STUDY ON ANTIOXIDANT ACTIVITIES AND ANTIFUNGAL PROPERTIES OF SOME MALAYSIAN TIMBERS FROM SELECTED HARDWOODS SPECIES

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by

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ABBREVIATIONS

CC	Column Chromatography
$CDCl_3$	Deuterated Chloroform
CD_3OD	Isotope methanol
CH_3OH	Methanol
$C_4H_{10}O$	Butanol
$CH_3\ (CH_2)_4CH_3$	*n*-Hexane
$CH_3COOCH_2CH_3$	Ethyl acetate
COSY	Total correlation spectroscopy
D_2O	Heavy water
DMSO-*d*6	Dimethyl sulphoxide (deuterated)
DPPH	1, 1-Diphenyl-2-Picryl-Hydrazyl
EC	Epicatechin
EGC	Epigallocatechin
EGCG	Epigallocatechin gallate
GC	Gas Chromatography
HHW	Heavy Hardwood
HMBC	Hetero-nuclear Multiple-Bond Connectivity
HPLC	High Performance Liquid Chromatography
H_3PO_4	Phosphorous acid
IBA	Indole butyric acid
LOO●	Lipid peroxyl radical
MHW	Medium Hardwood

NMR	Nuclear Magnetic Resonance
NP-HPLC	Normal-phase High Performance Liquid Chromatography
$OH^{\bullet}$	Hydroxyl radical
$O_2^{\bullet}$	Superoxide radicals
PAH	Polycyclic aromatic hydrocarbons
ROS	Reactive Oxygen Species
RP-HPLC	Reverse-phase High Performance Liquid Chromatography
SW	Softwood
TLC	Thin Layer Chromatography

ABSTRACT

Study on Antioxidant Activities and Antifungal Properties of Some Malaysian Timbers from Selected Hardwoods Species

The antioxidant activities, total phenolic compound and antifungal activities were investigated from selected Malaysian hardwood species. The species studied include keranji kuning kecil, durian, gerutu-gerutu, machang-machang, medang kemangi, medang daun lebar, sesendok, meranti pipit, meranti sarang punai, petai-petai, meranti melantai, ara berteh bukit, chengal, kembang semangkok jantung, mempelam, mersawa kuning and mersawa kuning kecil. All samples were divided into 3 parts; bark, heartwood and sapwood. The antioxidant activity was assessed by scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) while total phenolic compound was analyzed using Folin-Ciocalteu method. The antifungal activities were tested with *Gleophyllum trabeum* (MI-102) and *Pycnoperus sanguineus* (KUM 70097). The results showed that, mempelam heartwood were found the highest antioxidant activity with EC_{50} value 4.71 ± 0.89 μg/ml (n = 2). The antifungal activities with *Gloeophyllum trabeum* (MI-102) showed an inhibition zone on cengal (bark), mempelam (heartwood), sesendok (inner), meranti pipit (bark), and all parts of medang kemangi while in *Pycnoporus sanguineus* (KUM 70097) showed an inhibition zone on keranji kuning kecil (sapwood), machang (bark), meranti melantai (bark), cengal (bark), mersawa kuning (bark) and all parts of medang kemangi. Mempelam (*Mangifera indica*) heartwood was continued for further separation, isolation and purification using thin layer chromatography (TLC), column chromatography (CC), high performance liquid medium (HPLC) and nuclear magnetic

resonance (NMR). Two compounds were obtained from this sample which are 4",6"-*O*-*trans*-di-*p*-coumaroyl-4'-*O*-β-glucopyranosylgallocatechin and Iriflophenone-2-*O*-b-glucopyranoside.

ABSTRAK

Kajian Terhadap Aktiviti Antioksidan dan Sifat-sifat Antikulat Beberapa Kayu Keras Terpilih di Malaysia

Kajian dilakukan untuk mengetahui kandungan aktiviti antioksidan, jumlah fenol dan sifat-sifat antikulat yang terdapat di dalam kayu keras di Malaysia yang dipilih secara rawak. Spesies kayu yang terlibat di dalam kajian ini ialah keranji kuning kecil, durian, gerutu-gerutu, machang-machang, medang kemangi, medang daun lebar, sesendok, meranti pipit, meranti sarang punai, petai-petai, meranti melantai, ara berteh bukit, chengal, kembang semangkok jantung, mempelam, mersawa kuning and mersawa kuning kecil. Setiap sampel diasingkan kepada 3 bahagian iaitu kulit, kayu teras dan kayu gubal. Kaedah yang digunakan untuk mengetahui kandungan antioksidan ialah, dengan menggunakan 1, 1-diphenyl-2-picrylhydrazyl (DPPH), manakala kandungan fenol ditentukan dengan menggunakan kaedah Folin-Ciocalteu. Dua jenis kulat yang telah digunakan untuk mengkaji aktiviti antikulat terhadap setiap sampel kayu ialah *Gleophyllum trabeum* (MI-102) dan *Pycnoperus sanguineus* (KUM 70097). Keputusan menunjukkan bahawa bahagian kayu gubal mempelam mengandungi kandungan antioksidan yang paling tinggi berbanding dengan sampel yang lain dimana nilai EC_{50} ialah 4.71 ± 0.89 μg/ml (n = 2). Keputusan antikulat pula menunjukkan kayu cengal (kulit kayu), mempelam (kayu teras), sesendok (kayu bahagian dalam), meranti pipit (kulit kayu), dan semua bahagian pada medang kemangi tahan terhadap kulat *Gleophyllum trabeum* manakala kayu yang tahan terhadap kulat *Pycnoperus sanguineus,* ialah keranji kuning kecil (kayu gubal), machang (kulit kayu), meranti melantai (kulit

kayu), cengal (kulit kayu),mersawa kuning (kulit kayu) dan semua bahagian pada medang kemangi. Kayu yang mempunyai antioksidan yang paling tinggi iaitu Mempelam (*Mangifera indica*) kayu gubal diteruskan dengan menggunakan kaedah kromatografi iaitu TLC, CC dan HPLC, disusuli denagn penetuan struktur menggunakan NMR. Berdasarkan kaedah tersebut dua sebatian diperolehi daripada sampel ini iaitu 4'',6''-*O*-*trans*-di-*p*-coumaroyl-4'-*O*-β glucopyranosylgallocatechin and Iriflophenone-2-*O*-b- glucopyranoside.

1.0 INTRODUCTION

1.1 General Background

In wood extractive, there is also substantial amount of antioxidant and antifungal compound present. This antioxidant and antifungal are important part of extractive that could be utilized for other purpose such as medicinal, preservative, etc.

Antioxidant is a compound that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction (Zheng & Wang, 2001). It can be determined using pure extractive and tested with 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH). Source of natural antioxidants are primarily plant phenolics, which may occur in all parts of the plants such as fruits, vegetables, nuts, seeds, leaves, roots, and barks (Pratt, 1980).

Phenolic and polyphenolic compounds on the other hand constitute the main class of natural antioxidants (Aruna *et al.*, 2000; Cruz *et al.*, 2005). Phenols are known as one of the most important plant constituents and it has the ability to scavenge radicals. The total phenolic content may contribute directly to the antioxidant action. Most of the works dealing with natural products use gallic acid or catechin as standards (Price *et al.*, 1978; Duh *et al.*, 1999). Total phenol was usually analyzed using Folin-Ciocalteu method (Aruna *et al.*, 2000; Cruz *et al.*, 2005).

Plant pathogens include fungi, nematodes, bacteria, and viruses, which can cause diseases or damage in plants (Montesinos, 2003). Among these pathogens, fungi are the main pathogens and caused many diseases of plants. Pathogenic fungi also caused yield losses in numerous economically important crops (Fletcher *et al.*, 2006). Several fungi have been found to induce post-harvest spoilage of sweet potato, which is associated with decrease in starch, total sugar and organic acid (Ray and Ravi, 2005). Diseases caused by fungi are also the serious problem in forest management.

Due to biological nature, unprotected wood is susceptible to discoloration and biological deterioration, which reduce its mechanical and physical properties (Chang and Cheng, 2002). Developing methods that prolong the service life of wood has always been interest to wood researchers. From environmental perspective, finding naturally occurring constituent in highly durable tree species and understanding their mechanisms are the most appropriate approaches to achieve wood protection while preserving the environment (Chang *et al*., 2000). Therefore, extraction of natural compound having specific bioactive and/or medicinal properties from plants is an important application of natural product research.

Due to the unique ecosystem, there are many hardwoods species from Malaysian timbers in this Malaysia. Results obtained from previous studied showed that *Shorea macroptera* (Meranti melantai) had a significant weight loss against termites while *Neobalanocarpus heimii* (Cengal) showed no significant termite effect. Study by Kenzi (2000) indicated a weight loss against termites in *Shorea macroptera*. *Mangifera indica* (mempelam) commonly grown in many parts of the world. Its seed had been used for anti-diarrhoeal activity in Indian traditional medicine (Sairam *et al*., 2002).

To our best knowledge, there is not much research investigating the antioxidant activities and antifungal properties of methanol extracts from Malaysian timbers. For this reason, the study was conducted to study the antioxidant activity and antifungal properties from some of Malaysian timbers. The best result obtained from this antioxidant activity or antifungal properties was isolated and identified by column chromatography (CC), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). In addition, the structure of chemical compound was examined by nuclear magnetic resonance (NMR). In this study *Mangifera indica* (mempelam) heartwood was chosen for further separation, isolation, purification and identification.

1.2 Objectives

The objectives of the present work are

1. To evaluate the antioxidant activities from the selected hardwoods species of Malaysian Timbers.

2. To study the antifungal properties from the selected hardwoods species of Malaysian Timbers.

3. To study the structure of chemical compound from the highest antioxidant activities or antifungal properties among the selected hardwood species of Malaysian Timbers.

2.0 LITERATURE REVIEW

2.1 Malaysian Timbers

Malaysian timbers are classified into four categories, which are Heavy Hardwood (HHW), Medium Hardwood (MHW), Light Hardwood (LHW), and Softwood (SW). The characteristic between the terms, Hardwood and Softwood, is based on the normal botanical convention. The classification of the three categories of hardwoods is based on the average density of the timbers at 15% moisture content. The error to this rule is for the Heavy Hardwood category, which emplaces priority on natural durability over density. For example, Merbau, this has the characteristic of the heartwood being naturally durable, having an average air-dry density of 800 kg /m^3, and classify as a HHW. On the other hand, Kempas, having a higher average density of 890 kg/m^3, and classified as a MHW as the heartwood was found to be not durable. The difference between Medium and Light Hardwoods was based only on average density (Malaysia Timber Industry Board, 2002). The classification system was summarized in the Table 1.

Table 1. Classification of Malaysian Timbers

Classification	Density Range at 15% moisture content
Heavy hardwood	800 – 1120 kg/m^3
Medium hardwood	720 – 880 kg/m_3
Light hardwood	400 – 720 kg/m_3
Softwood	botanical distinction

In this study, randomly 15 species of Malaysian timbers were selected. The 15 species was chosen from hardwood species.

The terms hardwood usually is from trees of angiosperms class with broad leaves. This trees grown in tropical climate and grows faster than softwood, and have shorter fibers compared to softwood. Figure 1 showed the zones of wood within a tree.

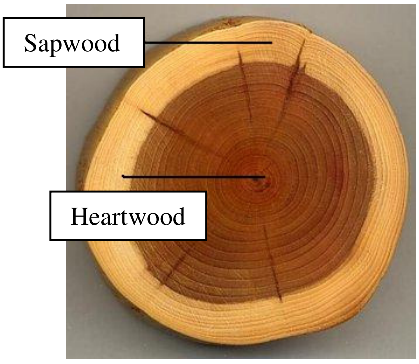


Figure 1. Heartwood and sapwood in the tree

The two zones above are distinguished as heartwood and sapwood. The center of the tree called heartwood, consisting of dormant wood and appear in dark colored compared to the sapwood. Sapwood is the fluid part of the tree that moves up from the roots through the outer portion of the trunk and branches and contributes to its growth. The acetyl content is higher in sapwood compared to heartwood (Cronquist, 1982).

Although heartwood and sapwood are so different in their properties, the only histological difference between them is that in the transformation from sapwood and heartwood the xylem vessel may become blocked by numerous bladder-like ingrowths (Cronquist, 1982).

2.1.1 Family of selected hardwoods species from Malaysian Timbers

In this study, seven families randomly selected from hardwood species of Malaysian Timbers, which are Anarcardiaceae, Dipterocarpaceae, Euphorbiaceae, Leguminosae, Moraceae, Sterculiaceae and Lauraceae.

Anarcardiaceae is a tree with caustic resin that usually turns black on exposure. Their leaves alternate or rarely opposite, also simple or compound, estipulate. Their flowers are small and in panicles. Sepals and petals in this family often have five (or three to seven) each. Their fruits very various and generally have one large seed or stone, and leathery or pulpy rind. Figure 2 below showed the properties of Anarcardiaceae (Hsuan, 1969).

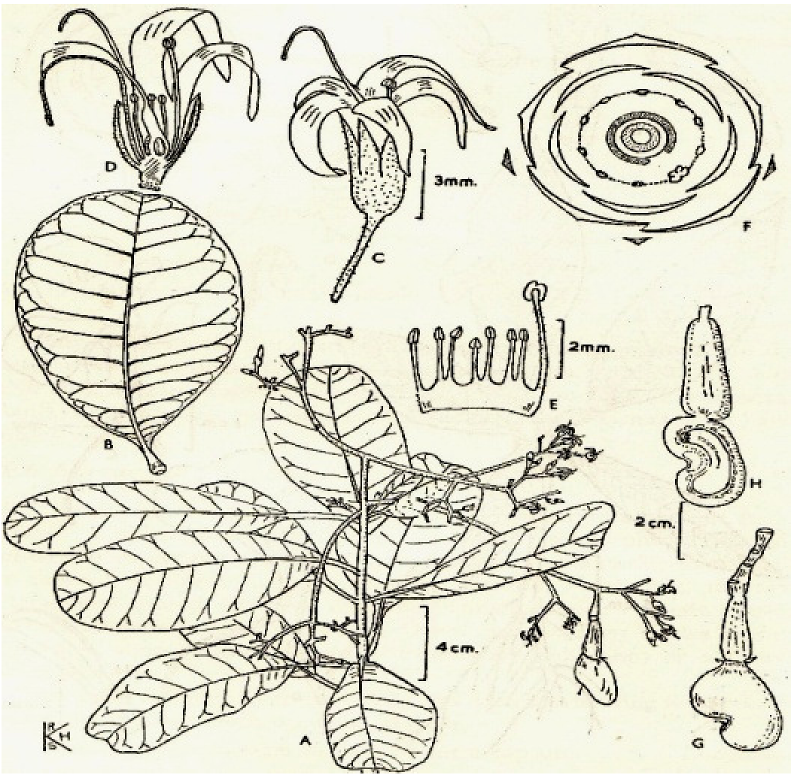


Figure 2. **A**. Branch with flowers and fruits; **B**. Leaf; **C**. Flower; **D**. Half-flower; **E**. Androecium expanded; **F**. Floral diagram; **G**. Fruit with swollen; **H**. Section of G

A few species yield edible fruits (e.g. the mango, *Mangifera indica* L. and the hog-plums). *Mangifera indica* (mempelam) and *Mangifera longipes* (machang-machang) were used in this study.

Mangifera indica grows in tropical and sub-tropical regions of the world and it is commonly use in folk medicine for a wide variety of remedies (Coe and Anderson, 1996). The chemical composition of this plant has been well studied and the extract yield triterpenes, flavonoids, phytosterols and polyphenols, in general (Anjaneyulu *et al*., 1994; Khan *et al*., 1994). The bark and seeds act as astringents (Nunez-Selles *et al*., 2002). In the traditional Indian system of medicine, mangiferin is also used for melancholia and nervous debility (Bhattacherya *et a*l., 1972). Mangiferin is also reported to possess antitumor, antiviral, antidiabetic, antibone resorption, immunological modulator (Moreira *et al*., 2001) and antioxidant activities (Sa'nchez *et al*., 2000). In this research *Mangifera indica* were tested for antioxidant activities and antifungal properties on their bark, heartwood, and sapwood. There was no previous research that have focused on parts of the wood.

Mangifera longipes (machang-machang) was not studied previously. From this study, we will investigate the ability of antioxidant activities and antifungal properties of *Mangifera longipes* (machang-machang) on their bark, heartwood, and sapwood.

Dipterocarpaceae is the most important in Malaysian timber, also known as resinous trees and often gigantic. Their leaves are simple and alternate and the flowers are bisexual, regular, and panicle. Five sepals were present that are free or connate and have five petals are contorted, free, or shortly connate at the base. They also have ten stamens to numerous (rarely five) and normally have three-ovulate; ovules two to several in each locule, on axile placentation; style base often enlarged forming a stylopodium. Their fruits mostly have one-seeded nut, usually winged by two, three, or five enlarged sepals. These families do not have endosperm, which are their cotyledons of the embryo often folded or twisted. Figure 3 showed the properties of Dipterocarpaceae and Figure 4 showed the properties of Dipterocarpaceae fruits (Hsuan, 1969).

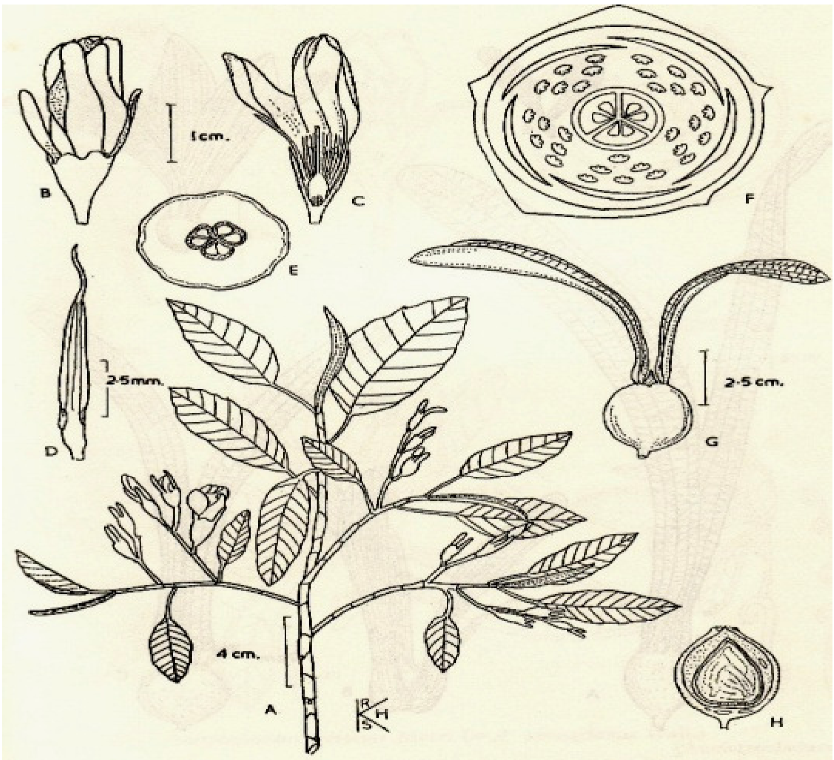


Figure 3. **A**. Branch with flowers; **B**. Flower; **C**. Half-flower; **D**. Stamen; **E**. Cross-section of ovary; **F**. Floral diagram; **G**. Fruit (a nut with winged calyx); **H**. Section of fruit

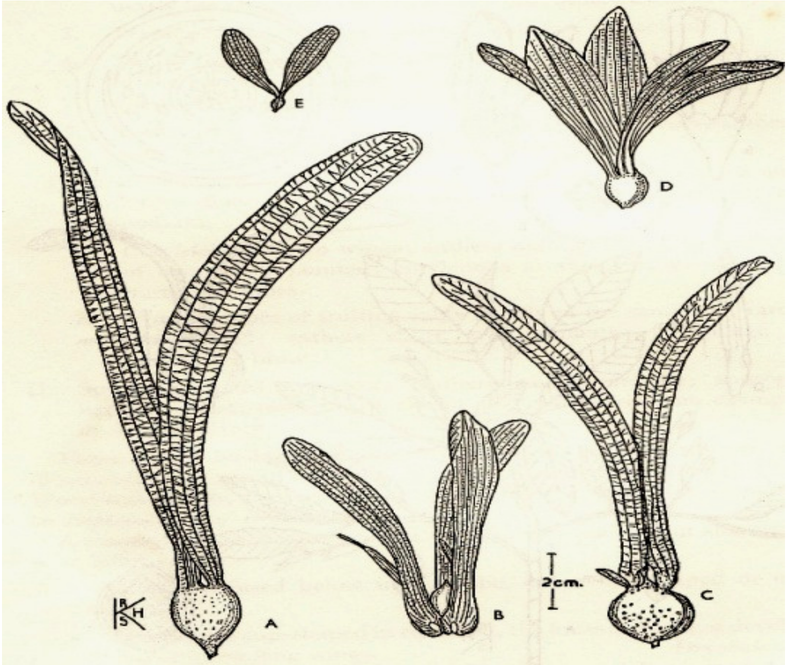


Figure 4. **A**. *Anisoptera megistocarpa* V. Slooten; **B**. *Shorea macroptera* Dyer;
C. *Dipterocarpus penangiana* Foxw. ; **D**. *Drybalanops aromatica* Gaertn;
E. *Hopea mengarawan* Miq.

Dipterocarpaceae have many species among the tallest trees in the lowland forests. They produce the most valuable timbers in Malaya, commercially known as meranti and balau (*Shorea*), keruing (*Dipterocarpus*), chengal (*Balanocarpus*), kapur (*Dryobalanops aromatica* Gaertn.), merawan (*Hopea*), mersawa (*Anisoptera*), resak (*Vatica*) etc. They yield very useful for resins as known as dammar and oil.

In this study, several species were investigated from this family, which are gerutu-gerutu (*Parashorea stellata*), meranti pipit (*Shorea assamica*), meranti sarang punai (*Shorea parvifolia*), mersawa kuning (*Anisoptera curtisii*), meranti melantai (*Shorea macroptera*) and chengal (*Neobalanocarpus heimii*).

Based on previous study, wood species of a heavy hardwood *Neobalanocarpus heimii* and a light hardwood *Shorea macroptera* Dyer were used in decomposition experiments with termite-exclusion and control trays on the forest floor of the Pasoh

Forest Reserve, West Malaysia to determine effect of wood quality on termite-mediated wood decay. *Shorea macroptera* (Meranti melantai) had a significant weight loss against termites while *Neobalanocarpus heimii* (Cengal) showed no significant termite effect. Study by Kenzi (2000) indicated a weight loss against termites in *Shorea macroptera*.

Previous research *Shorea macroptera* (Meranti melantai) and *Shorea parvifolia*) meranti sarang punai focused on the about production of planting stocks of several dipterocarp species through rooting of stem cutting. The hormone used for rooting was indole butyric acid (IBA). The commercial formulation suitable for rooting was either Seradix or Plantone-R depending on species. Rooting percentage between 60 to 80% could be obtained with *Dipterocarpus chartaceus, Shorea parvifolia*, *S. ovalis* and *S. sumatrana.* Low rooting of less than 50% was obtained with *S. exelliptica, S. macroptera, S. pinanga,* and *Parashorea densiflora* (Aminah *et al*., 2006).

Not much work has been reported on Gerutu-gerutu (*Parashorea stellata*) and relatively no work was reported on antioxidant activities and antifungal properties in this Dipterocarpaceae family.

Euphorbiaceae is the largest and the most complex family of woody plant in Malaysian, also known as herbs, shrubs or trees and often a laticiferous. Their leaves mostly alternate or stipulate. In this family, flowers usually small, regular, and unisexual. They have five-merous of perianth, one- or two-seriate and there have one stamens to numerous and the disc often present. Their ovary typically have three loculate; ovules one to two in each, pendulous and anatropous. The micropyle usually covered by a caruncle; styles typically three and each of them usually has two-lobed. There have various fruit and typically ripens into a septicidally dehiscent schizocarp (composed of three cocci) with three or six seed (Hsuan, 1969). Figure 5 showed the properties of Euphorbiaceae.

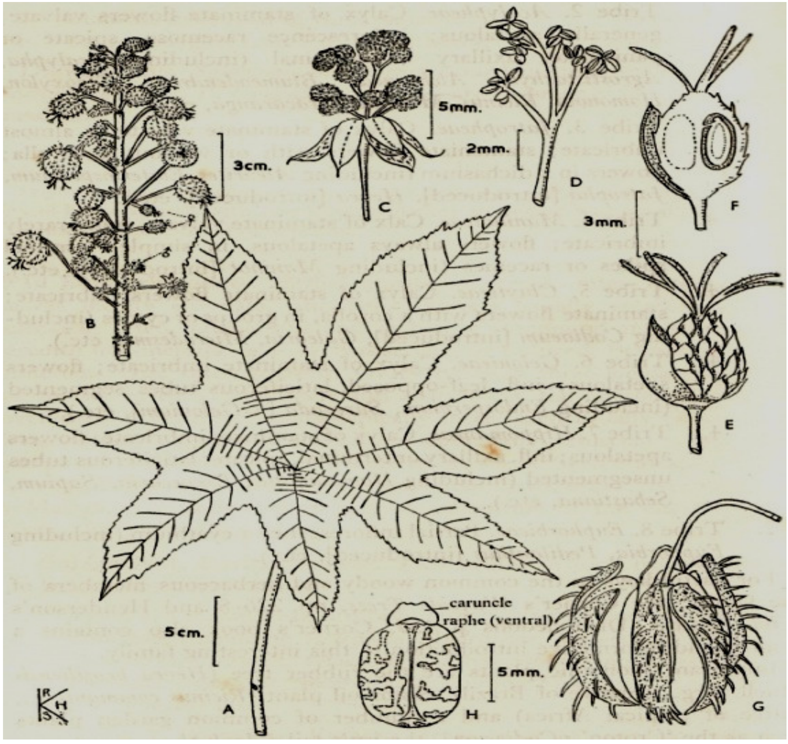


Figure 5. **A**. Leaf; **B**. upper part of panicle; **C**. staminaate flower; **D**. a fascicle of branched stamens; **E**.and **F**. carpellate flower and its section; **G**. fruit; **H**.seed.

In this study, *Endorspermum malaccense* (sesendok) was chosen after randomly selected from Malaysian timbers. This only one species occurs in Peninsular Malaysia, while in Sabah and Sarawak there is another species, *Endorspermum peltatum.* Heartwood of *E.malaccense* is yellow white or grey, without streaks and the color of sapwood is similar to the color of heartwood (Mohd Shukari, 1982). Based on the standard graveyard test of untreated specimens, the wood is classified as non-durable under Malaysian conditions. In a test conducted at the Forest Research Institute Malaysia (FRIM), the average service life for 29 untreated sesenduk was 1 year. When green, the timber is liable to blue-stain fungal infection (Mohd Shukari, 1982). No report has been made on the antioxidant activity of this species.

Leguminosae is the largest plants in Malaysia and the most important family. There have a bigger numbers and extraordinary variety. The plant have long been cultivated from the temperate to the tropical regions for their seed (peas and beans), for their wood, or for their superior ornamental qualities. The most important ones are, as food; the peas (*Pisum sativum* L.), groundnuts (*Arachis hypogaea* L.), soy bean (*Glycine max* Merr.), etc; useful timber; kempas (*Koompassia*), keranji (*Dialium*), merbau (Intsia), sena (*Pterocarpus*), sepetir (*Sindora*) etc; as common ornamentals; butterfly trees (*Bauhinia*), amherstia (*Amherstia nobilis* Wall.), flame-of-the-forest (*Delonix regia* Raf.) yellowflame (*Peltophorum*) many species of *Cassia*, *Caesalpinia*, etc (Hsuan, 1969).

Leguminosae also used as herbs, shrubs or trees. Their leaves showed alternate and often trifoliate or pinnately compound or stipulate. There have variety of flowers, which are, large or small, regular or irregular and usually bisexual and racemose or panicled. The sepals mostly have five (rarely four), free or connate and the petals also five or rarely, fewer or absent. Their stamens typically have ten and sometimes fewer, rarely and numerous. There are one carpel; ovary superior, gynophores often present and their ovules have one too many. The fruit typically a legume, dehiscent or indehiscent and their seed have one too many, usually exalbuminous. Figure 6 showed properties of Leguminosae (Hsuan, 1969).

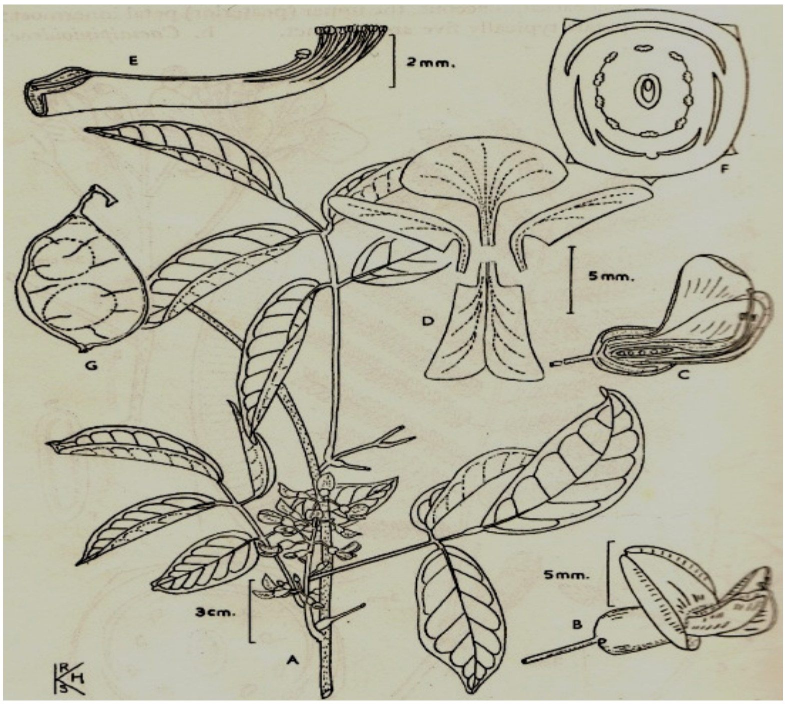


Figure 6. **A**. branch with inflorescence; **B**. flower; **C**. half- flower; **D.** petals separated (one standard; two wings and one keel composed of two united petals); **E**. androecium, showing diadelphous (nine plus one) structure; **F**. floral diagram; **G**. fruit.

In this study, two species were investigated from this family, which are keranji kuning kecil (*Diallium wallichii*) and petai-petai *(Parkia speciosa).*

Based on previous study, petai-petai (*Parkia speciosa*) has been used in folk medicine for its antibacterial activity on kidney, ureter and urinary bladder infection; its effect is due to the presence of several cyclic polysulphides (Fathaiya *et al*., 1994). The seeds have been eaten as food either cooked or raw in both Malaysia and Thailand. People in southern Thailand believe that the beans have an anti-diabetic effect (Wallie *et al*., 1995). Not much research about antioxidant activity and antifungal properties was reported from this species. From this study, we will be able their ability to antioxidant activity and antifungal properties.

No previous study has been conducted on Keranji kuning kecil (*Diallium wallichii*) for antioxidant and antifungal activities.

Moraceae generally is a tree and shrubs or laticiferous. Their leaves is simple and alternate which is the stipules is small or large and the forming a cap over the bud. The flowers are small, unisexual, in cymes or heads, or embedded in an urn-shaped receptacle. The perianths usually have four-lobed or rarely absent and have four stamens, which is opposite the perianth-lobes or reduced to one or two. The gynoecium has two carpellate, which are ovary one-loculate, and one ovulates; styles mostly two. The nut or drupe often formed in a solid mass, or enveloped within a fleshy receptacle. Figure 7 showed properties of Moraceae (Hsuan, 1969).

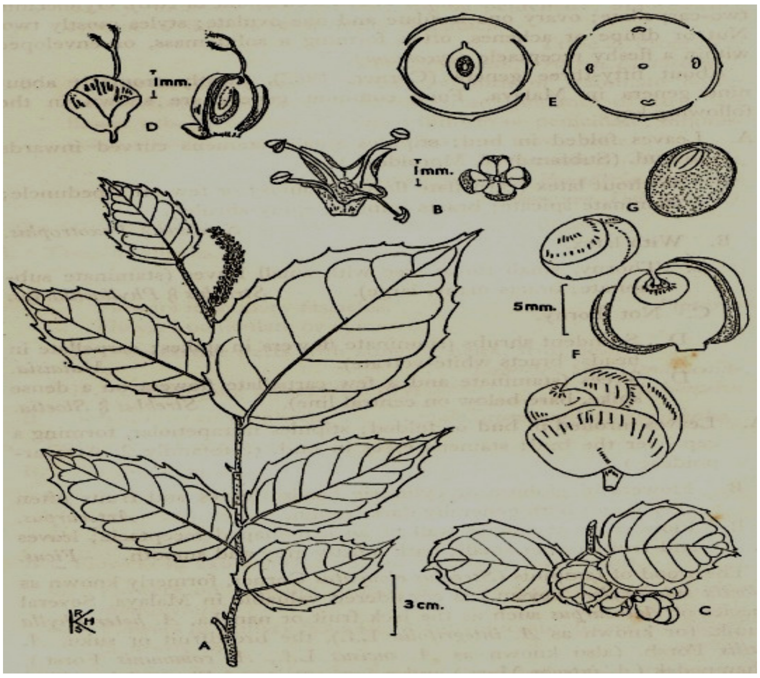


Figure 7. **A**. branch with staminate spike; **B**. staminate flower; **C**. branch with carpellate flowers; **D.** carpellate flower and its section; **E**.floral diagram; **F**.fruit and its section (with the seed removed); **G**. seed and its section.

In this study, ara berteh bukit (*Parartocarpus bracteatus*) was used to this research. No previous study has been reported on the antioxidant activities and antifungal properties on their bark, heartwood, and sapwood of this ara berteh bukit.

Sterculiaceae is a trees that shrubs, also known as herbs. Their leaves are simple or compound and the flowers shown bi- or unisexual. There have calyx in five lobed and have five petals which is small and reduce or lacking. The stamen in two whorls connate and the outer whorl either of staminode or absent and for the inner (antipetalous) which is fertile have two loculate anthers. There have five loculate in the ovaries with two ovules in each. The fruits have a capsule or follicle, and their seed an endospermous. About fifty genera, tropical and subtropical whereas about eighteen in Malaysian. The common Malaysian genera were classified with flower bisexual, andro-gynophore absent and stamina tube short. Figure 8 showed properties of Sterculiaceae (Hsuan, 1969).

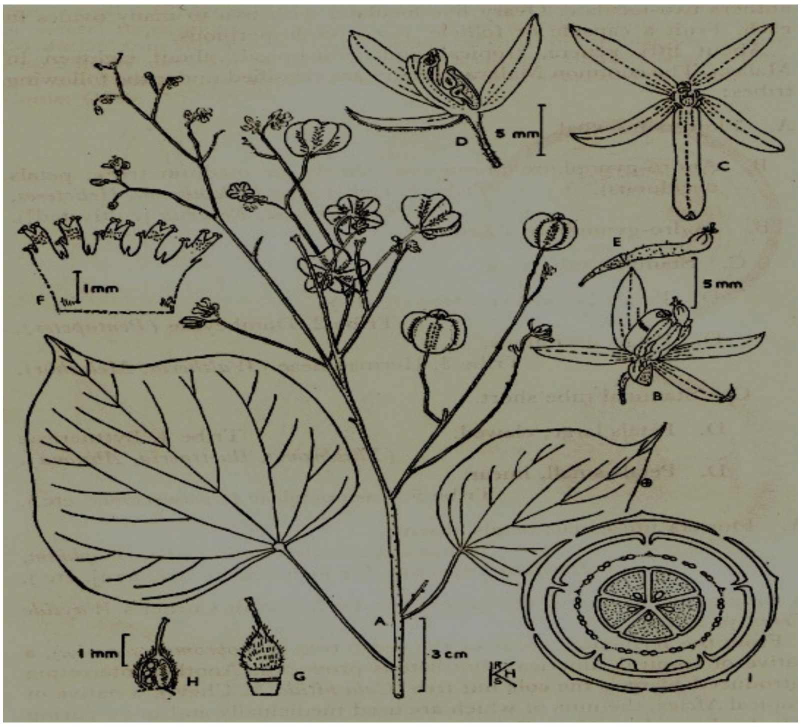


Figure 8. **A**. a branch with flowers and fruit; **B**. and **C**. flower in two different views;**D.**half-flower; **E**.androecium and gynoecium; **F**. filamental tube cut open ; **G**. and **H**. gynoecium and its section; **I**. floral diagram.

In this study, kembang semangkuk jantung (*Scaphium macropodum*) was used. Crown architecture was analyzed for (*Scaphium macropodum*), a common shade-tolerant emergent tree of a tropical rain forest in West Kalimantan, Indonesia. Saplings and poles shorter than 12m in height had no branches. Their leaves gathered at the ends of the stem. The leaves changed from entire to palmate-parted with increasing tree size. The parted leaves increased the light penetration through the clustered foliage. The weight of petiole per blade increased with leaf size and the leaf could not be enlarged. Crown architecture of *Scaphium macropodum* adapted to light environment (Toshihiro and Eizi, 1996). They (Toshihiro and Eizi, 1997) also studied about distribution of fruits and plants, mortality and growth rates of *Scaphium macropodum* (Sterculiacae) in four 1-ha plots in a tropical rain forest in West Kalimantan, Indonesia. The species is a large deciduous tree and produces wind-dispersed fruits on defoliated twigs. The density of dispersed fruits on the ground decreased with increasing distance from a parent tree. The area under the parent's crown had the highest density of the fruits and the highest mortality of the seedlings immediately after germination (Toshihiro and Eizi, 1997).

No research has been reported about antioxidant activity and antifungal properties on this species.

Lauraceae is an aromatic trees or shrubs, which is one species a leafless parasitic twines, *Cassytha*. Their leaves are simples and the flowers are regular, bisexual, or unisexual. The perianths usually have a six lobed, in two series. The stamens have six or nine that is in two to three series (rarely, three or twelve in one or four series) and the anthers open by two or four valves. Their ovary has one-loculate and one ovulates. The fruit in this family is drupaceous and often partly enclosed in the fleshy hypanthium where as the seed did not have an endosperm (Hsuan, 1969).

Over thirty genera, mainly in tropical and subtropical Asia and America and about fifteen were found in Malaysia. In this family include the avocado or alligator pear (fruit of *Persea Americana* Mill., a native of tropical America) which is a very useful product. The bark of *Cinnamomum verum* Presl, formerly C.*zeylanicum* Garc. Ex B1, camphor (from the wood of *Cinnamomum camphora* Nee & Eberm., a native of East Asia); and many valuable timbers, volatile oils, etc. Figure 9 showed properties of Lauraceae (Hsuan, 1969).

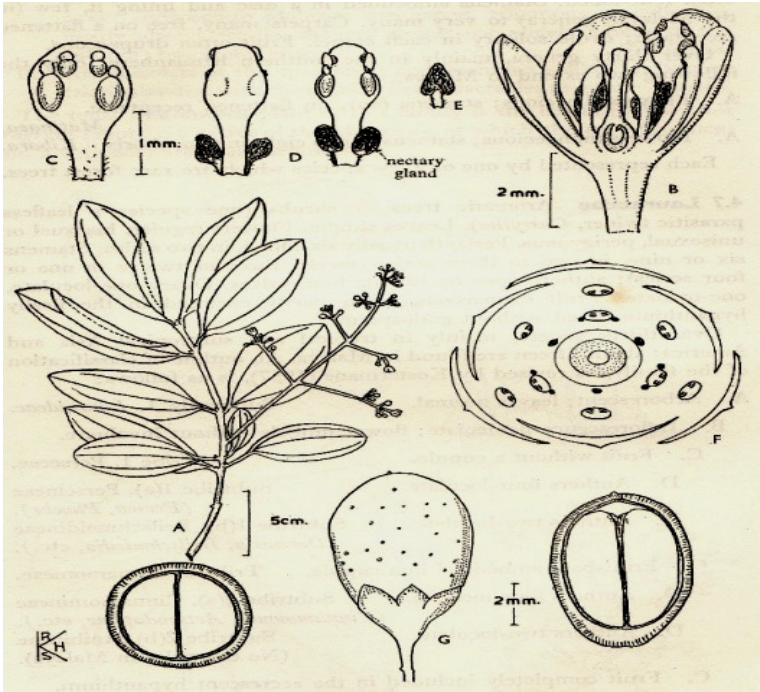


Figure 9. **A**. flowering branch; **B**. half flower; **C**. stamen from the first (or the outermost) whorl; **D.** two views of a stamen from the third whorl; **E**. staminode from the fourth (or the innermost) whorl ; **F**. floral diagram ; **G**. fruit and its sections.

In this study, two species were investigated from this family, which are medang kemangi (*Cinnamomum porrectum*) and medang daun lebar *(Litsea grandis)*.

Based on previous studies, the steam-distilled oil from the root of *Cinnamomum porrectum* was tested for its antimicrobial activity against human pathogens including bacteria, yeast and dermatophytes. It exhibited strongest activity against Streptococcus mutans followed by *Candida albicans* and dermatophytes, *Bacillus subtilis*, and susceptible strains of Staphylococcus aureus. It showed moderate activity *Crytococcus* neoformans but no activity against *Pseudomonas aeruginosa* and methicillin-resistant S.aureus. It can be conclude that *Cinnamomum porrectum* oil has antibacterial and antifungal activity. It showed that, *Cinnamomum porrectum* have an antifungal activity but not so much research about antioxidant activity yet (Hsuan, 1969).

Litsea grandis (medang daun lebar) is one of the Lauraceae families, usually on hillsides and ridges with sandy to clay soils, but also on limestone. For secondary forests, usually present as a pre-disturbance remnant tree. The wood is locally used for planks but has an unpleasant smell. Previous study did not found their antioxidant activity and antifungal properties. Therefore, in this study, we will know their ability for antioxidant activities and antifungal properties on their bark, heartwood, and sapwood (Hsuan, 1969).

2.2 Extraction

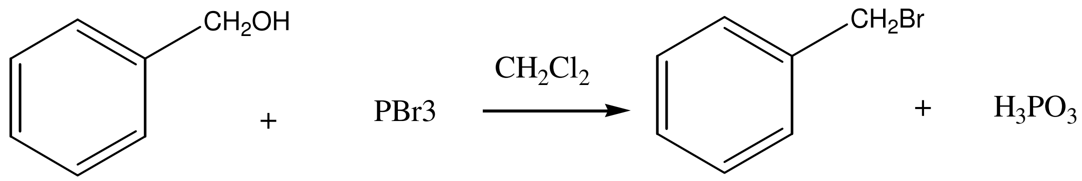
Extraction is a method used to separate compounds based on their solution preferences for two different immiscible liquids, usually water, and organic solvent (Sanchez *et al.*, 1999). Figure 10 showed the extraction process in laboratories that used in this study.

Figure 10. Extraction process

Usually one phase is water or based water (aqueous) solution and the other organic solvent that is immiscible with water. Extraction involves mixing of mutually insoluble materials, where a component of one of the phases moves into the other. For examples, to make a cup of tea, hot water is mixed with dried tea leaves. The leaves are not soluble in the water, so there are two immiscible phases, the solid and the water.

However, the water dissolves some of the compounds originally present in the tea leaves, these materials were extracted into the water. On the other hand, the preparation of a cup of instant coffee is not an extraction at all. Instant coffee powder is completely soluble in the water, and so there is no second phase. This is simply a process of solution (Balentine *et al.*, 1998).

Extractions are often classified according to the nature of the phases involved. The water-tea leaves system is an example of liquid-solid extraction. In this experiment, we will be concerned with liquid-liquid extraction, a very common laboratory operation that can be used as a separation or purification technique (Balentine *et al.*, 1998). Consider the following reaction:



(1)

This is a standard organic reaction, involving the conversion of an alcohol to an alkyl halide. For now, imagine that we have the crude reaction mixture, which contains the organic product benzyl bromide (1), and phosphorous acid in dichloromethane solvent. A very simple way to separate the phosphorous acid from the organic material is to add water. The dichloromethane solvent is immiscible with water, and separates as a second layer, the organic phase. Now the molecules of benzyl bromide can stay in the organic phase, or migrate to the water layer, the aqueous phase. The tendency for a compound to reside in a solution is, measured by its solubility. Benzyl bromide has a very much greater solubility in dichloromethane than in water, and so essentially this entire compound remains in the organic layer (Balentine *et al.*, 1998). For the phosphorous acid, on the other hand, the situation is just the opposite. As a polar, ionized material, phosphorous acid (H_3PO_4) is much more soluble in water than in dichloromethane. Consequently, the phosphorous acid molecules will migrate out of the dichloromethane phase into the aqueous layer, much like those water-soluble constituents of tea leaves. Extraction will only work for separation of materials, which have very different solubility. This technique was used most often for basic purification of crude reaction mixtures. Now, if the dichloromethane layer can be removed and the aqueous layer left behind, in a very simple way, a separation of the products of the reaction (Balentine *et al.*, 1998).

2.2.1 Extraction from plant material

The quantitative extraction of phenolics compound from plant tissue is a difficult problem that do not have satisfactory method. Although quantitative extraction is so difficult, the relative amount of the different pigments in each fraction is reasonably constant. The same result has been obtained with different glycosides (mono- and diglucosides of anthocyanins). It is possible to obtain information about the relative amounts of the different phenolics compounds in plant tissue, even though they have not been extracted completely from the tissue. This is probably true in the case of the flavonoid glycosides, although it may not be necessarily true of all phenolics constituent, especially the tannins (Oliver and Boyd, 1972).

Another important aspect of the problem of extraction of the phenolics constituent is the possibility that change may occur in the course of extraction. Plants cells contain many different enzymes liable to attack phenolics compounds, especially polyphenol oxidizes and glycosidase. Under the action of the latter, complex glycosides may be degrade into simpler ones, or even into aglycones, which appears as artifacts on paper chromatograms. To obtain a pure compound in crystal form, it is necessary that plant source should contain only a limited number of phenolic compounds. If this is not the case, purification processes are very difficult, if not in fact impossible (Oliver and Boyd, 1972).

2.2.2 Extraction by solvent

It is often recommended to make the first extraction of plant material with a non-polar solvent, such as light petroleum, which removes fats, waxes, chlorophyll and carotenoids more or less completely. If the extraction is not complete, this solvent does not extract the flavonoids. There are omissions, methyl flavones in leaves and fruits and heartwood flavonoids not glycosidically combined that are often easily extracted by light petroleum (Mackenzi, 2008).

The extraction was carried out which is, after disintegrator of the tissues in a blender, extract with methanol, ethanol or even with water. It is necessary to extract several times in order to achieve completely satisfactory result. Aqueous solvents have given the best result in the quantitative extraction of the anthocyanins of grapes. Methanol and ethanol have the advantage of being more easily removed, if it is desired to concentrate the solution in vacuo. If this is done, however, it is important not to proceed to complete dryness, because the residue is then difficult to dissolve. Water is to be preferred if fractionation is to follow by liquid-liquid extraction, for instance with ethyl acetate or ether, before or after hydrolysis (Oliver and Boyd, 1972).

Extraction of anthocyanins need a weak acid solvent (0.1% HCl is adequate) because these pigments are unstable in neutral or weakly alkaline solution. The acidity need to be adjusted because the presence of mineral constituents in the tissues may increase the pH. It is also necessary to make sure not to hydrolyze flavonoid glycosides because some of that are easily hydrolysable, especially if the extraction is carried out at the boiling point of the solvent. For instance, if plant tissue is treated with boiling alcohols, the enzymes may be inactive but the material is not protected against chemical hydrolysis by the action of the acids present in the tissue. Similarly, hydrolysis can occur in the course of a protracted extraction in apparatus of the Soxhlet type. The temperature required to keep the boiling solvent is low, it may be sufficient after long period to bring about changes in the phenolics constituents. With this method of extraction, it is advisable to add sodium carbonate to the flask containing the boiling solvent in order to lower the acidity (Mackenzi, 2008).

2.2.3 Methanol

Methanol was used as a solvent in this study. Methanol, also known as methyl alcohol, carbinol, wood alcohol or wood spirits, is a chemical compound with chemical formula CH_3OH showed in Figure 11. It is the simplest alcohol, light, volatile, colorless and flammable. It is also a poisonous liquid with a distinctive odor that somewhat milder and sweeter than ethanol (ethyl alcohol). It is used as an antifreeze, solvent, fuel, and as a denaturant for ethyl alcohol (Boyle, 1661).

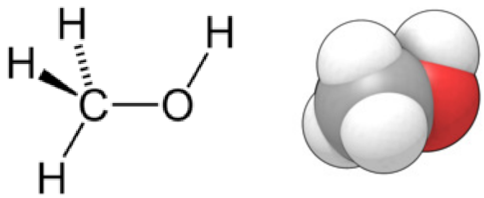


Figure 11. Structure of Methanol