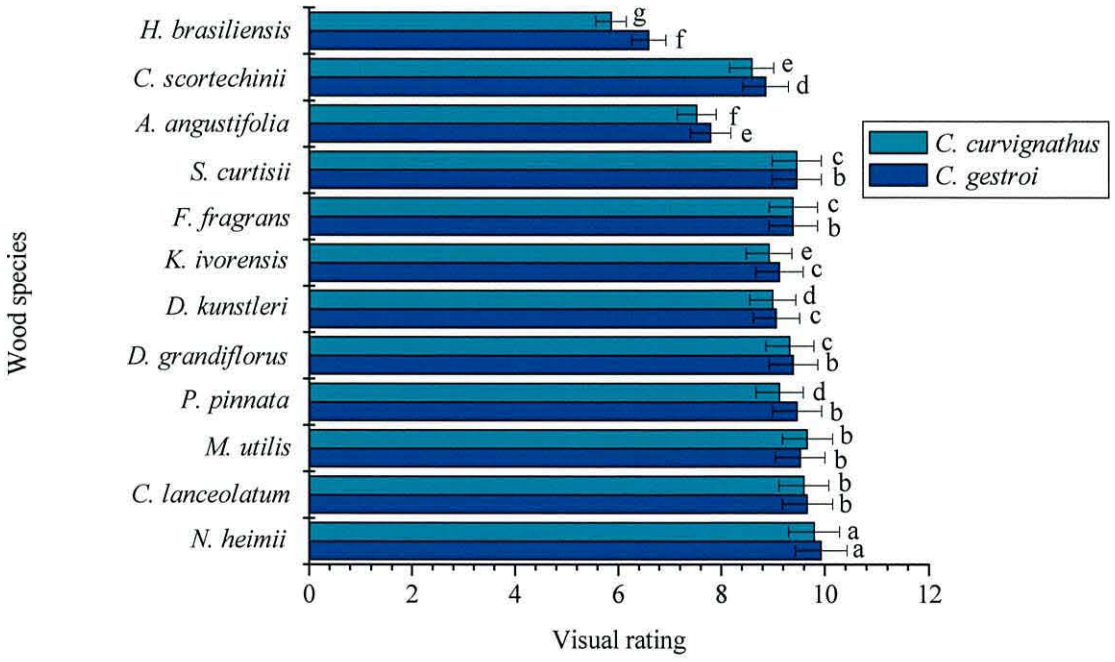



Figure 4.7 Visual rating of twelve Malaysian wood species after 28 days exposure to two subterranean termites. Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test in the same category

Table 4.1 A ranking using the X factor of the twelve Malaysian wood species based on the wood consumption. The product is the wood consumption values x 1000.

| Wood species | X factor | | |
|---|---|---|---|
| | *C. curvignathus* | *C. gestroi* | Product * 1000 |
| *H. brasiliensis* | 1.00 | 1.00 | 1000.00 |
| *A. angustifolia* | 0.67 | 0.67 | 442.37 |
| *D. kunstleri* | 0.45 | 0.33 | 148.06 |
| *C. scortechinii* | 0.49 | 0.25 | 120.45 |
| *K. ivorensis* | 0.39 | 0.24 | 95.14 |
| *D. grandiflorus* | 0.29 | 0.22 | 64.29 |
| *P. pinnata* | 0.31 | 0.15 | 45.51 |
| *F. fragrans* | 0.24 | 0.17 | 41.21 |
| *M. utilis* | 0.11 | 0.14 | 15.29 |
| *S. curtisii* | 0.12 | 0.12 | 15.00 |
| *C. lanceolatum* | 0.08 | 0.07 | 5.99 |
| *N. heimii* | 0.07 | 0.06 | 4.52 |

*this is the x factor of both species multiplied together

Analysis of variance (ANOVA) showed that only *A. angustifolia* and *H. brasiliensis* were highly significantly different in the amount of wood consumption with other timber species at $P \leq 0.001$ against *C. curvignathus* and *C. gestroi*. These two species also had highly significant differences in visual rating against both termite species. The other timber species are either significantly different or not significant at $P \leq 0.05$ or $P \leq 0.01$. The ANOVA table for both termite species are presented in Appendix Tables A4.3, A4.4, A4.5 and A4.6.

On the other hand, only *F. fragrans* (against *C. curvignathus*), *A. angustifolia*, *C. lanceolatum* and *S. curtisii* (against *C. gestroi*) were significantly different ($P \leq 0.05$) while others are not in total variation of wood consumption between the samples tested (Tables 4.2 and 4.3). Tables 4.2 and 4.3 also indicates that all timber species studied showed no significant difference in visual rating between samples when tested with *C. curvignathus* and *F. fragrans* is the only species that showed significant difference against *C. gestroi*. Even though the values are not significant, the other species still have differences to each other as shown by $r^2$ value. The ANOVA table is presented in Appendix Tables A4.7, A4.8, A4.9 and A4.10.

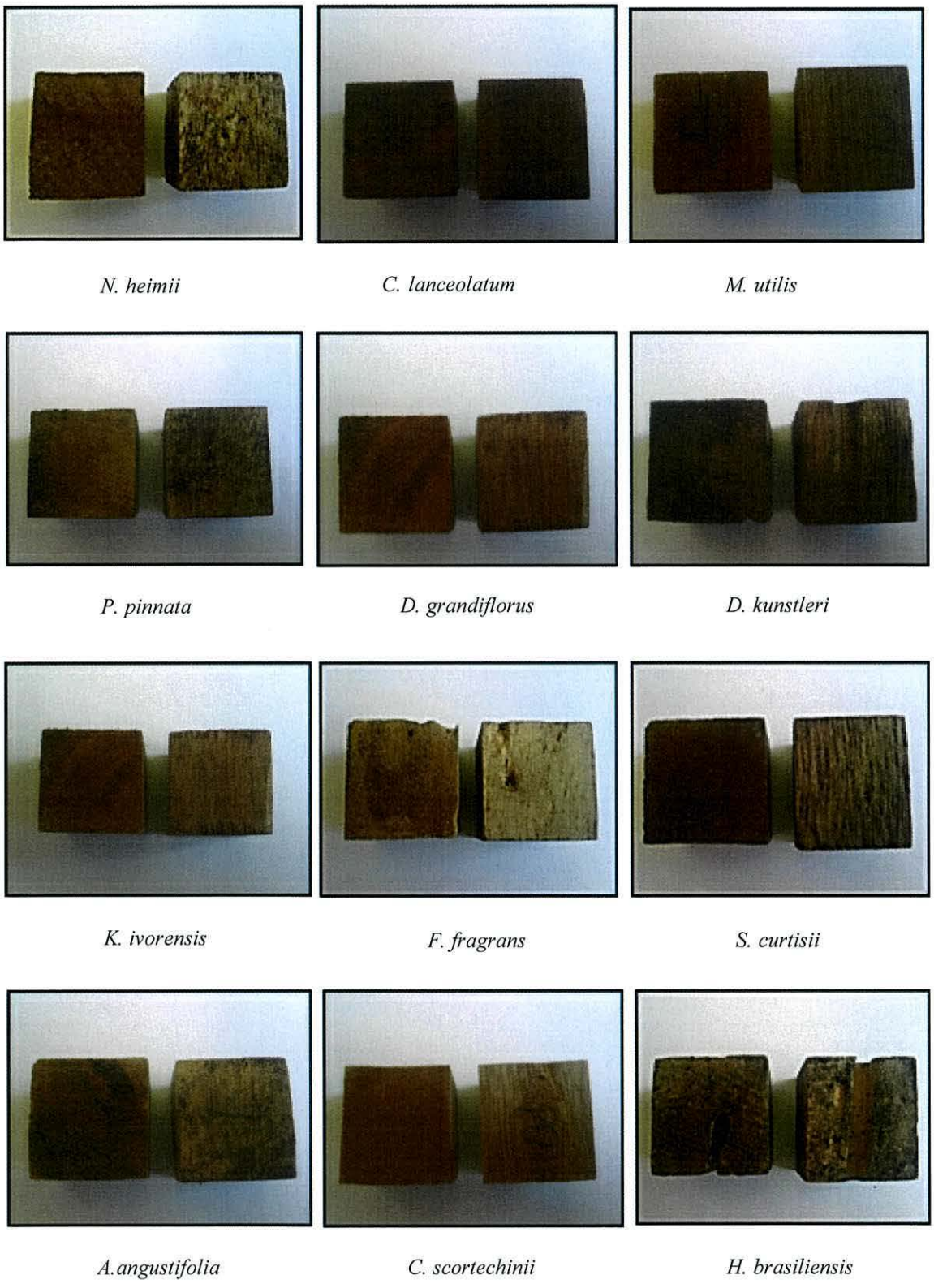



Figure 4.8 The appearance of wood blocks of twelve Malaysian hardwoods exposed to *C. curvignathus* after twenty eight days of incubation

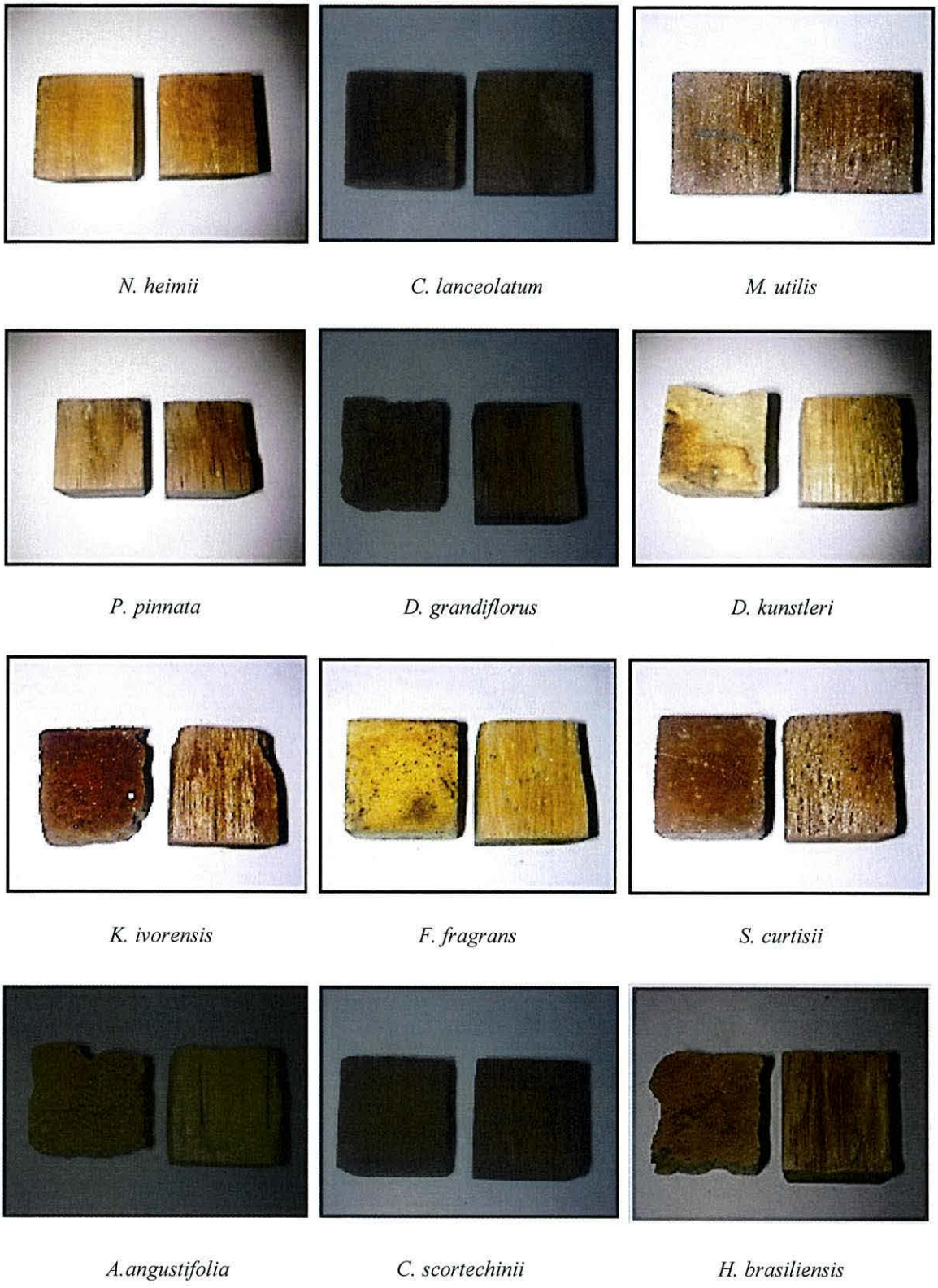



Figure 4.9 The appearance of wood blocks of twelve Malaysian hardwoods exposed to *C. gestroi* after twenty eight days of incubation

Table 4.2 ANOVA results showing the proportion (%) of total variation ($R^2$) in wood consumption (%), visual rating, termite mortality (%) and wood moisture content (%) accounted for within replicates tested (*C. curvignathus*)

| Species | Wood consumption | Visual rating | Termite mortality | Wood moisture content |
|---|---|---|---|---|
| *N. heimii* | 12.45ns | 16.67ns | na | 25.51ns |
| *C. lanceolatum* | 25.73ns | 7.41ns | na | 47.58ns |
| *M. utilis* | 7.15ns | 20.00ns | na | 22.01ns |
| *P. pinnata* | 50.43ns | 23.08ns | 53.14* | 15.06ns |
| *D. grandiflorus* | 12.07ns | 14.29ns | 15.79ns | 41.61ns |
| *D. kunstleri* | 25.97ns | 33.33ns | 35.43ns | 40.46ns |
| *K. ivorensis* | 28.38ns | 26.83ns | 30.79ns | 53.50* |
| *F. fragrans* | 51.35* | 25.93ns | 21.64ns | 37.97ns |
| *S. curtisii* | 33.22ns | 10.71ns | na | 30.19ns |
| *A. angustifolia* | 46.92ns | 31.82ns | 47.60ns | 79.12** |
| *C. scortechinii* | 8.69ns | 16.67ns | 31.80ns | 54.52* |
| *H. brasiliensis* | 16.04ns | 8.51ns | 65.79* | 44.12ns |

* $P \leq 0.05$, ** $P \leq 0.01$, ns – not significant, na – not available.

Table 4.3 ANOVA results showing the proportion (%) of total variation ($R^2$) in wood consumption (%), visual rating, termite mortality (%) and wood moisture content (%) accounted for within replicates tested (*C. gestroi*)

| Species | Wood consumption | Visual rating | Termite mortality | Wood moisture content |
|---|---|---|---|---|
| *N. heimii* | 26.46ns | 28.57ns | na | 35.50ns |
| *C. lanceolatum* | 54.33* | 20.00ns | na | 59.08* |
| *M. utilis* | 46.92ns | 28.57ns | 23.08ns | 15.57ns |
| *P. pinnata* | 8.14ns | 46.43ns | 5.47ns | 26.14ns |
| *D. grandiflorus* | 19.74ns | 23.61ns | 36.08ns | 25.24ns |
| *D. kunstleri* | 17.66ns | 2.06ns | 16.73ns | 43.49ns |
| *K. ivorensis* | 20.14ns | 17.48ns | 24.94ns | 64.52* |
| *F. fragrans* | 33.41ns | 62.96* | 24.94ns | 43.72ns |
| *S. curtisii* | 60.31* | 46.43ns | na | 64.00* |
| *A. angustifolia* | 56.90* | 50.29ns | 40.21ns | 29.46ns |
| *C. scortechinii* | 36.14ns | 38.36ns | 29.44ns | 41.06ns |
| *H. brasiliensis* | 33.90ns | 34.21ns | 44.69ns | 32.77ns |

* $P \leq 0.05$, ** $P \leq 0.01$, ns – not significant, na – not available.

### 4.3.3 Termite mortality

Throughout the test period of four weeks, the number of living termites feeding on all the wood species reduced significantly (Figure 4.10) with the highest decline occurred

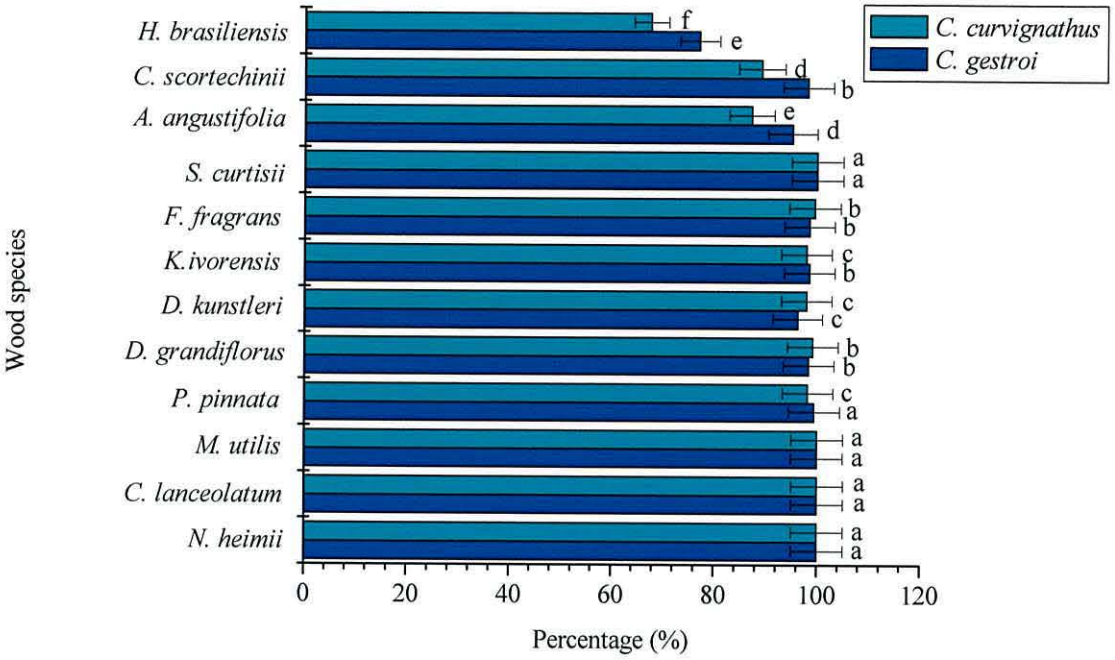



Figure 4.10 Mortality of two subterranean termites (%) after testing on twelve Malaysian wood species. Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test.

to termites feeding on *M. utilis* (0.03%) or none (0%) of the termites survived (*N. heimii*, *C. lanceolatum* and *S. curtisii*) while the average number of days for 100% (total) mortality was 10.9, 9.1, 11.1 and 12.6, respectively; against *C. curvignathus* and about 9.3, 7.8, 8.9 and 10.1 against *C. gestroi*, respectively.

*C. scortechinii* and *A. angustifolia* had nearly 90% (89.2% and 87.3%, respectively) termite mortality after the 4 weeks test and the remaining species were between the range of 98.0 % to 99.5% against *C. curvignathus*. About 32.3% of the termites were healthy and active at the end of the test in *H. brasiliensis* blocks. However, when dealing with *C. gestroi*, *H. brasiliensis* was the only timber species that had termite mortality below 80% while the other species had more than 95%. The great contrast in mortality of termites on four timber species discussed earlier (*M. utilis*, *N. heimii*, *C. lanceolatum* and *S. curtisii*) either against *C. curvignathus* or *C. gestroi* indicates the resistance of these timber against subterranean termites.

Pairwise comparison showed that only three timber species (*C. scortechinii*, *A. angustifolia* and *H. brasiliensis*) were significantly different to the other timber species in termite mortality when tested against *C. curvignathus*. Meanwhile, only *P. pinnata* and *H. brasiliensis* were significantly different against *C. gestroi*. The other timber species were

not significantly different with each other at P≤ 0.05. The ANOVA tables are presented in the Appendix Tables A4.11 and A4.12.

Analysis of variance (Tables 4.2 and 4.3) showed that four timber species (*M. utilis*, *N. heimii*, *C. lanceolatum* and *S. curtisii*) were identical to each other in terms of their termite mortality with *C. curvignathus*. The same also occurred for *N. heimii*, *C. lanceolatum* and *S. curtisii* against *C. gestroi*. Only *P. pinnata* and *H. brasiliensis* showed huge variation at 53.14% and 65.79%, respectively, between samples against *C. curvignathus*. No timber species showed this variation with *C. gestroi*. The ANOVA table are presented in Appendix Tables A4.13 and A4.14.

### 4.3.4 Moisture content

The average moisture content of twelve Malaysian wood species after exposure to *C. curvignathus* and *C. gestroi* are presented in Figure 4.11.

The highest moisture content was found in *H. brasiliensis* blocks (80.50%) and the lowest in *P. pinnata* (27.83%) against *C. curvignathus*. *H. brasiliensis* again had the highest moisture content (91.05%) against *C. gestroi* and the lowest was found in *N. heimii* blocks (31.41%).

Pairwise comparison of the results indicates that *P. pinnata* is the only timber species that is significantly different (P≤0.05) for moisture content to other timber species against *C. curvignathus*. *N. heimii*, *K. ivorensis* together with *H. brasiliensis* were significantly different against *C. gestroi*. The other timber species are not significantly different at P≤0.05. *A. angustifolia* was highly significantly different in between sample variation (P≤0.01) against *C. curvignathus*. While *K. ivorensis* and *C. lanceolatum* were significantly different at P≤0.05, the others are not against *C. gestroi*. Against *C. gestroi*, only *K. ivorensis* (64.5%) and *C. lanceolatum* (64%) were significantly different in the variation of moisture content between samples tested. The ANOVA tables are presented in Appendix Tables A4.15, A4.6 A4.17 and A4.18.

Table 4.4 shows that a correlation exists between the moisture content and wood consumption against both subterranean termites. Majority of wood species had a positive correlation with the moisture content at P≤0.05. Meanwhile Table 4.5 shows that a correlation exists between the moisture content and mortality of both subterranean termites. All of wood species showed significantly negative correlation at P≤0.05 and some of them at P≤0.01. However, the correlations between both variables are not

available for four wood species against *C. curvignathus* and three against *C. gestroi* due to the identical data, i.e. all survive.

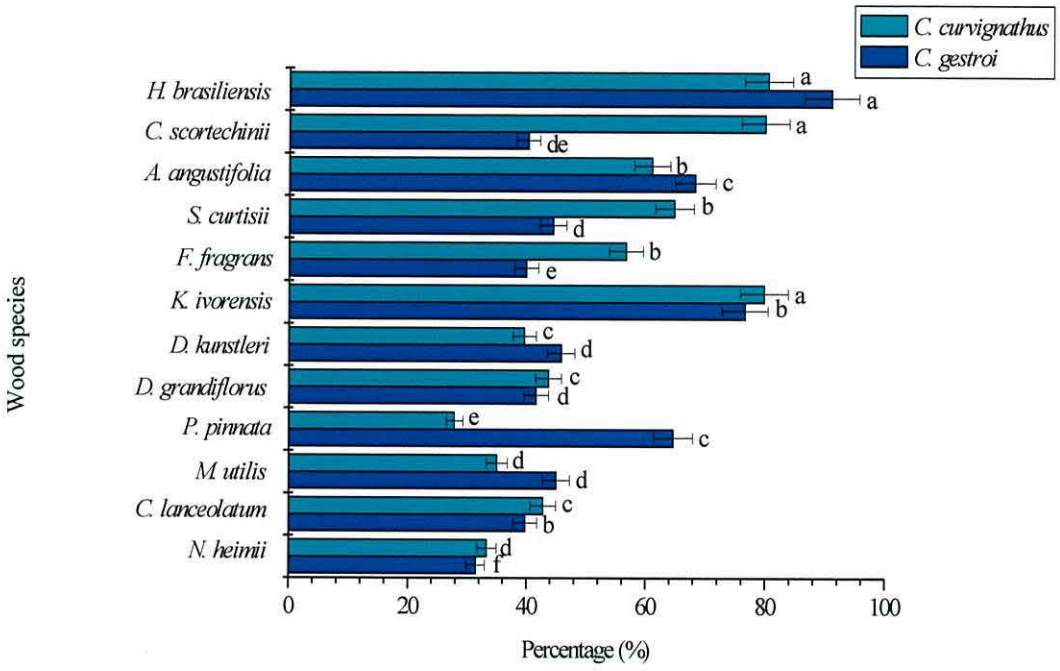



Figure 4.11 Moisture content of the test blocks after testing of twelve Malaysian wood species against two subterranean termites. Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test.

Table 4.4 Correlation (R) between wood consumption by subterranean termites and moisture content of twelve Malaysian hardwood species (within species) (n = 5)

| Wood species | *C. curvignathus* | | *C. gestroi* | |
|---|---|---|---|---|
| | R | P value | R | P value |
| *N. heimii* | 0.95 | 0.013 | 0.97 | 0.002 |
| *C. lanceolatum* | 0.06 | 0.921 | 0.76 | 0.182 |
| *M. utilis* | 0.84 | 0.075 | 0.91 | 0.056 |
| *P. pinnata* | 0.94 | 0.017 | 0.82 | 0.072 |
| *D. grandiflorus* | 0.86 | 0.061 | 0.69 | 0.201 |
| *D. kunstleri* | 0.58 | 0.304 | 0.71 | 0.188 |
| *K. ivorensis* | 0.89 | 0.041 | 0.67 | 0.212 |
| *F. fragrans* | 0.35 | 0.562 | 0.54 | 0.321 |
| *S. curtisii* | 0.79 | 0.112 | 0.89 | 0.067 |
| *A. angustifolia* | 0.95 | 0.012 | 0.95 | 0.013 |
| *C. scortechinii* | 0.86 | 0.062 | 0.86 | 0.071 |
| *H. brasiliensis* | 0.86 | 0.062 | 0.81 | 0.076 |

Table 4.5 Correlation (R) between moisture content of wood blocks and termite mortality of twelve Malaysia wood species (within species) (n = 5)

| Wood species | R | |
|---|---|---|
| | *C. curvignathus* | *C. gestroi* |
| *N. heimii* | na | na |
| *C. lanceolatum* | na | na |
| *M. utilis* | na | -0.702* |
| *P. pinnata* | -0.705* | -0.827** |
| *D. grandiflorus* | -0.812** | -0.838** |
| *D. kunstleri* | -0.689* | -0.611* |
| *K. ivorensis* | -0.882** | -0.902** |
| *F. fragrans* | -0.811** | -0.721** |
| *S. curtisii* | na | na |
| *A. angustifolia* | -0.738* | -0.901** |
| *C. scortechinii* | -0.721* | -0.806** |
| *H. brasiliensis* | -0.503* | -0.850** |

***, **, *, ns, na: correlation respectively significant at 0.001%, 1%, 5%, not significantly and not available due to the identical data.

## 4.4 Discussion

Mohd Dahlan and Tam (1987) classified that these twelve Malaysian woods species were under three durable classes; durable (*N. heimii* and *C. lanceolatum*), moderately durable (*M. utilis*, *P. pinnata*, *D. grandiflorus*, *D. kunstleri*, *K. ivorensis*, *F. fragrans*, *S. curtisii*) and non-durable (*H. brasiliensis*, *A. angustifolia* and *C. scortechinii*).

### 4.4.1 Resistance against *C. curvignathus*

This termite showed some aggression to all twelve timbers tested. It was found that only two test blocks from *M. utilis*, twelve from *N. heimii*, eight from *C. lanceolatum* and one from *S. curtisii* had wood consumption less than 1%. The remaining had more than 1% up to 16%. Meanwhile, only one sample from *D. kunstleri* and *D. grandiflorus*, two from *K. ivorensis*, three from *C. scortechinii* and eleven from *A. angustifolia* had a visual rating of 7.0 (moderate attack and penetration). Four out of fifteen *H. brasiliensis* blocks had failed in the test as shown by the visual rating of 4.0 and the remaining had either 7.0 or 9.0.

*C. curvignathus* has been found as the primary pest for agricultural and forestry tree crops (young and mature trees) in Malaysia (Tho, 1975; Tho and Kirton, 1992; 1998; Kirton *et al*, 1999). In addition Lee (2002a; 2002b) and Kirton (1995) reported that this species together with *C. travians* are the two most aggressive species that readily attack

urban structures and buildings in Malaysia. Sornnuwat (1996) also found that *C. curvignathus* also occasionally affected buildings in Thailand.

### 4.4.2 Resistance against *C. gestroi*

This termite species is less aggressive than *C. curvignathus*. Out of fifteen test blocks tested for each timber species, two from *M. utilis*, thirteen from *N. heimii*, one from *P. pinnata* and *D. kunstleri*, twelve from *C. lanceolatum* and four from *S. curtisii* that had wood consumption less than 1% while the remaining test blocks were between 1% to 18%. Subsequently, only one test block from *D. grandiflorus*, and two from *D. kunstleri*, *K. ivorensis* and *C. scortechinii*, had a visual rating of 7.0. The others had a visual rating range from 9.0 to 10.0. However, it is interesting that *A. angustifolia* had three failed test blocks (visual rating of 4.0) compared to none against *C. curvignathus*. *C. gestroi* showed a similar pattern to attacking the *H. brasiliensis* test blocks as *C. curvignathus*.

*Coptotermes gestroi* which is commonly known as the Asian subterranean termite is found to be particularly destructive and poses a constant threat to wooden structures wherever it occurs (Scheffrahn and Su, 2000). Furthermore, this species of termite is the most serious pest termite in Southeast Asia (Thailand, Malaysia, Singapore, Burma, Indo China, Brunei and Indonesia) (Kirton and Brown, 2003). This species is almost similar to the well-known *C. formosanus* Shiraki, the Formosan subterranean termite (Su and Scheffrahn, 1998). The life history, damage caused and pest management of *C. formosanus* is similar as they related to *C. gestroi*.

### 4.4.3 Variation in wood consumption and visual rating between species and samples

Consumption rates are generally expressed as weight of wood removed by termites (number or biomass) over time (Smythe and Carter, 1970; Mannesmann, 1973; Watson *et al*, 1978; Wood, 1978; Lenz and Barrett, 1982; Su and La Fage, 1984).

The mode of attack differed in each individual timber and termite species which influences the wood consumption and visual rating. Table 4.2 showed that *F. fragrans* had a huge (51.35%) variation of proportion between samples of the same timber species of wood consumption and significantly different at $P \leq 0.05$ against *C. curvignathus.* Nevertheless, *P. pinnata* also had more than 50% (50.4%) of the total variation in wood consumption even though this was not significantly different. None of the timber was significantly different in variation within the trees for visual rating. However, among the

twelve timber species, *D. kunstleri* had a huge variation (33.3%) against *C. curvignathus* and followed by *A. angustifolia* (31.8%).

Table 4.3 indicates that a large variation of wood consumption against *C. gestroi* occurred in *S. curtisii* (60.31%). Again, it was followed by *A. angustifolia* (56.90%) and *C. lanceolatum* (54.33%) while the others gave less than 50% variation. *F. fragrans* showed the highest percentage (63.0%) of variation for visual rating between sample (significantly different at $P \leq 0.05$). Contrarily, low variation in visual rating occurred in *D. kunstleri* (only 2.1%) and the other timber species variations were between 17% to 51%.

Heavy termite infestation is the major cause of damage for only three wood species and *H. brasiliensis* was clearly preferred in the no-choice test followed by *A. angustifolia* and *C. scortechinii* for both subterranean termites. This is because *H. brasiliensis* is sapwood (Lim *et al*, 1999) and it may contain high quantities of sugars (2%) and starch, up to 10% (Kadir and Sudin, 1989). Other studies (Hong *et al*, 1983; Mohd Dahlan and Tam, 1987) have also demonstrated the susceptibility of *H. brasiliensis.* In addition *A. angustifolia* has been reported as highly susceptible to *C. curvignathus* by these authors.

Martawijaya (1965), Rudman *et al* (1967) and Wong *et al* (1998) in their studies have noted that durability of hardwoods varies between and within species. Gay *et al* (1955) also noted that different species of termites may have different food preferences and results obtained for tests with one species do not necessarily apply to other species and may be misleading. Furthermore many studies (Becker, 1965; Smythe *et al,* 1971; Haverty and Nutting, 1974) had found that the factors effecting the wood consumption by termites are numerous and show complex interactions. Nevertheless, the most important are the wood species and hardness, extractives contents, feeding inhibitors, presence or absence and degree of fungal decay, moisture content of wood and soil and last, but not least, is temperature.

### 4.4.4 Variation in termite mortality between species and samples

Mortality of *C. curvignathus* in *M. utilis*, *N. heimii*, *C. lanceolatum* and *S. curtisii* were greater than other timber species (Table 4.2). A similar situation also occurred with *C. gestroi* in *N. heimii*, *C. lanceolatum* and *S. curtisii*. As discussed before, total mortality of termites in the four timber species earlier died before the test ended which make all the cases identical. Bultman *et al* (1979) suggested that when damage was limited only to

surface etching during exposure, death was caused either by starvation or a volatile toxic substance in the wood since a limited amount of wood was ingested.

Even though the variation between wood samples against *C. gestroi* was not significantly different to each other, it still happened which is shown by the $r^2$ value. *H. brasiliensis* was found to have the highest percentage (44.7%) while *P. pinnata* was lowest at 5.5%. The variation of termite survival between and within the wood species has been confirmed before by previous studies (Carter *et al*, 1975; 1979).

### 4.4.5 Variation in wood moisture content between species and samples

One of the most important factors has been shown to affect wood consumption by termites is wood moisture content (Smythe *et al*, 1971; Haverty and Nutting, 1974; Carter and Smythe, 1974). As stated before, many studies (Becker, 1965; Smythe *et al,* 1971; Haverty and Nutting, 1974; Delaplane and La Fage, 1989; Delaplane, 1991; Nakayama *et al*, 2004; 2005; Indrayani *et al,* 2007) have reported that termite feeding activity is also influenced by the moisture content in wood.

### 4.4.6 Correlation between wood consumption and moisture content

It has been reported (Morimoto, 1980; Yamano, 1997; Woodrow and Grace, 1998) that either damp-wood termites or subterranean termites in particular, are well known for their high water requirements.

This study observed correlation between moisture content and wood consumption in each wood species which was significant. A highly positively correlation between moisture content and wood consumption was only found in *N. heimii* against *C. gestroi* (Table 4.4). Meanwhile, eight wood species showed that wood consumption increased significantly with moisture content against *C. curvignathus* (*N. heimii*: r = 0.95, *M. utilis*: r = 0.84, *P. pinnata*; r = 0.94, *D. grandiflorus*; r = 0.86, *K. ivorensis*; r = 0.89, *A. angustifolia*; r = 0.95, *C. scortechinii*; r = 0.86 and *H. brasiliensis*; r = 0.86). However, the other four wood species showed invalid correlations between the above two variables (*C. lanceolatum*: r = 0.06, *D. kunstleri*: r = 0.58, *S. curtisii*: r = 0.79 and *F. fragrans*: r = 0.35). With *C. gestroi*, moderately positive correlations were found in all species tested but these were only significant for *M. utilis, P. pinnata, C. scortechinii, A. angustifolia, H. brasiliensis* and *S. curtisii*. No negative correlation occurred between moisture content and wood consumption for both termite species.

From the above results, it can be concluded that correlations exist between the wood moisture content and wood consumption. In many cases the wood consumption by both termites was greater when the wood had higher moisture content, generally above 46%. The moisture content of the wood has been reported to affect the feeding of different termite species (Peace, 1997; Nakayama *et al*, 2005). This observation explains why, in service, damage by subterranean termites generally occurs in wood at higher temperatures and high humidity (Yamano, 1997; Nakayama *et al*, 2005).

Previous studies (Smythe, 1972; Smythe and Williams, 1972; Haverty and Nutting, 1974; Williams, 1976; Ahmad Khan, 1980; Lenz and Barrett, 1982; Steward, 1983; Delaplane and La Fage, 1989; Delaplane, 1991; Nakayama *et al*, 2004; 2005; Indrayani *et al*, 2007) reported that moisture content is one of the factors that influenced the termite behaviour including feeding activity, nesting behaviour, colonizing flights, post-flight behaviour, seasonal reproductive cycles and distribution patterns of termites within buildings. Nakayama *et al* (2005) in their laboratory based studies showed that subterranean termites (*C. formosanus* and *Reticulitermes speratus*) prefer higher moisture contents between 70% to 200%.

### 4.4.7 Correlation between termite mortality and moisture content

Water content had been reported to effect the survival of insects (Bursell, 1974; Zabel and Morrell, 1992) and also foraging behaviour (Smith and Rust, 1993a; 1994; Haagsma and Rust, 1995; Evans and Gleeson, 2001). This is true especially for subterranean termite, which have very little resistance to the dehydration. In other words, they should maintain contact with the soil or other moisture resources.

Table 4.5 showed that highly negatively significant correlation between the mortality and the wood moisture content occurred in *D. grandiflorus* (r = -0.81), *K. ivorensis* (r = -0.88) and *F. fragrans* (r = -0.81) forwards *C. curvignathus* and, in *P. pinnata* (r = -0.837), *D. grandiflorus* (r = -0.84), *K. ivorensis* (r = -0.90), *C. scortechinii* (r = -0.81), *A. angustifolia* (r = - 0.90), *H. brasiliensis* (r = -0.85) and *F. fragrans* (r = -0.72) forwards *C. gestroi*. The others showed moderately negatively correlation either against *C. curvignathus* (*P. pinnata*: r = -0.71, *D. kunstleri*: r = -0.69, *C. scortechinii*: r = -0.72 and *A. angustifolia*: r = -0.74) or *C. gestroi* (*M. utilis*: r = -0.70 and *D. kunstleri*: r = -0.61). The correlation between the above two variables are not available for *M. utilis*, *N. heimii*, *C. lanceolatum* and *S. curtisii* against *C. curvignathus* and *C. gestroi* (accept *M. utilis*) due to the identical data of termite mortality.

In similar result, Smith and Rust (1993b) found that, the wood consumption by *R. hesperus* were decreased due to higher mortality at high temperature (26.7 °C to 32.2 °C). Cooler temperatures (15.6 °C) (higher moisture content) protected this species from death.

## 4.5 Conclusions

1. A laboratory no-choice test for natural durability showed that *C. curvignathus* is more aggressive subterranean termites than *C. gestroi.*
2. *N. heimii* was the most resistant wood species while *H. brasiliensis* was susceptible against both termite species. Other wood species were moderately resistant.
3. Pairwise comparisons showed significant differences in wood consumption, visual rating, termite mortality and wood moisture content between majority of the timber species against *C. curvignathus* and *C. gestroi.* The difference in consumption by both termites of *A. angustifolia* and *H. brasiliensis* were highly significant.
4. With *C. curvignathus*, high between sample variations only occurred in *F. fragrans* (wood consumption), *P. pinnata* and *H. brasiliensis* (termite mortality), *K. ivorensis, C. scortechinii* and *A. angustifolia* (wood moisture content). No significant differences in visual rating occurred. With *C. gestroi* significant variation occurred in *A. angustifolia*, *C. lanceolatum* and *S. curtisii* (wood consumption), *F. fragrans* (visual rating) and, *K. ivorensis*, *C. lanceolatum* and *S. curtisii* (wood moisture content). There were no significant differences in termite mortality.
5. Although the wood moisture contents at the end of the test were not uniform, majority of the timber tested showed significantly positive correlation between moisture content and wood consumption with both termite species.

# CHAPTER 5: LABORATORY TESTING OF NATURAL DURABILITY AGAINST FUNGI

## 5.1 Introduction

Beside insects or termites, fungal decay resistance is also the main factor that affects the durability of wood in service. Common methods of obtaining information about natural durability are either by graveyard stake (field) tests or using accelerated laboratory tests. Laboratory testing is faster but does not expose the specimens to a mixed microbial flora, cyclic weather conditions and the UV from sunlight. Field tests select a microflora into the wood substrate (Scheffer and Cowling, 1966; Bravery, 1968; Behr, 1973; Baines, 1982; Butcher and Nilsson, 1982; Butcher, 1983; Eaton and Hale, 1993).

Many studies (Takahashi and Kishima, 1973; Wong and Sabri, 2000; Jusoh and Kamden, 2001) have been conducted on the natural durability of Malaysian timbers. However, these studies only focused on the variation between timber species rather than intensively investigating the between-tree variability for decay resistance.

An early study by Wangaard and Muschler (1952) showed there was a significant variation in decay susceptibility of timber but the available data were insufficient to reliably predict these variations on tropical timbers especially Malaysian timbers. Thus, this chapter was designed to study the variability of the natural durability of twelve Malaysian wood species between species and samples. The purpose of this was to employ *in vitro* laboratory decay tests to estimate the ability of *Pycnoporus sanguineus, Trametes versicolor* and *Lentinus sajor-caju* to degrade the heartwood of these Malaysian hardwoods alongside susceptible reference species, *H. brasiliensis* and a European reference species, *Fagus sylvatica.*

## 5.2 Materials and methods

### 5.2.1 Preparation of test blocks

Twelve replicates from each timber species measuring 19 x 19 x 19 mm were prepared for each fungus. A sample was taken from the outer heartwood as well as a little bit closer to the pith representing the rest of the heartwood. With *H. brasiliensis* and *F. sylvatica* only sapwood was tested because of the difficulty defining heartwood. The wood samples were weighed and dried at 103 °C for 24 hours. They were then wrapped in polyethylene bags and sterilised by irradiation according to EN113. The total layout of the test block numbers used for each decay test is shown in Table 5.1.

### 5.2.2 Decay test

The natural durability test on twelve Malaysian timber species were carried out according to ASTM D2017 (1993). This standard is also widely applied for the testing of the natural durability of untreated wood according to EN 350-1 Standard (Adopted European standard, 1994).

Three types of white-rot fungus, *Pycnoporus sanguineus* (L.ex.Fr.); culture collection – KUM 70117 (*Trametes versicolor* (L.Fries) Pilat; culture collection – CTB 863A and *Lentinus sajor-caju*; culture collection – KUM 70097 were obtained from the Forest Research Institute Malaysia (FRIM) Malaysia. *P. sanguineus* and *L. sajor-caju* are local fungi commonly found in Malaysia while *T. versicolor* is a European fungus that has been used to test decay resistance in most parts of the world.

4% malt agar was prepared as a culture medium. 60 ml were poured into a series of 500 ml squat jars, lids were applied and the jars were sterilised by autoclaving. When cooled the jars were inoculated with the test fungi. All the jars were then incubated at 22 °C at 65% relative humidity for 2 weeks for fungus growth.

Sterilized polypropylene fabric was introduced on the surface of the agar medium before the wood samples were placed inside the testing jars. Four (two controls and two of each timber species) blocks were introduced into these jars by placing the wood blocks on top of the fabric. They were maintained at 22 °C and 70% RH for 16 weeks. Weekly observations were made to ensure that there was no contamination by the wrong fungi.

Table 5.1 Total number of test samples in the decay test

| Test | Fungi | Wood species | Replicates | Blocks |
|---|---|---|---|---|
| Decay test-1 | 3F | 12 | 12 | 432 |
| Decay test-2 | 1SC | 12 | 12 | 144 |
| Time check test | 3 | 2 | 12 blocks in 6 incubation periods | 432 |
| Reference test species | 3 | *H. brasiliensis* | 12 | 36 |
| | | *F. sylvatica* | 12 | 36 |
| | | | Total | 1080 |

F – No. of fungi, SC – Sterile control.

The rate of fungal attack was estimated on a separate series of test jars only containing *H. brasiliensis* or *F. sylvatica*. Weight loss was determined after 2, 4, 6, 8, 10, 12 (time check tests) and 16 weeks (main tests) of exposure. After each time period, the

test blocks were taken out and the mycelium removed from the blocks prior to weighing and drying at 103 °C for 24 hours. The test blocks were then reweighed and the average percentage weight losses and moisture contents (dry weight basis) were calculated for each individual timber species and test fungus.

Essentially the same procedure was followed for the main tests. Average weight losses were used to calculate the X factor as used in the termite wood consumption test. As a means of producing an overall durability figure for each wood species there have been further multiplied by each other to give a product of the 3 x factors and to make the data more readable they have been multiplied by 1000 to give an overall figure. This is essential the same as was performed for the termite durability tests.

An analysis of variance (ANOVA) was carried out using the MINITAB 15 software to test the between- and within-tree differences in decay resistance.

## 5.3 Results

### 5.3.1 Incubation time check test

The average weight loss and wood moisture content subjected to decay by the three fungi on *H. brasiliensis* and beech are shown in Figures 5.1 and 5.2. Details of the result are presented in Appendix Table A5.1. The most aggressive fungus to attack *H. brasiliensis* was *Pycnoporus sanguineus* while *Lentinus sajor-caju* was the least aggressive. Contrarily, *Trametes versicolor* was the most aggressive on beech and the least was also *L. sajor-caju.*

The weight losses of the three fungi were noted to be slightly low (less than 3%) in the first 2 weeks' incubation. The subsequent rate of weight loss of *H. brasiliensis* with *P. sanguineus* was rapid. The rate of weight losses caused by *T. versicolor* were only slightly slower; it was initially faster on *H. brasiliensis* but by the end of the experiment the weight loss on *F. sylvatica* was similar. *P. sanguineus* was much less capable on *F. sylvatica* than it was on rubberwood. Meanwhile, *L. sajor-caju* gave lower overall rates of decay on both *H. brasiliensis* and *F. sylvatica.* Target weight losses of 40-60% were deemed desirable as they would be within the linear phase of decay so that, based on these rates of decay, it was decided to extend the other, main decay experiment to the full 16 weeks, despite the small block sizes. The moisture content of test blocks exposed to different fungi for 12 weeks showed a general increase throughout the experiment consistent with an initial increase and then a slow increase as a result of lowered wood density (Figure 5.2).

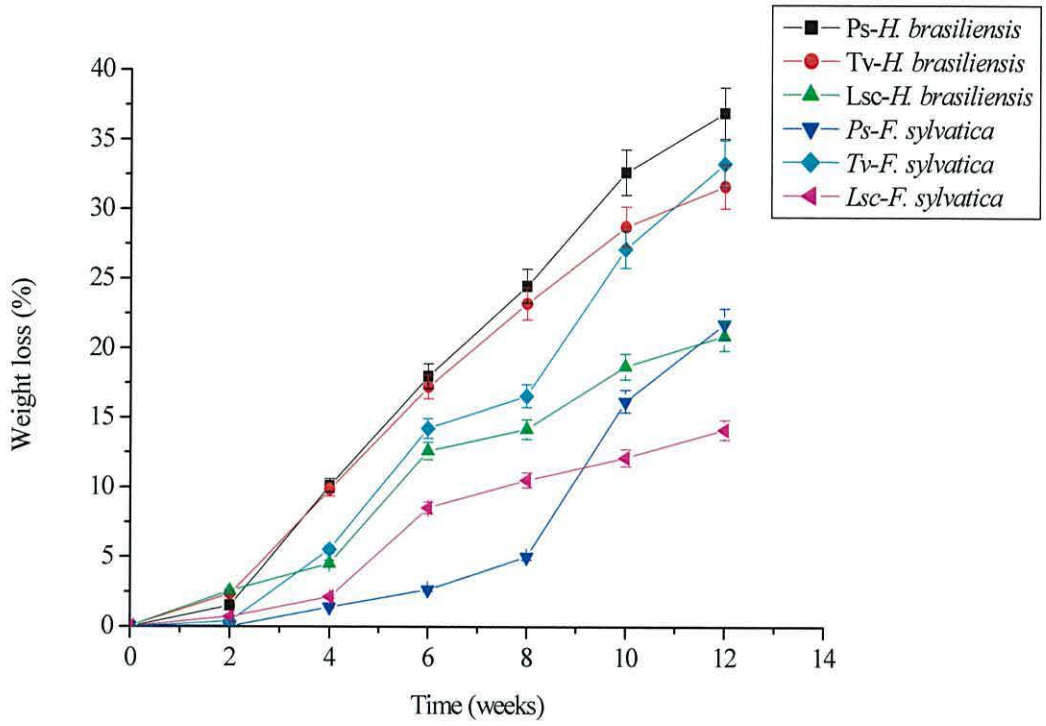



Figure 5.1 Change in average weight loss (%) during incubation.

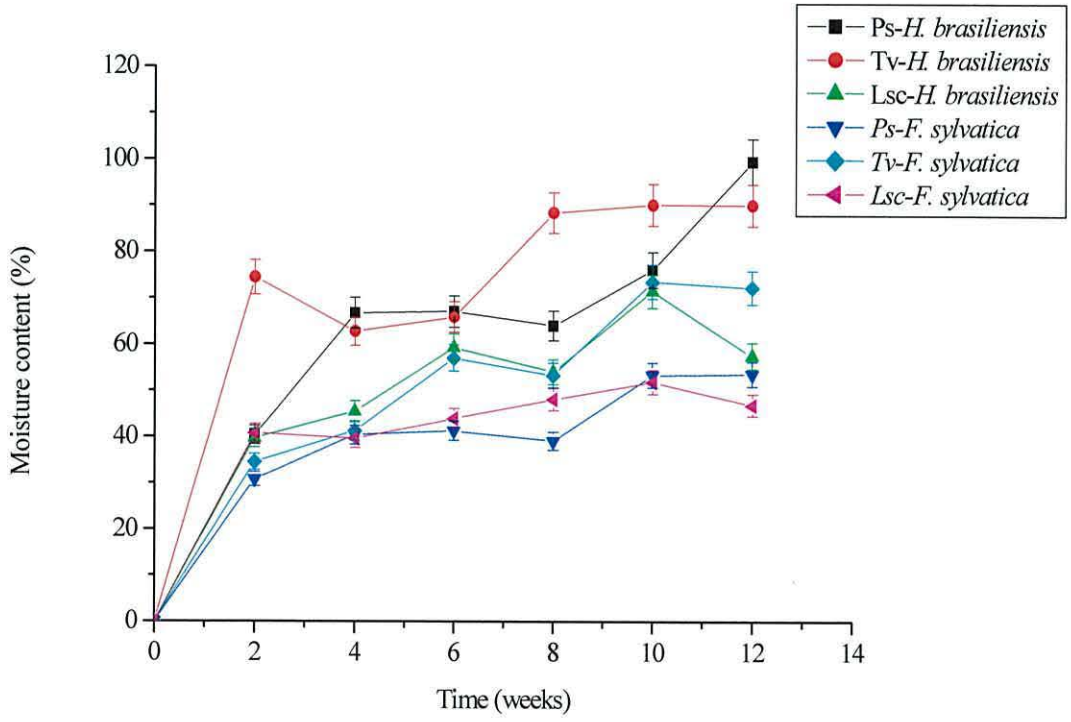



Figure 5.2 Change in moisture content of wood (%) during incubation.

### 5.3.2 Decay test 1 (12 wood species, 3 fungi, 16 week test)

After one week of exposure, almost all the wood samples were covered with fungus but the colonisation was less with *P. sanguineus*. The weight losses caused by the three fungi are presented in Figures 5.3, 5.4 and 5.5. The details of each fungus and wood species tested are presented in Appendix Table A5.2.

Among the three fungi tested, *T. versicolor* (except in *C. lanceolatum*, *D. kunstleri* and *N. heimii*) was the most aggressive fungus while *Pycnoporus sanguineus* was the least aggressive (except in *N. heimii* and *D. kunstleri*). Average weight losses of samples ranged from 0.56 to 30.45% for *P. sanguineus*, 0.34 to 36.95% for *T. versicolor* and 0.57 to 25.35% for *L. sajor-caju*. *N. heimii* was the only timber that showed an excellent level of resistance against all the three test fungi. Even though three other timber species (*M. utilis*, *D. grandiflorus* and *S. curtisii*) showed some weight loss values less than 1%, their weight losses varied between 0.14% to 1.83 for *P. sanguineus*, 0.51% to 11.93% for *T. versicolor* and 0.23% to 5.50% for *L. sajor-caju* (Appendix Tables A5.2, 5.3 and 5.4). Visually all of the test samples from these four species (*N heimii, M. utilis*, *D. grandiflorus* and *S. curtisii*) were free of fungal attack at the end of the test period.

Other wood species were less durable and all three fungi decayed *P. pinnata*, *C. scortechinii*, *C. lanceolatum* and *F. fragrans* heavily but less than *H. brasiliensis*, which was the most heavily decayed (over 25%). The weight losses of *A. angustifolia* and *K. ivorensis* were much higher for *T. versicolor* than for the two other fungi while decay by *P. sanguineus* was more on *D. kunstleri* than that of *T. versicolor* and *L. sajor-caju.*

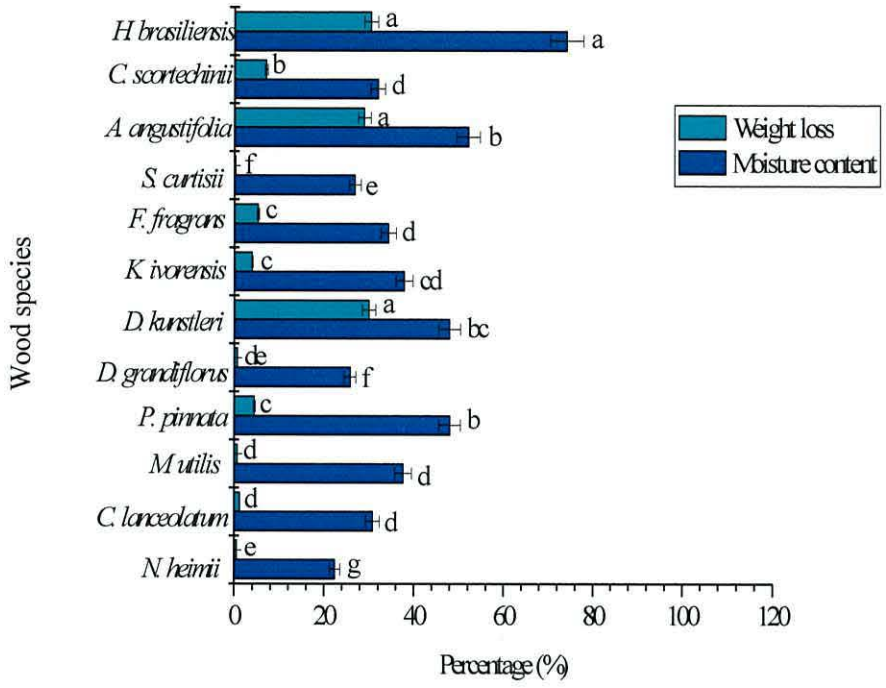



Figure 5.3 Average weight loss (AWL, %) and moisture content (MC, %) of 12 Malaysian woods species (decay test 1) towards *P. sanguineus.* Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test

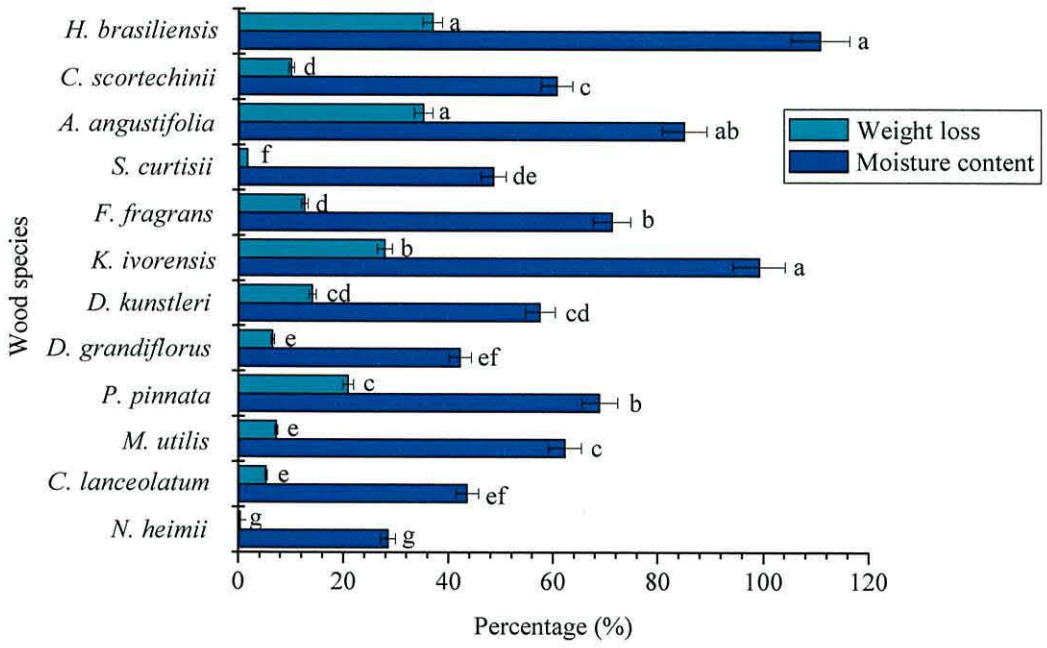



Figure 5.4 Average weight loss (AWL, %) and moisture content (MC, %) of 12 Malaysian woods species (decay test 1) towards *T. versicolor.* Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test

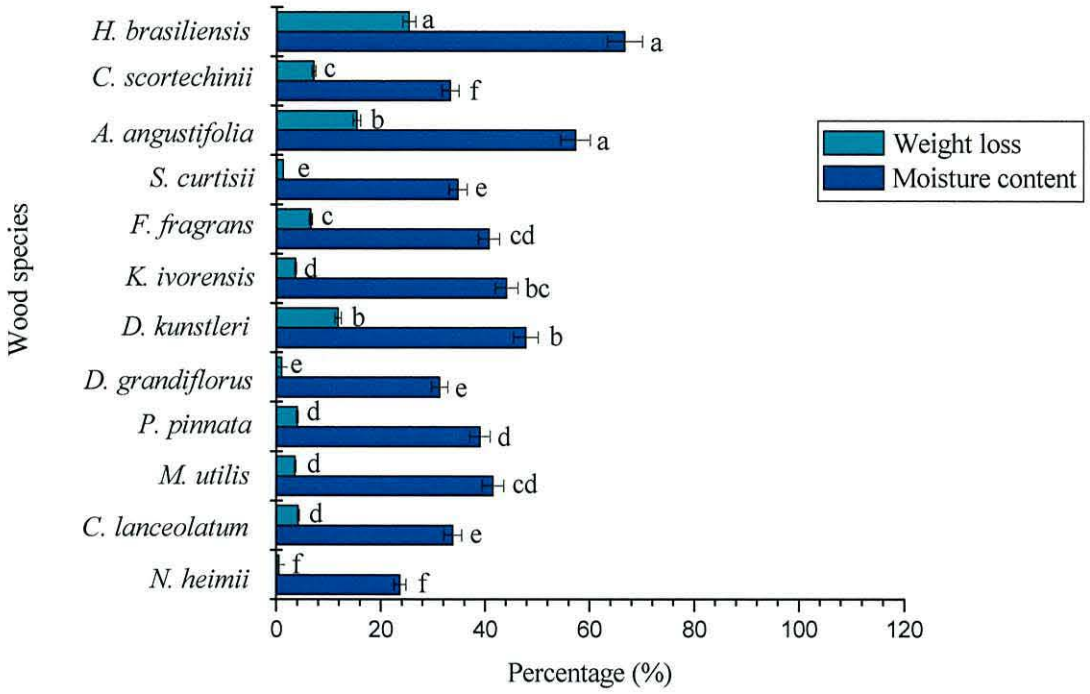



Figure 5.5 Average weight loss (AWL, %) and moisture content (MC, %) of 12 Malaysian woods species (decay test 1) towards *L. sajor-caju.* Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test

Table 5.2 presents the X factor of each wood species against fungi. As mentioned earlier, the results are indexed against a susceptible species *H. brasiliensis*. On this basis *N. heimii* and *S. curtisii* performed extremely well in the fungal decay tests among the twelve wood species. To even out the variation between fungi and get an overall score the product of the X factors (x1000) is presented. This clearly separates *N. heimii* from the other species (0.0038), gives a series of four durable species (0.019 to 0.94), a third group of moderately durable species (12.83 to 17.56) and a final, not durable group (174.79 to 1000).

Analysis of variance was conducted to show the difference in weight loss among the species tested against the three fungi. Pairwise comparisons of the ANOVA results showed that only *C. scortechinii* and *S. curtisii* were significantly different with other timber species against *P. sanguineus*, *N. heimii*, *K. ivorensis* and *S. curtisii* against *T. versicolor* and, *N. heimii* and *H. brasiliensis* against *L. sajor-caju*. The other wood species were not significantly different at $P \leq 0.05$ (Table 5.2, 5.3 and 5.4). The ANOVA tables are presented in Appendix Tables A5.3, A5.4 and A5.5.

Table 5.2 A ranking using the X factor of the twelve Malaysian wood species based on the weight loss. The product is the weight loss values x 1000

| Wood species | X factor | | | Product X1000 |
|---|---|---|---|---|
| | *P. sanguineus* | *T. versicolor* | *L. sajor-caju* | |
| *H. brasiliensis* | 1.00 | 1.00 | 1.00 | 1000.0000 |
| *A. angustifolia* | 0.95 | 0.95 | 0.60 | 547.5790 |
| *D. kunstleri* | 0.98 | 0.38 | 0.47 | 174.7851 |
| *C. scortechinii* | 0.23 | 0.27 | 0.28 | 17.5642 |
| *F. fragrans* | 0.17 | 0.34 | 0.26 | 14.9707 |
| *K. ivorensis* | 0.13 | 0.75 | 0.14 | 13.9808 |
| *P. pinnata* | 0.14 | 0.57 | 0.16 | 12.8320 |
| *C. lanceolatum* | 0.04 | 0.14 | 0.17 | 0.9403 |
| *M. utilis* | 0.02 | 0.20 | 0.14 | 0.6525 |
| *D. grandiflorus* | 0.02 | 0.18 | 0.04 | 0.1615 |
| *S. curtisii* | 0.01 | 0.04 | 0.05 | 0.0191 |
| *N. heimii* | 0.02 | 0.01 | 0.02 | 0.0038 |

The analysis of variance within wood species for average weight loss (Table 5.3) showed that only *A. angustifolia* and *H. brasiliensis* were significantly different to others at $P \leq 0.05$ against *P sanguineus*, two wood species (*D. grandiflorus* at $P \leq 0.01$ and *K. ivorensis* at $P \leq 0.05$) towards *T. versicolor*. Three wood species were found to show significant variation in average weight loss against *L. sajor-caju* (*A. angustifolia*, *H. brasiliensis* and *F. fragrans*). The ANOVA tables are presented in Appendix Tables A5.6, A5.7 and A5.8. Moisture content in all test blocks exposed to different fungi for 16 weeks showed a different trend for different decay types (Figures 5.3, 5.4 and 5.5).

Wood species with lower range of moisture content had lower weight loss while those with higher range of moisture content had higher weight loss. Moisture content increases with weight loss for a variety of reasons but partly because the block density decreases. With *T. versicolor* all the timber species tested had higher moisture contents (range between 25.7% to 274.4%) followed by *L. sajor-caju* (range between 21.1% to 92.9%) and *P. sanguineus* (range between 19.9% to 145.8%). The highest moisture content of test blocks for all three fungus tested were found in *H. brasiliensis* (110.8%, 66.7% and 74.2%, respectively, and the lowest was in *N. heimii* (28.5%, 23.7% and 22.4%, respectively).

All moisture content values were shown to vary with timber species at the 5% level of significance. The result of pairwise comparison indicated that *N. heimii*, *D. grandiflorus*, *H. brasiliensis* and *S. curtisii* had a highly significantly different values of moisture content ($P \leq 0.001$) to other timber species for *P. sanguineus*. In addition *N. heimii* had significantly different moisture content at the end of the test to *T. versicolor* and

*L. sajor-caju*. The other timber species had no significant different either at P≤0.05 or P≤0.01. The ANOVA tables are presented in Appendix Tables A5.9, A5.10 and A5.11.

Table 5.3 shows that there is considerable variation in the moisture contents of some of the sets of the blocks (i.e. fungus wood species combinations) and these were significant at different levels, e.g. *P. pinnata* against *P. sanguineus and A. angustifolia* against *L. sajor-caju* (P≤0.001), *C. scortechinii* and *S. curtisii* against *P. sanguineus* and, *M. utilis* and *C. lanceolatum* against *L. sajor-caju* (P≤0.01) and four wood species showed significantly different against *P. sanguineus* (*N. heimii*, *D. kunstleri*, *A. angustifolia* and *F. fragrans*), only one against *T. versicolor* (*D. grandiflorus*) and five species against *L. sajor-caju* (*P. pinnata*, *D. kunstleri*, *D. grandiflorus*, *C. scortechinii* and *F. fragrans*) (P≤0.05). The others are not significantly different. The ANOVA tables are presented in Appendix Tables A5.12, A5.13 and A5.14. The causes of these differences will be discussed later but they may relate either to test variation as a statistical artefacts (e.g. *N. heimii* and *P. sanguineus*, 67% variation but range of 19.9 to 23.3% moisture contents) or decay severity.

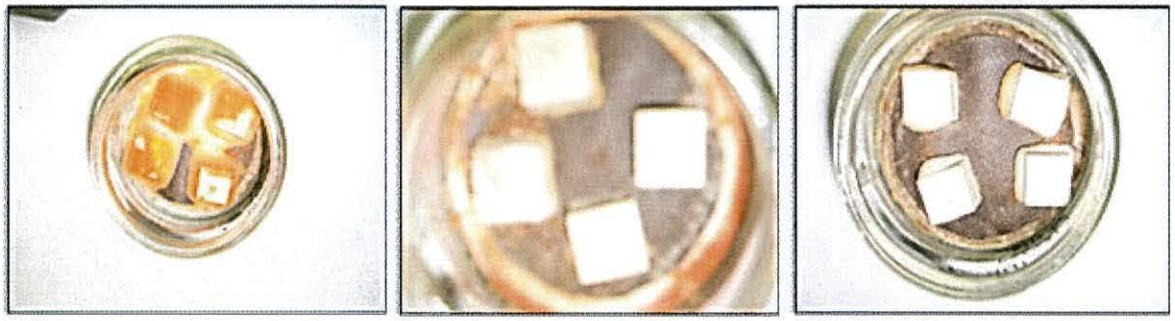
Table 5.3 ANOVA results showing the proportion (%) of total variation ($R^2$) in average weight loss and moisture content accounted for within samples tested

| Species | Average weight loss | | | Average moisture content | | |
|---|---|---|---|---|---|---|
| | PS | TV | LSC | PS | TV | LSC |
| *N. heimii* | 36.11ns | 9.06ns | 38.44ns | 67.54* | 21.41ns | 27.76ns |
| *C. lanceolatum* | 23.14ns | 2.19ns | 11.57ns | 13.87ns | 15.83ns | 86.30** |
| *M. utilis* | 31.33ns | 46.41ns | 38.27ns | 31.59ns | 11.93ns | 87.90** |
| *P. pinnata* | 20.80ns | 12.74ns | 35.25ns | 91.53*** | 16.63ns | 56.76* |
| *D. grandiflorus* | 29.69ns | 76.09** | 45.30ns | 41.28ns | 58.77* | 53.92* |
| *D. kunstleri* | 36.12ns | 25.13ns | 12.10ns | 67.48* | 14.04ns | 60.03* |
| *K. ivorensis* | 8.86ns | 55.94* | 2.44ns | 21.43ns | 40.18ns | 14.65ns |
| *F. fragrans* | 48.52ns | 7.27ns | 68.04* | 63.88* | 25.79ns | 65.16* |
| *S. curtisii* | 23.68ns | 9.42ns | 31.11ns | 74.38** | 9.45ns | 32.78ns |
| *A. angustifolia* | 64.47* | 23.66ns | 61.09* | 59.60* | 37.13ns | 89.09*** |
| *C. scortechinii* | 26.21ns | 43.10ns | 31.67ns | 77.56** | 25.90ns | 56.25* |
| *H. brasiliensis* | 68.85* | 40.30ns | 64.31* | 38.75ns | 50.90ns | 6.95ns |

PS - *P. sanguineus*, TV - *T. versicolor*, LSC – *L. sajor caju*

*N. heimii* *C. lanceolatum* *M. utilis*

*P. pinnata* *D. grandiflorus* *D. kunstleri*



*K. ivorensis* *F. fragrans* *S. curtisii*

*A.angustifolia* *C. scortechinii* *H. brasiliensis*

Figure 5.6 Appearance of some of the test jars containing wood blocks of twelve Malaysian wood species after sixteen weeks of exposure to *P. sanguineus*

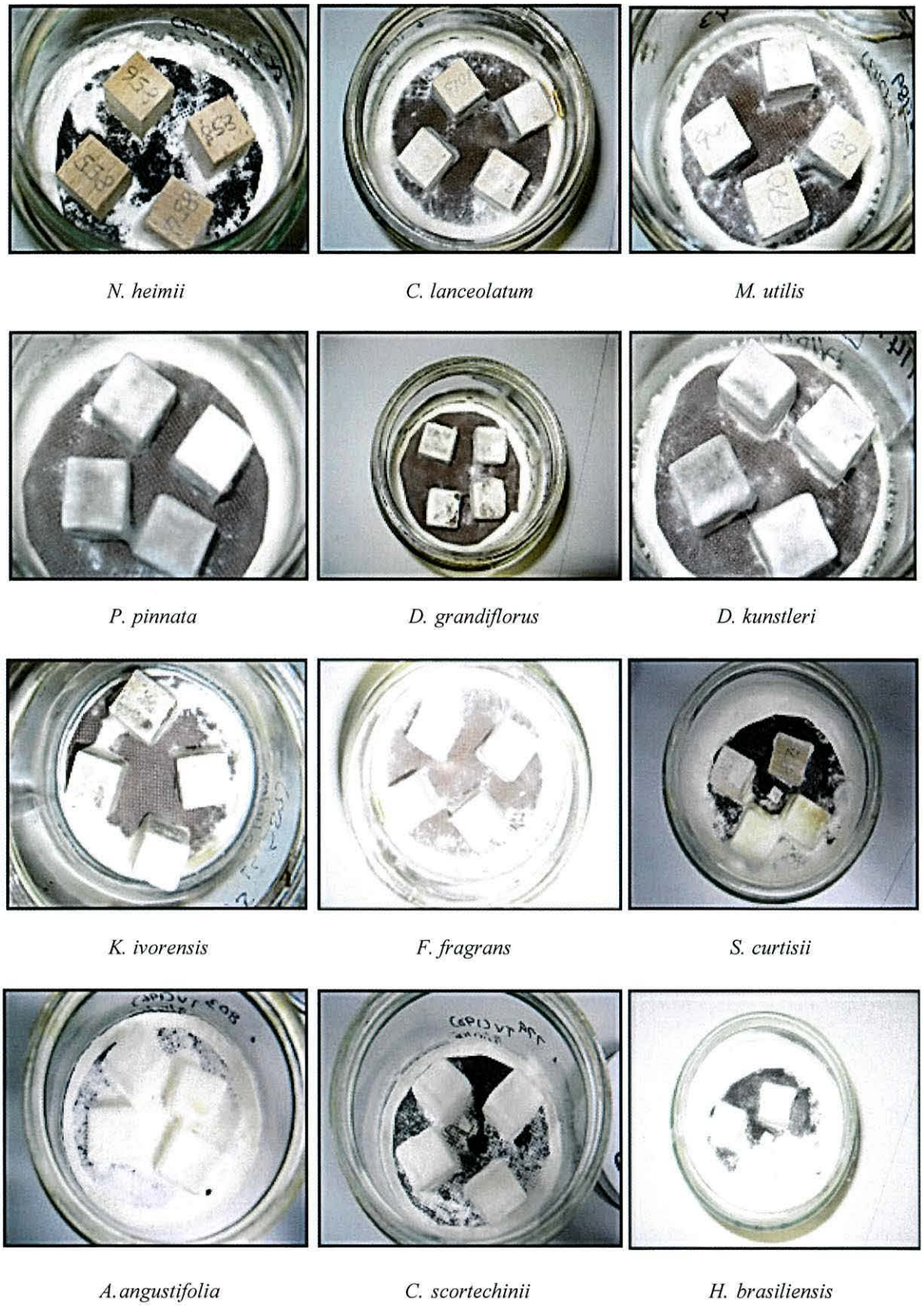
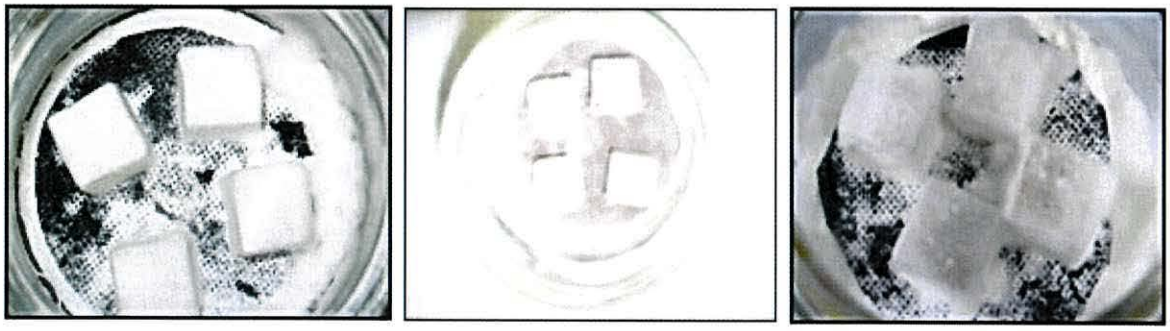



Figure 5.7 Appearance of some of the test jars containing wood blocks of twelve Malaysian wood species after sixteen weeks of exposure to *T. versicolor*

*N. heimii* *C. lanceolatum* *M. utilis*

*P. pinnata* *D. grandiflorus* *D. kunstleri*

*K. ivorensis* *F. fragrans* *S. curtisii*



*A.angustifolia* *C. scortechinii* *H. brasiliensis*

Figure 5.8 Appearance of some of the test jars containing wood blocks of twelve Malaysian wood species after sixteen weeks of exposure to *L. sajor-caju*

### 5.3.3 Decay test 2 (12 species, 0 fungi) – Sterile control

Weight losses for sterile wood blocks for different wood species are summarized in Table 5.4 below. Table 5.4 showed that weight losses were generally very low, and within that normally expected for a decay test, the highest (0.5%) average weight loss was found in *N. heimii* and the lowest (0.1%) was in *D. kunstleri* samples. Of all the blocks of twelve Malaysian hardwoods the sterile controls ranged from 0.01% to 0.70%. Although *N. heimii* had the highest average weight loss, it had the lowest moisture content (22.0%) at the end of 16 weeks test. *H. brasiliensis*, second after *D. kunstleri* having the least weight loss, had the highest of moisture content (43.0%). All of the moisture contents of the blocks were adequate for initiation of decay, i.e. above fibre saturation point, although *N. heimii* may be close to the threshold.

Pairwise comparison showed that only *N. heimii* was highly significantly different at $P \leq 0.001$ in all cases of average weight loss of sterile samples. Other comparisons (34 cases) are not significantly different to each other at $P \leq 0.05$. The ANOVA table is presented in Appendix Table A5.16. Meanwhile, Table 5.5 indicates that only *C. scortechinii*, *A. angustifolia, D. kunstleri* and *P. pinnata* are the four species that have significantly different in all comparisons for blocks moisture content either at $P \leq 0.05$, $P \leq 0.01$ or $P \leq 0.001$. The other are significantly different at $P \leq 0.05$. The ANOVA table is presented in Appendix Table A5.17

Table 5.4 Average weight loss (AWL, %) and moisture content (MC, %) of 12 Malaysian wood species (decay test 2 – sterile samples)

| Timber species | AWL | Range of AWL | MC | Range of MC |
|---|---|---|---|---|
| *N. heimii* | 0.45 (0.11)$^{a}$ | 0.23 - 0.61 | 21.98 (0.94)$^{h}$ | 21.10 - 24.36 |
| *C. lanceolatum* | 0.17 (0.02)$^{cdf}$ | 0.14 - 0.20 | 25.48 (0.21)$^{g}$ | 25.13 - 25.89 |
| *M. utilis* | 0.20 (0.06)$^{cd}$ | 0.12 - 0.30 | 28.99 (2.57)$^{d}$ | 24.87 - 34.25 |
| *P. pinnata* | 0.20 (0.06)$^{cd}$ | 0.10 - 0.28 | 27.48 (0.55)$^{e}$ | 26.49 - 28.49 |
| *D. grandiflorus* | 0.22 (0.12)$^{bc}$ | 0.13 - 0.54 | 24.44 (0.45)$^{h}$ | 24.08 - 25.78 |
| *D. kunstleri* | 0.11 (0.05)$^{i}$ | 0.05 - 0.18 | 25.83 (1.28)$^{fg}$ | 24.12 - 28.61 |
| *K. ivorensis* | 0.19 (0.15)$^{cde}$ | 0.01 - 0.47 | 27.75 (2.33)$^{de}$ | 23.30 - 30.83 |
| *F. fragrans* | 0.17 (0.07) $^{cdg}$ | 0.06 - 0.23 | 22.74 (3.54)$^{h}$ | 14.44 - 28.76 |
| *S. curtisii* | 0.16 (0.07)$^{ch}$ | 0.08 - 0.25 | 26.28 (0.82)$^{f}$ | 24.45 - 27.06 |
| *A. angustifolia* | 0.31 (0.17)$^{b}$ | 0.11 - 0.70 | 33.59 (1.85)$^{b}$ | 31.03 - 36.05 |
| *C. scortechinii* | 0.22 (0.09)$^{bd}$ | 0.11 - 0.36 | 31.18 (2.85)$^{c}$ | 27.72 - 37.86 |
| *H. brasiliensis* | 0.13 (0.10)$^{efghi}$ | 0.01 - 0.30 | 42.96 (10.93)$^{a}$ | 20.23 - 58.80 |

Mean (± SD) of 12 replicates for each species. Means within each column followed by the same letter are not significantly different at the 5% level of ANOVA test.

Table 5.5 ANOVA results showing the proportion (%) of total variation ($R^2$) average weight loss and moisture content accounted for sterile samples between samples tested

| Species | Average weight loss | Moisture content |
|---|---|---|
| *N. heimii* | 40.68ns | 26.90ns |
| *C. lanceolatum* | 91.70*** | 7.27ns |
| *M. utilis* | 5.04ns | 37.65ns |
| *P. pinnata* | 47.61ns | 79.76** |
| *D. grandiflorus* | 28.50ns | 31.93ns |
| *D. kunstleri* | 71.95* | 56.41* |
| *K. ivorensis* | 18.01ns | 17.53ns |
| *F. fragrans* | 36.75ns | 40.84ns |
| *S. curtisii* | 28.38ns | 41.54ns |
| *A. angustifolia* | 49.62ns | 67.18* |
| *C. scortechinii* | 9.90ns | 75.93** |
| *H. brasiliensis* | 13.14ns | 15.63ns |

P≤0.05, **P≤0.01, *** P≤0.001, ns – not significant.

### 5.3.4 Correlation between moisture content and durability

A highly significant positive correlation between moisture content and average weight loss was only found in three species against *T. versicolor* and four against *L. sajor-caju* (Table 5.6). Moderate significantly positive correlation occurred in majority of wood species against *P. sanguineus*. Inconsistent correlations between average weight loss and density were found against all white-rot fungi. Some had positive correlations and some were negative but were not significant.

Table 5.6 Correlation (R) between weight loss and moisture content of twelve Malaysian hardwood (within species) (n = 5)

| Wood species | R | | |
|---|---|---|---|
| | *P. sanguineus* | *T. versicolor* | *L. sajor-caju* |
| *N. heimii* | 0.47ns | 0.94* | 0.56ns |
| *C. lanceolatum* | 0.95* | 0.98** | 0.97** |
| *M. utilis* | 0.83* | 0.89* | 0.97** |
| *P. pinnata* | 0.85* | 0.98** | 0.99** |
| *D. grandiflorus* | 0.91* | 0.79ns | 0.90* |
| *D. kunstleri* | 0.76ns | 0.99** | 0.77ns |
| *K. ivorensis* | 0.95* | 0.68ns | 0.83* |
| *F. fragrans* | 0.88* | 0.80ns | 0.89* |
| *S. curtisii* | 0.50ns | 0.85* | 0.84* |
| *A. angustifolia* | 0.89* | 0.95* | 0.76ns |
| *C. scortechinii* | 0.85* | 0.85* | 0.46ns |
| *H. brasiliensis* | 0.73ns | 0.71ns | 0.99** |

***, **, *, ns: correlation respectively significant at 0.001%, 1%, 5% and not significantly.

## 5.4 Discussion

The time control test (Figure 5.1) gave rapid decay results for all of the fungi tested. *H. brasiliensis* was more rapidly decayed than *F. sylvatica* (beech). The plots of weight loss were more or less linear with time and, despite the small size of the decay blocks, it was decided to run the test for the full 16 weeks to achieve weight losses suitable for the standard methods. The maximum sustained average decay rate was for *P. sanguineus* on *H. brasiliensis* at about 3.7% weight loss per week, which should have given a 60% weight loss in 16 weeks.

The decay rates were somewhat slower than those for the time control test. The main decay tests were successful and all three decay fungi gave high weight losses on the susceptible species *H. brasiliensis*, with a 30.45% weight loss for *T. versicolor*, a 25.35% weight loss for *P. sanguineus* and a 36.95 % weight loss for *L. sajor-caju*. It is thus valid to compare the weight loss results for the other timber species, although *H. brasiliensis* should be classified in the not durable class with a mass loss of 30% or more for all test fungi. The main test was done at a slightly later time and some problems were experienced with the conditioning room controls which may explain this. In order to correct for this the X-factor, using *H. brasiliensis*, has been used to normalise the data (Table 5.2). A slightly more generous rating could have employed *A. angustifolia* for this purpose. Had this not been done, species like *H. brasiliensis* would have been classed in durable classes.

### 5.4.1 Decay with *P. sanguineus*

Among the three fungus tested, *P. sanguineus* showed a huge variation (-0.20% to 44.80%) in weight losses (Appendix Table A5.2). From the twelve wood species tested, four (*M. utilis*, *N. heimii*, *D. grandiflorus*, and *S. curtisii*) of them had a weight loss less than 1%, a good performance which showed a good level of resistance to *P. sanguineus*.

As regards the durability classes as listed in Tables 2.7 (EN350) and 2.8 (ASTM-D2017) the results give different durability ratings due to the differences in the standards and the non-standardised weight losses, something which is corrected through the use of the X-factor. Thus from the X-factor durability ratings have been derived following Table 2.7 (Table 5.7). The results with this fungus class these four species together with *C. lanceolatum, P. pinnata* and *K. ivorensis*, under very durable while *C. scortechinii*, and *F. fragrans* can be categorized under durable and the other three (*D. kunstleri*, *A. angustifolia* and *H. brasiliensis*) species were under not durable against *P. sanguineus.*

Table 5.7 Durability ratings of the twelve wood species exposed to the 3 different decay fungi based on the X-factor in EN 350-1 (1994)

| Timber species | Durability class | | | Worst case rating |
|---|---|---|---|---|
| | *P. sanguineus* | *T. versicolor* | *L. sajor-caju* | |
| *H. brasiliensis* | 5 | 5 | 5 | 5 |
| *A. angustifolia* | 5 | 5 | 4 | 5 |
| *D. kunstleri* | 5 | 3 | 3 | 5 |
| *C. scortechinii* | 2 | 2 | 2 | 4 |
| *F. fragrans* | 2 | 2 | 2 | 4 |
| *K. ivorensis* | 1 | 4 | 1 | 4 |
| *P. pinnata* | 1 | 3 | 2 | 3 |
| *C. lanceolatum* | 1 | 1 | 2 | 2 |
| *M. utilis* | 1 | 2 | 1 | 2 |
| *D. grandiflorus* | 1 | 1 | 1 | 1 |
| *S. curtisii* | 1 | 1 | 1 | 1 |
| *N. heimii* | 1 | 1 | 1 | 1 |

### 5.4.2 Decay with *T. versicolor*

*T. versicolor* was the most aggressive fungus giving appreciable decay in the majority of the wood species tested (Appendix Table A5.3). Out of twelve wood species, four (*N. heimii*, *S. curtisii D. grandiflorus* and *C. lanceolatum* ) were under very durable, three (*M. utilis*, *F. fragrans* and *C. scortechinii* ) under the durable class, *P. pinnata* and *D. kunstleri* under the moderately durable class, *K. ivorensis* under the slightly durable class and the remaining species, *A. angustifolia* and *H. brasiliensis*, under non-durable.

### 5.4.3 Decay with *L. sajor-caju*

Malaysia is reported to be rich with *Lentinus* spp. (Chin, 1981; Corner, 1981; Oldridge *et al*, 1986; Lee *et al*, 1995) and one of the species that is usually used for tests in Malaysia is *L. sajor-caju*. *L. sajor-caju* belongs to the Aphyllophorales and is commonly found decaying wood. In tests it is aggressive and can cause more than 50% of weight loss (Salmiah, 1997).

For this fungus *M. utilis*, *N. heimii*, , *D. grandiflorus*, *K. ivorensis* and *S. curtisii* were very durable while *P. pinnata, C. lanceolatum F. fragrans* and *C. scortechinii* were classed as durable, *D. kunstleri* as moderately durable, and *A. angustifolia* as slightly durable and *H. brasiliensis* as not durable.

### 5.4.4 Variation in durability loss between wood species

In Figures 5.3, 5.4 and 5.5 it is apparent that there is a variation between the weight losses caused by the decay fungi (data in Appendix Tables A5.2, A5.3 and A5.4), for each timber species. Using the X factor (Table 5.2) to normalise the data to *H. brasiliensis* direct comparisons can be made and it is clear that there is a large variation in durability between the test fungi, with, for example, *K. ivorensis* failing badly to *T. versicolor* and *L. sajor-caju* being less aggressive on a number of wood species. When the product is examined *N. heimii, S. curtisii, D. grandiflorus* and *M. utilis* all show good performance, which in the case of *N. heimii* is exceptional. This is discussed more fully in Chapter 8, where the correlations between extractives and decay are examined more fully.

In the figures it is apparent that there is little variation in the decay resistance between the samples tested (12 replicates). This may be because they were all obtained from the same region in the tree (the base).

Under European Standard (EN 350-1, 1994) (Table 5.7), only two timber species (*N. heimii* and *S. curtisii*) are classified very durable against all fungi on weight loss criteria, whereas three species are very durable when the x factor is used. Many studies (Wong *et al*, 2005) have reported the high durability of *N. heimii* (Foxworthy and Wooley, 1930; Jackson, 1957; Mohd Dahlan and Tam, 1985; Mohd Dahlan and Azlan, 1994). Yamamoto and Hong (1988) reported that this is because of the high extractive content.

However, under Malaysian standard, *M. utilis*, *N. heimii* and *C. lanceolatum* were classified as durable timbers while a group of moderately durable will contained *P. pinnata*, *D. kunstleri*, *D. grandiflorus*, *K. ivorensis*, *S. curtisii* and *F. fragrans* Mohd Dahlan and Tam, 1985). The remaining timbers (*C. scortechinii*, *A. angustifolia* and *H.*

*brasiliensis*) would probably be too susceptible for use in any situation of high decay hazard, such as ground contact, but a few less susceptible species could possibly give satisfactory service as exposed woodwork, as does *C. scortechinii*. However, those species which are comparable with the highly susceptible *H. brasiliensis* heartwood would be unsuitable for external use in humid climatic conditions, unless they were impregnated with a suitable preservative.

### 5.4.5 Variation in wood moisture content between species

The relationship between moisture, fungal growth and decay has been widely discussed (Christensen and Kaufmann, 1965; Ayerst, 1968; Griffin, 1963; 1969; 1972). For successful colonisation, the wood moisture content should be around or above fibre saturation point but not saturated and if the sterile controls indicate an intermediate moisture content, then adequate moisture has been supplied for the fungus to colonise. In this test a geotextile mat was used rather than glass rods which are common in agar block tests. This was done to force wet the more moisture resistant species. During decay, moisture contents generally increase to above that of the controls due to transport of water by fungi, reductions in wood density caused by decay and water generated by wood breakdown (i.e. carbohydrates). The moisture content of the sterile control blocks (Figures 5.3, 5.4 and 5.5, Appendix A5.16) are all around or above fibre saturation point, although in some instances low values were encountered, e.g. *N. heimii* (22%) in other case high, *H. brasiliensis* (43%). In those species which gave very low durability the moisture contents of the sterile controls were elevated but some species with low moisture contents the moisture contents may be considered marginal. In similar species with high extractive content (Skaar, 1988) the fibre saturation points are lowered due to blocking of wood cell wall hydroxyl groups, so even in these species there is likely to be sufficient free water available for fungal colonisation. For optimal decay however many studies (Scheffer, 1993; Milberg, 1987; Eaton and Hale, 1993; Nicholas and Crawford, 2003) have found that moisture contents between 30 to 80% are optimal for decay for the most active decay fungi.

### 5.4.6 Correlation between weight loss and moisture content

In Chapter 8 the relationships between extractives contents, moisture content and decay resistance will be examined.

## 5.5 Conclusions

1. *T. versicolor* proved the most aggressive while *P. sanguineus* was the least.
2. Wood species which had high end of test moisture contents also had high decay rates. Those showing little decay had enough water to initiate decay but had low moisture contents at the end of test.
3. Of the twelve hardwood species used, five showed little decay namely *N. heimii, C. lanceolatum, M. utilis, D. grandiflorus* and *S. curtisii.* Of these only *N. heimii* and *S. curtisii* are very durable on the basis of weight loss and X-factor criteria.

# CHAPTER 6: BIOACTIVITY OF BARK AND HEARTWOOD EXTRACTIVES OF MALAYSIAN WOODS

## 6.1 Introduction

One of the most important factors stated for the natural durability of wood is toxicity of extractive compounds (Scheffer and Cowling, 1966; Bamber and Fukazawa, 1985; Hillis, 1987b). A number of woods have been shown to contain extractable substances which are toxic or deterrent for bacteria, beetles, termites and fungi (Becker, 1965; Weissmann and Dietrichs, 1975; Bauch *et al*, 1977). Even though these compounds are present in only small amounts (3 to 20%) (Hillis, 1987a; Dorado *et al*, 2000; Ishida *et al*, 2007; Zhang *et al*, 2007), they play an important role in determining other wood properties too. Therefore their study is of considerable technical significance.

In recent years, much interest has developed in role of extractives and their role in wood protection. Early work showed relationships between durability and extractives (Anderson, 1961; Rudman, 1965a; 1965b; Hart and Hillis, 1974; Reis, 1972; Takahashi and Kishima, 1973). Some studies included tropical wood species such as teak (*Tectona grandis*), granadillo (*Platymiscum yucatanum*) and African padauk (*Pterocarpus soyauzii* Taub) (Waterman, 1946; Hillis, 1962; Rudman, 1963b; Chudnoff, 1980; Bezuidenhout *et al*, 1988; Scalbert, 1991; Reyes-Chilpa *et al*, 1998; Thevenon *et al*, 2001) and temperate woods; osage orange, black locust and redwood (Kamdem, 1994; Schultz *et al*, 1995). However, few studies have specifically reported on Malaysian hardwood species. Studies by Buckley (1932), Yatagai and Takahashi (1980), Rasadah *et al* (1987), Yamamoto and Hong (1988; 1989) found that wood extractives were a significant factor of decay resistance against insects and fungi. However, most of these studies only reported quantitative data of extractive amounts. Few studies addressed the question of which components in the extractives are of importance or how they protected the wood against termites and fungi. This section of the study was initiated to answer these questions.

The aims of this chapter were to examine the ability of extractives from *M. utilis*, *N. heimii*, *C. lanceolatum* and *S. curtisii* to limit the growth of decay fungi, decrease their ability to decay *H. brasiliensis* and increase the termite mortality. These wood species have been shown in Chapter 3 to have high extractive quantities and show both good resistance to termites (Chapter 4) and decay fungi (Chapter 5). Other work has confirmed that these species contain high quantities of extractives (Hong and Abdul Razak, 1983; Yamamoto and Hong, 1988; 1989).

## 6.2 Materials and methods

### 6.2.1 Extraction process

A hot water extraction was used to extract the four selected (*N. heimii, C. lanceolatum, M, utilis* and *S. curtisii*) wood species as described before. A weighed amount of about 120 g (total) of air-dried sawdust was used in order to get sufficient extractives for the termite and fungus tests. 40 g of sawdust were mixed with 2 litre deionised water in a round bottomed flask and attached to reflux condenser. The flask was placed in a boiling water bath such that the fluid level was lower than the bath level and the flask was gently heated for 3 hours. After extraction and cooling the solution was filtered and dried to a constant weight at 40 to 50 °C. The extracts were put into stoppered glass bottles and stored at room temperature and kept for 5 days for further tests.

### 6.2.2 Bioactivity of extractives

#### 6.2.2.1 Antitermitic activity

The bioassay method used by previous studies (Tellez *et al*, 2002; Ganapaty *et al*, 2004; Kusumoto *et al*, 2009) with slightly modified was used to evaluate the antitermitic activity of wood extracts against *C. curvignathus* and *C. gestroi*. The four wood species (*C. lanceolatum, M. utilis*, *N. heimii* and *S. curtisii*) heartwood were selected because of antitermitic properties that observed in earlier studies. Samples of 5.0 mg, 10 mg and 20 mg of wood extract from four different wood species were dissolved in 60 $\mu$l of methanol (MeOH) to obtain solutions of 0.5%, 1.0% and 2.0%, respectively. Then the solutions were applied to 30 mg filter paper samples (Advantec, 8 mm diameter and 1.5 mm thickness) and dried in a vacuum desiccator for 24 hours. The paper discs were weighed before and after drying. Untreated paper discs were used as a controls and each of the tests contained 10 replicates. 20 active termite workers were introduced into each petri dish (90 mm diameter and 16 mm height) which contained 3 g of sterile sand. A few drops of water were added periodically to the basal edge of each petri dish. All the petri dishes with covers were placed into an incubator (maintained in darkness) at 27$\pm$1 °C and 80% RH for 10 days. The mortality of the termites was counted and recorded daily.

The susceptibility index was obtained by dividing the filter paper consumption for any one species by the filter paper consumption of the most susceptible species. So the wood species can be compared and ranked to each other to determine the suitability of end products (Curling and Murphy, 2002).

### 6.2.2.2 Antifungal assay

*Bioassays with extractives*

The toxicity of four different extractives was tested against *T. versicolor* (CTB 863A), *L. sajor-caju* (KUM 70097) and *C. puteana* (FPRL 11E). *T. versicolor and L. sajor-caju* were chosen because both are the most two aggressive fungi in the decay test (Chapter 5) while *C. puteana* is for comparison between white-rot and brown-rot fungi. This test was designed to give a basic understanding of performance of the extractives. All extractives were prepared at concentrations of 1.5%. The test was performed by putting a small plug of fungus into the middle of 90 mm of petri-dishes containing 2.5% malt agar. Then drops containing 50 $\mu$l of extractives were put around the mycelium plug, with five replicate plates. Malt agar to which no extractive was applied was used as a control. All the petri-dishes then incubated at 22 °C, 70% relative humidity. The diameters (mm) of the fungi were measured every 2 days. Where the growth was not circular, two measurements were taken and averaged. The controls were used to compare the toxic effect of the extracts.

*Bioassay with treated wood blocks*

The test rapidly evaluated the performance of different types of extractives in a jar assay. The tested extractives were not purified or separated into individual components. Thus the test provided just a basic understanding of performance of the total components present in the particular types of extractives.

Wood specimens 19 x 19 x 5 mm, were cut from sapwood of *H. brasiliensis* and were oven-dried at 103±5 °C. The wood specimens were vacuum (60 mins, 20 mm Hg) treated with hot water extractives from the four different wood species at four different treatment strengths (2%, 4%, 6% and 8%). Then, the wood specimens were dried at 60 °C for 24 hours and reweighed. Blocks were then sterilised by irradiation as previously.

Jars were prepared, inoculated and precultured prior to specimen support and wood block planting, as previously (section 5.2.2). Five wood blocks (4 treated and 1 untreated controls) were placed into each culture jar. Then all the test jars were maintained at 22 °C, 70% RH for 12 weeks. All samples were subjected to decay by two aggressive white-rot fungi (*Trametes versicolor* and *Lentinus sajor-caju*) and one brown-rot fungus (*Coniophora puteana*) according to the ASTM standard (ASTM D1413, 1998).

After exposure the mycelium was removed from the test blocks prior to weighing and oven drying to a constant weight (nearest 0.01 g) at 103±5 °C for 24 hours and then

reweighed to determine both moisture content and weight loss. The same method as in 6.2.2.1 was used to calculate the susceptibility index for fungi. Susceptibility index was obtained by dividing the weight loss for any one species by the weight loss of the most susceptible species.

One way analysis of variance (ANOVA) was performed on all data to determine the significance of variation in extractive compounds, antitermitic and antifungal between wood species as well as between samples.

## 6.3 Results

### 6.3.2 Bioactivity of extractives

#### 6.3.2.1 Further termite feeding test

A response was noted when the termites were introduced into the bottles; they avoided the treated filter paper discs but not the control samples. All extracts reduced the survival of both termites compared to the corresponding control, showing significant activity against *C. curvignathus* and *C. gestroi*. Observation at 2 days intervals found that both termites did not feed under or on the treated paper discs with the concentration more than 1.0% wood extractives. However, they did feed under the untreated paper discs (controls) and try to make tunnels. Two types of data are presented in this section, survival and filter paper consumption.

*Termite survival*

In no-choice tests on absorbent paper discs treated with heartwood extracts from the four selected different tree species, the survival of both termite species varied with extractive source. The trend was similar in tests either against *C. curvignathus* or *C. gestroi* with the same wood extracts. Minimal mortality (not more than 20%) occurred in *C. curvignathus* and *C. gestroi* on control samples. This indicates that the filter paper cellulose had little effect on termite mortality. On the other hand, *C. curvignathus* looked more aggressive than *C. gestroi* which is similar when tested on solid wood (Chapter 4).

The bioassays results indicate that the survival of both termite species was significantly worse on paper discs with *N. heimii* extracts and the best survival was with *S. curtisii* (Table 6.1). At the lowest treatment level (0.5%), only test samples treated with *N. heimii* extracts gave a result of less than 50% survival for both termites' species. The other three extracts species gave a survival between 55 to 80% for *C. curvignathus* and 54 to 76% for *C. gestroi*.

Table 6.1 Mean percentage survival rates of subterranean termites against solution concentration

| Extracted species | Solution concentration (%) | Survival days | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 |
| | | | ***C. curvignathus*** | | | | |
| *N. heimii* | 0 | 100(0) | 100(0) | 100(0) | 96(2.11) | 95(3.33) | 90(0) |
| | 0.5 | 100(0) | 97(2.58) | 90(3.33) | 79(3.16) | 60(4.08) | 50(2.36) |
| | 1.0 | 100(0) | 80(4.08) | 65(4.08) | 48(2.58) | 40(5.27) | 25(2.36) |
| | 2.0 | 100(0) | 60(4.08) | 48(4.22) | 40(4.08) | 32(5.37) | 0(0) |
| *C. lanceolatum* | 0 | 100(0) | 99(2.11) | 97(4.22) | 92(4.83) | 90(3.94) | 88(3.94) |
| | 0.5 | 100(0) | 100(0) | 94(3.16) | 86(3.94) | 78(5.87) | 55(4.08) |
| | 1.0 | 100(0) | 88(2.58) | 79(3.16) | 65(3.33) | 58(4.22) | 36(2.11) |
| | 2.0 | 100(0) | 78(2.58) | 65(2.36) | 55(0) | 46(4.59) | 25(4.08) |
| *M. utilis* | 0 | 100(0) | 100(0) | 100(0) | 97(2.58) | 94(3.16) | 92(2.58) |
| | 0.5 | 100(0) | 100(0) | 98(4.22) | 93(4.22) | 85(5.77) | 79(4.59) |
| | 1.0 | 100(0) | 98(2.58) | 90(4.08) | 82(4.22) | 66(3.94) | 37(2.58) |
| | 2.0 | 100(0) | 92(3.50) | 87(4.83) | 75(3.33) | 63(5.87) | 30(3.33) |
| *S. curtisii* | 0 | 100(0) | 98(2.58) | 97(2.58) | 95(0) | 92(2.58) | 90(3.33) |
| | 0.5 | 100(0) | 100(0) | 95(4.08) | 90(3.33) | 86(3.94) | 72(2.58) |
| | 1.0 | 100(0) | 95(3.33) | 87(3.50) | 72(2.58) | 54(3.94) | 40(4.08) |
| | 2.0 | 100(0) | 82(2.58) | 71(2.11) | 53(2.58) | 36(2.11) | 30(4.08) |
| | | | ***C. gestroi*** | | | | |
| *N. heimii* | 0 | 100(0) | 96(3.16) | 92(4.83) | 90(4.08) | 85(4.08) | 80(5.27) |
| | 0.5 | 100(0) | 92(2.58) | 76(3.16) | 61(4.59) | 54(4.59) | 42(5.87) |
| | 1.0 | 100(0) | 84(3.94) | 65(4.71) | 48(2.58) | 40(4.71) | 25(3.33) |
| | 2.0 | 100(0) | 68(3.50) | 55(4.08) | 40(6.24) | 28(5.87) | 0(0) |
| *C. lanceolatum* | 0 | 100(0) | 96(3.16) | 90(2.36) | 86(3.16) | 84(5.16) | 80(4.08) |
| | 0.5 | 100(0) | 90(3.33) | 86(3.94) | 78(4.22) | 70(4.08) | 54(5.68) |
| | 1.0 | 100(0) | 88(2.58) | 80(5.27) | 72(4.83) | 60(4.08) | 32(2.58) |
| | 2.0 | 100(0) | 80(4.08) | 72(3.50) | 52(4.22) | 34(3.94) | 24(4.59) |
| *M. utilis* | 0 | 100(0) | 100(0) | 98(2.58) | 95(0) | 86(2.58) | 81(4.08) |
| | 0.5 | 100(0) | 92(2.58) | 88(3.50) | 84(4.83) | 77(4.22) | 70(0) |
| | 1.0 | 100(0) | 86(3.94) | 77(2.58) | 62(2.58) | 40(3.33) | 20(2.36) |
| | 2.0 | 100(0) | 80(0) | 68(2.58) | 54(3.94) | 38(2.58) | 20(3.33) |
| *S. curtisii* | 0 | 100(0) | 99(2.11) | 95(0) | 92(2.58) | 90(2.36) | 82(2.36) |
| | 0.5 | 100(0) | 90(4.08) | 85(4.08) | 82(4.22) | 80(4.08) | 76(2.11) |
| | 1.0 | 100(0) | 90(2.36) | 82(3.50) | 70(4.08) | 62(4.22) | 37(3.50) |
| | 2.0 | 100(0) | 88(2.58) | 70(3.33) | 65(3.33) | 42(2.64) | 30(4.71) |

Mean (± SD) of 10 replicates for each species.

There is a general trend where the survival of both termite species decreased significantly with increased extractives concentrations. At the highest treatment level (2.0%), complete mortality of *C. curvignathus* and *C. gestroi* was also observed in test samples pads treated with *N. heimii* extracts. Only 1.10% and 0.69% had been eaten by both termite species, respectively (Table 6.2). This may due to the toxic action on the intestinal protozoa after ingesting the treated cellulose pad because of heavy feeding. Survivals of *C. curvignathus* at 2% concentrations were about 60% lower than those in the controls for *M. utilis* and *S. curtisii*, and 50% for *C. lanceolatum* while this was similar

with *C. gestroi* tests ((*M. utilis*, 70% and *C. lanceolatum* and *S. curtisii,* 60%). Both termite species only consumed less than 2% of paper discs at this concentration, thus at a 2% concentration the extracts had antitermitic properties against both subterranean termite species. Meanwhile the 1.0% trials showed that more than 60% of termite mortality occurred during the 10 days exposure for all timber extracts.

*Filter paper consumption*

Further evidence is gained by examining the filter paper consumption after 10 days exposure (Table 6.2). Different concentrations gave significantly different percentages of consumption. The consumption decreased significantly with the concentrations of wood extractives. The lowest concentration (0.5%) showed some consumption decrease, generally less than half the consumption of the controls (0%) against both termite species and this improved as the concentrations increased.

*C. curvignathus* consumed more than *C. gestroi* for every extractive, indicating they are more toxic to *C. gestroi* than *C. curvignathus*. Among the four wood extracts, *N. heimii* extract was very toxic to both subterranean termites followed by *C. lanceolatum*, *M. utilis* and *S. curtisii.* However, higher concentration of wood extracts (more than 2%) was needed in order to achieve 0% of wood consumption especially for *C. curvignathus*. Pairwise comparison between each concentrations also showed that wood consumption varied very significantly between each level of concentrations ($P \leq 0.01$). The ANOVA tables are presented in Appendix Tables A6.1 and A6.2.

Figures 6.1 and 6.2 further clearly shows that among the extracts from four wood species, *N. heimii* had higher susceptibility index against both termite species (*C. curvignathus* and *C. gestroi*) in all level of extractive concentrations (0%, 0.5%, 1% and 2%). Even though the index of *C. lanceolatum* (90%) was bigger than *N. heimii* (89%) at 2% of concentration, it was not significant. The same situation also occurred between *M. utilis* and *S. curtisii* extracts. As a conclusion, *N. heimii* extracts were more toxic followed by *C. lanceolatum*, *S. curtisii* and the least toxic was *M. utilis* extracts against both termite species.

Table 6.2 Mean percentage of filter paper consumption after 10 days against subterranean termites

| Extracted species | Solution concentration (%) | | | |
|---|---|---|---|---|
| | 0 | 0.5 | 1.0 | 2.0 |
| *C. curvignathus* | | | | |
| *N. heimii* | 10.09(0.32)$^{a}$ | 2.53(0.34)$^{b}$ | 1.44(0.03)$^{c}$ | 1.10(0.13)$^{d}$ |
| *C. lanceolatum* | 10.79(0.55)$^{a}$ | 3.97(0.66)$^{b}$ | 2.09(0.54)$^{c}$ | 1.09(0.11)$^{d}$ |
| *M. utilis* | 10.45(0.32)$^{a}$ | 5.05(0.27)$^{b}$ | 3.88(0.23)$^{c}$ | 1.47(0.08)$^{d}$ |
| *S. curtisii* | 10.78(0.75)$^{a}$ | 5.60(0.40)$^{b}$ | 3.78(0.65)$^{c}$ | 1.72(0.16)$^{d}$ |
| *C. gestroi* | | | | |
| *N. heimii* | 8.11(1.09)$^{a}$ | 1.42(0.18)$^{b}$ | 0.90(0.17)$^{c}$ | 0.69(0.05)$^{d}$ |
| *C. lanceolatum* | 8.13(0.83)$^{a}$ | 3.79(0.97)$^{b}$ | 1.72(0.45)$^{c}$ | 0.95(0.09)$^{d}$ |
| *M. utilis* | 8.01(0.77)$^{a}$ | 4.67(0.80)$^{b}$ | 2.34(0.39)$^{c}$ | 1.23(0.25)$^{d}$ |
| *S. curtisii* | 8.09(0.65)$^{a}$ | 4.29(1.26)$^{b}$ | 2.16(0.14)$^{c}$ | 1.40(0.22)$^{d}$ |

Mean (± SD) of 10 replicates for each species. Means within each column followed by the same letter are not significantly different in the same group at the 1% level of ANOVA test.

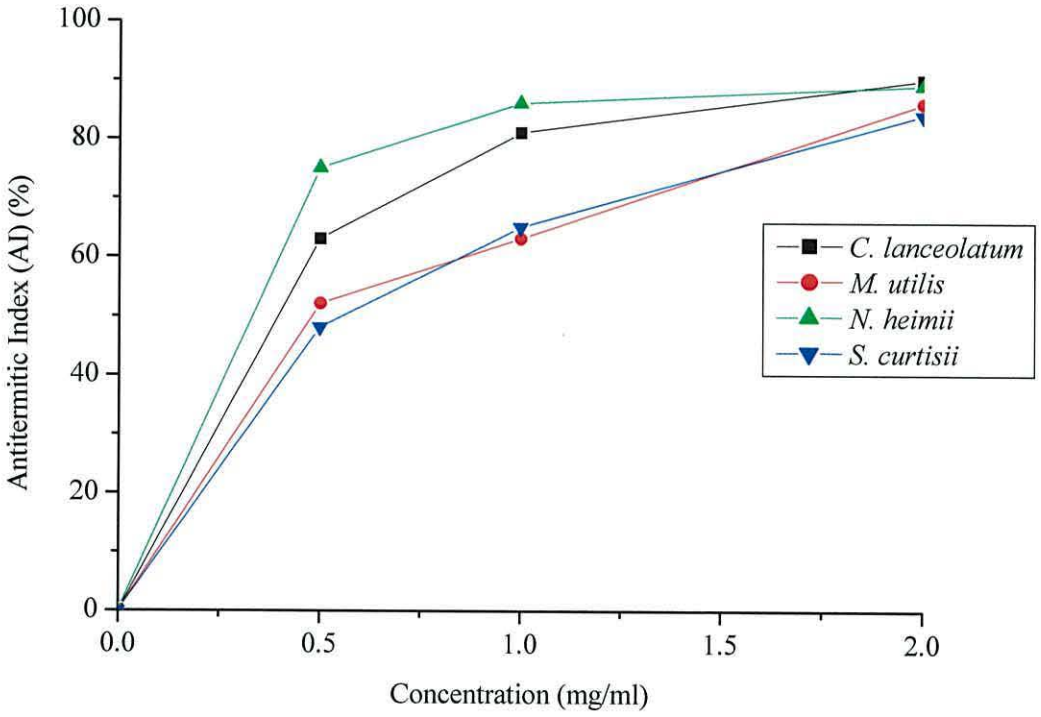



Figure 6.1 Antitermitic index of four Malaysian wood species extracts against *C. curvignathus*

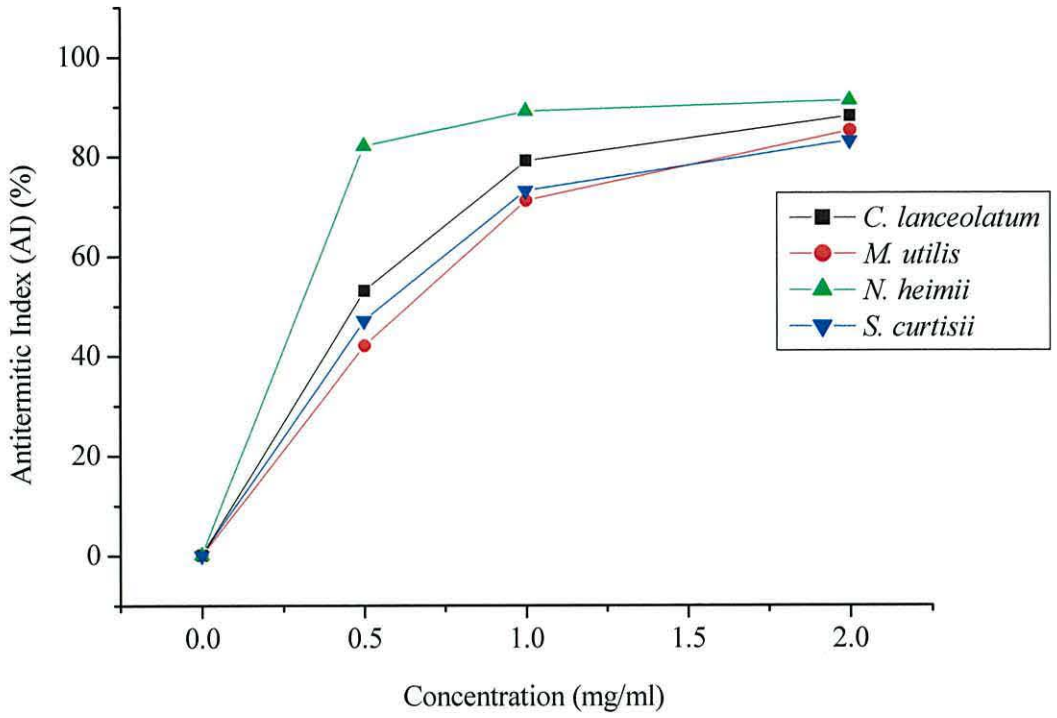



Figure 6.2 Antitermitic index of four Malaysian wood species extracts against *C. gestroi*

### 6.3.2.2 Further decay test

*Toxicity screening of the wood extractives*

The screening test of different wood extracts was done by the spot tests. The growth was recorded every 2 days and an example plot of the results (Table 6.3) shows that it is difficult to compare meaningfully. Accordingly the linear regressions of days 4 to 10 have been made to give growth rates in mm $day^{-1}$. The appearance of the petri-dishes at the end of 10 days is shown in Figures 6.3 to 6.5.

All three fungi had covered the 90 mm diameter of plates within 10 days with Tol:IMS extract from four wood species whereas their controls had not, suggesting some inhibition by the solvent which may have substantially flashed off in the extract. Considering this the water extractive controls have been used for comparison. Tol:IMS extracts presented higher activities (faster growth rates) than hot water extracts. When the data are compared it is apparent that the hot water extracts have a greater effect in reducing the fungal growth rates. Generally, they took about 12 to 14 days to reach 100% full growth on agar plates. *N. heimii* extracts gave the slowest the fungus growth. Growth was much faster with the *M. utilis* extracts followed by *S. curtisii* and *C. lanceolatum*.

Figures 6.3, 6.4 and 6.5 showed the growth of fungi against different wood extractives extracted with different solvent (Tol:IMS and hot water). The figures clearly shows that the fungi grew over some of the extracts (e. g. all fungi for *S. curtisii*) and not some of the others (*T. versicolor* for *N. heimii*). This is due to the toxicity of extractives from particular species to fungi. Colour reactions were also noted around some of the spots for the white rot fungi, which is typical for phenol oxidation by an enzyme like laccase, but no such reaction was noted for the brown rot fungus, which do not produce laccases. Some of the extractives also diffused out because some of them are water soluble and some not. Figures 6.7, 6.8 and 6.9 also showed that hot water extracts better inhibit fungal growth than the Tol:IMS extracts.

Table 6.3 Diameter growth (mm) of fungi in 2.5% malt agar containing four species of wood extractives

| Wood species | | *T. versicolor* | | | | *L. sajor-caju* | | | | *C. puteana* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Day | Tol: IMS (Control) | Hot water: (Control) | Tol:IMS extractives | Hot water extractives | Tol: IMS (Control) | Hot water (Control) | Tol:IMS extractives | Hot water extractives | Tol: IMS (Control) | Hot water (Control) | Tol:IMS extractives | Hot water extractives |
| *N. heimii* | 2 | 16.8 | 15.8 | 14.0 | 17.0 | 11.0 | 12.25 | 10.5 | 10.5 | 17.0 | 17.8 | 16.8 | 17.0 |
| | 4 | 42.3 | 46.0 | 29.8 | 33.5 | 25.8 | 28.75 | 28.8 | 25.5 | 22.0 | 44.8 | 41.5 | 47..3 |
| | 6 | 50.0 | 55.0 | 44.5 | 45.0 | 36.0 | 42.0 | 37.0 | 37.5 | 24.0 | 54.5 | 53.0 | 54.0 |
| | 8 | 52.0 | 71.3 | 58.8 | 57.8 | 38.0 | 50.8 | 47.0 | 49.8 | 26.0 | 84.0 | 72.8 | 70.3 |
| | 10 | 55.0 | 90.0 | 73.3 | 70.5 | 40.0 | 74.5 | 64.5 | 67.5 | 32.0 | 90.0 | 84.0 | 90.0 |
| *C. lanceolatum* | 2 | 17.0 | 17.8 | 17.0 | 16.8 | 9.5 | 11.0 | 9.5 | 10.0 | 16.8 | 15.8 | 17.0 | 17.3 |
| | 4 | 42.3 | 46.0 | 35.0 | 35.3 | 24.5 | 28.8 | 27.0 | 33.0 | 22.5 | 42.8 | 44.3 | 40.0 |
| | 6 | 45.0 | 57.0 | 46.0 | 47.0 | 28.0 | 40.5 | 37.8 | 37.5 | 27.0 | 60.0 | 55.0 | 52.0 |
| | 8 | 50.0 | 72.0 | 60.0 | 58.0 | 30.0 | 52.0 | 49.0 | 52.0 | 31.0 | 85.0 | 78.0 | 76.0 |
| | 10 | 58.5 | 90.0 | 73.25 | 73.0 | 38.3 | 76.0 | 66.0 | 69.0 | 34.0 | 90.0 | 90.0 | 90.0 |
| *M. utilis* | 2 | 14.5 | 15.8 | 13.0 | 17.5 | 11.0 | 12.5 | 10.5 | 11.3 | 18.0 | 16.0 | 15.8 | 18.3 |
| | 4 | 40.0 | 41.5 | 36.0 | 37.3 | 29.0 | 28.5 | 32.0 | 29.8 | 20.0 | 47.5 | 41.5 | 47.3 |
| | 6 | 50.0 | 54.0 | 53.0 | 49.5 | 35.0 | 39.5 | 39.3 | 38.5 | 24.0 | 66.0 | 63.3 | 62.5 |
| | 8 | 52.0 | 74.0 | 63.0 | 60.8 | 36.0 | 59.5 | 55.0 | 55.5 | 30.0 | 75.0 | 82.0 | 84.0 |
| | 10 | 54.0 | 90.0 | 77.5 | 76.5 | 38.0 | 73.0 | 71.3 | 70.3 | 34.0 | 80.0 | 90.0 | 90.0 |
| *S. curtisii* | 2 | 16.0 | 16.5 | 14.0 | 17.0 | 10.0 | 12.0 | 11.0 | 11.0 | 15.0 | 16.0 | 15.0 | 16.0 |
| | 4 | 41.0 | 42.0 | 36.0 | 37.0 | 28.0 | 26.0 | 30.0 | 30.0 | 28.0 | 43.0 | 40.0 | 40.0 |
| | 6 | 45.0 | 56.0 | 50.0 | 48.0 | 32.0 | 41.0 | 38.0 | 36.0 | 32.0 | 62.0 | 58.0 | 57.0 |
| | 8 | 50.0 | 73.0 | 62.0 | 61.0 | 36.0 | 55.0 | 52.0 | 56.0 | 36.0 | 88.0 | 80.0 | 82.0 |
| | 10 | 57.0 | 90.0 | 75.0 | 75.0 | 40.0 | 77.0 | 68.0 | 69.0 | 38.0 | 90.0 | 90.0 | 90.0 |

Mean of 5 replicates of each species

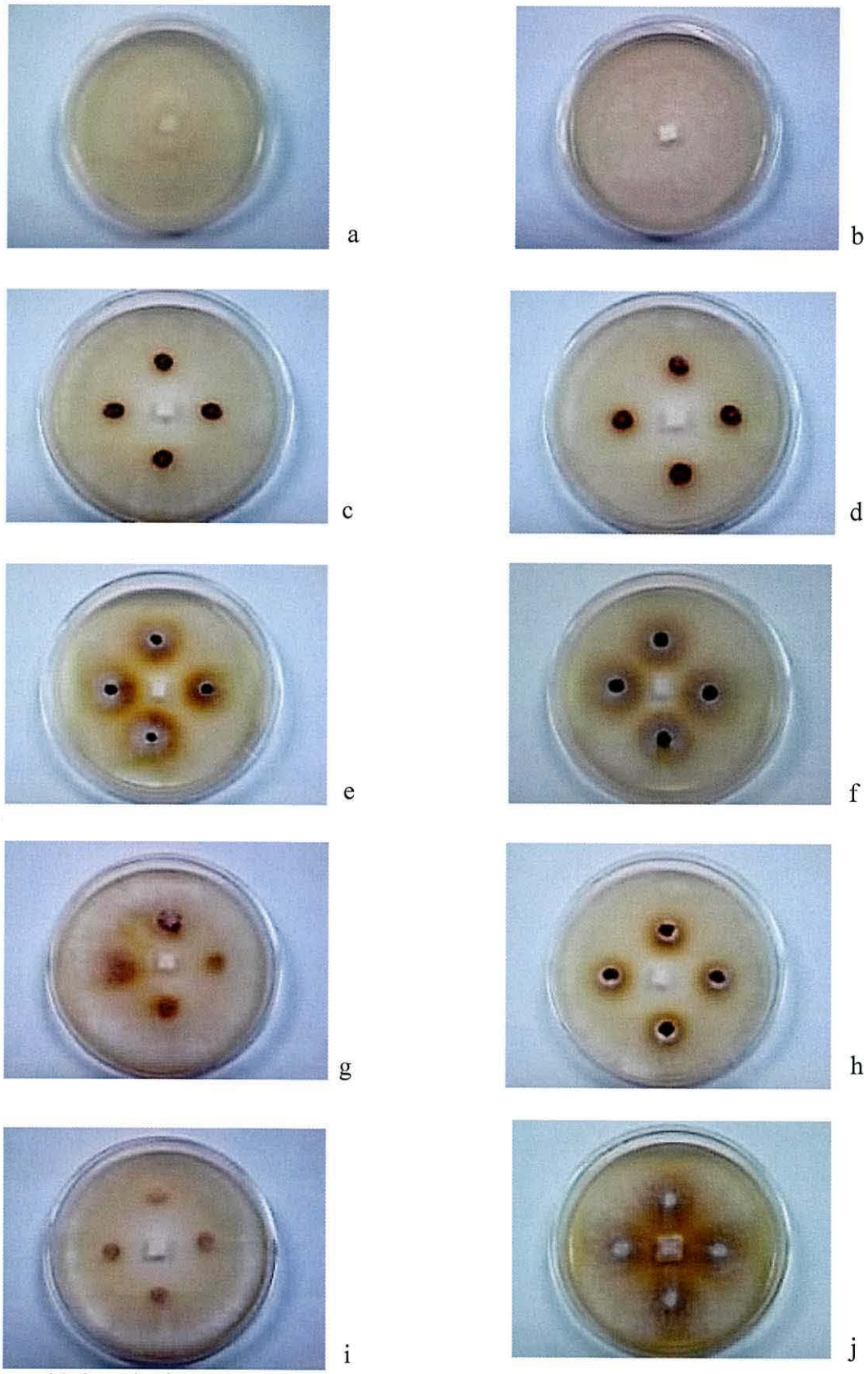



Figure 6.3 Growth of *T. versicolor* after 10 days exposure to different wood extractives; a-Tol:IMS (control), b-Hot water (control), c-Tol:IMS (*M. utilis*), d-Hot water (*M. utilis*), e-Tol:IMS (*N. heimii*), f-Hot water (*N. heimii*) g-Tol:IMS (*C. lanceolatum*), h-Hot water (*C. lanceolatum*), i-Tol:IMS (*S. curtisii*), j-Hot water (*S. curtisii*)

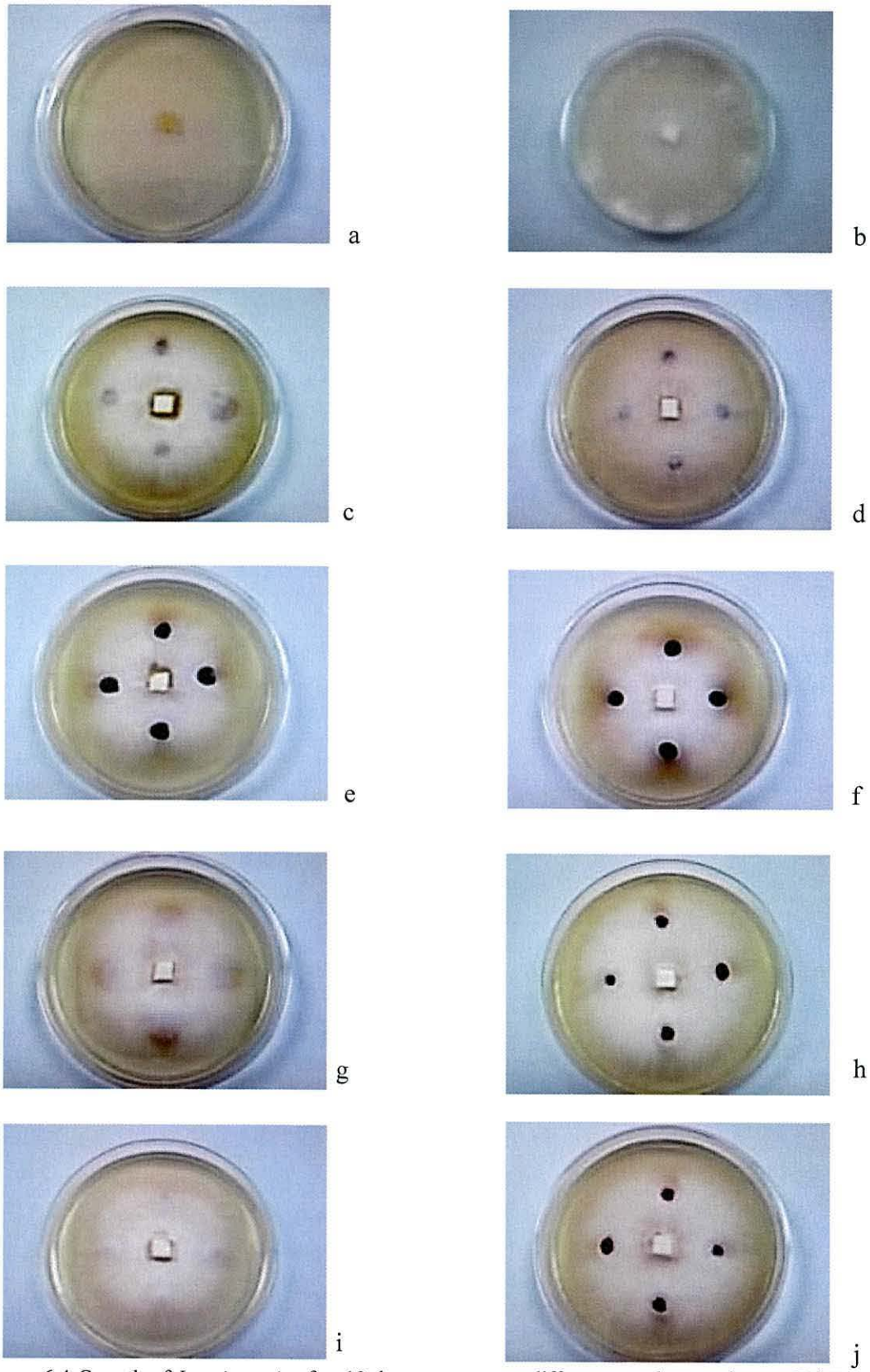



Figure 6.4 Growth of *L. sajor caju* after 10 days exposure to different wood extractives; a-Tol:IMS (control), b-Hot water (control), c-Tol:IMS (*M. utilis*), d-Hot water (*M. utilis*), e-Tol:IMS (*N. heimii*), f-Hot water (*N. heimii*) g-Tol:IMS (*C. lanceolatum*), h-Hot water (*C. lanceolatum*), i-Tol:IMS (*S. curtisii*), j-Hot water (*S. curtisii*)

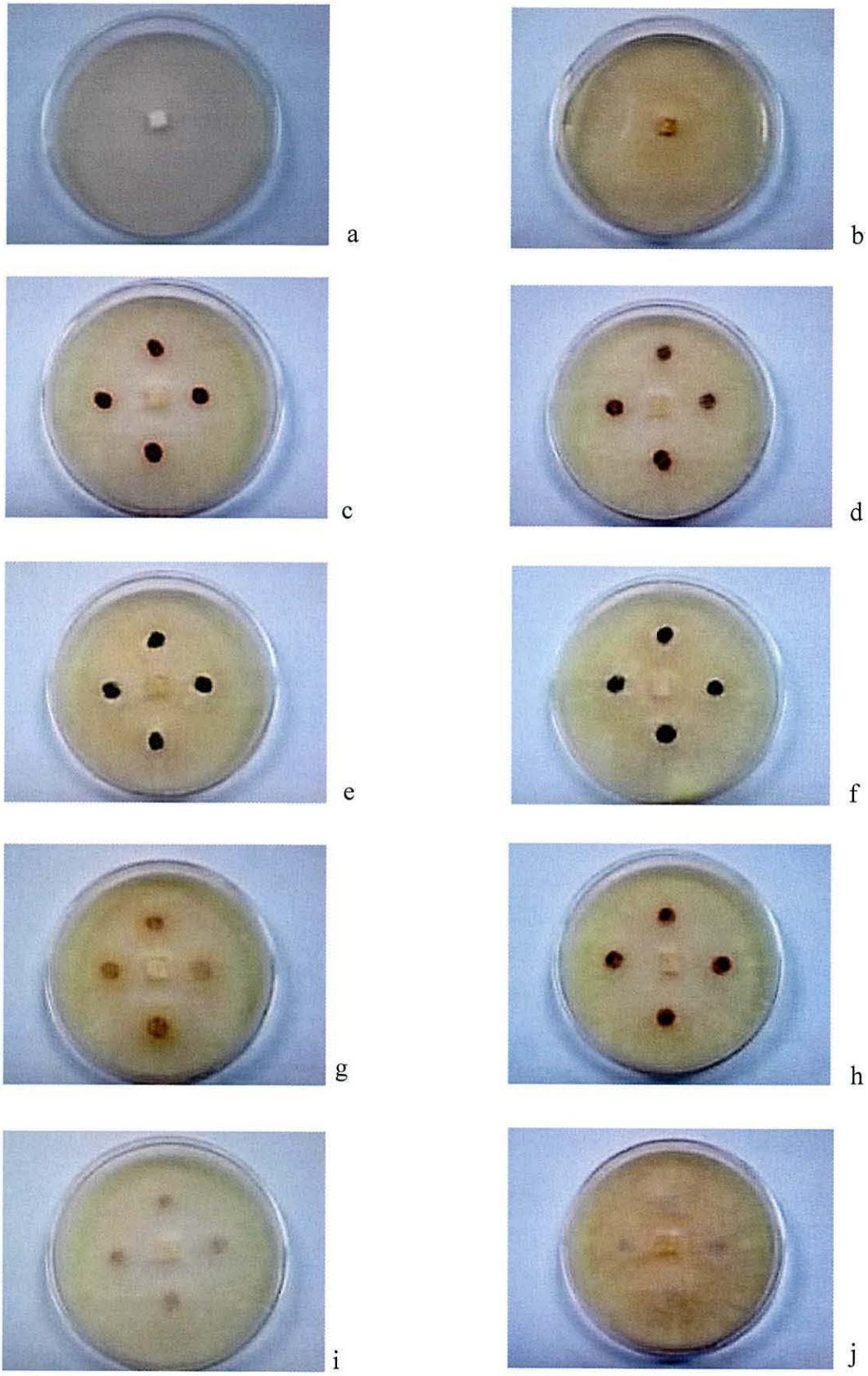



Figure 6.5 Growth of *C. puteana* after 10 days exposure to different wood extractives; a-Tol:IMS (control), b-Hot water (control), c-Tol:IMS (*M. utilis*), d-Hot water (*M. utilis*), e-Tol:IMS (*N. heimii*), f-Hot water (*N. heimii*) g-Tol:IMS (*C. lanceolatum*), h-Hot water (*C. lanceolatum*), i-Tol:IMS (*S. curtisii*), j-Hot water (*S. curtisii*)

*Effectiveness in protecting H. brasiliensis*

The average weight losses and moisture content of the hot water extractive treated specimens an after exposure to white rot (*Trametes versicolor* and *Lentinus sajor-caju*) and brown rot (*Coniophora puteana*) for 12 weeks, and results of statistical analysis, are shown in Figures 6.6, 6.7, 6.8 and 6.9. Detailed data are presented in Appendix Table A6.3.

Higher extractive concentrations are known to give a good natural durability against biological agents. As shown in Figures 6.6, 6.7, 6.8 and 6.9, there is a surprisingly close correlation between the average weight loss in the decay tests with all fungus and the heartwood extractives content of the corresponding four wood species. Differences in weight loss varied depending on the specific species of fungi used in the decay tests, the extractive contents from specific species and the level of concentration used.

The weight loss of unimpregnated *H. brasiliensis* control blocks, inoculated with *T. versicolor* was 50.3% where decay of wood by *L. sajor-caju* and *C. puteana* was 33.0% and 34.0%, respectively. *C. puteana* (brown-rot fungi) is more aggressive on softwoods while the white rots, especially *T. versicolor* has proved especially aggressive on untreated blocks. Figures 6.6, 6.7, 6.8 and 6.9 also clearly show that all wood extracts reduced decay with increasing concentrations. *N. heimii* extracts displayed better protection against all fungi tested. One way of looking at this is to examine the gradients of the graphs and to average these for all of the fungi. *N. heimii* gave an average of 2.05, *C. lanceolatum*, 1.63, *M. utilis*, 1.35 and *S. curtisii*, 1.6. This also demonstrates that *N. heimii* is the most effective.

The other extracts were less effective and were similar if the different performances between the different fungi are considered. In particular *C. lanceolatum* and *S. curtisii* extracts were similar against all fungi. *S. curtisii* extracts were better than *C. lanceolatum* extracts against *T. versicolor* but not with *L. sajor-caju*. It was also more effective against *C. puteana*. *M. utilis* extracts were less effective at lower concentrations but higher concentrations were similar to the other less effective extracts. In all cases *T. versicolor* proved more difficult to protect against.

Results of a Tukey pairwise comparison test revealed that impregnation of *H. brasiliensis* by all four wood extracts at concentrations level of 2, 4, 6 and 8% was partially effective in suppressing decay of all fungi (Appendix Tables A6.3). Generally, the results for antifungal assays mimic with the results of the antitermite test. In the termite tests high mortality (lower weight loss of treated cellulose pad) occurred on *N. heimii* extracts followed by *C. lanceolatum*, *S. curtisii* and *M. utilis*.

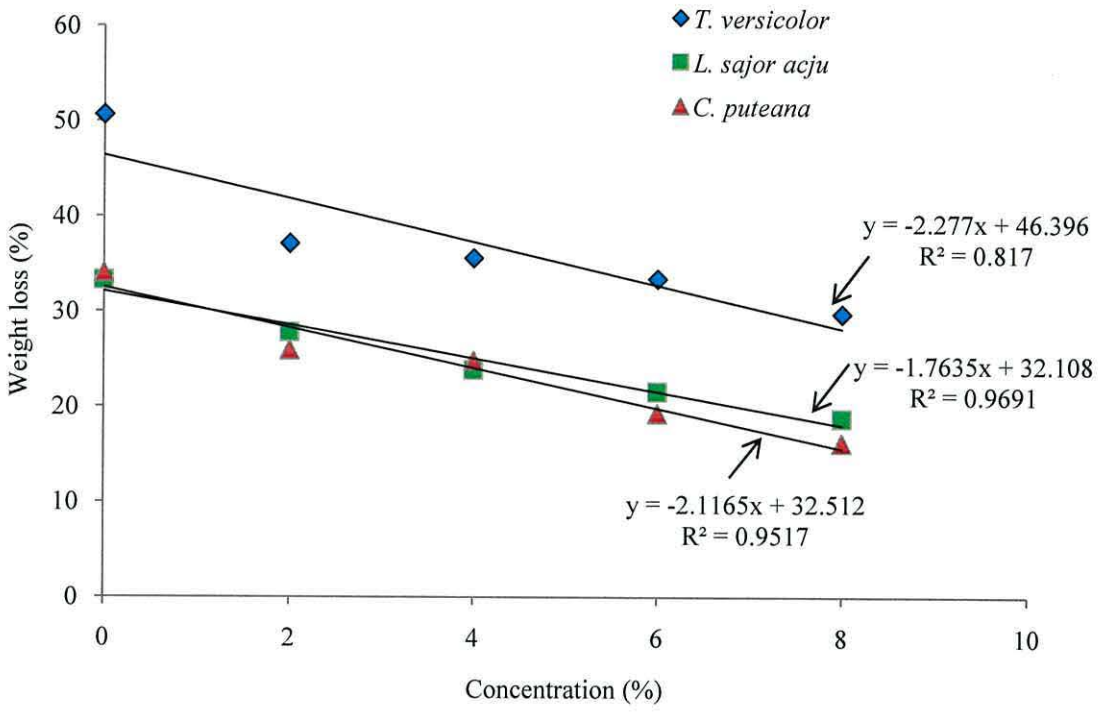



Figure 6.6 Effectiveness of extracts N. heimii in H. brasiliensis against T. versicolor, L. sajor-caju and C. puteana

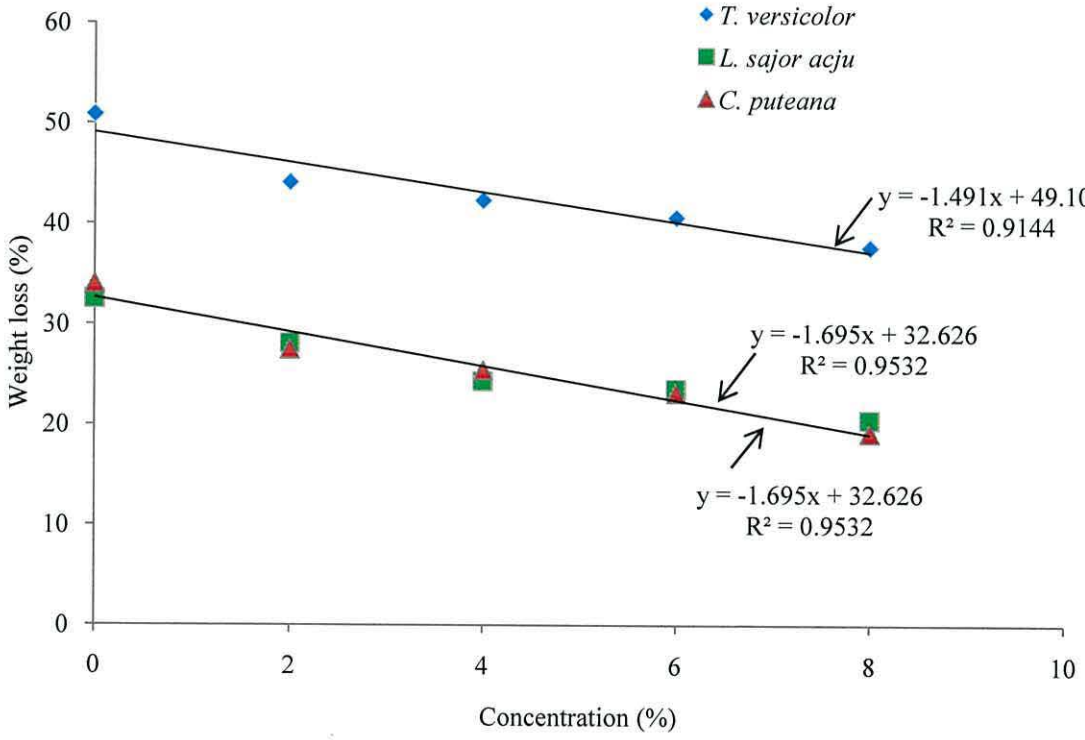



Figure 6.7 Effectiveness of C. lanceolatum extracts in H. brasiliensis against T. versicolor, L. sajor-caju and C. puteana

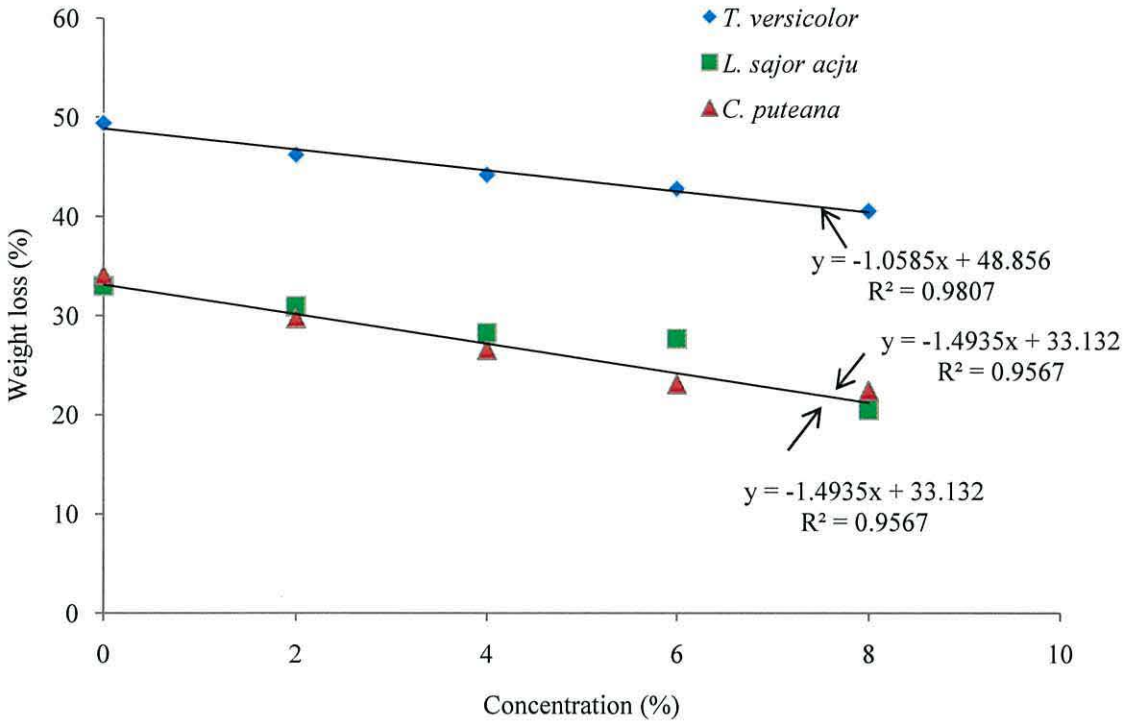



Figure 6.8 Effectiveness of M. utilis extracts in H. brasiliensis against T. versicolor, L. sajor-caju and C. puteana

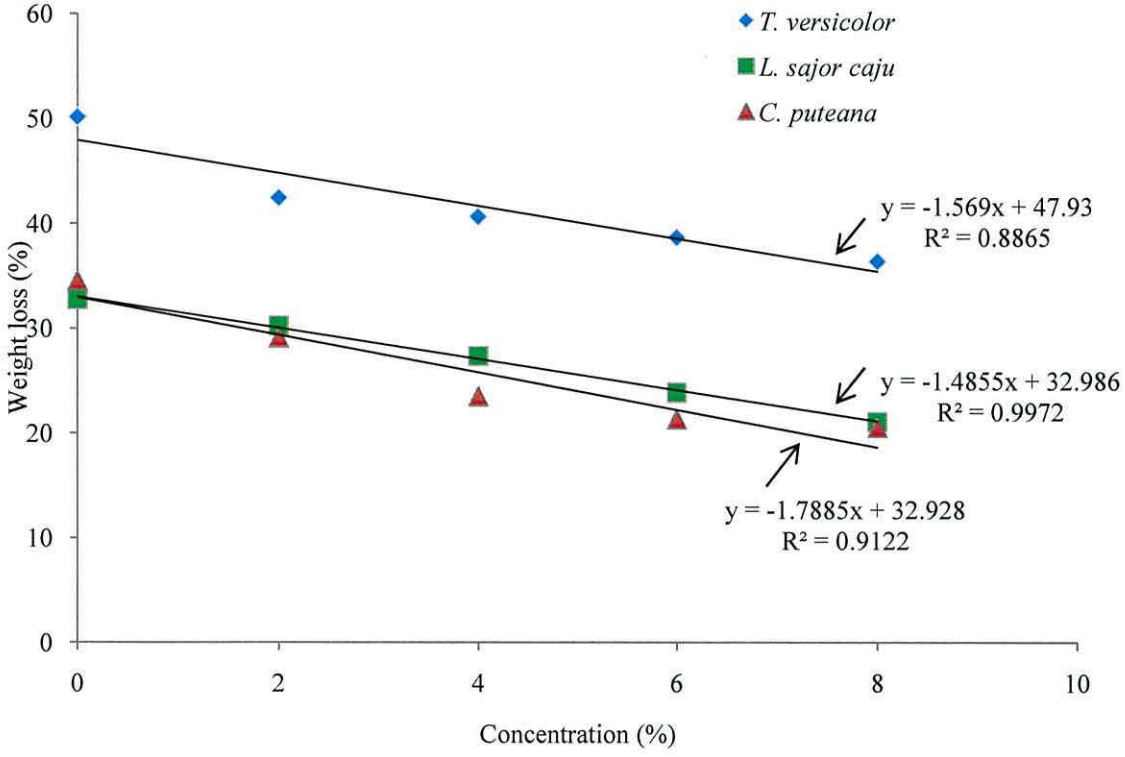



Figure 6.9 Effectiveness of S. curtisii extracts in *H. brasiliensis* against *T. versicolor, L. sajor-caju and C. puteana*

Table 6.4 shows that moisture content is related to decay severity for the different fungal wood species combinations and each showed different behaviour. Generally the higher the moisture content, the greater the weight loss. There was a wide range for all fungi (*T. versicolor*: 63% to 129%, *L. sajor-caju*: 51% to 126% and *C. puteana*: 36% to 75%).

Figure 6.10 clearly shows that the decay inhibition properties of these extracts exhibited concentration dependent activities, normalised for the different decay rates of the fungi. The *N. heimii* extract showed the best antifungal index against all fungi (41% for *T. versicolor*, 56% for *L. sajor-caju* and 52% for *C. puteana*) which were significantly better than the other three extracts at the 8% concentration. *C. lanceolatum* showed a lower (26%) antifungal index than *S. curtisii* (28%) with *T. versicolor,* similar with *L. sajor-caju* (26%) but higher than *S. curtisii* with *C. puteana* (44% and 41%, respectively). However all of them are not significantly different. *M. utilis* clearly had a lower antifungal index with all fungi (18%, 32% and 34%, respectively). The details of data are presented in Appendix Table A6.4.

Table 6.4 Average moisture content of test blocks of *H. brasiliensis* impregnated with the extracted of 4 Malaysian woods species extractives (n = 12)

| Extracted species | Solution concentration (%) | Moisture content (%) | | |
|---|---|---|---|---|
| | | *T. versicolor* | *L. sajor-caju* | *C. puteana* |
| *N. heimii* | 0 (control) | 63.71 (13.32)$^{c}$ | 78.68 (5.93)$^{b}$ | 69.69 (9.20)$^{a}$ |
| | 2 | 71.02 (26.38)$^{b}$ | 100.91 (36.79)$^{a}$ | 60.51 (7.22)$^{b}$ |
| | 4 | 72.55 (27.60)$^{b}$ | 111.97 (45.95)$^{a}$ | 55.78 (4.20)$^{b}$ |
| | 6 | 77.49 (37.44)$^{a}$ | 51.01 (19.92)$^{c}$ | 44.20 (18.35)$^{c}$ |
| | 8 | 62.70 (34.42)$^{c}$ | 75.88 (42.02)$^{b}$ | 36.49 (5.23)$^{d}$ |
| *C. lanceolatum* | 0 (control) | 94.29 (9.12)$^{a}$ | 72.66 (8.70)$^{c}$ | 56.48 (2.78)$^{a}$ |
| | 2 | 79.06(25.97)$^{c}$ | 90.56 (37.31)$^{a}$ | 53.37 (3.46)$^{b}$ |
| | 4 | 93.79 (31.27)$^{a}$ | 67.09 (30.01)$^{d}$ | 44.52 (4.60)$^{c}$ |
| | 6 | 85.04 (20.14)$^{b}$ | 78.52 (40.73)$^{b}$ | 52.97 (9.13)$^{b}$ |
| | 8 | 75.55 (24.10)$^{c}$ | 73.08 (35.83)$^{c}$ | 38.53 (5.47)$^{d}$ |
| *M. utilis* | 0 (control) | 80.33 (32.06)$^{b}$ | 71.86 (5.92)$^{d}$ | 62.20 (8.79)$^{a}$ |
| | 2 | 82.05 (40.55)$^{b}$ | 125.68 (30.18)$^{a}$ | 58.55 (13.50)$^{ab}$ |
| | 4 | 129.06 (33.70)$^{a}$ | 97.19 (27.84)$^{c}$ | 53.70 (6.22)$^{c}$ |
| | 6 | 41.18 (16.47)$^{c}$ | 105.34 (42.08)$^{b}$ | 48.81 (4.32)$^{d}$ |
| | 8 | 125.09 (33.91)$^{a}$ | 72.68 (23.87)$^{d}$ | 48.76 (2.83)$^{d}$ |
| *S. curtisii* | 0 (control) | 94.33 (16.05)$^{a}$ | 79.50 (9.63)$^{d}$ | 75.38 (8.17)$^{a}$ |
| | 2 | 89.91 (25.38)$^{ab}$ | 88.54 (29.11)$^{c}$ | 53.58 (3.73)$^{b}$ |
| | 4 | 87.63 (30.68)$^{b}$ | 95.93 (27.27)$^{b}$ | 46.91 (3.87)$^{c}$ |
| | 6 | 80.29 (23.88)$^{c}$ | 102.74 (43.96)$^{a}$ | 36.05 (5.87)$^{d}$ |
| | 8 | 82.52 (29.64)$^{c}$ | 76.14 (34.56)$^{d}$ | 43.59 (10.67)$^{c}$ |

Mean (± SD) of 12 replicates for each species. Percentage values followed by the same letter are not significantly different in the same group at the 0.01 level of probability.

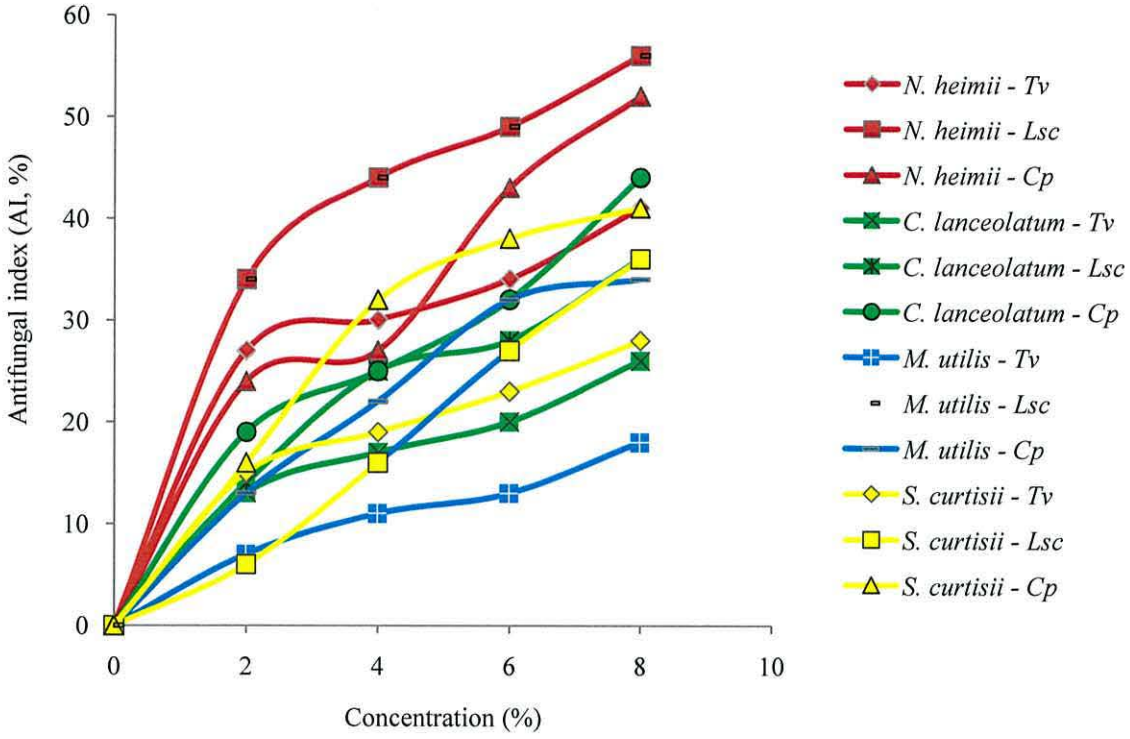



Figure 6.10 Anti-fungal index of four Malaysian wood species extracts against *T. versicolor, C. lanceolatum* and *C. puteana*

## 6.4 Discussion

### 6.4.1 Termite feeding test

The function of wood extractives as antitermitic compounds has been investigated for many years because of their promising as termite control preservatives (Scheffrahn, 1991; Escoubas *et al*, 1995; Chen *et al,* 2004; Santana *et al*, 2010).

The data obtained in this study suggest that all extracts tested contained antitermitic or biologically active compounds that influence feeding activities of both species; *C. curvignathus* and *C. gestroi.* However, the suitable loadings depend on the source of wood extractives as the active compounds vary with wood species. *N. heimii* extracts were more effective than the other three wood extracts against both subterranean termites. *C. lanceolatum* extract was not as good as *N. heimii* but was better than *M. utilis* and *S. curtisii* extracts (*N. heimii* > *C. lanceolatum* > *M. utilis* > *S. curtisii*). It is believed that if the exposure time was longer, the mortality of the termites would increase. As mentioned by Su *et al* (1987), non-repellent termiticides are slow acting and required longer time to express their lethal effects.

As reported in a previous study (Logan *et al*, 1990), several plant extracts are known as being repellent or toxic against termites. The toxicity or repellency of compounds present can explain their high activity. Previous chemical analyses from these four wood species (*C. lanceolatum*, *M. utilis*, *N. heimii* and *S. curtisii*) has revealed high concentrations of phenolic compounds (Wong, 1993; Kim *et al*, 2006), including hopeaphenol ($C_{56}H_{42}0_{12}$) (Coggox *et al*, 1965) from *N. heimii*, and saponins and triterpenes/steroids from all four wood species (Abdul Razak *et al,* 1982). The extracts of some woods from this study are not toxic but are repellent or distasteful to both termite species, thereby resulting in termite mortality from starvation. It is possible that factors other than extractive toxicity are important, including antioxidant properties. These could act together to achieve high durability against biodeteriogens.

### 6.4.2 Fungal decay test

At the end of 12 weeks, all the test blocks had been completely covered by the test fungi. Interestingly *T. versicolor* (white-rot) caused more weight loss than *C. puteana* (brown-rot) and the other white-rot; *L. sajor-caju*. The differences may be due to their differences of decay mechanisms in respect of phenolic degradation and the selectivity of these fungi to different cell wall components. In another study (Eberhardt *et al*, 1994) showed that *T. versicolor* is capable of degrading extractive components.

This study has showed that extracts from Malaysian tropical wood species contained compounds which slowed the growth of decay fungi and reduced the amounts of decay of a susceptible wood species, *H. brasiliensis*. The activity varied with concentration, the fungal species and the bioassay test used. Generally, low concentrations retarded the growth of all the fungi tested. These variations were similar as reported by Anderson *et al* (1963) and, Eaton and Hale (1993).

The differences of behaviour against fungi between the extractive contents from the selected four wood species are likely to be due to the differences in chemical composition. The result of impregnation of *H. brasiliensis* with extractives from this study are similar to those reported elsewhere (Yamamoto and Hong, 1988; 1989; 1994; Eslyn *et al* 1981; Ejechi and Obuekwe, 1993; Onuorah, 2000). It is difficult to relate the extractive performance with the original performance from heartwood as the actual loadings in the *H. brasiliensis* test blocks were not estimated, only a solution concentration was used, but the extractive additions are apparently less effective than those within heartwood. This may be due to degradation during the extraction process. This issue will be examined in the

Chapter 7, where extractives have been analysed. Another aspect, not dealt with in this thesis, may be due to their location in the wood cell wall.

### 6.4.3 Comparison of antitermitic/antifungal activities

From the four selected wood species (*C. lanceolatum, M. utilis, N. heimii* and *S. curtisii*) that has been tested for antitermitic and antifungal, results showed that *N. heimii* extracts had the strongest antitermitic and antifungal effects on the survival of subterranean termites, and the growth and decay by the white- and brown-rot fungi. *N. heimii* extracts also had the best antioxidant properties (Chapter 3). The *S. curtisii* extract had more of an effect on reducing fungal growth than *M. utilis* but it was weaker as an antitermitic. The performance of *C. lanceolatum* was intermediate to *N. heimii* and the latter two woods.

Many plants from Dipterocarps have been reported to contain a variety of terpenoids (Diaz *et al*, 1966; Messer *et al*, 1990) which are suspected as a defence compounds against fungi and insects. For example, sesquiterpenoids from *Dipterocarpus kerrii* King had antitermitic properties and inhibited fungal growth (Richardson *et al*, 1989) and many Dipterocarps are resistant to biological attack (Sen-Sarma, 1963; Bakshi *et al*, 1967; Sen-Sarma and Chatterjee, 1968).

## 6.5 Conclusions

1. The reponses of termites and the white and brown rot fungi to the extracts from the four selected Malaysian heartwoods were very different, with better responses against the termites. However, the study found that the heartwood extracts showed some promise as wood preservatives.
2. Wood durability against both termites is closely related to the extractives; they are toxic to both termite species. They show some inhibition to fungal growth but this is insufficient to explain their performance as wood preservatives on a toxic basis.
3. *N. heimii* heartwood extractive was very effective against subterranean termites and fungal decay followed in order of efficacy by *C. lanceolatum*, *S. curtisii* and *M. utilis*. However, repellent or non-repellent characteristics of termiticides and fungicides were dependent on the concentrations of wood extractives used.

# CHAPTER 7: MALDI-TOF ANALYSIS OF *N. HEIMII* METHANOL EXTRACTS

## 7.1 Introduction

*N. heimii* is a highly durable Malaysian hardwood timber from the Dipterocarpaceae. It is resistant to termites (Ahmad Said and Mohd Hamami; 1983, Takamura, 2001) and fungi (Yamamoto and Hong, 1988; Wong *et al*, 2005, Kawamura *et al*, 2010b) due to its high extractive content. These may contain a variety of terpenoids. Diaz *et al* (1966) have shown that Dipterocarp woods contain a variety of terpenoids. These are suspected as a defence to biological agents.

Phenolic phytochemicals are the largest classes of compounds widely distributed in the plant kingdom (Umezawa, 2001). This class which includes flavonoids, phenolic acids (hydroxybenzoic and hydroxycinnamic acids), stilbenes and polyphenols (hydrolysable and condensed tannins) are of considerable physiological and morphological importance. The flavonoids are the most studied because of their properties. They have a wide range of activities including flower colouring, UV protection in plants, insect antifeedants, antifungals, anticancer agents and antivirals (Elford *et al*, 1987; Frankel *et al,* 1993; Croteau *et al*, 2000; Harborne and Williams, 2000). However, stilbenes have also been studied due to their structural complexity and diverse bioactivites including antioxidant, antimutagenic and antifungal (Bokel *et al*, 1988; Uenobe *et al*, 1997; Fang *et al*, 2002).

The flavan-3-ols (+)-catechin, (-)-epicatechin, (-)-epigallocatechin and their gallate esters have been reported widely distributed in plants. Due to their astringency, these catechins and other flavonols (including isoquercetin, condensed tannin, kaempferol and isoflavonoids) present a plant defence system against harmful fungi and insects (Mazza and Miniati, 1993; Croteau *et al*, 2000). However, there is wide variety of structures and quantities in plants and their identification and quantification is limited by lack of rapid methods for characterization identification.

There are many way to analyse the compounds of wood extractives, including Thin layer Chromatography (TLC) (Yusiasih *et al*, 2003), Column Chromatography (CC) (Wong, 1978), Gas Chromatography (GC) coupled with either standard, (flame ionisation detector, FID) or mass spectrum (MS) detectors (GC/MS) (GC/MS) (Gao *et al*, 2008) and High Performance Liquid Chromatography (HPLC) (Chang *et al*, 2000) with various detectors, including MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Maldi-TOF) (Hoong *et al*, 2010) to separate and identify the chemical

components from the extracts. Generally, gas chromatography is widely used to analyse volatile compounds or compounds derivatised to make them volatile and HPLC is widely used for the fractionation and isolation of the more polar and non-volatile compounds. Where compounds are characterised, their mass spectra can be compared with available libraries (reference substances recorded under same experimental parameters) and to a degree of some certainty, the substances can be identified by retention time on the column and mass data. Liquid chromatography and mass spectrometry (LC-MS) also use the similar methods. When a substance has been purified (e.g. by preparative HPLC) useful information about chemical structures using fragmentation patterns in MS combined with nuclear magnetic resonance (NMR) can be used for structural determination of natural products (Wang and Lee, 2005).

Mass spectrometric techniques have been proven successful in analysis of polydisperse oligomers (Hanton, 2001). Matrix-assisted laser desorption/ionization coupled with MS (MALDI-MS) is suitable for identifying flavonol glycosides in food sources (Frison-Norrie and Sporns, 2002; Wang and Sporns, 2000) and MALDI-TOF (MALDI-time of flight) has been used for the separation of large phenolic compounds (Karas *et al*, 1987) for a number of reasons. It is capable of detecting intact molecular ions of more than 100,000 Daltons (Reed *et al*, 2005), it forms only one molecular ion from each compound in a complex mixture irrespective of molecular size (Montaudo *et al*, 2002), it has high sensitivity across a broad range of masses, it allows detection of oligomeric series of compounds (Reed *et al*, 2005), it gives fast and accurate information on molecular masses and molecular weight distribution (Almkvist and Persson, 2008). MALDI-TOF also is broadly applied for analysis of inorganic or organic compounds (Cohen and Gusev, 2002), other biomolecules (including lipids and /or phospholipids) (Petković *et al* 2005). Fragmentation patterns by MALDI-TOF also are similar to those obtained by FAB (Fast Atom Bombardment) (Cai *et al*, 2003; Zaia, 2004). Details of how MALDI-TOF works can be found in Schiller *et al* (2004).

The aim of this study was to characterise the *N. heimii* heartwood extracts using MALDI-TOF MS.

## 7.2 Materials and methods

### 7.2.1 Extraction of samples

A 2 g (air-dried) of *N. heimii* sawdust (the moisture content has been determined before) were placed in 400 ml beaker and covered with 300 ml methanol according to the

standard method ASTM D1110-84 (1984) – cold water solubility. The mixture was then stirred for 48 hours at a room temperature. After 48 hours, the browny-yellow mixture were filtered through a fritted-glass crucible, washed and dried using vacuum rotary evaporation at 45 °C and re-weighed. The samples were not fully dried to avoid condensation and insolubilisation.

### 7.2.2 MALDI-TOF MS analysis

MALDI-TOF-MS was carried out in the positive ion mode on a Bruker Reflex III instrument using a pulsed nitrogen laser at a wavelength of 337 nm for ionisation. In the positive reflection mode, an accelerating voltage of 25.0 kV and a reflection voltage of 26.5 kV were used. 2,5-dihydroxybenzoic acid (DHB) was used as the matrix as it is the most appropriate matrix for lipid analysis (Schiller *et al*, 1999). The sample solution (10 mg/ml 10% methanol solution) was mixed with the matrix solution at a volumetric ratio of 1:3. The mixture (1$\mu$L) was spotted to the steel target. Inulin was used as a standard because the oligosaccharide gives a range of regular peaks with increasing polymer length. A Tol:IMS sample, extracted using Soxhlet and a product from earlier work (Chapter 3) was also analysed for comparative purposes.

## 7.3 Results

MALDI-TOF MS is very sensitive to molecular weight and has been considered a method of choice in analysis of oligomeric plant polyphenols exhibiting large structural heterogeneity. $Li^+$ or $Na^+$ have been found the best cationic agents for polymers with electronegative elements such as oxygen or nitrogen (Pasch and Schrepp, 2003).

The results of MALDI-TOF MS are effected by the matrix used in terms of signal-to-noise ratio and resolution. The best matrix shows spot-to-spot and sample-to-sample repeatability and reproducibility. Figure 7.1 shows the inulin chromatogram. The expected deprotonated molecules $[M_x - H]^-$ are the main components of the inulin mass spectra.

Figure 7.2 shows the MALDI-TOF mass spectrum trace of hot Soxhlet Tol:IMS extract of *N. heimii* heartwood. This was an early examination of the extractives. This shows two main clusters of peaks, one at 373.74 *m/z* and one at 452.75 *m/z*. These are similar to some of the lower molecular weight components found in the cold methanol extract but no high molecular weight components were found, so it is suggested that these are degradation products. Their precise molecular weights may not be correct as the

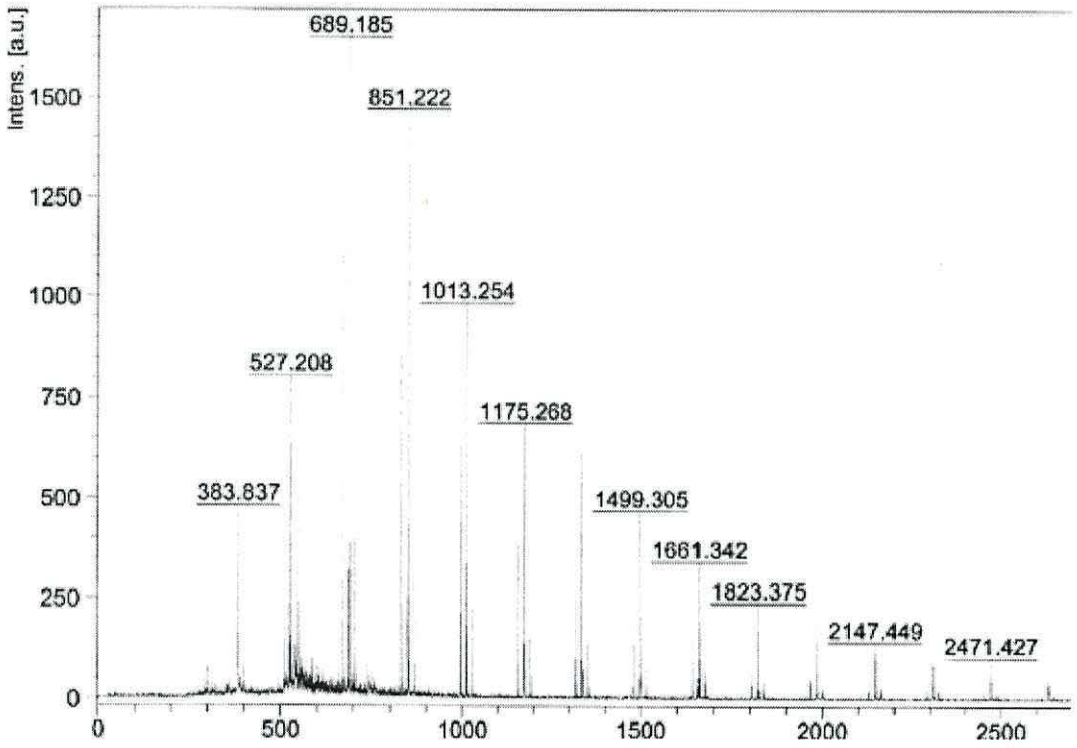



Figure 7.1 MALDI-TOF positive reflection mode mass spectra of the inulin

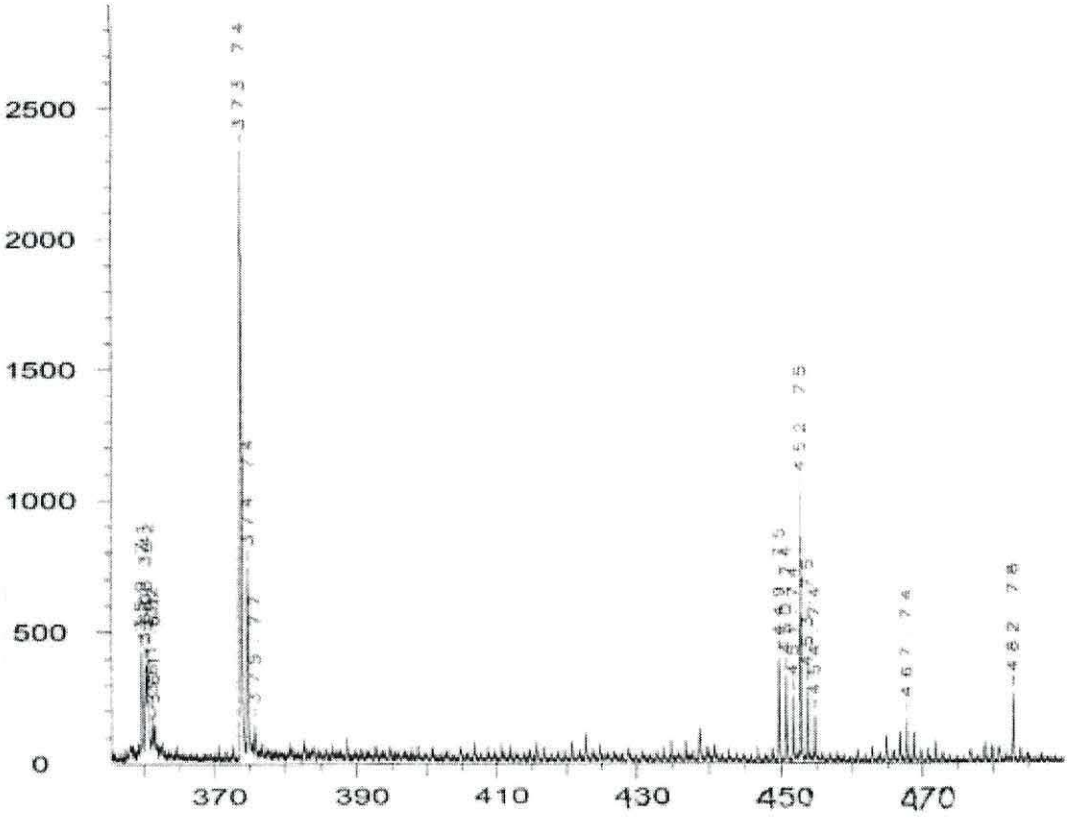



Figure 7.2 MALDI-TOF MS positive reflection mode mass spectra of the tol:IMS polyphenolic compounds from *N. heimii* heartwood.

instrument may not have been calibrated for these measurements. Better calibration was done for later work.

Figure 7.3 shows the MALDI-TOF mass spectrum of the polyphenols isolated from *N. heimii* heartwood using cold methanol extraction. Absolute identification requires considerable extra work (e.g. preparatory HPLC and NMR) so these are only tentative identifications. The interpretation is that the chromatogram shows a resveratrol series (M + Na+) from monomer (376.005 *m/z*) to the tetramer (973.468 *m/z*, Table 7.1). The highest peak/response is observed for protonated ions. The molecular weight of the highest peaks among the resveratrol polymers with identical degree of polymerization increased at the distance of 228 Dalton, corresponding to one resveratrol ($C_{14}H_{12}O_3$) monomer (Filip *et al*, 2003 ). The masses indicate the presence of resveratrol units which may be containing one more hydroxyl group at the aromatic ring B than kaempferol units. Given the absolute masses of each peak, it is suggested that *N. heimii* extracts may contain resveratrol and kaempferol oligomers. The possible polyphenols from the Figure 7.3 could be as follows; peak no. 1 (376.005 *m/z*) is monokaempferol + 2K + $C^+$, peak no.2 (451.15 *m/z*) is resveratrol dimer – $H^+$, balanocarpol ($C_{28}H_{20}0_6$), peak no. 3 (507.355 *m/z*) is resveratrol dimer + K + $CH_2$, peak no. 4 (587.381 *m/z*) is kaempferol dimer + $CH_3$, ($C_{31}H_{23}O_{12}$), peak no. 5 (680.393 *m/z*) is resveratrol trimer + $2H^+$, copalliferol or stemonoporol ($C_{42}H_{32}0_9$), peak no. 6 (717.334 *m/z*) is resveratrol trimer + Na, peak no. 7 (785.427 *m/z*) is kaempferol trimer + 3Na + $4H^+$, peak no. 8 (929.447 *m/z*) is resveratrol tetramer + $CH_5^+$, hopeaphenol ($C_{57}H_{47}0_{12}$) and peak no. 9 (973.468 *m/z*) is resveratrol tetramer + Na + K.

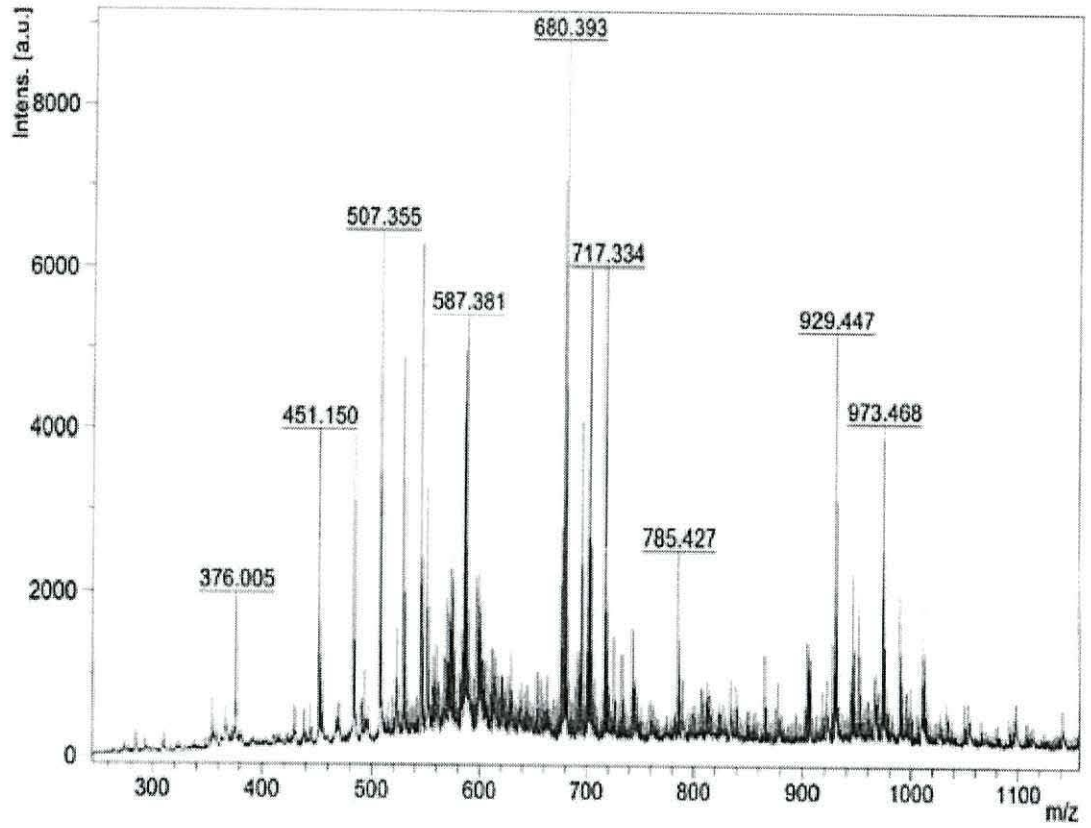



Figure 7.3a MALDI-TOF MS positive reflection mode mass spectra of the cold methanol polyphenolic compounds from *N. heimii* heartwood

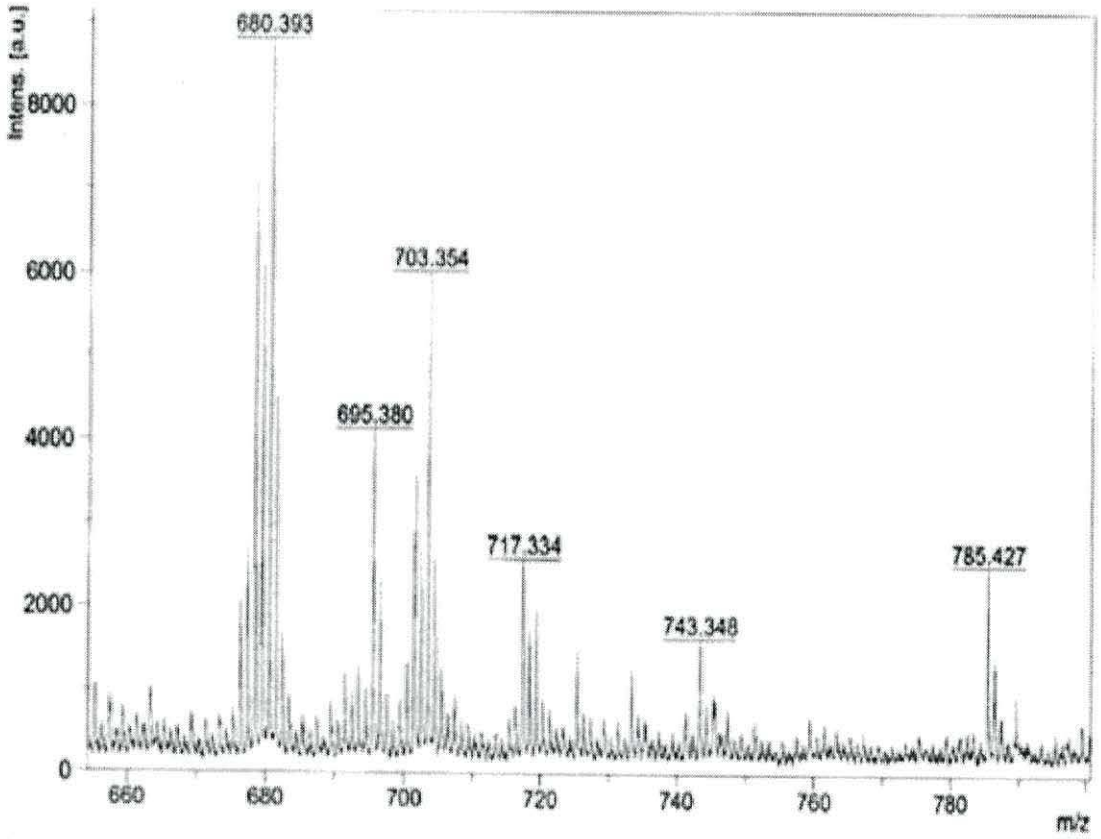
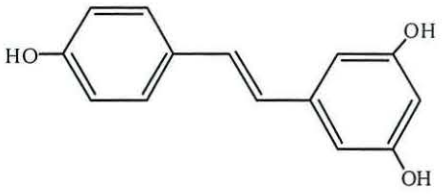


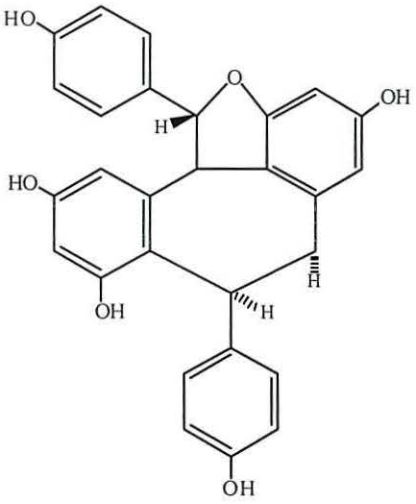

Figure 7.3b MALDI-TOF MS positive reflection mode mass spectra of the cold methanol polyphenolic compounds from *N. heimii* heartwood, detail of 660 to 790 m/z region

Table 7.1 MALDI-TOF MS of polyphenolic compounds from cold methanol extracts of *N. heimii* heartwood

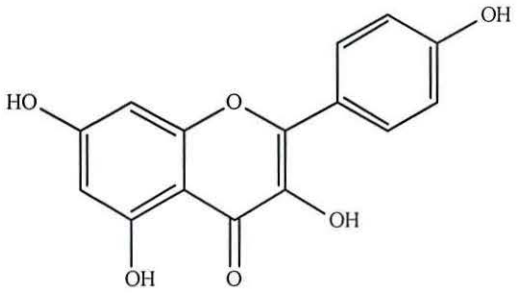
| Polymer | Observed $(M + Cs)^+$ |
|---|---|
| Dimer | 451.15 |
| | 483.69 |
| | 507.36 |
| Trimer | 676.38 |
| | 677.38 |
| | 678.39 |
| | 679.39 |
| | 680.39 |
| | 695.38 |
| | 701.36 |
| | 703.35 |
| | 717.33 |
| | 719.31 |
| | 743.35 |
| | 785.43 |
| Tetramer | 904.44 |
| | 927.44 |
| | 929.45 |
| | 945.41 |
| | 951.46 |
| | 973.47 |
| | 989.41 |



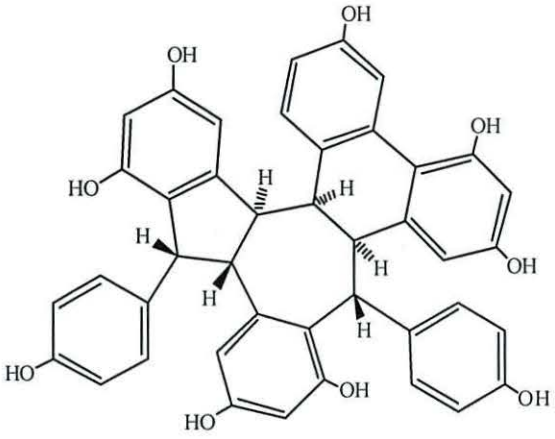
Resveratrol



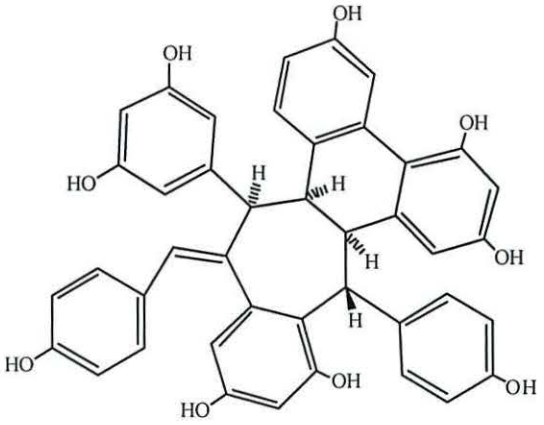
Balanocarpol



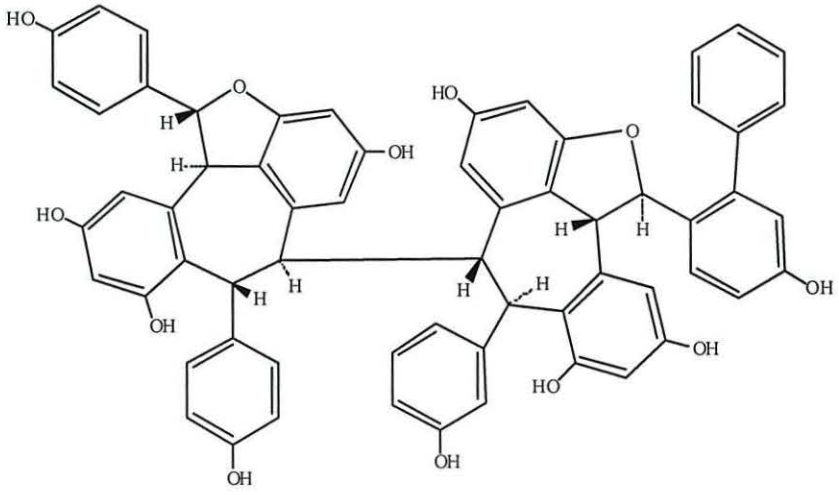
Kaempferol



Copalliferol A



Stemonoporol



Hopeaphenol

Figure 7.4 Chemical structures of major resveratrol and kaempferol derivatives isolated from *N. heimii*

## 7.4 Discussion

The yellow colour in the browny yellow of the methanol extract may indicate that the solution may contain flavonol glycosides (Frison and Sporns (2002). MALDI-TOF chromatograms (Figure 7.3) indicated that the main chemical compounds in *N. heimii* extracts are resveratrols (balanocarpol, copallliferrol A or stemonoporol, and hopeaphenol or vaticaphenol) and kaempferol. Further simple confirmation could be by the use of standards and other chromatographic techniques. Both groups of compounds are the polyphenolic groups of stilbenes (resveratrol) and flavonoids (kaempferol). The results from this study confirmed previous findings by Coggon *et al* (1965) (resveratrol tetramer; hopeaphenol) and Weber *et al* (2001) (stilbenes; hopeaphenol, vaticaphenol A, copalliferol A, balanocarpol and heimiol A). However, heimiol A was not detected in this study. The interesting finding from this study was the presence of kaempferol and derivatives as a putatively new chemical compound in *N. heimii.*

The results from this study describe for the first time of the isolation and identification of stilbenes and flavonoid compounds from MALDI-TOF analysis. These compounds have been isolated from many tropical woods such as *Balanocarpus zeylanicus* (Diyasena *et al*, 1985), *Shorea* sp. (Madhav *et al*, 1967) and Stemonoporol A (Sotheeswaran *et al*, 1983) for resveratrol and, *Afzelia bipindensis* Harms (Kilic and Niemz, 2010) and *Siparuna apiosyce* (Leitäo, 2000) for kaempferol. This class of flavonoid (kaempferol) has not previously been described in the case of *N. heimii* extracts but is known to exist in nature. The role of flavonoids in plant defence is widely accepted either against insects (Treutter, 2006) or fungi (Grayer and Harborne, 1994).

Kaempferol is one of the flavonoids that inhibits the growth of the fungal rice blast pathogen (*Pyricularia oryzae*) (Padmavati *et al*, 1997) and also has antifeedant activity against *C. formosanus* (Ohmura *et al*, 2000). The performance of kaempferol as an antifeedant could be due to hydroxyl group at C-4' in B-rings as suggested by Ohmura *et al* (2000) and carbonyl group at C-4 in pyran rings has been necessary for higher antifeedant activity. The presence of hydroxyl group at C-3 in pyran rings also contributed to the activities. These types of flavonoids also protect plant against UV-B irradiation (Croteau *et al*, 2000).

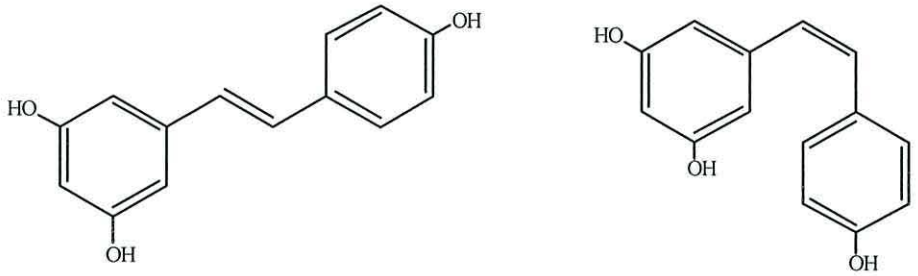
Stilbenes are polyphenolic compounds which are associated with the natural durability of plants and trees (Seppänen *et al*, 2004; Aslam *et al*, 2006). They are widely distributed in liverworts and higher plants especially in grapes and peanuts (Gorham, 1989), in the monomeric form or as dimers, trimers and polymeric stilbenes (Cassidy *et al*,

2000). Resveratrol is a monomeric stilbenoid. It is also known as 3,5,4'-trihydroxystilbene and is abundant in the plant families of Dipterocarpaceae, Vitaceae, Cyperaceae, Gnetaceae and Leguminosae where it occurs from the monomer to octamers (Sotheeswaran and Pasupathy, 1993; Ohguchi *et al*, 2003). In the other families Haemodoraceae, Musaceae and Paeoniaceae they occur from dimers to tetramers (Hirano *et al,* 2001). Resveratrol is produced by several plants only when under attack by pathogens (mainly *Botrytis cinerea*) (Langcake and Pryce, 1976; Frémont, 2000). It exists in two geometrical isomers; E- *(trans-)* and *Z-(cis-)* as shown in Figure 7.5 (Filip *et al*, 2003). Resveratrol is often discussed for its effectiveness on biological properties such as antioxidizing effect, effect on the cardiovascular system, anti-mutagenic effect and chemo-protective advantage against cancer proliferation (Kimura *et al*, 1983; Frankel *et al*, 1995; Uenobe *et al*, 1997; Jang *et al*, 1997) but it also has antitermitic and antifungal properties.

The resveratrol trimer (canaliculatol) from bark of *Stemonoporus canaliculatus* (Dipterocarpaceae) is antifungal against *Cladosporium cladosporioides* (Bokel *et al*, 1988) and from *Shorea belangaren* against six fungi: *Fusarium oxysporum, Corynespora cassiicola, Cochiliobolus miyabeanus, Trichoderma harzianum, Aspergillus niger* and *Penicillium italicum* (Kusuma and Tachibana, 2007).

There are well over 4000 unique flavanoid structures that have been isolated and identified in the literature (Harborne *et al*, 1975) and flavonoids are found in every plant. This group of secondary metabolites have pronounced biological activities even though they may occur in small amounts (Giesman, 1962). They may be responsible for the yellow pigmentation of many fruits, vegetables and grains (Frison and Sporns, 2002) and generally, are significant in terms of plant defence (Treutter, 2006). There is abundant evident that flavonoids had antifeedant activities. For examples, taxifolin has been found to exhibits antitermitic activity against *Cryptotermes brevis* (Wolcott, 1953b) and ten other flavonoids also had antifeedant activity against *Coptotermes formosanus* (Shiraki) (Ohmura *et al*, 2000). Taxifolin also shows antifungal activity against several wood-rotting fungi (Elliger *et al*, 1980).

As the results from Chapter 6 shown that *N. heimii* extracts had significant antitermitic against two subterranean termites and antifungal activities against one brown and two white rot fungi. This could be due to these two groups of chemical compounds (resveratrol and its polymers and kaempferol) found by MALDI-TOF.




*trans*-resveratrol
*E-resveratrol*

*cis*-resveratrol
Z-resveratrol

Figure 7.5 Structure of E-resveratrol and Z-resveratrol (Filip *et al*, 2003)

In comparison the main constituents derived from the *N. heimii* extracts heartwood were different from those obtained by Coggan *et al* (1965) and Weber *et al* (2001). The result from this study indicate that the chemical components are influenced by extrinsic and intrinsic factors such as extraction methods, genus, wood species, location of the samples in the stem, environmental, geographical site and seasonal variation (Dahm, 1970; Hillis, 1971; Snajberk and Zavarin, 1976; Fengel and Wegener, 1984; Hillis, 1987a; Su *et al*, 1993; Matsunaga *et al*, 1999; Baeza and Freer, 2001; Sakai, 2001; Willför *et al,* 2003; Schultz *et al*, 2008; Xu *et al*, 2009). In this study cold methanol extraction methods were used, following an earlier pilot study using the Tol:IMS extracts. These earlier extracts showed only two sets of peaks in the molecular mass regions of 373.74 *m/z* (monokaempferol) and 452.75 *m/z* (resveratrol dimer) which indicates that considerable depolymerisation has occurred coupled with possible further degradation. The impact of this on the biological activity tests will be discussed later in Chapter 9.

This study has only looked into the cold methanolic extract from the heartwood of the most durable species selected in this study. Much further work remains to be done on this species and the other species of this study. In this species it is not clear where the extractives are located and whether they are far more polymerised within the wood cell wall, giving an extra form of wood cell wall modification, possibly imparting substantial dimensional stability and decay resistance. Thus subtle but powerful extraction methods may be useful at determining the true nature of the extractives with this and other durable wood species.

## 7.5 Conclusions

1. The molecular masses of the peaks gained in the MALDI-TOF analyses are consistent with the major phenolics from the cold methanol extract being a series of resveratrol oligomers ranging in DP from 1 to 4, including (hopeaphenol, vaticaphenol A, copalliferol A and balanocarpol). Flavonoids were also detected and these have been tentatively identified as kaempferol.
2. Hot extraction systems should be used with caution where features other that quantitative determinations are involved.

# CHAPTER 8: CORRELATIONS BETWEEN NATURAL DURABILITY AND WOOD PROPERTIES

## 8.1 Introduction

Correlation coefficients are commonly and widely used in sciences in order to know strength of linear dependence between two variables. The correlation coefficient *r* is a measure of the linear relationship between two attributes or columns of data. The correlation coefficient is also known as the Pearson product-moment correlation coefficient. The value of *r* can range from -1 to +1 and is independent of the units of measurement. A value of *r* near 0 indicates little correlation between attributes; a value near +1 or -1 indicates a high level of correlation. In general significant means important, while in statistics analysis, significant means probably true (not due to chance). A research finding may be true without being important. When statisticians say a result is "highly significant" they mean it is very probably true. They do not (necessarily) mean it is highly important. Sometimes, data did not show any significant correlation even though the values are nearly +1 or -1 due to the data distributions. In this case, common sense was always applied to explain the situations that happened.

However, the correlation coefficient only measures linear relationships between X and Y and for any relationship to exist, any change in X has to have a constant proportional change in Y. If the relationship is not linear then the result is inaccurate. In addition to this, the correlation may be meaningless if it is about categorical data, such as hair colour or gender. It is important to remember that the correlation coefficient does not imply causality, that is it may show that two variables are strongly correlated, however it does not mean that they are responsible for each other (Cohen *et al*, 2002).

The aims of this chapter were to discuss the other correlations that had not been covered before.

## 8.2 Materials and methods

The correlations between physical properties (wood density) were done with durability (termites and fungi) for all 12 Malaysian wood species in order to examine the influence durability. Further correlations were done between total extractive contents and durability with focus on the wood consumption (termites) and weight loss (fungi). Lastly, combination of correlations between extractive contents and durability were done in order to better understand of their influence to each others. All the individual data (replicates)

have been combined together according to the species because the relation of wood properties did not depend on between-tree variation but depended on only the values of the wood properties (Ona *et al*, 1998). For correlation between wood extractives and wood consumption by termites or average weight loss by fungus, all the individual species data has been combined together because quantification of the extractives was not from the same blocks as were used to determine durability.

Analyses of covariances were also done to know how all variables (physical properties, durability and extractive contents) interacted with each other.

## 8.3 Results

Statistical analyses were done in order to determine the relationships between all variables (wood density and extractives contents) and wood consumption by termites and also weight loss by fungi. Summary results of each correlation for both subterranean termites and all decay fungi are summarized in Tables 8.1, 8.2, 8.3 and 8.4 below.

Table 8.1 Correlation (R) between wood consumption (after 28 days) and wood density of basal of twelve Malaysian wood species for samples tested (within species) (n = 5)

| Wood species | *C. curvignathus* | | *C. gestroi* | |
|---|---|---|---|---|
| | R | P value | R | P value |
| *N. heimii* | -0.09 | 0.743 | -0.06 | 0.826 |
| *C. lanceolatum* | -0.13 | 0.655 | -0.52 | 0.046 |
| *M. utilis* | -0.23 | 0.414 | -0.24 | 0.384 |
| *P. pinnata* | -0.99 | 0.002 | -0.50 | 0.056 |
| *D. grandiflorus* | -0.58 | 0.024 | -0.87 | 0.008 |
| *D. kunstleri* | -0.54 | 0.087 | -0.42 | 0.119 |
| *K. ivorensis* | -0.83 | 0.005 | -0.85 | 0.005 |
| *F. fragrans* | -0.66 | 0.008 | -0.26 | 0.342 |
| *S. curtisii* | -0.56 | 0.029 | -0.48 | 0.068 |
| *A. angustifolia* | -0.36 | 0.188 | -0.42 | 0.121 |
| *C. scortechinii* | -0.37 | 0.181 | -0.70 | 0.023 |
| *H. brasiliensis* | -0.98 | 0.001 | -0.50 | 0.060 |

Table 8.2 Correlation (R) between weight loss and wood density of twelve Malaysian wood species for samples tested (within species) (n = 5)

| Wood species | *P. sanguineus* | | *T. versicolor* | | *L. sajor-caju* | |
|---|---|---|---|---|---|---|
| | R | P value | R | P value | R | P value |
| *N. heimii* | 0.36 | 0.255 | 0.23 | 0.479 | 0.61 | 0.035 |
| *C. lanceolatum* | -0.30 | 0.339 | -0.09 | 0.776 | -0.19 | 0.548 |
| *M. utilis* | -0.41 | 0.192 | -0.82 | 0.001 | -0.37 | 0.233 |
| *P. pinnata* | -0.23 | 0.480 | -0.84 | 0.001 | -0.33 | 0.300 |
| *D. grandiflorus* | 0.91 | 0.000 | -0.44 | 0.149 | 0.38 | 0.223 |
| *D. kunstleri* | 0.05 | 0.869 | -0.66 | 0.020 | -0.02 | 0.954 |
| *K. ivorensis* | 0.45 | 0.142 | 0.25 | 0.427 | 0.58 | 0.046 |
| *F. fragrans* | 0.12 | 0.705 | 0.25 | 0.429 | 0.03 | 0.926 |
| *S. curtisii* | 0.06 | 0.857 | -0.05 | 0.870 | -0.67 | 0.016 |
| *A. angustifolia* | 0.28 | 0.384 | 0.08 | 0.795 | -0.56 | 0.060 |
| *C. scortechinii* | 0.61 | 0.034 | -0.51 | 0.092 | -0.21 | 0.516 |
| *H. brasiliensis* | -0.29 | 0.354 | -0.26 | 0.413 | -0.22 | 0.498 |

Table 8.1 showed that wood density is negatively correlated with wood consumption in all of the wood species studied against both termite species although these were not strong or significant in all cases. Table 8.2 shows that correlations between weight loss and wood density were positive in some cases, negative in others so that little overall conclusions can be gained from this. Only one species shows highly significant positive slope at $P<0.001$ and a few at $P<0.05$ (significantly positive or negatively slopes).

Majority of wood consumption by both termite species; *C. curvignathus* and *C. gestroi* was significantly negatively correlated with either type of heartwood extractives and also with the total wood extractives (Table 8.3). Weight loss by all white rot fungi (*P. sanguineus, T. versicolor* and *L. sajor-caju*) also had significant negative correlations with heartwood extractives in most cases (Table 8.4). As shown by Figure 8.1, both wood consumption by termites and mass loss by fungus had negative correlations with total phenols.

Table 8.3 Correlation (R) between wood consumption and heartwood extractives contents of twelve Malaysia wood species (within species) (n = 5)

| Wood species | Termites | Tol:IMS | | Hot water | | Total extractives | |
|---|---|---|---|---|---|---|---|
| | | R | P value | R | P value | R | P value |
| *N. heimii* | *C. curvignathus* | -0.49 | 0.121 | -0.80 | 0.009 | -0.80 | 0.009 |
| | *C. gestroi* | -0.27 | 0.396 | -0.74 | 0.015 | -0.87 | 0.006 |
| *C. lanceolatum* | *C. curvignathus* | -0.64 | 0.024 | -0.52 | 0.037 | -0.31 | 0.323 |
| | *C. gestroi* | -0.79 | 0.011 | -0.98 | 0.003 | -0.88 | 0.005 |
| *M. utilis* | *C. curvignathus* | 0.45 | 0.193 | -0.03 | 0.987 | -0.11 | 0.736 |
| | *C. gestroi* | -0.50 | 0.119 | -0.91 | 0.003 | -0.35 | 0.289 |
| *P. pinnata* | *C. curvignathus* | -0.73 | 0.016 | -0.94 | 0.001 | -0.72 | 0.017 |
| | *C. gestroi* | -0.88 | 0.005 | -0.79 | 0.012 | -0.21 | 0.412 |
| *D. grandiflorus* | *C. curvignathus* | 0.11 | 0.701 | -0.44 | 0.210 | -0.22 | 0.399 |
| | *C. gestroi* | -0.23 | 0.389 | -0.76 | 0.014 | -0.57 | 0.032 |
| *D. kunstleri* | *C. curvignathus* | -0.14 | 0.689 | -0.76 | 0.014 | -0.22 | 0.399 |
| | *C. gestroi* | -0.73 | 0.016 | -0.53 | 0.035 | -0.76 | 0.014 |
| *K. ivorensis* | *C. curvignathus* | 0.19 | 0.652 | -0.52 | 0.037 | -0.69 | 0.019 |
| | *C. gestroi* | -0.71 | 0.018 | -0.53 | 0.036 | -0.24 | 0.390 |
| *F. fragrans* | *C. curvignathus* | -0.13 | 0.683 | -0.91 | 0.003 | -0.92 | 0.003 |
| | *C. gestroi* | -0.83 | 0.008 | -0.81 | 0.009 | -0.45 | 0.193 |
| *S. curtisii* | *C. curvignathus* | -0.69 | 0.020 | -0.47 | 0.178 | -0.42 | 0.200 |
| | *C. gestroi* | -0.55 | 0.033 | -0.63 | 0.025 | -0.85 | 0.007 |
| *A. angustifolia* | *C. curvignathus* | 0.33 | 0.301 | -0.79 | 0.011 | -0.97 | 0.006 |
| | *C. gestroi* | -0.42 | 0.231 | 0.22 | 0.390 | -0.64 | 0.025 |
| *C. scortechinii* | *C. curvignathus* | -0.92 | 0.002 | -0.82 | 0.008 | -0.72 | 0.017 |
| | *C. gestroi* | -0.74 | 0.016 | -0.44 | 0.214 | -0.62 | 0.026 |
| *H. brasiliensis* | *C. curvignathus* | 0.40 | 0.245 | -0.39 | 0.287 | -0.73 | 0.016 |
| | *C. gestroi* | -0.18 | 0.666 | -0.93 | 0.002 | -0.92 | 0.003 |

Table 8.4 Correlation (R) between weight loss and heartwood extractives contents of twelve Malaysia wood species (within species) (n = 5)

| Wood species | Fungus | Tol:IMS | | Hot water | | Total extractives | |
|---|---|---|---|---|---|---|---|
| | | R | P value | R | P value | R | P value |
| *N. heimii* | *P. sanguineus* | -0.54 | 0.035 | -0.84 | 0.008 | -0.65 | 0.023 |
| | *T. versicolor* | -0.34 | 0.295 | -0.52 | 0.037 | -0.88 | 0.005 |
| | *L. sajor-caju* | -0.74 | 0.015 | -0.54 | 0.035 | -0.85 | 0.007 |
| *C. lanceolatum* | *P. sanguineus* | 0.02 | 0.943 | -0.88 | 0.005 | -0.50 | 0.147 |
| | *T. versicolor* | -0.51 | 0.143 | -0.33 | 0.312 | -0.66 | 0.023 |
| | *L. sajor-caju* | -0.74 | 0.016 | 0.20 | 0.453 | -0.09 | 0.094 |
| *M. utilis* | *P. sanguineus* | -0.98 | 0.000 | -0.68 | 0.021 | -0.71 | 0.018 |
| | *T. versicolor* | -0.57 | 0.032 | -0.65 | 0.024 | -0.08 | 0.954 |
| | *L. sajor-caju* | -0.09 | 0.094 | -0.81 | 0.001 | -0.18 | 0.540 |
| *P. pinnata* | *P. sanguineus* | -0.93 | 0.000 | -0.57 | 0.031 | -0.41 | 0.188 |
| | *T. versicolor* | -0.16 | 0.924 | -0.64 | 0.025 | -0.20 | 0.521 |
| | *L. sajor-caju* | -0.67 | 0.022 | -0.51 | 0.038 | -0.49 | 0.156 |
| *D. grandiflorus* | *P. sanguineus* | -0.82 | 0.009 | -0.66 | 0.023 | -0.78 | 0.012 |
| | *T. versicolor* | -0.83 | 0.008 | -0.75 | 0.014 | -0.84 | 0.007 |
| | *L. sajor-caju* | -0.94 | 0.000 | -0.76 | 0.013 | -0.88 | 0.005 |
| *D. kunstleri* | *P. sanguineus* | -0.82 | 0.009 | -0.01 | 0.983 | -0.89 | 0.004 |
| | *T. versicolor* | -0.66 | 0.022 | -0.66 | 0.023 | -0.71 | 0.018 |
| | *L. sajor-caju* | -0.65 | 0.025 | -0.55 | 0.033 | -0.65 | 0.024 |
| *K. ivorensis* | *P. sanguineus* | -0.73 | 0.017 | -0.24 | 0.432 | -0.70 | 0.019 |
| | *T. versicolor* | -0.67 | 0.021 | -0.03 | 0.848 | -0.43 | 0.156 |
| | *L. sajor-caju* | -0.78 | 0.012 | -0.46 | 0.174 | -0.29 | 0.412 |
| *F. fragrans* | *P. sanguineus* | -0.78 | 0.012 | -0.05 | 0.808 | -0.85 | 0.007 |
| | *T. versicolor* | -0.83 | 0.008 | -0.59 | 0.029 | -0.84 | 0.008 |
| | *L. sajor-caju* | -0.94 | 0.000 | -0.82 | 0.009 | -0.87 | 0.006 |
| *S. curtisii* | *P. sanguineus* | -0.15 | 0.735 | -0.41 | 0.189 | -0.40 | 0.231 |
| | *T. versicolor* | -0.25 | 0.311 | -0.89 | 0.004 | -0.02 | 0.863 |
| | *L. sajor-caju* | -0.59 | 0.030 | -0.24 | 0.422 | -0.98 | 0.000 |
| *A. angustifolia* | *P. sanguineus* | -0.36 | 0.232 | -0.32 | 0.287 | -0.15 | 0.542 |
| | *T. versicolor* | -0.22 | 0.321 | -0.67 | 0.022 | -0.86 | 0.006 |
| | *L. sajor-caju* | -0.50 | 0.145 | -0.88 | 0.005 | -0.61 | 0.028 |
| *C. scortechinii* | *P. sanguineus* | -0.84 | 0.008 | -0.90 | 0.004 | -0.79 | 0.011 |
| | *T. versicolor* | -0.95 | 0.000 | -0.61 | 0.027 | -0.2 | 0.211 |
| | *L. sajor-caju* | -0.12 | 0.754 | -0.65 | 0.023 | 0.37 | 0.222 |
| *H. brasiliensis* | *P. sanguineus* | -0.12 | 0.785 | -0.90 | 0.004 | -0.03 | 0.896 |
| | *T. versicolor* | -0.83 | 0.009 | -0.04 | 0.946 | -0.99 | 0.000 |
| | *L. sajor-caju* | -0.75 | 0.014 | -0.57 | 0.031 | -0.81 | 0.001 |

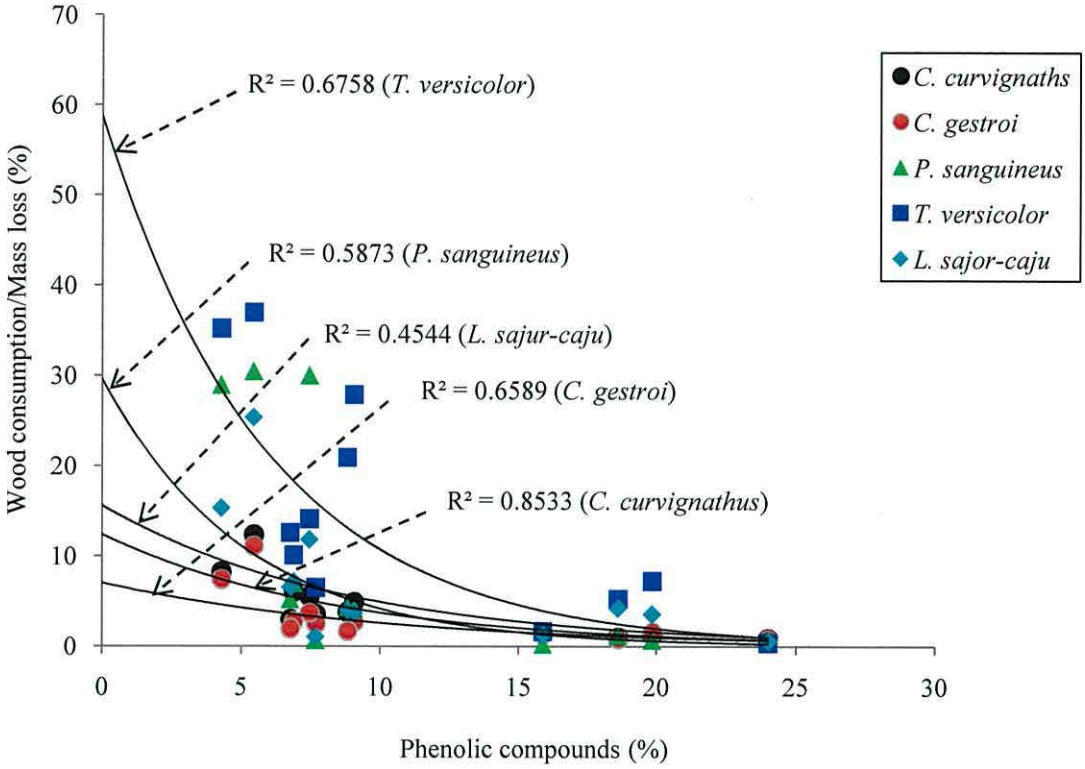



Figure 8.1 Relationship between wood consumption against termites/mass loss against fungus and amount of phenolic compounds in the extract of twelve Malaysian wood species

## 8.4 Discussion

### 8.4.1 Durability (wood consumption of Cc and Cg) and wood density

Termites used their mandibles to fragment the wood mechanically. In this case, the hardness which is related to density of the wood is important in order to know the ability of the termites to consume the wood.

Statistical analysis was done in order to determine the correlations between wood consumption and density ratio using the Pearson rank correlation coefficient. Result (Table 8.1) showed that the percentage of wood consumption and density appear significantly inverse related for *P. pinnata* (r = -0.99), *D. grandiflorus* (r = -0.58), *D. kunstleri* (r = -0.46), *K. ivorensis* (r = -0.83), *F. fragrans* (r = -0.66), *S. curtisii* (r = -0.56) and *H. brasiliensis* (r = -0.98) with *C. curvignathus*. Meanwhile, a significantly negatively correlation were found in *C. lanceolatum* (r = -0.52), *P. pinnata* (r = -0.50), *D. grandiflorus* (r = -0.87), *K. ivorensis* (r = 0.85), *S. curtisii* (r = -0.48), *C. scortechinii* (r = -0.69) and *H. brasiliensis* (r = -0.50) for *C. gestroi*.

This study also found that no valid correlation between the above two variables (wood consumption and density) in others: *N. heimii* (r = -0.09), *C. lanceolatum* (r = -0.13), *M. utilis* (r = -0.23), *A. angustifolia* (r = -0.36) and *C. scortechinii* (r = -0.37) against *C. curvignathus*. This situation also exist against *C. gestroi* in *N. heimii* (r = -0.06), *M. utilis* (r = -0.24), *D. kunstleri* (r = -0.42), *F. fragrans* (r = -0.26) and *A. angustifolia* (r = -0.42).

The results from this study showed the correlation exist between the durability and wood density against both subterranean termites. This agrees with the conclusions by six previous studies (Behr *et al,* 1972; Coulson and Lund, 1973; Bultman *et al,* 1979; Abreu and Silva, 2000; Ahmad Said *et al,* 1982; Ahmad Said and Mohd Hamami, 1983). Behr *et al* (1972) reported that the test against *Reticulitermes flavipes* give a negative correlation between the wood consumption and hardness of the wood. Coulson and Lund (1973) agreed with the inverse correlation between the above two variables with the combination of extractive content and wood hardness. Bultman *et al* (1979) found a general inverse correlation between these two variables when dealing with *Coptotermes formosanus.* Abreu and Silva (2000) also found the same results against *Nasutitermes macrocephalus*. A significant correlation (r = -0.83**) was reported in study by Ahmad Said *et al* (1982) between the wood consumption and wood density of ten Malaysian hardwood species against *C. curvignathus.* In other studies, Arango *et al* (2006) found that mass loss or wood consumption of tropical hardwoods had negative correlation with specific gravity or wood density against *R. flavipes*. In a parallel study, Roszaini *et al* (2009) found that this correlation occurred in nine Malaysian wood species against *C. gestroi*. They reported that wood density had higher significantly negative correlation (-0.84**) with wood consumption by this aggressive subterranean termite. They also found that the high mortality of *C. gestroi* occurred in jars that contained samples with high density.

From the above result, it can be concluded that wood densities have some correlation with durability against subterranean termites. However, this alone cannot be the only factor that influences the wood consumption by the Isoptera as other factors are involved in wood durability. Previous studies (Arndt, 1968; Jalaluddin and Labosky, 1985; Nagnan and Clement, 1990; Lemaire *et al*, 1990; Scheffrahn, 1991; Grace, 1997; Taylor *et al*, 2006; Roszaini *et al,* 2009) have reported that extractives content have a greater impact on durability against termites than wood density. Haygreen and Bowyer (1989) confirm that majority of wood species that have higher basic density also have a higher extractive

content. Extractives act as a termiticide and these are composed a large range of chemical compounds such as terpenoids, quinines (Arndt 1968; Dietrichs and Hausen, 1971; Carter *et al*, 1978; Ganapaty *et al*, 2004), flavonoids (Reyes-Chilpa *et al*, 1995; Doi *et al*, 2002; Chen *et al*, 2004; Morimoto *et al*, 2006), tannins (Fava *et al*, 2006), stilbene chlorophorin (Arndt, 1968).

As far as durability is concerned, it is difficult to expect that differences in wood density between species, genera or within species could influence termite attack sufficiently to offset the differences of extractive compounds. For this, the possible effect of wood density may be can be used only within a single species.

Essenthers (1977) studied the natural durability of 21 native north American and exotic woods and found a positive correlation between specific gravity and resistance. Other study by Arango *et al* (2004) found that *R. flavipes* had a higher mortality rates in jars containing higher wood density samples. This implies that wood with higher density had higher extractive contents than wood with lower density. High mortality was generally caused by starvation as the both termites species did not feed to any extent on the resistant woods. That why in this study, higher percentage of termite mortality occurred when tested with higher wood density due to high wood material (extractive contents).

### 8.4.2 Durability (weight loss of Ps, Tv and Lsc) and wood density

Analysis has been done to show the correlation between the weight loss and wood density (Table 8.2). Highly positive correlations between durability (average weight loss) and wood density only occurred in *D. grandiflorus* (r = 0.91) and *C. scortechinii* (r = 0.61) with *P. sanguineus* and moderately positive correlations were found in *N. heimii* (r =0.61) and *K. ivorensis* (r = 0.58) with *L. sajor-caju*. Contrarily, highly negative correlations were found in *M. utilis* (r = -0.82) and *P. pinnata* (r = -0.84) with *T. versicolor*. Moderately negative correlations occurred in 2 cases each with *T. versicolor* and *L. sajor-caju* (*D. kunstleri*; r =-0.660 and *C. scortechinii*, r =-0.51 and *A. angustifolia*; r = -0.56 and *S. curtisii*; r = -0.67, respectively). Out of twelve timber species studied, only 2 timber species showed high correlations and 4 species showed moderate correlations between durability and wood density.

The majority of the timber species showed no significant correlation between these two variables either by *P. sanguineus* [in *N. heimii* (r = 0.36), *C. lanceolatum* (r = -0.30), *M. utilis* (r = -0.41), *P. pinnata* (r = -0.23), *D. kunstleri* (r = 0.05), *K. ivorensis* (r = 0.45), *S. curtisii* (r = 0.06), *F. fragrans* (r = 0.12), *A. angustifolia* (r = 0.28) and *H.*

*brasiliensis* (r = -0.29)], by *T. versicolor* [in *N. heimii* (r = 0.23), *C. lanceolatum* (r = -0.09), *D. grandiflorus* (r = -0.44), *K. ivorensis* (r = 0.25), *F. fragrans* (r = -0.25), *S. curtisii* (r = -0.05), *A. angustifolia* (r = 0.08) and *H. brasiliensis* (r = -0.26)] or by *L. sajor-caju* [in *C. lanceolatum* (r = -0.19), *M. utilis* (r = -0.37), *P. pinnata* (r = -0.33), *D. grandiflorus* (r = 0.38), *D. kunstleri* (r = -0.02), *F. fragrans*, r = 0.03), *C. scortechinii* (r = -0.21) and *H. brasiliensis* (r = -0.217, 0.498)].

Results from this study clearly show that there was no correlation between weight loss and density of the wood. The same result has previously been reported in hardwoods (Jorgensen, 1953; Bultman and Southwell, 1976; Paes *et al*, 2004; Bhat *et al,* 2005). The result of the trend that denser wood had more resistance against all fungi may be because denser wood has lower porosity which is more difficult for hyphal penetration during the limited time of the test (16 weeks). Higher density is also related to the quantity of wood extractives present which are known to inhibit decay (Martínez-Inigo *et al*, 1999).

The reason has been more closely related to the chemical composition of the extractives and their effects such as fungitoxicity as well as their presence and quantity and thus the effect on density. Early work by Hawley *et al* (1924) found that a close correlation between the toxicity of extracts and the resistance to decay in wide range of timbers. The hot water extract from mulberry, catalpa and redwood inhibited the growth of the test fungi. Other studies (Englerth and Scheffer, 1954; Scheffer and Cowling, 1966; Rao, 1982), point out the presence and amounts of chemical components are much responsible for resistance rather than specific gravity itself.

In addition, Buckley (1932) showed that the durable timbers in Malaysia contained higher quantities of extractives than non-durable timbers. He made a comparison between giam (*Hopea nutans*) that had 18.5% of alcohol-benzene extract (Tol:IMS equivalent) and jelutong (*Dyera costulata*) that had only 4.1%, and suggested that over 5.0% of extractives, the timber can considered as durable (phenols are mainly extracted with alcohol extraction). A obvious point has been made regarding this by Zabel and Morrell (1982) who stated that wood density is not a good indicator for natural decay resistance, commenting that white oaks are durable while beech and maple are non durable even though they have similar densities.

Besides that extractive content, decay resistance has been attributed to anatomical structure (shape and dimension of cells, abundance and structural of pits) (Southam and Ehrlich, 1943; Highley, 1978) and chemical composition [proportion of structural materials – cellulose, hemicelluloses and lignin (Scheffer and Cowling, 1966; Highley, 1982) and

non-structural – carbohydrates, nitrogen and various minerals (Cochrane, 1958; Levi and Cowling, 1968; Hulme and Shields, 1970).

Until now, the correlation between wood density/specific gravity and decay resistance has been seriously questioned. For this reason, the possible effect of wood density on decay resistance is of interest only within a single timber/fungus species and density, *per se*, is not a factor in preventing fungal decay.

### 8.4.3 Durability (wood consumption of Cc and Cg) and extractive contents

In general, wood extractives (phenolic contents) serve as plant defence mechanisms (prevent molecular damage and damage from microorganisms, insects etc.) by counteract reactive oxygen species (ROS) (Vaya *et al*, 1997). Extractives contents had a big impact on durability rather than physical properties of wood (Sen-Sarma, 1963). Early studies (Kofoid and Bowe, 1934; Da Costa *et al*, 1958; 1961b; Rudman *et al*, 1967) reported that the type and amount of extractives in wood are factors that affect the selection of food source by termites. This is related to wood species, tree age, heart and sapwood but other factors such as moisture content, , hardness of the wood and the extent of any previous attack by fungi or other insects may be considered.

This study found that negative correlation occurred between majority of the heartwood extractives and wood consumption by both subterranean species (Table 8.3). Highly significant negative correlations ($P<0.001$) were found in *C. lanceolatum* (hot water extracts; r = -0.98) against *C. gestroi* and *A. angustifolia* (total extractives: r = -0.97) against *C. curvignathus*. Only three wood species (*P. pinnata*: r = -0.88, *F. fragrans*: r = -0.83 and *C. scortechinii*: r = -0.92) had moderately significant negative correlations ($P<0.01$) for Tol:IMS extracts, six wood species for hot water extracts (*N. heimii*: -0.80, *M. utilis*: r = -0.91, *P. pinnata*: r = -0.94, *F. fragrans*: r = -0.91 and r = -0.81, *C. scortechinii*: r = -0.82 and *H. brasiliensis*: r = -0.93) and five species for total extractives (*N. heimii*: r = -0.80 and r = 0.87, *C. lanceolatum*: r = -0.88, *F. fragrans*: r = -0.92, *S. curtisii*: r = -0.85 and *H. brasiliensis*: r = -0.92).

Table 8.3 also showed that significantly negative correlations ($P<0.05$) occurred in six species (*C. lanceolatum*: r = -0.64 and -0.79, *P. pinnata*: r = -0.73, *D. kunstleri*: r = -0.73, *S. curtisii*: r = -0.69 and -0.55, *C. scortechinii*: r = -0.74 and *K. ivorensis*: r = -0.71) for Tol:IMS extracts, eight species for hot water extracts (*N. heimii*: r = -0.74, *C. lanceolatum*: r = -0.52, *P. pinnata*: r = -0.79, *D. grandiflorus*: r = -0.76, *D. kunstleri*: r = -0.76 and -0.53, *K. ivorensis*: r = -0.52 and -0.53, *S. curtisii*: r = -0.63 and *A. angustifolia*: r

= -0.79,) and seven species for total extractives (*P. pinnata*: r = -0.72, *D. grandiflorus*: r = -0.57, *D. kunstleri*: r = -0.76, *K. ivorensis*: r = -0.69, *A. angustifolia*: r = -0.64, *C. scortechinii*: r = -0.72 and -0.62 and, *H. brasiliensis*: r = -0.73,). The others show no valid correlation between extractive contents and durability (wood consumption) against both subterranean termites.

These results seem to be in accordance with earlier investigations (Bultman and Southwell, 1976; Lukmandaru and Takahashi, 2008) in that resistance against termites is strongly related to heartwood extractives. Similar corroboration is provided in many previous studies (Oshima, 1919; Wolcot, 1953; Sandermann and Dietrichs, 1957; Rudman and Da Costa, 1959, Bultman and Southwell, 1976; Ahmad Said and Mohd Hamami, 1983; Simatupang *et al.*, 1996). Rudman and Da Costa (1959), and Simatupang *et al* (1996) showed that wood extractives (tectoquinone; 2-methylanthraquinone) from *Tectona grandis* had antitermitic properties which are toxic to termite gut symbionts. Ahmad Said and Mohd Hamami (1983) reported that due to higher extractives content (20.60%), *N. heimii* has been classified as durable timber against drywood termites.

The results suggest that the use of wood species with higher extractives content might be an acceptable strategy in minimising attack of both subterranean termites on timbers in service. As more of the wood samples were degraded, the greater amount of extractives present in the woods was ingested by the termites which could be fatal.

### 8.4.4 Durability (weight loss of Ps, Tv and Lsc) and extractive contents

The relationship between chemical composition in heartwood extractives and decay resistance was first reported by Hawley *et al* (1924). Results from this study (Table 8.4) showed that negative correlations between the amount of extractives (by Tol:IMS, hot water or total extractives) and durability occurred in the majority of the wood species against all fungi, to at least $P<0.05$ level of significance, with the only exceptions being *S. curtisii* and *A. angustifolia* with *P. sanguineus* .

Highly negatively significant correlation with Tol:IMS extracts were found in *M. utilis* (r = -0.98), *P. pinnata* (r = -0.93), *C. scortechinii* (r = -0.95), *D. grandiflorus* (r = -0.94) and *F. fragrans* (r = -0.94) and, *H. brasiliensis* (r = -0.99) and *S. curtisii* (r = -0.98) with total extractives.

Moderately negatively significant correlations were found in *D. grandiflorus* (r = -0.82 and -0.83), *D. kunstleri* (r = -0.82), *F. fragrans* (r = -0.83) *C. scortechinii* (r = -0.84), and *H. brasiliensis* (r = -0.83) with Tol:IMS extracts, *N. heimii* (r = -0.84), *C. lanceolatum*

(r = -0.88), *M. utilis* (r = -0.81), *F. fragrans* (r = -0.82), *S. curtisii* (-0.89), *A. angustifolia* (r = -0.88), *C. scortechinii* (r = -0.90) and *H. brasiliensis* ( r = -0.90) with hot water extracts and, *N. heimii* (r = -0.88 and -0.85), *D. grandiflorus* ( r = -0.84 and -0.88), *D. kunstleri* (r = -0.89), *F. fragrans* (r = -0.85, -0.84 and 0.87), *A. angustifolia* (r = -0.86), and *H. brasiliensis* ( r = -0.81) with total extracts.

Nine species showed significantly negative correlation ($P<0.05$) between extractives content and durability (weight loss) with Tol:IMS extracts (*N. heimii*: r = -0.54, and -0.74, *C. lanceolatum*: r = -0.74, *M. utilis*: r = -0.57, *P. pinnata*: r = -0.67, *K. ivorensis*: r = -0.73, -0.67 and -0.78, *D. kunstleri*: r = -0.66 and -0.65, *F. fragrans*: r = -0.78, *S. curtisii*: r = -0.59 and *H. brasiliensis*: r = -0.75), nine species also with hot water extracts (*N. heimii*: r = -0.52 and -0.54, *M. utilis*: r = -0.68 and -0.65, *P. pinnata*: r = -0.57, -0.64 and -0.51, *D. grandiflorus*: r = -0.66, -0.75 and -0.76, *D. kunstleri*: r = -0.66 and -0.55, *F. fragrans*: r = -0.59, *A. angustifolia*: r = -0.67, *C. scortechinii*: r = -0.61 and -0.65 and, *H. brasiliensis*: r = -0.57) and eight species with total extractives (*N. heimii*: r = -0.65, *C. lanceolatum*: r = -0.66, *M. utilis*: r = -0.71, *D. grandiflorus*: r = -0.78, *D. kunstleri*: r = -0.71 and -0.65, *K. ivorensis*: r = -0.70, *A. angustifolia*: r = -0.61 and *C. scortechinii*: r = -0.79).

It is thus concluded here that the results of this study confirm numerous previous studies which point to extractive contents as being the significant factor limiting white rot decay.

### 8.4.5 Durability and total phenols; durability and antioxidant actvity

Figure 8.1 shows that when the mean of the wood consumption (termites) and mass loss (fungus) to each of the wood species compared with total phenols measured from the same wood species, the correlation between durability and total phenols was clear. The resistance of twelve Malaysian wood species increased with the increase of total phenols in the extract. In other words, the wood consumption by termites and mass loss by fungus decreased with the increase of total phenols. The relationship between both durability and total phenols content was hyperbolic (*C. curvignathus*: $R^2$ = 0.8533, *C. gestroi*: $R^2$ = 0.6589, *P. sanguineus*: $R^2$ = 0.5873, *T. versicolor*: $R^2$ = 0.6758 and *L. sajor-caju*: $R^2$ = 0.4544). Therefore, it is shown that the antitermitic and antifungal fractions of four selected wood species may toxic to termites and inhibit the fungal decay.

Furthermore, it was also found that the antitermitic and antifungal fractions that inhibit termite and fungi activity also showed high DPPH radical scavenging activity

(Chapter 6). This supports the hypothesis of Schultz and Nicholas (2000) and Schultz *et al* (2008). They suggested that the extractives may have a dual function; possess termiticidal or fungicidal activity as well as being excellent antioxidants.

Other studies have also found negative correlations between the total phenols and the durability against termite and fungus. Antioxidant activity may be one of the factors that contribute to the antifungal/antitermitic activity (Mihara *et al*, 2005) even though some studies have failed to show this (Huang *et al*, 2009).

Further strengthened to results from this study is by Adfa *et al* (2010). They found polyphenols (scopoletin and coumarin derivatives) from *Protium javanicum* were significantly correlated with antitermitic activity against *Coptotermes formosanus* Shiraki. Harju *et al* (2002; 2003) and Harju and Venäläinen (2006) found a fairly strong correlation on Scots pine against brown-rot (*C. puteana*). Gierlinger *et al* (2004b) found a strong negative correlation in all investigated larch origins of *Larix* sp. against the brown-rot fungi (*Poria placenta* and *Coniophora puteana*). In addition, Harju *et al* (2002) found that resin acids had minor contributions to durability of Scots pine compared to total phenols.

## 8.5 Conclusions

1. The correlation between wood densities, extractive content of twelve Malaysian timber species on durability was investigated

   Majority of wood species studies showed significant negative correlations between the wood density and durability of wood against subterranean termites. The denser the wood, the more durable it is. However, the correlation was not valid for decay fungi. The durability against fungus is more correlated with extractive contents than wood density.

2. Extractives content had a highly significant inverse correlation with durability in majority of wood species studies (except a few cases) torwards termites and fungal decay. The higher extractive content, the more durable the timbers.

3. The study also found the positive correlation between extractive contents and total phenolic compounds in majority of the wood species studied. The linear correlation also occurred between total phenolic compounds and free radical scavenging antioxidant activity in many wood species. However, is should be remembered that antioxidant activities not only depend on the composition of the wood but also the conditions and type of the test used.

4. Total phenolic content also had a correlation with the natural durability of the wood. The resistance of the wood increased with the increasing total phenols.
5. The results are from one tree per species only. However, a large number of trees per species could have shown more significance between all correlations.

# CHAPTER: 9 GENERAL DISCUSSION

The results of this study can be summarised in a simple table (Tables 9.1 and 9.2), where the important data has been grouped together.

Table 9.1 Average values of properties tested of the twelve Malaysian wood species

| Properties | *N. heimii* | *C. lanceolatum* | *M. utilis* | *P. pinnata* | *D. grandiflorus* | *D. kunstleri* | *K. ivorensis* | *F. fragrans* | *S. curtisii* | *A. angustifolia* | *C. scortechinii* | *H. brasiliensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Density (kg/m$^3$), Chapter 3** | | | | | | | | | | | | |
| Basal | 797 | 803 | 712 | 711 | 783 | 730 | 487 | 668 | 543 | 411 | 490 | 583 |
| Middle | 775 | 744 | 697 | 713 | 766 | 714 | 482 | 644 | 527 | 402 | 483 | 580 |
| Top | 732 | 710 | 672 | 633 | 732 | 686 | 472 | 598 | 490 | 385 | 464 | 556 |
| **Extractives (%), Chapter 3** | | | | | | | | | | | | |
| **a. Bark** | | | | | | | | | | | | |
| Tol:IMS | 23.5 | 8.8 | 14.7 | 11.2 | 4.2 | 2.85 | 8.0 | 3.3 | 6.1 | 2.4 | 3.1 | 1.6 |
| Hot water | 10.9 | 13.3 | 6.2 | 6.6 | 2.3 | 7.8 | 5.4 | 6.3 | 13.6 | 2.4 | 2.2 | 2.3 |
| Total | 34.4 | 22.1 | 20.9 | 17.9 | 6.5 | 10.6 | 13.3 | 9.6 | 19.7 | 4.8 | 5.3 | 3.9 |
| **b. Heartwood** | | | | | | | | | | | | |
| Tol:IMS | 9.2 | 5.3 | 5.6 | 4.6 | 2.2 | 2.8 | 3.3 | 4.4 | 2.8 | 2.2 | 2.2 | 1.3 |
| Hot water | 5.6 | 4.0 | 3.4 | 4.6 | 2.3 | 2.2 | 5.1 | 4.4 | 4.8 | 2.3 | 2.3 | 1.5 |
| Total | 14.8 | 9.3 | 9.0 | 9.1 | 4.5 | 5.0 | 8.4 | 8.8 | 7.58 | 4.6 | 4.5 | 2.8 |
| **Antioxidant (AO), Chapter 3** | | | | | | | | | | | | |
| **a. Total phenols (%)** | | | | | | | | | | | | |
| Bark | 29.1 | 23.2 | 22.1 | 13.9 | 8.9 | 10.4 | 18.1 | 8.7 | 18.8 | 4.9 | 7.5 | 8.2 |
| Heartwood | 24.1 | 20.1 | 18.8 | 8.8 | 7.7 | 7.6 | 9.5 | 6.8 | 16.0 | 4.3 | 6.8 | 5.5 |
| **Total extractable phenols, heartwood, Chapter 3** | | | | | | | | | | | | |
| Tol:IMS | 2.21 | 1.06 | 1.06 | 0.41 | 0.17 | 0.21 | 0.32 | 0.30 | 0.45 | 0.10 | 0.15 | 0.07 |
| Total basis | 3.57 | 1.87 | 1.70 | 0.81 | 0.35 | 0.38 | 0.79 | 0.60 | 1.21 | 0.20 | 0.31 | 0.15 |
| **b. DPPH assay, Chapter 3** | | | | | | | | | | | | |
| Bark | 93.6 | 86.3 | 83.6 | 72.9 | 51.9 | 66.8 | 56.1 | 71.8 | 84.1 | 22.6 | 46.1 | 70.1 |
| Heartwood | 83.8 | 81.4 | 75.3 | 63.1 | 16.7 | 34.7 | 27. 6 | 66.2 | 76.8 | 7.5 | 28.5 | 54.4 |
| **AO capacity, heartwood** | | | | | | | | | | | | |
| | 12.4 | 7.6 | 6.8 | 5.8 | 0.8 | 1.7 | 2.3 | 5.8 | 5.8 | 0.3 | 1.3 | 1.5 |

Table 9.2 Average values of the durability data of the twelve Malaysian wood species

| Properties | *N. heimii* | *C. lanceolatum* | *M. utilis* | *P. pinnata* | *D. grandiflorus* | *D. kunstleri* | *K. ivorensis* | *F. fragrans* | *S. curtisii* | *A. angustifolia* | *C. scortechinii* | *H. brasiliensis* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Durability** | | | | | | | | | | | | |
| **a. Termites (% wood consumption), Chapter 4** | | | | | | | | | | | | |
| *C. curvignathus* | 0.9 | 1.0 | 1.4 | 3.8 | 3.6 | 5.5 | 4.9 | 3.0 | 1.5 | 8.2 | 6.0 | 12.4 |
| *C. gestroi* | 0.7 | 0.8 | 1.5 | 1.6 | 2.5 | 3.7 | 2.7 | 1.9 | 1.4 | 7.4 | 2.7 | 11.1 |
| **b. Termite mortality (%), Chapter 4** | | | | | | | | | | | | |
| *C. curvignathus* | 100 | 100 | 100 | 98.2 | 99.2 | 98.0 | 98.0 | 99.5 | 100 | 87.3 | 89.2 | 67.7 |
| *C. gestroi* | 100 | 100 | 99.9 | 99.4 | 98.4 | 96.3 | 98.5 | 98.5 | 100 | 95.2 | 98.2 | 77.1 |
| **c. Fungi (% weight loss), Chapter 5** | | | | | | | | | | | | |
| *P. sanguineus* | 0.56 | 1.2 | 0.7 | 4.4 | 0.69 | 30 | 3.9 | 5.2 | 0.26 | 29.0 | 7.0 | 30.5 |
| *T. versicolor* | 0.34 | 5.2 | 7.2 | 20.9 | 6.5 | 14.1 | 27.9 | 12.6 | 1.7 | 35.2 | 10.1 | 37.0 |
| *L. sajor-caju* | 0.57 | 4.2 | 3.6 | 4.0 | 1.0 | 11.8 | 3.7 | 6.5 | 1.3 | 15.3 | 7.1 | 25.4 |
| **Resistance class (weight loss basis) against, Chapter 5** | | | | | | | | | | | | |
| *P. sanguineus* | HR | HR | HR | HR | HR | MR | HR | HR | HR | MR | HR | MR |
| *T. versicolor* | HR | HR | HR | R | HR | R | MR | R | HR | MR | HR | MR |
| *L. sajor-caju* | HR | HR | HR | HR | HR | R | HR | HR | HR | HR | HR | MR |
| **X factor, Chapters 4 and 5** | | | | | | | | | | | | |
| *C. curvignathus* | 0.07 | 0.08 | 0.11 | 0.31 | 0.29 | 0.45 | 0.39 | 0.24 | 0.12 | 0.67 | 0.49 | 1.0 |
| *C. gestroi* | 0.06 | 0.07 | 0.14 | 0.15 | 0.22 | 0.33 | 0.24 | 0.17 | 0.12 | 0.67 | 0.25 | 1.0 |
| *P. sanguineus* | 0.02 | 0.04 | 0.02 | 0.14 | 0.02 | 0.98 | 0.13 | 0.17 | 0.01 | 0.95 | 0.23 | 1.0 |
| *T. versicolor* | 0.01 | 0.14 | 0.20 | 0.57 | 0.18 | 0.38 | 0.75 | 0.34 | 0.04 | 0.95 | 0.27 | 1.0 |
| *L. sajor-caju* | 0.02 | 0.17 | 0.14 | 0.16 | 0.04 | 0.47 | 0.14 | 0.26 | 0.05 | 0.60 | 0.28 | 1.0 |
| **Durability class (X factors basis) against, Chapters 4 and 5** | | | | | | | | | | | | |
| *C. curvignathus* | 1 | 1 | 1 | 3 | 2 | 3 | 3 | 2 | 1 | 4 | 3 | 5 |
| *C. gestroi* | 1 | 1 | 2 | 2 | 2 | 3 | 2 | 2 | 1 | 4 | 2 | 5 |
| *P. sanguineus* | 1 | 1 | 1 | 1 | 1 | 5 | 1 | 2 | 1 | 5 | 2 | 5 |
| *T. versicolor* | 1 | 1 | 2 | 3 | 1 | 3 | 4 | 2 | 1 | 5 | 2 | 5 |
| *L. sajor-caju* | 1 | 2 | 1 | 2 | 1 | 3 | 1 | 2 | 1 | 4 | 2 | 5 |

HR = Highly Resistance, R = Resistance, MR = Moderately Resistance.

## 9.1 Natural durability of Malaysian timbers

Measures of decay are often the same for fungi and termites, and weight loss and wood consumption are effectively the same measurement. Also when normalising data, the use of an index for normalising data is common. With fungal decay testing an X factor is used, whereas for termite testing this is termed the susceptibility index. In Table 9.2 they have both been termed the X factor for consistency. This system is, in effect, better than that used in some standard treatments of test data which is heavily dependent on the

severity of decay in tests and conformity of different test organisms. For completeness, a rating, based on weight loss, is included in Table 9.2.

In Chapters 4 and 5, the variation in natural durability of twelve Malaysian timbers species was highly significant for wood species but the wood species performed differently against termites and fungal species. With the fungi, wide differences occurred in the ratings of the wood species to different fungi, with the highest rating difference for *K. ivorensis* (Table 9.2). In the laboratory tests, only two species were rated as very durable against both termites and fungi (*N. heimii* and *S. curtisii*) and although *N. heimii* is rated very durable in field tests and in service, *S. curtisii* is only rated moderately durable under a Malaysian system, two classes down from the very durable category (Mohd Dahlan and Tam, 1985; Table 2.10). In this laboratory test data various species can be rated as durable which agrees with the Malaysian ranking, including *M. utilis*, *C. lanceolatum* and *F. fragrans*, while *P. pinnata* has a durable rating in field tests but in laboratory tests was rated lower in both termite and fungal tests. In terms of good resistance in laboratory tests, *D. grandiflorus* was ranked very durable against fungi and durable against termites whereas in field data it is only moderately durable. Other species broadly were in agreement between field and laboratory test data and were generally of low durability.

## 9.2 Effects of density on durability

Wood density (Chapters 3 and 8) had an effect on durability of the heartwoods of the Malaysian wood species studied. Generally denser heartwoods were associated with less termite attack, but the most attacked species, *H. brasiliensis*, falls into the mid-range for density and *S. curtisii* has lower density but shows little attack. With termites, denser wood is a barrier to chewing during a limited time, no choice test. The effects of wood density are more obvious when combined with other properties (e.g. extractive content). Wood density showed some correlation with durability against fungi but a number of species showed considerable decay despite high densities including *D. kunstleri* (density 730 kg $m^{-3}$ but high weight loss, 30%, X = 0.98 vs. *P. sanguineus*). One species which has good durability against termites and fungi, *D. grandiflorus,* may have done well in these tests because of density or density related aspects, e.g. permeability, because it was shown to have low extractive contents. Experimental approaches to look at extractive free wood would have allowed a closer examination of this but this was considered outside the range

of this study and considerable difficulties would have been experienced in obtaining extractive free material, particularly for *N. heimii*.

## 9.3 Effects of extractive contents on durability

In terms of sample location, bark had higher extractive contents than heartwood. In some wood species, high extractive contents were associated with durability of Malaysian timbers either against subterranean termites or decay fungi but in some instances high extractives only imparted moderate durability against both termites and fungi (e.g. a number of those with extractive totals around 9%; *D. kunstleri, F. fragrans, P. pinnata* and *K. ivorensis*). All extractive contents are significantly different between timber species and within tree (bark and heartwood). Very durable and many durable timber species had higher extractive contents but not all species with high extractive contents had high durability and not all of those with low extractive contents had low durability (viz. *D. grandiflorus* very durable vs fungi, but only durable performance vs termites). However, the effect of extractives depends not only on the quantity but on its composition. The quantity is a product of the type of tissue extracted (bark, heartwood) method of extraction, and the solvents used.

## 9.4 Effects of extractives, phenols and antioxidants

Examination of the extractives of twelve different wood species showed that majority of the extractives had high total phenols contents and high free radical scavenging activity (Table 9.1). Generally, higher extractive yielding wood species produced higher total phenols but some, *P. pinnata, F. fragrans* and *K. ivorensis* had moderately high extractive contents but lower total phenol contents and lower total extractable phenols. There are problems however in attempting to get values for the total extractable phenols because only the Tol:IMS extractives were assayed for total phenols and there is no reason to suggest that this system has extracted all of the phenols or that the subsequent hot water extraction is devoid of phenols. However, if this shortcoming is accepted and the calculations are done with both the Tol:IMS and the total extractives, an attempt can be made to look at total extractable phenols. The four species with the best durability (*N. heimii, S. curtisii, C. lanceolantum* and *M. utilis*) have high total extractable phenols on the total basis (i.e. >1.21) and this separates them from the other species. They still separate out with Tol:IMS total extractable phenols but with *S. curtisii*, the separation is marginal due to large proportion of its extractives as hot water soluble. This may have some

influence on the difference between it field performance and the laboratory test data, and it may lose extractives in a real situation due to leaching loss. Meanwhile, low durability wood species have low extractive contents, low total phenols and also low extractable phenol.

Despite this a number of the extracts from low durability heartwood had high antioxidant activity (*H. brasiliensis*). However, when the antioxidant capacity is calculated, based on the Tol:IMS extractive amounts and the antioxidant activity (DPPH assay, Table 9.1), the durable species once again show high values, which in *N. heimii* is very high (12.4) and *H. brasiliensis* is low (1.5). The other three durable species (*C. lanceolatum*, *M. utilis* and *S. curtisii*) were also high (7.6, 6.8 and 5.8, respectively) but the value of *S. curtisii* overlapped *P. pinnata* and *F. fragrans*. Of the remaining species, *K. ivorensis* has the highest antioxidant capacity value (2.3) while the others are considerably lower.

However, even though this study showed that majority of durable Malaysian wood species tested possess high antioxidant capacity, it is premature to draw strong conclusions since the results are only based on testing the Tol:IMS extractives and does not include the hot water extractives. It is also apparent from the MALDI-TOF study of *N. heimii* Tol:IMS extractives, that the Tol:IMS extractives were degraded during the extraction process. So it is likely that the results presented here represent a partial picture. Thus wood extractives from these species should be more fully analysed for further exploitation of potential new natural resources.

## 9.5 Comparisons of wood properties between test organisms

For durability against subterranean termites, *C. curvignathus* was more aggressive than *C. gestroi*. *N. heimii* was the most resistant hardwood against both termites' species. Four timber species are considered as having only cosmetic damage (less than 3% wood consumption) against *C. curvignathus* and nine species against *C. gestroi*. Higher mortality (100%) of *C. curvignathus* was observed on *C. lanceolatum, N. heimii, S. curtisii* and *M. utilis* and in the first three wood species for *C. gestroi*. The lowest mortality rate (67.70% and 77.13%, respectively) was on *H. brasiliensis*.

In the case of durability against decay fungus, *T. versicolor* had the higher ability to degrade tropical hardwoods followed by *L. sajor-caju* rather than *P. sanguineus*. *N. heimii*, *C. lanceolatum, M. utilis*, *D. grandiflorus*, and *S. curtisii* were the five species that had significant resistance against *P. sanguineus*. However, for *T. versicolor*, only *N. heimii*

and *S. curtisii* are the most resistant. Only *N. heimii*, *D. grandiflorus* and *S. curtisii* showed resistance against *L. sajor-caju*.

The fungi selected in these tests, as pointed out above, have given slightly different results to those from field test data. In this study there was insufficient time to do field tests to select the optimum fungi for these tests and in some cases this might have given closer agreement. However, as discussed above, microbial differences are a part of the picture and features such as permeability and water solubility become important in field performance.

## 9.6 Bioassay tests results

A series of bioassays were performed on filter papers and wood blocks with hot water extracts from the four most durable wood species. The termite tests showed good activity with *N. heimii* at 2% treatment solution but less activity at lower treatment strengths and with the other species tested. Significant mortality occurred with the *N. heimii* 1.0% solution.

The fungal spots tests on agar plates failed to give clear inhibition zones but did demonstrate growth rate reductions which were most pronounced for *N. heimii*. This may have been a result of degradation during extraction or it may indicate that the extractives protect the hardwoods by a different mechanism, possibly involving antioxidants. Tests on impregnated *H. brasiliensis* blocks showed the best activity with *N. heimii* extracts but these were less effective than was hoped. The reasons for this could again be due to degradation of the activity of the extractives during extraction but it is likely that they work by a more subtle mechanism than can be achieved by simple impregnation alone. Previous studies have failed to mimic the effects of extractives because when extracted and reimprengated into a different species, they are different chemicals, are not polymerised in the cell wall and are put back into the wood in a different manner.

## 9.7 Detailed study on the composition of *N. heimii* extractives

The MALDI-TOF study revealed that hot extraction in Tol:IMS can degrade the extractive from the wood (boiling at about 110 °C) and is thus quite effective at removing some of the extractive from the wood. However, when others have performed exhaustive extraction of *N. heimii*, they have shown that up to 32.6% can be extracted (Yamamoto and Hong, 1988) in hot methanol (boiling at 65 °C) which suggests that less than half has been removed during the current study. The importance of knowing that the extraction method

may have an impacted on a range of the tests, is important as what is seen here is only a partial picture of the potential of the extractable materials from these species. The degraded extract revealed fragments of compounds which were suggested to have derived from polymers.

When cold methanol was used to extract and the extract was examined by MALDI-TOF the trace revealed various polymeric components of 1-4 DP, derived from resveratrol and kaempferol.

## 9.8 Proposed mechanism of protection of *N. heimii.*

The wood cell wall and structure of *N. heimii* appears to be very well protected against fungi and termites and the levels and types of protection can be speculated, with reason, as to their nature. At one level, the wood is high density and probably because of this and its extractive content, it has low permeability to water and air, which fungi need for decay. The wood cell wall is probably also well protected and is dimensionally stable and will probably show a low fibre saturation point, similar to other high extractive species. Further studies on the sorption behaviour will reveal its fibre saturation point. The cell wall modification is probably by a bulking mechanism, which blocks the wall. The blocking probably blocks the wall not only against water but to the low molecular degradative agents of fungi, i.e. free radical systems. Thus some further studies could be conducted to examine the nature and porosity of the cell wall microvoids, possibly by using solute exclusion or other appropriate techniques ($N_2$ sorption).

However it may be more subtle than that alone. When the extractives are formed inside the wood, they are deposited inside the wood cell lumens and inside the wood cell wall, where they probably polymerise, forming a leach proof or slowly leaching protection. Possibly some of the extractive deposited inside the wood cell wall forms a secondary lignin-like structure. When fungi challenge the cell wall, their free radicals are quenched and in so doing some of the adsorbent is released. This, if fungitoxic (as shown in Yamamoto and Hong, 1988) could control the fungi or as demonstrated here, could reduce their rate of growth. Further, the phenolics released have been shown in this work to be involved in protection and to possess high radical quenching capacity. Further interesting work could be done by looking at the cell walls by microscopy to examine extractive distribution and by looking at extracted cell walls, and possibly by looking at decay patterns inside extracted walls.

With termite resistance the effects may be that the wood is dense and thus very hard and that it does not soften significantly when wetted to its lowered fibre saturation point. The extractives shown to be toxic to termites in the filter paper study and they are probably also antifeedants. The effects of the phenolics on their gut microflora may also be significant and could be studied further.

## 9.9 Performance of the study

The results from this study provide valuable results of both academic and industrial interest for concerning wood durability. The results give some promise that wood extractives can be evaluated as a potential source for industrial compounds. The wood extractives can offer substantial advantages for wood protection against both biological agents (termites and fungi). The findings also point to the wood and bark as a resource for several avenues of natural products of commercial interest.

## 9.10 Conclusions

1. The properties of twelve Malaysian timber species studied differ with timber species.
2. Basic density showed a huge variation (385 $kgm^{-3}$ to 803 $kgm^{-3}$) among the species tested.
3. The test against subterranean termites showed that wood resistance depends on the termite species. *C. curvignathus* is a more aggressive subterranean termite than *C. gestroi*. *N. heimii* and *H. brasiliensis* are respectively the most and least durable timber species against both termite species.
4. Wood decay also depends on the fungus species. Different fungi had different abilities to decay woods. *T. versicolor* was the most aggressive fungus and *P. sanguineus* was the least.
5. Extractive contents are significantly different with timber species and solvent used. All the timber species showed that extractive contents were abundant in bark and less in heartwood. Durable timber (bark/heartwood) had a larger amount of extractives than the non durable timbers.
6. General trends in the property values/characteristics for twelve Malaysian timber species studied include: 1) higher susceptibility/resistance of timbers to biological agents (termites and fungi) associated with extractive contents, 2) the more dense the more resistance of the wood (termites) and 3) the higher extractive yields were

accompanied by higher amounts of total phenols and antioxidants. These were much greater when the results were multiplied to give total extractable phenols and antioxidant capacity.

7. This study confirm that the differences of relative durability of twelve Malaysian timber species could be due to the differences in a) the presence of antitermitic and antifungal compounds in the wood, b) the amount of extractives contents and c) the wood hardness.

8. Antioxidants properties are stronger in the majority of wood species studied thus giving a promising potential alternative for synthetic antioxidants, from natural resources.

## 9.11 Scope for further studies

The recent withdrawal and restricted use of a number of key synthethic pesticides and wood preservatives has created a critical need for safe alternatives. The requirement of biologically-based technologies and products has been recognized universally as an alternative to synthetic organic pesticides. Since there is a vast amount of silvichemicals from the Malaysian forest (over 15000 higher plants species), it is essential that systematic wood extractive and natural product research should be implemented to identify chemical components for potential commercial application. This is a promising way to avoid environmental pollution and health problems through the use of natural sources.

However, long roads of research and practice must be travelled before the maximum or optimum utilization of wood extractives can be attained. On the other hand, the complex and varied components of wood extractives do hold possibilities. Some wood extractives which today serve only a limited purpose will be in time explored for a much wider field of utilization. Beside that, more information and a better understanding concerning the process of microbial colonization and succession of wood is needed especially for tropical hardwood. This can help to develop the test methods for prediction of wood and wooden constructions behaviour in the future.

The success of wood extractives as a green (natural) preservative can be achieved by the combinations of different extracts into one product which can give a wider spectrum of protection activity. In addition with the integrated bio-control strategies, it can also result in increased efficacy and consistency of the biological treatment.

Last but not least, further toxicological and pharmacological studies on tropical hardwoods should now be performed to assess the therapeutic interest of these compounds

as antioxidants. It is hoped that the isolation and identification of new bioactive compounds from tropical wood especially from Malaysian forest species will contribute to development of new preservative against termite and fungus as well as insects and always remembered that the method used to control wood deterioration must be effective without having a negative impact on the environment and public health.

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