BIOASSAY GUIDED ISOLATION OF ANTI-TERMITE COMPOUNDS FROM THE BARK OF *RHIZOPHORA APICULATA*

KONG NEIN HING

UNIVERSITI SAINS MALAYSIA

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by

KONG NEIN HING

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LIST OF ABBREVIATIONS

AR	aqueous residue
ASTM	American Society for Testing and Materials
B	*n*-butanol treated wood blocks
BE	*n*-butanol extract
C	chloroform treated wood blocks
CE	chloroform extract
E1-E4	ethyl acetate treated wood blocks used in subsequent termite tests
EE	ethyl acetate extract
EtOAc	ethyl acetate
EtOH	ethanol
eV	electron volt
F1-F3	fractions from column chromatography of ethyl acetate extract
F2A	acidic fraction of ethyl acetate extract
F2AI-F2AIII	fractions from column chromatography of F2A extract
F2R	neutral/basic fraction of ethyl acetate extract
FA-FD	fractions from column chromatography of phenolic extract
FB1-FB3	fractions from column chromatography of FB extract
m/z	mass/charge
M1-M3	methanol treated wood blocks used in subsequent termite tests
MeOH	methanol
n	number of replication
n-BuOH	*n*-butanol
NIST	National Institute of Standards and Technology mass spectral database
P	petroleum ether treated wood blocks
PE	petroleum ether extract
Pet-ether	petroleum ether
ppm	part per million
sp.	species
STDEV	standard deviation
t_R	retention time (min)
U1-U7	untreated wood blocks used in subsequent termite tests
WILEY	Atlas of Mass Spectral Data, John Wiley & Sons, N. Y.
WPG	Weight Percent Gain

PENGASINGAN BERPANDUKAN UJIAN MAKMAL SEBATIAN ANTI ANAI-ANAI DARIPADA KULIT POKOK *RHIZOPHORA APICULATA*

ABSTRAK

Serbuk kulit kayu *Rhizophora apiculata* pada mulanya diekstrak dengan petroleum eter (PE) dan metanol. Ekstrak metanol dalam bentuk larutan akueus telah diekstrak semula ke dalam pecahan kloroform (CE), etil asetat (EE) dan *n*-butanol (BE). Pengasingan berpandukan ujian makmal (ASTM D3345-74) telah dijalankan dengan mempertimbangkan kehilangan berat kayu minimum (LWL). Aktiviti anti anai-anainya (ATA) adalah EE>CE>PE>BE, LWL_{EE} = 10.54% pada 500 ppm. Secara perbandingan, kawalan telah haus dimakan. Fraksi-fraksi EE menunjukkan ATA dalam turutan F2>F3>F1, LWL_{F2} = 5.53%. Analisis kimia dan spektroskopi menentukan asid fenolik adalah komponen aktif dalam EE. Fraksi F2 seterusnya diekstrak dengan kaedah asid-bes (pH ~3) dan hasilnya dikromatografkan. Sifat-sifat kimia dan biologi tidak berubah selepas pemisahan dan ATAnya adalah F2AII>F2AIII>(F2R≈F2AI), $LWL_{F2AII, F2AIII}$ = 6.86, 9.12%. Asid vanilik (Va) dan asid siringik (Sa) akhirnya diasingkan daripada F2AII dan F2AIII. Strukturnya dikenalpasti berdasarkan bukti-bukti spektroskopi. Kedua-duanya bertindak sebagai penghindar serangan dan kepekatan terendah menunjukkan serangan visual adalah 200 ppm (Va) dan 300 ppm (Sa). Sebagai tambahan, pengekstrakan fenolik (pH 6~7) dan turus kromatografi (CC) hasil selanjutnya memberikan 4 fraksi. Turutan ATAnya adalah FB>FC>FA>FD, LWL_{FB} = 17.24%. Fraksi FB kemudiannya ditulenkan melalui CC bagi memberikan metil vanilat (Mv) dan siringol (Sy) masing-masing daripada FB2 dan FB3. Turutan ATA sebatian-sebatian aktif ini adalah (Mv≈Va)>Sa>Sy.

BIOASSAY GUIDED ISOLATION OF ANTI-TERMITE COMPOUNDS FROM THE BARK OF *RHIZOPHORA APICULATA*

ABSTRACT

The powdered bark of *Rhizophora apiculata* was initially extracted with petroleum ether (PE) and methanol. The aqueous methanol extract was re-extracted into chloroform (CE), ethyl acetate (EE) and *n*-butanol (BE). Bioassay (ASTM D3345-74) guided fractionation was carried out on the basis of the least wood loss (LWL). The anti-termite activity (ATA) was in the order of EE>CE>PE>BE, $\mathrm{LWL}_{\mathrm{EE}}$ = 10.54% at 500 ppm. In contrast, controls were severely damaged. Fractions of EE showed ATA in the order of F2>F3>F1, $\mathrm{LWL}_{\mathrm{F2}}$ = 5.53%. Chemical and spectral studies proposed that the active compounds in EE were of phenolic acids. Fraction F2 was then partitioned into aqueous and organic layers at pH ~3 and the organic residue was subjected to chromatographic separation. The chemical and biological properties of the F2 fractions were not altered and the ATA sequence was F2AII>F2AIII>(F2R≈F2AI), $\mathrm{LWL}_{\mathrm{F2AII,\ F2AIII}}$ = 6.86, 9.12%. Vanillic (Va) and syringic (Sa) acids were finally isolated from F2AII and F2AIII. Their structures were characterized by means of spectroscopic evidences. They acted as repellent rather than toxicant and the lowest concentration for preventing visible damage was 200 ppm (Va) and 300 ppm (Sa). Additionally, phenolic extraction (pH 6~7) and subsequent column chromatography (CC) of the extract afforded 4 fractions. The ATA of the fractions was FB>FC>FA>FD, $\mathrm{LWL}_{\mathrm{FB}}$ = 17.24%. Fraction FB was further fractionated by repeated CC to yield methyl vanillate (Mv) and syringol (Sy) from FB2 and FB3, respectively. The sequence of ATA for these potential biocides was (Mv≈Va)>Sa>Sy.

CHAPTER 1

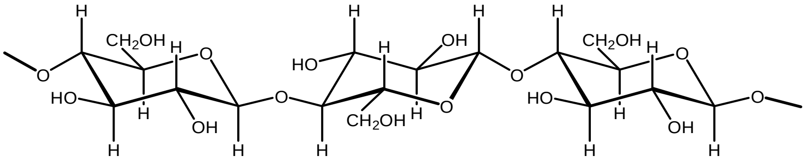
INTRODUCTION

1.1 Wood Origin, Chemical and Physical Properties

Wood is a three-dimensional polymeric composite whose biological and technical properties are mainly determined by the chemical composition of the cell wall. Wood cell walls are made up primarily of cellulose **(1.1)**, hemicelluloses **(1.2)** and lignin **(1.3)** (Deka & Saikia, 2000; Deka *et al.*, 2002; Cheng *et al.*, 2004). There is some variation in the relative abundance of these constituents in different species of wood but as rough guideline, cellulose is taken to be 50% (by dry weight) of wood and the other two components contribute 25% each to the dry weight of wood (Fengel & Wegener, 1983; Shafizadeh, 1984; Sjöström, 1993). The tensile strength of wood fibres is primarily determined by cellulose and hemicelluloses, while lignin (nitrogen free co-polymer) mediates adhesion between the fibres to impose rigidity and minimizes water permeation (Marchand *et al.*, 2005).

Cellulose **(1.1)** is a linear polymer of high molecular weight (average 100,000), exclusively built up by 1–4 glycoside-linked β-D-glucose molecules. The empirical formula for cellulose is based on polymerized glucose units $(C_6H_{10}O_5)_n$, where n may be up to about 3,000. The molecular chains are unbranched, but appear to be bound laterally into fibrils. The chains of the more complex hemicelluloses **(1.2)** are much shorter than those of cellulose but they usually bear side groups, such as monosaccharides and acetyl groups, and in some cases they are branched. The constituents of hemicelluloses are glucose **(1.4)**, mannose **(1.5)**, galactose **(1.6)**, xylose **(1.7)** and arabinose **(1.8)**. Lignin **(1.3)** is amorphous in nature and can be characterized by general empirical formula $C_9H_{8-x}O_2\,[H_2]\,\{1.0[OCH_3]_x\}$. It is highly resistant to attack by microorganisms (Ksibi *et al.*, 2003; Lima *et al.*, 2007) and is hardly decomposed by termites (Katsumata *et al.*, 2007) because of its high molecular weight and the presence of various biological stable β-*O*-4-ether bonds, β-5 carbon-to-carbon and ether linkages (Raj *et al.*, 2007). The inducible deposition of lignin in cell walls represents an effective barrier to pathogen entrance and spread (de Ascensao & Dubery, 2003). Due to the hardly accessible and relatively stable biopolymer features, lignin, a unique tracer for vascular plant matter is suitable even for the chemotaxonomic distinction between angiosperms, gymnosperms, and non-woody vascular plants (Opsahl & Benner, 1995; Dittmar & Lara, 2001; Marchand *et al.*, 2005). Lignin is a three-dimensional aromatic polymer formed by dehydrogenative polymerization of three cinnamyl alcohols which are precursors of *p*-hydroxyphenyl (*H*; **1.9**), guaiacyl (*G*; **1.10**) and syringyl (*S*; **1.11**) lignin units. Lignin composition varies in different groups of vascular plants, being *G*, *GS* and *HGS* lignins characteristic for softwoods (woody gymnosperms), hardwoods (woody angiosperms) and grasses (non-woody angiosperms) respectively (Martinez *et al.*, 2001; de Ascensao & Dubery, 2003; Lima *et al.*, 2007).

The properties of wood are not only influenced by the cell wall polymers but also extractives, accessory compounds extractable by solvents of different polarity. These compounds comprise of phenolics, terpenes, carbohydrates, fats, waxes and other substances (Fengel & Wegener, 1983; Kinyanjui *et al.*, 2000; Cheng *et al.*, 2004; Mai *et al.*, 2004).



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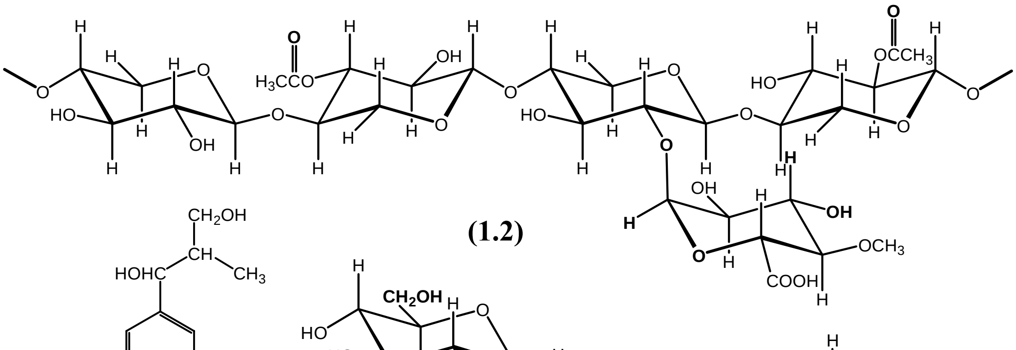

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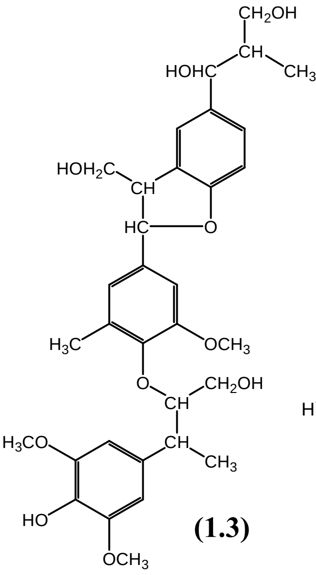

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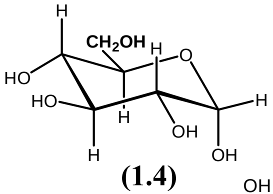

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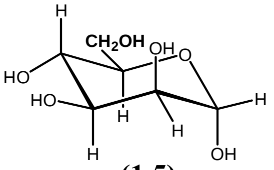

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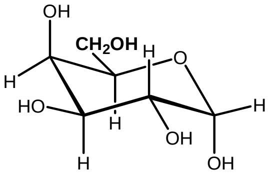

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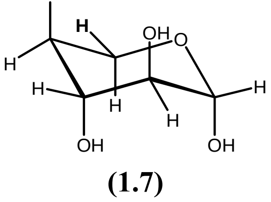

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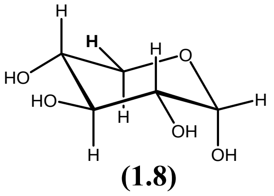

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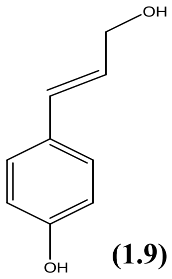

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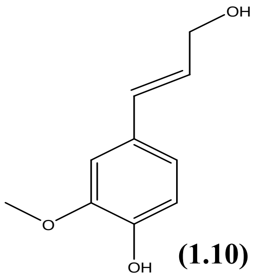

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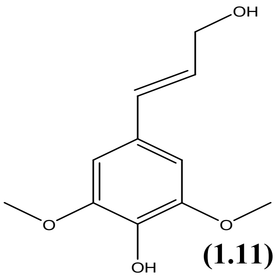

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1.1.1 The nature of wood

Wood is the most preferred building material because of its strong physical strength, aesthetically pleasing properties and low processing cost. It has many potential functions in human societies, for example as an indoor building material and in outdoor constructions. However, it has some troublesome inherent properties like changes in dimension with time due to environmental modulation and decomposition by some agents of animate and inanimate origin (Fengel & Wegener, 1983; Shafizadeh, 1984; Sjöström, 1993). The natural weathering factors such as heat, light, oxygen as well as other variables are undoubtedly cause the physical and chemical changes in wood substance which result in loss of strength making it difficult to use for constructional purposes (Deka *et al.*, 2002; Marney & Russell, 2007).

The moisture content of timber is crucial with respect to destructive microbial and insecticidal confrontations (Schultz & Nicholas, 2002; Cheng *et al.*, 2004). In wood cellulose, the glucose units are joined by glycosidic linkage and the free OH groups absorb atmospheric moisture that causes swelling of wood. This process is reversible, because on drying the wood shrinks (Deka & Saikia, 2000). Wood with water content of 12% or below, as typically found within buildings, is not susceptible to biological threats. Wood moisture of 12–18% enables attack by insects (beetle, wood borer, termite, etc.). Infestation by fungi (moulds, staining fungi) is also possible when the moisture content rises (sporadically) above 18%, as for example roof-protected wood observed outdoors and indoors in humid rooms. Wood degradation by basidiomycetes (white-rot, brown-rot) requires moisture content above the fibre saturation point (about 28–33%). Beyond this point, unbound water is found in cell lumina. Permanently wet wood in direct contact with soil is vulnerable to fungi (soft-rot) and bacteria (Mai *et al.*, 2004).

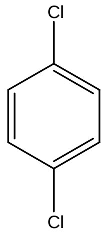
1.1.2 Wood preservation and the impact on environment

Developing methods that prolong the service life of wood has always been the interest of wood researchers. A number of methods have been devised to reduce degradation of wood by biotic and abiotic factors via appropriate chemical treatments. It may be necessary to use inorganic compounds or synthetic pesticides to preserve the woods and lengthen their application life (Cheng *et al.*, 2007). In the recent years, up to about the year 1945, the toxicants in general used by agricultural authorities and other interested agencies were mercuric dichloride, Paris green, other arsenical compounds, sodium fluosilicate, zinc chloride, potassium dichromate, *p*-dichlorobenzene **(1.12)**, lime, crude or fuel oil emulsions, coal-tar creosote, carbolic acid (phenol; **1.13**), etc. (Roonwal, 1979). The compositions were applied by spraying, dipping, brushing, immersing or pressure impregnation, depending on the material being treated and its intended purpose (Hickin, 1971; Pearce, 2000).

Some researchers have utilized the inherent chemical functionality of wood to assist in its modification. They have gone beyond a simple impregnation process and have performed reaction between wood preservatives with the available hydroxyl sites within the wood structure (Marney & Russell, 2007). The dimensional changes due to atmospheric moisture can be minimized either by reducing water absorption and swelling, bulking the fibres to reduce water holding capacity or by cross-linking the cellulose chains of the component fibres. These methods include treating wood with various etherifying and esterifying agents, acetals, alkylene oxides and alkoxysilane coupling agents (Deka & Saikia, 2000; Deka *et al.*, 2002).

However, serious environmental pollution has been caused by our excessive dependence on synthetic chemicals. Biodegradation products of insecticides, sometimes displaying much higher toxicity than their parent insecticides themselves, have entered the environment, including water resources, and have caused important damage and health risk (Wimmer *et al.*, 2006). Recently, the use of insecticides such as organochlorine and organophosphor has been restricted due to the impacts on our health and the environment (Ohmura *et al.*, 2000; Yatagai *et al.*, 2002). For example, Malaysia Pesticide Board has banned the import of chlordane **(1.14)** because it affects nervous system, digestive system and liver in people and animals. Large amounts of chlordane taken by mouth can cause convulsions and death in people (Lee & Chung, 2003).

Although the preservative chromated copper arsenate (CCA) is highly effective to protect wood against wood-destroying organisms, the perceived environmental hazards of the disposal of such metals may limit the future use of it. Indeed, the availability of CCA-treated lumber has been greatly reduced in Hawaii, and CCA use has been restricted in many European countries, the United States, and Japan (Schultz & Nicholas, 2002; Arango *et al.*, 2006; Schultz *et al.*, 2006). Moreover, extensive use of insecticides has led to insecticide resistance. In Malaysia, insecticide resistance in *Culex quinquefasciatus* mosquitoes, German cockroaches and house flies has been reported. To date, many populations of the German cockroaches in Malaysia are resistant to pyrethroid **(1.15)** and carbamate insecticides (Lee & Yap, 2003).



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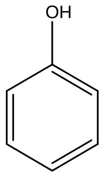

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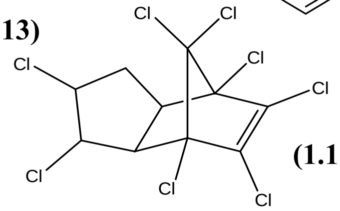

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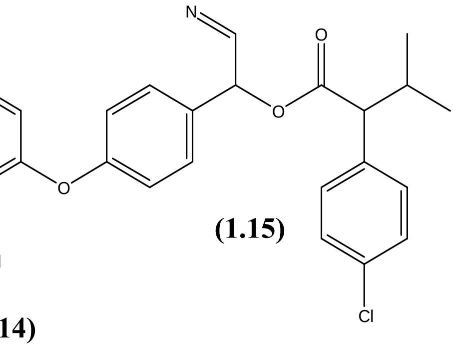

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Now, due to growing environmental concern and increasing scientific knowledge, legal restrictions to some conventional processes have altered the situation. Several alternatives to commonly used synthetic pesticides have been suggested, including cultural methods, biological control, selection of resistant crops, physical barriers and the use of natural insecticides or repellents. Bio-pesticides can be biochemical or microbial. Biochemical may include plant-derived pesticides (botanicals) that can interfere with the growth, feeding, or reproduction of pests or insect pheromones applied for mating disruption, monitoring or attract-and-kill strategies. Microbial pesticides contain a microorganism such as a bacterium, virus, fungus, protozoan or an alga as an active ingredient to control pests (Akhtar *et al.*, 2007). For example, three different species of hydrogen cyanide-producing rhizobacteria were found to be effective in killing the subterranean termite, *Odontotermes obesus* under *in vitro* conditions

(Devi *et al.*, 2007). Since microbials are mostly living organisms and might be more sensitive to climatic conditions, adequate handling requires a greater knowledge (Mai *et al.*, 2004).

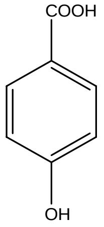
More information on termite control, including wood and soil treatment, fumigation, baiting, physical and biological control as well as safety guide can be found in the reviews by Hickin (1971), Roonwal (1979), Olkowski *et al.* (1991), Pearce (2000), Lee and Chung (2003).

1.2 Plant Ecology

Insects are the most widespread animals, representing one of the most important components of the ecosystem. With increase in the human population, some insect species have become important food competitors of humans and/or vectors of dangerous diseases. Insect species, often vectors of serious diseases, have migrated to the new climatic areas, where their natural predators are not yet present. To ensure food resources and health protection, humans have always tried to control insect pest population densities (Wimmer *et al.*, 2006).

A number of insect species are dependent on plants, either as symbiotes or predators. In turn, plants and trees have evolved elaborate biochemical defence mechanisms to protect themselves from pathogen invasion and insect herbivory. This is especially true in tropical environments because there is no frost season to keep pest populations down. Often these biochemicals are synthesized when the plant is under attack in order to keep invaders out and may accumulate throughout the plant in response to a local injury (Edwards & Wratten, 1980; Bradshaw *et al.*, 1991; Arango *et al.*, 2006). For instance, many monocotyledon plants have lignin **(1.3)** to which various aromatic acids, predominantly *p*-hydroxybenzoic **(1.16)**, ferulic **(1.17)** and *p*-coumaric **(1.18)** acids are esterified. Evidence strongly suggests that esterification of phenols to cell-wall materials is a common theme in the expression of resistance and the presence of phenols in host cell walls is usually taken to imply an increase in resistance to fungal enzymes as well as a physical barrier against pathogens and insects penetration (de Ascensao & Dubery, 2003; Nutt *et al.*, 2004; Dey *et al.*, 2005; Cvikrova *et al.*, 2006).

Due to the fast reproductive process in insects resulting in several or even many generations per year, insects have become resistant to most conventional insecticides which display medium to high toxicity towards warm-blooded animals, fish and different invertebrates. By using plant-derived extracts (phytochemicals) which have long been a subject of research in an effort to develop alternatives to conventional insecticides, the environmental impact would be minimal, yet still toxic to the insects (Arango *et al.*, 2006; Wimmer *et al.*, 2006; Akhtar *et al.*, 2007). It is, however, the analytical problem in plant-animal interaction is difficult because of the very limited amounts of biological material at the disposal of the phytochemist. For example, in following the fate of the phytochemicals in insects feeding on plants, it is necessary to analyse different organs of the insects to see where the compounds are stored; such analyses are often complicated and time-consuming (Harborne, 1998).



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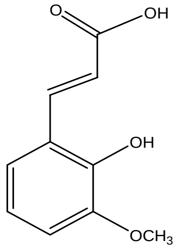

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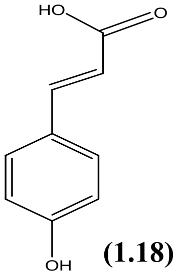

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1.2.1 Diversity in secondary plant substances

Plants are very rich in chemicals which are apparently not directly connected with the normal metabolic processes of photosynthesis, respiration and growth. These secondary chemicals, as they are called, also occur in animals but over 80% of all known natural products are of plant origin. This richness of phytochemicals must be related at least partly to the immobility of plants; since they cannot escape environmental pressures by moving, the only defensive mechanisms are their physical structures and chemical composition (Edwards & Wratten, 1980). Secondary metabolites are present in all plants generally as mixtures that can be highly diverse. Secondary metabolite diversity could be a resistance trait in two ways. One is that each compound in the mixture has a particular herbivore or pathogen target. Another is that the compounds are functioning all together as a mixture that is effective against one or several plant consumers. In this case, compounds interact synergistically or additively (Millar & Sims, 2000). If the compounds work additively, plant consumer adaptation to the mixture would be very difficult or delayed. If the compounds work synergistically, the plant could use the mixture to yield the same effect as a higher concentration of a single compound (Castellanos & Espinosa-Garcia, 1997).

1.2.2 Variation in plant vigour on resistance

Although the capacity to produce particular secondary metabolites is genetically determined, plants show a wide variation in the quantities of these substances produced at different stages in their life and under different environmental conditions. Such variation may have important implications for their ability to resist insect attack (Edwards & Wratten, 1980). Variation occurs in biologically active compounds, specifically flavonoids, sterols, alkaloids and phenolic acids, which are known to influence host plant acceptance, selection, and feeding by phytophagous insects (Jordon-Thaden & Louda, 2003).

The natural resistance of Alaskan yellow-cedar wood is mostly caused by the nootkatone oil **(1.19)** and the presence of allelochemicals, such as terpenoids, quinones and phenolics. The oil, which is a known biocide, creates a distinct aroma in heartwood. It is thought that as the tree gets older, the oil will dry up or evaporate, making the tree more susceptible to attack by fungi and termites (Arango *et al.*, 2006). Besides, Machrafi *et al.* (2006) also reported that the toxicity of the phenolic extracts decreased from fresh to the older bark residues. The highest concentration of total phenolic compounds was from fresh bark; most of these were soluble in water or 0.1M NaOH. For older bark residues, the total phenolic content depended on solvent strength, generally in the order of 2.0M NaOH> 0.1M NaOH = hot water > cold water.

In general, plants which are under stress and so growing less than optimum are those most susceptible to attack. Recently, it has been suggested that in poorly growing plants, the production of protective secondary compounds is greatly altered and reduced. If the function of certain secondary compounds is to protect a plant against insect attack, then we may expect that a plant under stress will be less able to devote photosynthetic metabolites to producing these chemicals and so will be more vulnerable to attack. In forests, for example, isolated outbreaks of phytophagous insects usually occur on the least vigorous and slowest-growing trees (Edwards & Wratten, 1980).

The important role of stand characteristic (the altitude, steepness and orientation of the slopes, soil type) in the decline of spruce *Picea sylvestris* caused by pathogenic fungi *Gremmeniella abietina* was already established. The nutrient conditions of the soil are important, and it is evident that massive outbreaks of defoliating insects usually occur on the most nutrient-poor soils. Vulnerability of Norway spruce and Scots pine to pathogen invasion and insect herbivory was also reported to depend on the contents of Cu and Ni in the humus horizon and on its pH (Edwards & Wratten, 1980; Cvikrova *et al.*, 2006).

Thus, it is difficult to separate cause and effect; a plant may be growing badly because it is heavily infested by insects or it is under some other stress. Probably in many cases both factors are operating at the same time (Edwards & Wratten, 1980).

1.3 The Natural Durability of Timber

Concerns over the safety and environmental impacts of chemically treated wood have increased demand for naturally durable wood products. While the demand is increasing, the nature of the forest resource that will be used to meet this demand is changing from older and larger trees, which contain wood with a proven history of durable performance, to younger and smaller trees whose wood properties are not as well understood. Changes in the use of wood products in housing applications, and expansion of the areas that can be threatened by termites are putting additional demands on the performance criteria for wood products. Research to better understand the effects of changes in wood properties on resistance to termites and decay fungi could lead to more rational utilization of naturally durable timber (Taylor *et al.*, 2006).

Beal *et al.* (1974) has categorized two species of mangroves, i.e. *Laguncularia racemosa* and *Rhizophora brevistyla* to be resistant and moderately resistant, respectively, against feeding of subterranean termites *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki. Their result of 8-week force-feeding test on 97 species of tropical woods is given in Table 1.1. The higher survival is reflected in the greater weight loss of wood samples. From changes in their protozoan population, termites that survived on the resistant and moderately resistant woods appeared to be starving. The population was generally normal for termites in tests having high survival. In addition, they also reported that *C. formosanus* survived better and damaged more wood than did *R. flavipes*. In starvation checks, *R. flavipes* lived only for approximately 4 weeks, whereas *C. formosanus* had 56% survival after 8 weeks. Pearce (2000) has also mentioned that more aggressive termites such as *Coptotermes* will replace other termites such as *Reticulitermes* where food resources are restricted.

Anti-termite activity has been observed in many hardwood (Angiosperm) species (Grace *et al.*, 1996). Unfortunately for the users of naturally durable wood products, termite and decay resistance varies significantly among individual pieces of wood and this variability can be difficult to predict (Taylor *et al.*, 2006). Natural resistance of wood to termite attack seems to be correlated with wood species that have a higher specific gravity (Arango *et al.*, 2006). A study carried out by Peralta *et al.* (2004), which focused on wood consumption rates of different forest species by termites under field conditions, did not find a strong correlation between wood density and termite resistance. The authors did, however, acknowledge the importance of wood hardness as a deterrent to termite damage, concluding that wood density alone cannot be considered the single most important factor in determining termite resistance.

Carter *et al.* (1975) and Yalinkilic *et al.* (1998) have stated that the resistance of wood to termite attack is mainly determined by the presence of chemicals such as phenolics in the lignocellulosic tissue. The heartwood of some tree species, e.g. *Chamaecyparis nootkatensis* and *Thuja plicata* is naturally resistant to termite and fungal attack, largely due to the presence of non-structural chemical “extractives” such as quinones, flavonoids and terpenoids that possess natural repellent and toxic properties (Harborne *et al.*, 1999; Arango *et al.*, 2006; Taylor *et al.*, 2006). It has also been suggested that extractives from naturally durable woods could be applied to susceptible timbers or used as models for new wood preservatives (Grace *et al.*, 1996). For instance, phenolic extractives which are well known to have fungicidal, antioxidant and metal chelating properties can be envisaged to protect wood against fungi and termites attack through combination with an organic biocide. This is because wood degradation by fungi often involves various metals, either in free form or as key components of enzymes (Yalinkilic *et al.*, 1998; Schultz & Nicholas, 2002; Schultz *et al.*, 2006; Gao *et al.*, 2007).

Table 1.1 Resistance levels of 97 species of tropical woods after 8-week force-feeding test by subterranean termites *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki.*

Resistant (little or no damage to wood blocks)		
Group 1[a]	**Group 2**[b]	
Caryocar costaricense	*Brosimum* sp. (prob.)	*Myroxylon balsamum*
Caryocar sp.	*Calycophyllum candidissimum*	*Ocotea dendrodaphne*
Diphysa robinioides	*Cariniana pyriformis*	*O. rodiei*
Enterolobium cyclocarpum	*Cedrela mexicana*	*Paramachaerium gruberi*
Eschweilera sp. (prob.)	*Centrolobium orinocense*	*Pithecellobium mangense*
Gliricidia sepium	*Colubrina glandulosa*	*P. saman*
Guarea longipetiola	*Conocarpus erectus*	*Platymiscium pinnatum*
Hippomane mancinella	*Cordia alliodora*	*Pouteria campechiana*
Manilkara chicle	*Dalbergia retusa*	*P. chiricana*
Pentaclethra macroloba	*Guajacum officinale*	*Swartzia simplex*
Swartzia panamensis	*Hymenaea courbaril*	*Sweetia panamensis*
Terminalia catappa	*Laguncularia racemosa*	*Swietenia macrophylla*
Vitex floridula	*Lecythis ampla*	*Tabebuia chrysantha*
Vochysia ferruginea	*Licaria pittieri*	*T. guayacan*
	Luehea seemannii	*Trichilia tuberculata*
	Minquartia guianensis	
Moderately Resistant (little or no damage to wood blocks)[c]		
Andira inermis	*Copaifera aromatica*	*Manilkara bidentata*
Aspidosperma megalocarpon	*Coumarouna oleifera*	*Manilkara* sp.
Astronium graveolens	*Dialium guianense*	*Rhizophora brevistyla*
Brosimum panamense (prob.)	*Dicorynia paraensis*	*Symphonia globulifera*
Calophyllum brasiliense	*Genipa americana*	*Terminalia amazonia*
Cassia moschata	*Lafoënsia punicifolia*	*Ternstroemia seemannii*
Chlorophora tinctoria	*Licania arborea*	*Vatairea* sp. (prob.)
Chrysophyllum cainito	*Lonchocarpus* sp.	
Susceptible		
Moderate damage to wood blocks[d]	**Heavy damage to wood blocks**[e]	
Avicennia marina	*Anacardium excelsum*	*Trattinickia aspera*
Byrsonima crassifolia	*Bursera simaruba*	*Virola koschnyi*
Carapa slateri	*Carapa* sp.	*V. sebifera*
Cornus disciflora	*Cedrela* sp.	
Hura crepitans	*Croton panamensis*	
Hyeronima alchorneoides	*Cupania* sp.	
Magnolia sororum	*Dialyanthera otoba*	
Mora oleifera	*Erythrina glauca*	
Nectandra whitei	*Phoebe johnstonii*	
Pelliciera rhizophorae	*Pinus caribaea*	
Terminalia myriocarpa	*Prioria copaifera*	
Tetragastris panamensis	*Quercus* sp.	
Zanthoxylum belizense	*Sterculia apetala*	

[a] No survival for both the species.

[b] 23% survival for *C. formosanus*; 0% for *R. flavipes*.

[c] 50% survival for *C. formosanus*; *R. flavipes* survived only on *R. brevistyla* (9%) and *S. globulifera* (14%).

[d] 61% survival for *C. formosanus*; *R. flavipes* survived only on *T. myriocarpa* (3%), *M. oleifera* (6%), *C. disciflora* (33%) and *N. whitei* (52%).

[e] 68% survival for *C. formosanus*; 44% for *R. flavipes*.

* Beal *et al.* (1974).

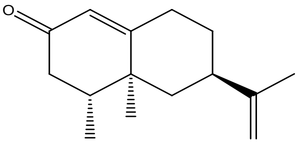
Owing to the great diversity of the extractives, no generalized procedures are adequate for isolation and determination of individual compounds. Moreover, the constituent which imparts resistance may be confined to a particular genus or species of wood and may be present only in minute quantities (Carter *et al.*, 1975; Roonwal, 1979; Pearce, 2000).

1.4 Secondary Plant Substances as Toxins

A large number of plant products have shown different type of biological activities, either in their natural forms or in their conjugated forms (glycosides, glycoproteins, etc.) which enable easier internal transportation of these natural products (secondary metabolites) in plant organisms (Wimmer *et al.*, 2006). Some of the defensive secondary metabolites are low molecular weight phenolic or terpenoid compounds; others are proteins, which are harmful to the invading pathogen or grazing herbivore (Bradshaw *et al.*, 1991; de Ascensao & Dubery, 2003; Cvikrova *et al.*, 2006). A brief summary of the major groups of secondary plant chemicals, their taxonomic distribution and biological activity is given in Table 1.2.

Extractives within a piece of wood can range from low molecular weight volatile compounds to large polymers and it appears that not all of these components are equally important in determining natural durability. There is evidence that the volatile fraction (essential oil) is particularly effective in reducing insect and termite attack because it is likely to be the front line defence of a plant to herbivores (Cheng *et al.*, 2004; Cheng *et al.*, 2007). For instance, essential oil from yellow-cedar heartwood, i.e. nootkatone **(1.19)** and carvacrol **(1.20)** are having insecticidal and/or acaricidal activity toward various agricultural, stored products, or medicinal arthropod pests (Kelsey *et al.*, 2005).

Lower molecular weight terpenoids have been identified as the most common anti-termite compounds in various wood species by Pearce (2000), Zhu *et al.* (2001) and Watanabe *et al.* (2005). For example, thujaplicin **(1.21)** in *T. plicata* and nootkatin **(1.22)** in *C. nootkatensis* are the active substances. In addition to these terpenoids, there are many other compounds in *T. plicata* and *C. nootkatensis* heartwood that make up the extractive mixture which may also contribute to extensive natural resistance (Taylor *et al.*, 2006).



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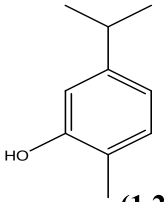

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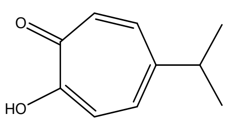

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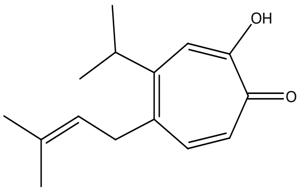

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Table 1.2 Major classes of secondary plant compounds involved in plant-animal interactions.*

Class**	Distribution	Physiological Activity
	Nitrogen compounds	
Alkaloids (5500)	Widely in angiosperms, especially in root, leaf and fruit	Many toxic and bitter-tasting
Amines (100)	Widely in angiosperms, often in flowers	Many repellent smelling; some hallucinogenic
Amino acids (non-protein; 400)	Especially in seeds of legumes but relatively wide-spread	Many toxic
Cyanogenic glycosides (30)	Sporadic, especially in fruit and leaf	Poisonous (as HCN)
Glucosinolates (75)	Cruciferae and ten other families	Acrid and bitter (as isothiocyanates)
	Terpenoids	
Monoterpenes (1000)	Widely, in essential oils	Pleasant smells
Sesquiterpene lactones (600)	Mainly in Compositae, but found increasingly in other angiosperms	Some bitter and toxic, also allergenic
Diterpenoids (1000)	Widely, especially in latex and plant resins	Some toxic
Saponins (500)	In over 70 plant families	Haemolyse blood cells
Limonoids (100)	Mainly in Rutaceae, Meliaceae and Simaroubaceae	Bitter-tasting
Cucurbitacins (50)	Mainly in Cucurbitaceae	Bitter-tasting and toxic
Cardenolides (150)	Especially common in Apocynaceae, Asclepiadaceae and Scrophulariaceae	Toxic and bitter
	Phenolics	
Simple phenols and phenolic acids (200)	Universal in leaf, often in other tissues as well	Anti-microbial and insect antifeedant
Flavonoids and tannins (1000)	Universal in angiosperms and ferns	Bitter-tasting and feeding deterrent
Xanthones (36)	Occasional occurrence in Leguminosae, Loganiaceae, Lythraceae, Moraceae, Polygalaceae and Rhamnaceae	Insecticidal and anti-termite activity
Quinones (500)	Widely, especially Rhamnaceae	Coloured, irritant and insect/termite repellent

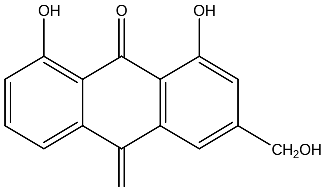
* Harborne *et al.* (1999); modified.
** Approximate number of structures in parentheses.

This phenomenon is mentioned in the report by Regnault-Roger *et al.* (2004). Plants of the family Lamiaceae (Labiatae) exhibited the most pronounced insecticidal effects among aromatic plants. This plant family is well known for its high essential oil content besides a large number of polyphenolic compounds. Even after extraction of essential oils which had an insecticidal effect, some residual extracts were still capable to protect the plants against insect damage. Consequently, it appeared that other plant components, considerably polyphenolics were responsible for this effect. In fact, antifeedant effect has been observed for polyphenolic acids, like ferulic acid **(1.17)** and *p*-coumaric acid **(1.18)** against the maize weevil, *Sitophilus zeamaìs* Motschulsky, and the large grain borer, *Prostephanus truncatus* (Horn).

1.4.1 Phenolics as natural insecticides/termiticides

In the plant kingdom, phenolic compounds play diverse roles and transform into several derivatives, including free radical scavengers (antioxidants), phytoalexins (antimicrobial), coumarins (oral anticoagulants), lignin (cell-wall strength/fortification), various flavonoids, phenolic acids and condensed tannins (feeding deterrents). As such, the branches of phenolic metabolism are under complex regulation mediated by plant development and biotic/abiotic stress. It has become evident in recent years that phenolic compounds play a widespread role in natural plant protection against phytophagous insects and phytopathogenic fungi by affecting the activity of various enzymes. It reduces insect damage through antifeedant effects. A large range of insects belonging to different orders appearing to be sensitive, including aphids, Isoptera, Lepidoptera, Orthoptera, and Diptera (Lege *et al.*, 1995; Regnault-Roger *et al.*, 2004). Besides, it is evident that the more abundant of phenolics in the leaves and other tissues, the more distasteful they are to animals. Thus, phenolics serve a useful role in deterring herbivory (Harborne *et al.*, 1999). Higher concentrations of some of these compounds can be induced in plants by insect feeding (Nutt *et al.*, 2004).

Several publications devoted to plant phenolics underline their role in termite resistance. Quinones such as aloe-emodin **(1.23)**, chrysophanol **(1.24)**, deoxylapachol **(1.25)**, 1,4-dihydroxy-2-methylanthraquinone **(1.26)** and tectoquinone **(1.27)**; and xanthone such as macluraxanthone **(1.28)** have shown potent anti-termite activity (Harborne *et al.*, 1999). Natural stilbene, pinosylvin (3,5-dihydroxystilbene; **1.29**) reportedly had feeding deterrent activity against the West Indian dry wood termite *Crytotermes brevis* (Walker). Stilbene glycosides and methylated stilbenes extracted from the bark of *P. glehnii* revealed large feeding deterrent ability against *Reticulitermes speratus* (Kolbe) when experimented using paper disc tests (Shibutani *et al.*, 2004). Furthermore, flavonoid, castillen E **(1.30)** showed concentration-dependent feeding deterrent, but was not toxic to *C. brevis*. Fecundity, mortality, and food consumption of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki were also evaluated in response to glyceollin **(1.31)**, genistein **(1.32)**, biochanin A **(1.33)**, apigenin **(1.34)** and quercetin **(1.35)** (Boue & Raina, 2003). Additionally, Ohmura *et al.* (2000) and Chen *et al.* (2004) documented that other flavonoids such as taxifolin **(1.36)** and dihydroflavonols were also effective in controlling *C. brevis* and *C. formosanus* Shiraki.



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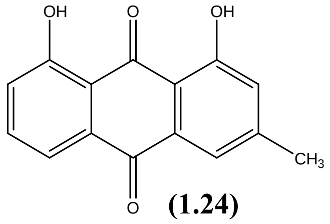

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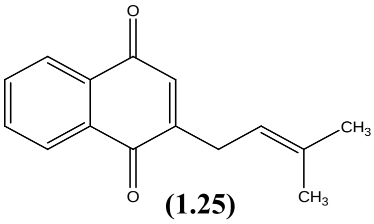

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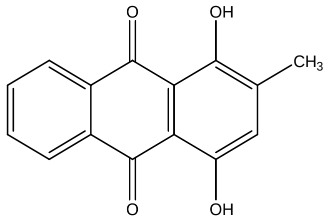

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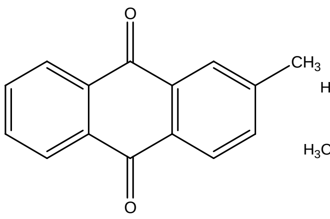

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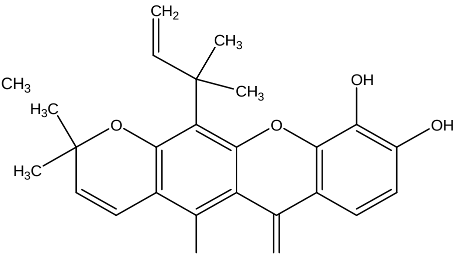

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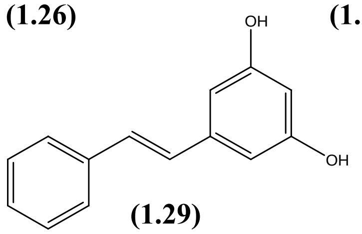

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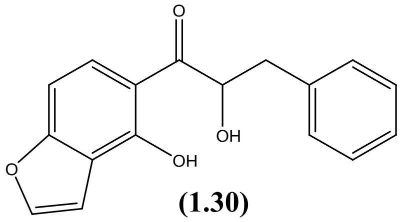

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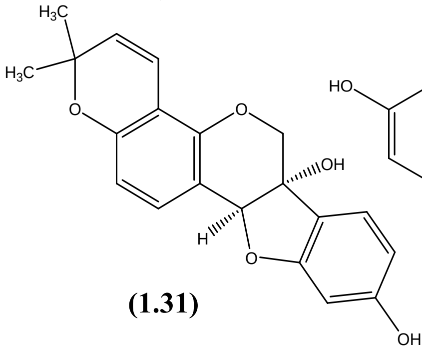

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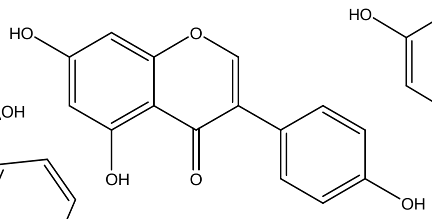

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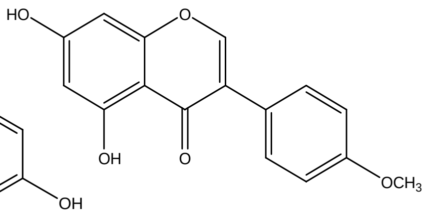

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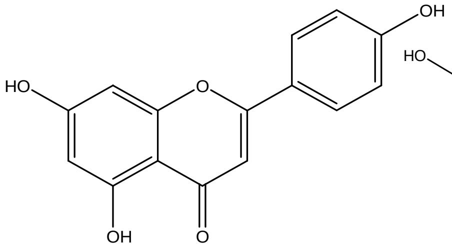

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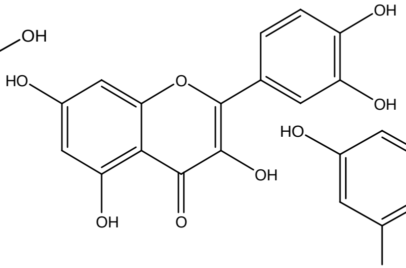

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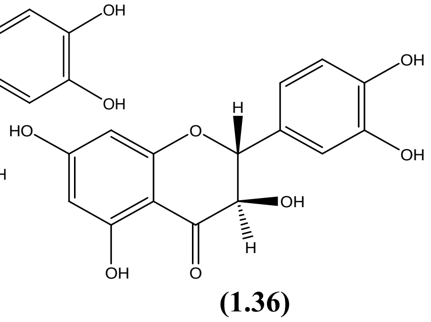

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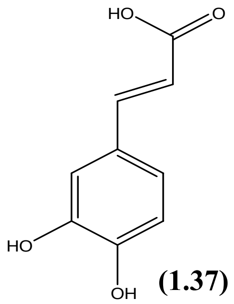
1.4.1.1 Phenolic acids

To the best of our knowledge, there is no or scarce documentation about termiticidal activity of phenolic acids although their insecticidal effects have been studied so far. Of non-volatile compounds at least phenolic acids may contribute to sensory properties. Phenolic acids are known to have sour, bitter and astringent flavours and the non-bound (free) phenolic acids are suggested to be most flavour-active (Dimberg *et al.*, 1996; Koseki *et al.*, 1996; Heinio *et al.*, 2007). These objectionable and repellent tastes are thus thought to act as insect feeding deterrents (Lege *et al.*, 1995; Jordon-Thaden & Louda, 2003; Bitzer *et al.*, 2004).

Phenolic acids can affect insect behaviour. For example, reduced feeding by various grasshoppers and planthopper, *Peregrinus maidis*, was correlated with high concentrations (dry weight) of total phenolic acids. Ferulic acid **(1.17)** was also shown to be involved in maize resistance to the weevil *Sitophilus zeamais*. More than half of the common phenolic acids tested reduced feeding of the Mexican bean beetle (Coccinellidae) when sprayed on to the leaves of its preferred host, the common bean *Phaseolus vulgaris*. *Cirsium vulgare* contains phenolic acids such as *p*-hydroxybenzoic **(1.16)**, ferulic **(1.17)**, *p*-coumaric **(1.18)**, caffeic **(1.37)**, protocatechuic **(1.38)** and vanillic **(1.39)** acids that could influence host choice behaviour and show biological activity towards insects as antifeedant (Jordon-Thaden & Louda, 2003). Additionally, Lege *et al.* (1995) suggested that resistance in cotton (*Gossypium hirsutum*) to spider mite (*Tetranychus urticae*) might be attributed to high levels of the condensed tannins and phenolic acids such as *p*-coumaric **(1.18)** and syringic **(1.40)** acids.



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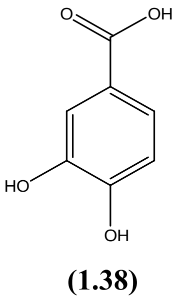

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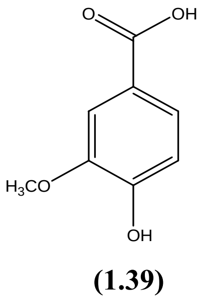

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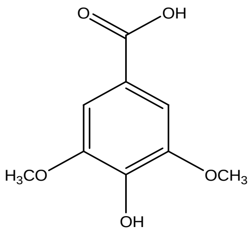


(1.40)

The granary weevil is a pest in granaries throughout the world. It attacks wheat, maize, sorghum, rice and other cereals. All these grains have phenolic acids that have been implicated in cereal resistance against stored grain insects. High concentrations of these acids cause lower grain consumption and affect survival, weight gain and larval development in stored grain insects negatively (Castellanos & Espinosa-Garcia, 1997).

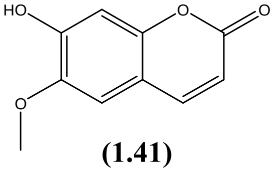
Many phenolic acids are effective pest deterrents or are precursors to more complex molecules that convey resistance. Caffeic acid **(1.37)** concentrations increase in *Verticillium*-infected cotton plants, which suggest that this compound may serve as a stress metabolite. Plants infected by any of a number of microorganisms reportedly have increased concentrations of scopoletin **(1.41)**, a derivative of ferulic acid **(1.17)**. In addition, cotton squares infested with *Helicoverpa zea* larvae had higher concentrations of cinnamic and benzoic acid derivatives. The larvae which fed on synthetic diets containing cinnamic acid **(1.42)** had higher mortality, lower pupa weights and delayed pupation compared to the control larvae (Lege *et al.*, 1995).

Regnault-Roger *et al.* (2004) had conducted a study on Mediterranean aromatic plants with insecticidal properties. The hydro distillate fraction of five species of Lamiaceae showing insecticidal effects on *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) was analyzed to determine if polyphenols were involved in the toxic effect. The polyphenols tested were toxic to the beetles to different degrees. Caffeic **(1.37)**, ferulic **(1.17)** acids and vanillin **(1.43)** induced a knockdown effect on the first day, gallic acid **(1.44)** on the second day. Quercetin **(1.35)** significantly decreased natural mobility from the first day; syringaldehyde **(1.45)**, vanillic **(1.39)** and syringic **(1.40)** acids after the 4th day. On the 8th day all compounds caused significant mortality. The beetles were either paralyzed or were dead at the end of the experiment. Enclosed in a limited area and without any possibility of escape, the insects experienced a cumulative toxic effect. On the other hand, they also found that sensitivity differs between species. The aphid *Rhopalosiphum padi* (L.) was more sensitive to methoxy compounds like estragole **(1.46)** or anethole **(1.47)** than to phenolic compounds, while the fruit fly *Ceratitis capitata* (Wiedemann) reacted equally to both types of compounds. In contrast, *A. obtectus* appears to respond particularly to compounds with a phenolic configuration.

Furthermore, Bitzer *et al.* (2004) carried out bioassays with collembolan *Ceratophysella denticulate* as well as with the specialized collembolan predator *Stenus comma*

(Staphylinidae). They found that starved *S. comma* attacked living *C. denticulate* would immediately spit them out after contact. This underlined the existence of deterrent substances either in the integument or on the body surface of *C. denticulata*. Two phenolic acids were identified, i.e. 3-hydroxy-4,5-dimethoxybenzoic acid **(1.48)** and 4-hydroxy-3,5-dimethoxybenzoic acid or syringic acid **(1.40)**. Staphylinid beetles topically treated with these acids try to clean their mouthparts by rubbing them on the ground significantly more often than do control beetles. Both compounds individually and as a natural mixture have deterrent effects towards the predator *S. comma*. In other insects, syringic acid also shows deterrent effects. Adams and Bernays (1978) who used syringic acid in feeding experiments with *Locusta migratoria* observed that the feeding rate was reduced at the high concentrations.

Glucose **(1.4)** and quinic acid esters **(1.49)** of several hydroxybenzoic **(1.16)** and cinnamic **(1.42)** acids were identified in methanolic extracts of the leaves of 15-day-old *Sorghum bicolour* together with two *O*-glycosides. Some of the phenolic acids were also found as insoluble esters of cell wall polysaccharides. While these derivatives did not affect the feeding of *Locusta migratoria* on sorghum, the free acids, as a mixture, were markedly inhibitory. It was found that *Sorghum* contained a mixture of hydrolases which could effect the transformation of "inactive" phenolic esters and glycosides into "active" phenolic acids at a high enough concentration to significantly reduce feeding by *Locusta* (Woodhead & Cooper-Driver, 1979).



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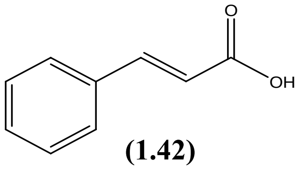

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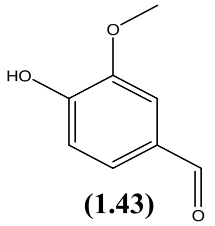

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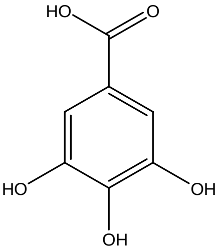

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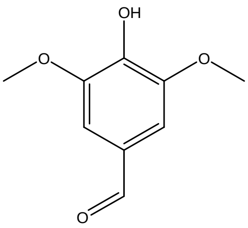

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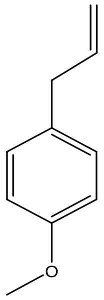

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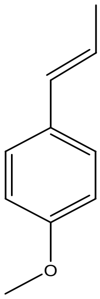

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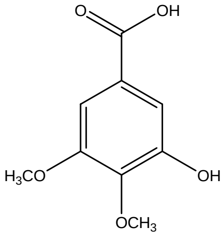

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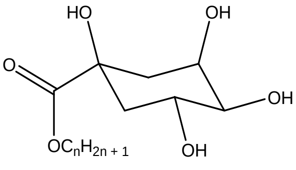

(1.49)

1.5 Urbanization and Pest Control in Malaysia

Over the last two decades, Malaysia has been transformed from an agricultural-based country to one that emphasizes on manufacturing sector. In the midst of this transformation, many areas in this country have been urbanized, and migrations of human population from rural to urban areas have been rampant. Many plantation lands are converted to erect urban structures and buildings. With the rapid increase in physical development and population in many towns in Malaysia, urban pest problems have also increased in a prolific manner (Lee & Chung, 2003; Lee & Yap, 2003). A typical example is with *Coptotermes* in Malaysian rainforest, originally a damp forest genus that has become a serious pest of houses (Pearce, 2000).

Urban pest control started about 50 years ago with the establishment of the first pest control company in Malaysia. However, there are not many institutions in this country researching on urban pests. Currently, only two institutions have active urban/medical pest control research programmes, namely Universiti Sains Malaysia and the Institute for Medical Research (Lee & Chung, 2003; Lee & Yap, 2003).

1.6 Termites as Pests

Termites are considered to be amongst the most destructive insect pests in the world. It is estimated that the annual damage caused to buildings and structures is amount to several billions of US dollars (Olkowski *et al.*, 1991; Cheng *et al.*, 2007). In Malaysia, termite-related pest control efforts alone amounted to 50% of the total pest control operations. It was estimated at RM40 million yearly. To effectively control termites, it is important to understand the biology and behaviour of these insects. This will also help pest control operators to utilize the appropriate chemicals and application techniques to prevent buildings and structures from damage (Lee & Chung, 2003; Lee & Yap, 2003).

1.6.1 Biology and behaviour of termites

Termites are social insects in the order Isoptera – from the Greek “iso,” meaning equal, and “ptera,” meaning wing – so named for the equal length and similar texture of the wings of the reproductive termites. It is generally known that termites damage a variety of materials ranging from paper fabrics to even non-cellulosic materials such as asbestos, asphalt bitumen, lead, and metal foils (Cheng *et al.*, 2007). The habit of eating cellulose **(1.1)** and lignin **(1.3)** is different among termites owing to the presence of specific symbiotic protozoa and bacteria in a particular termite genus (Hickin, 1971; Pearce, 2000; Katsumata *et al.*, 2007). They are highly organized as a big colony, relying on chemical and sensory messages for communication and defence which enable them to exist in total darkness. Termites' sense organs (e.g. chemoreceptor) are able to distinguish between salt and sugar, as well as between many plant chemicals. This is one reason why some woods are resistant to termites (Pearce, 2000). To the general public, termites are very often misidentified as ants. The characteristics which can be used to differentiate between the two different insects are listed in Table 1.3. They inhabit approximately 70% of the world geographical areas, mainly in the tropical and subtropical regions, extending to some areas in temperate latitudes. There are about 2500 species of termites; however, only a few species are known to be pests to urban structures. The Rhinotermitidae (especially *Reticulitermes* and *Coptotermes* in America, Europe and Asia) and Macrotermitinae (especially *Macrotermes*, *Odontotermes* and *Microtermes* in Africa and Asia) constitute most of the pest species (Pearce, 2000).

Table 1.3 Major differences between ants and termites.*

Features	Ants (Hymenoptera)	Termites (Isoptera)
Metamorphosis	4 stages	3 stages
Longevity of Fertile Males	Short-lived	Long-lived
Workers	Females only	Both sexes
Integument (colour)	Mostly sclerotized (dark)	Mostly unsclerotized (light)
Antennae	Elbowed	Straight, thread- or beadlike
Front and hind wings	Dissimilar size	Equal size
Cerci	Absent	Present
Waistline	Constricted	Broad
Food habits	Variable	Cellulose-based
Distribution	Cosmopolitan	Warm climates

* Schabel (2006); Table 6.1, p. 180.

Ecologically, termites are important recyclers of cellulosic materials, returning the carbonaceous and other compounds in trees to the soil for reuse. They serve also as food source to other insects, animals and even humans, thus they play an important role in nutrient recycling in the forest. In fact, termites are the most dominant organisms in tropical rain forests.

Their populations can be as high as 2000–10000 individuals per m^2. Termites are polymorphic, i.e. one species has several castes, each differing in appearance and performing different tasks with the indisputable logic of a computer. These include workers, soldiers and reproductive members. Factors affecting caste formation include gender, pheromones, nutrition and/or sensory stimuli. With the exception of some queens, that may reach a length of over 100 mm at maturity, termites tend to be small to medium-sized insects of less than 25 mm (Hickin, 1971; Olkowski *et al.*, 1991; Lee & Chung, 2003; Schable, 2006).

1.6.1.1 The life cycle of termites

Termite colonies are initiated when swarms of winged male and female reproductives leave a mature nest, usually in summer in warm temperate areas and during the rainy season in tropical and warm desert areas. Termites pair off, shed their wings and excavate a nest in or near a source of wood. After constructing a chamber, the pair mates and the female begins laying eggs, producing from a few hundred to tens of thousands (depending on the species) over her many-year lifetime. The eggs hatch within a few weeks or months (depending on the species), and the emerging larvae, or nymphs, mature over a period of two or six months. Most become workers or soldiers. When the nest reaches its maximum population, some of the nymphs mature into winged reproductives, and the cycle is repeated (Olkowski *et al.*, 1991).

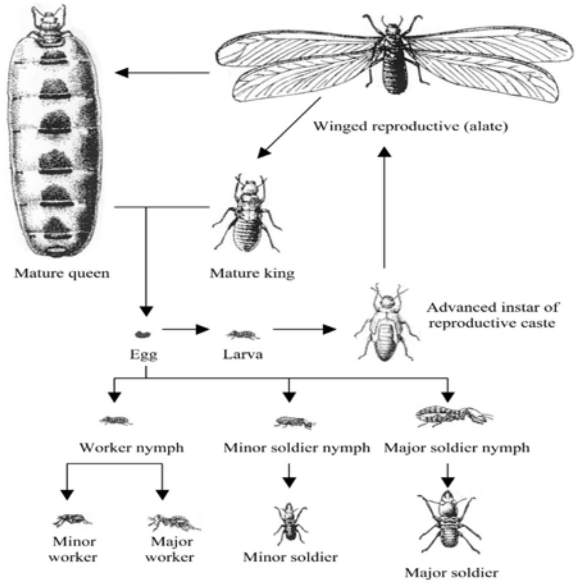

Figure 1.1 Schematic life cycle of *Macrotermes* sp. (Termitidae), showing all castes except secondary reproductives (Schabel, 2006; Figure 6.11, p. 196).

1.6.2 Classification of termites (nest type)

Termites can be classified as dry-wood (Kalotermitidae), damp-wood (Holotermitidae and Termopsidae) or subterranean termites (Rhinotermitidae and Termitidae), depending on their habitat. The subterranean termite is probably the most important among the three groups as they cause the most damage to human property. In the USA, approximately US$2 billion is spent annually on termite control, out of which 80% is attributed to the control of subterranean termites (Lee & Chung, 2003; Lee & Yap, 2003).

1.6.2.1 Subterranean termites and their infestation in Malaysia

About 175 species have been discovered, but less than 10% are pest species. In the urban and suburban environment, five species of termites are important, namely *Coptotermes gestroi*, *Coptotermes curvignathus*, *Coptotermes havilandi*, *Coptotermes kalshoveni* and *Coptotermes sepangensis*. A study conducted in 1998 demonstrated that more than 90% of all termite damages in buildings in central Peninsular Malaysia were caused by *Coptotermes*, while the remaining were by other peridomestic species (e.g. *Macrotermes gilvus*, *Microtermes pakistanicus*, *Globitermes sulphureus*, *Schedorhinotermes* sp. and *Microcerotermes* sp.).

Table 1.4 Common locations of infestation of subterranean termites within buildings and structures in Malaysia (n=124).*

Location	% total infestations found
Door/window frame	35
Parquet floor	30
Baseboard/skirting area	5
Built-in wall cabinet	5
Roof/ceiling	10
Bathroom area	10
Others	5

* Lee and Chung (2003); Table 9.1, p. 104.

Termite infestation is currently a major problem of residential premises in Malaysia. This is clearly reflected by the fact that 65% of the pest control services provided for termite control were carried out in residential premises, compared to 20% in the industrial sectors (e.g. factories and warehouses), 10% in commercial buildings (office complexes, shopping malls, etc.) and 5% for other locations. On common locations of infestation by subterranean termites within buildings and structures in Malaysia, wooden doors and window frames, and parquet floors were most prone to attack (Table 1.4). Another common location is the wooden frames around the bathroom door where moisture is high (Lee & Chung, 2003).

1.6.2.2 Genus *Coptotermes*

The genus *Coptotermes* (family Rhinotermitidae) is widespread in the tropical and subtropical regions of the world. All species of *Coptotermes* consume wood, and 28 species are important structural pests, the largest number for any termite genus (Jenkins *et al.*, 2007). This subfamily of "milk exuding" termites consists of small, pale yellowish brown species whose soldiers have a pear-shaped head with a small mid-dorsal tubular projection from which a white liquid (a liquid in a mucopolysaccharide) is exuded for defence purposes. These termites are less than 5 mm long and highly destructive to wood-work (Roonwal, 1979; Pearce, 2000; Schabel, 2006). *Coptotermes* feeding rate is greater than that of the other main subterranean pests, such as *Reticulitermes* (Beal *et al.*, 1974). This means that *Coptotermes* can cause more damage. It was found that one colony of *Coptotermes* in the USA could produce over 60,000 night-flying alates and therefore pose a major pest problem if they become established and form colonies (Pearce, 2000).

Coptotermes species are readily transported by human commerce in part, e.g. ships' timbers, due to their habit of constructing carton material for nests and for filling aboveground excavations and voids. Carton is comprised of soil and cellulose admixed with faecal and salivary secretions, and its ability to retain moisture is likely to enhance the survival of these termites when inadvertently transported in infested materials. As a result, some of them have become pests far from their place of origin (Schabel, 2006; Jenkins *et al.*, 2007).

Coptotermes gestroi (Wasmann) (synonym, *Coptotermes havilandi* Holmgren per), the Asian subterranean termite (AST), is the primary pest species of *Coptotermes* originating from the Indo-Malayan Region; its native distribution is from Assam through Burma and Thailand down to Malaysia and the Indonesian archipelago. It is an important structural and agricultural pest that has become established in many areas of the world and is one of the most important urban pests in Southeast Asia (Jenkins *et al.*, 2007).

1.6.3 Determinants in the natural resistance of trees/woods

The factors which determine the natural durability of various trees/woods to termites are not well understood, but several factors seem to be involved. It should be borne in mind that no tree species carries absolute immunity against termites. Specific decay factor and condition of a particular resistant and durable timber must also be stated. In general, these factors include species and condition of trees, species of termite, soil type, food alternatives, moisture content, altitude, temperature and other climatic elements; and very often more than one factor is operating (Hickin, 1971; Roonwal, 1979; Schabel, 2006). Some of them have been discussed previously in Section 1.2.2. Preferences and resistance will vary with the hardness, lignin content, chemical constituents and even pH of the wood content. Lower extractive content has been correlated with reduced termite and fungal resistance (Taylor *et al.*, 2006). Sapwood, which has more starch and sugar, is generally preferred by termites than heartwood. *Trinervitermes* have been known to eat wood when grass availability was reduced. In such condition, the termites have no choice and must eat the wood fibre or die. Besides, salt-tolerant shrubs, such as species of *Sueda* and *Halopeltis*, can also be resistant (Pearce, 2000).

1.6.4 Termite defence and adaptation

Termites have evolved a complex social structure, formidable defence responses, and adaptive behaviour toward pathogen infected individuals (Devi *et al.*, 2007). Cannibalism is common in termites where the food source is low, and often appendages are bitten off. If dead termites are not eaten, they may be sealed off or become part of the nest in mounds of soil. This is one reason why low doses of biological agents have been unsuccessful as the uninfected individuals seal themselves off from those which have been infected (Pearce, 2000).

Several excellent details of termite biology, ecology, economic significance, food selection, etc. are given in Hickin (1971), Roonwal (1979), Pearce (2000) and Schabel (2006).

1.7 Mangroves' Ecological, Socio-Economic Values and their Challenges

Mangrove forests are specific wetlands, developing in the inter-tidal zone, that have a high adaptation capacity to extreme environmental conditions (from fresh to hyper saline