UNIVERSITI PUTRA MALAYSIA

ISOLATION, IDENTIFICATION AND BIOACTIVITY OF NATURAL PRODUCTS FROM *MELALEUCA CAJUPUTI (MYRTACEA)*

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ISOLATION, IDENTIFICATION AND BIOACTIVITY OF NATURAL PRODUCTS FROM *MELALEUCA CAJUPUTI (MYRTACEA)*

By

VAQAR UL HASSAN

Thesis submitted in Fulfilment of the Requirements for the Degree of Master of Science at the Faculty of Science and Environmental Studies Universiti Putra Malaysia.

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DEDICATION

This research project, is dedicated to my family, brothers, sister and to my late parents.

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In the name of Allah, the most benevolent and most merciful in giving me the strength and patience to complete this project.

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ir	infrared
br	broad
s	sharp
wk	weak
tlc	thin layer chromatography
MS	mass spectrometry
NMR	nuclear magnetic resonance
dd	doublet of doublet
GC	gas chromatography
mp	melting point

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science.

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August, 1996

Chairman: Associate Professor Faujan bin Haji Ahmad, Ph.D.

Faculty: Science and Environmental Studies

In this study the components of aerial and subterranean parts of *Melaleuca cajuputi* were isolated and identified. Chemical investigation on the leaves resulted in the isolation of two known triterpenoids, betulinic acid and ursolic acid ester. The oily fractions contained two phenolic components, a naphthalene dione, an anthracene carboxal-dehyde and a phenenthracene analogy.

Methanol extracts of the bark, seeds, and flowers allowed for the isolation and identification of betulinic acid, urolic acid and betuline, respectively. Their structures were determined using modern spectroscopic techniques such as IR, NMR, MS and by comparison with literature.

The bioassay was carried out on crude extracts of leaves, roots, seeds, and bark against first instar mosquito larvae (*Aedes albopictus*) according to the procedure described in the literature. These extracts were found to be acute or relatively toxic to the target organisms. Extract from leaves exhibited the highest toxicity followed by extracts from roots, seeds, and bark respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat ijazah Master Sains.

PENGASINGAN, PENGENALAN DAN BIOAKTIVITI BAHAN HASIL SEMULAJADI DARIPADA *MELALEUCA CAJUPUTI (MYRTACEA)*

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Pengerusi: Prof. Madya Faujan bin Haji Ahmad, Ph.D.

Fakuliti: Sains dan Pengajian Alam Sekitar

Penyelidikan ini telah dicapai dengan mengasing dan mengenal pasti komponen-komponen daripada bahagian pucuk hingga akar tumbuhan *M. cajuputi*. Penyelidikan sebatian kimia terhadap daun telah berjaya mengasingkan dua triterpenoid yang dikenalpasti sebagai asid betulinik dan ester bagi asid ursolic. Pecahan berminyak dari ekstrak daun mengandungi dua sebatian fenol, satu sebatian naftalena dion, satu antrasena berkarboksi aldehid, dan satu analogi kepada fenantrasena.

Ekstrak metanol terhadap kulit batang, biji benih dan bunga telah memisahkan asid betulinik, asid ursolik dan betulina. Struktur-struktur bahan ini telah dikenal pastikan dengan manggunakan teknik-teknik spektroskopi moden seperti IR, NMR, MS dan perbandingan dengan data daripada rujukan.

Bioesei telah dijalankan untuk ekstrak mentah daripada daun, akar, biji benih dan kulit batang terhadap fasa pertama larva nyamuk (*Aedes albopictus)* mengikut prosedur yang telah diterangkan di dalam rujukan. Estrak-estrak ini didapati menunjukan ketoksikan terhadap organisma yang diuji. Ekstrak daun menunjukkan kadar toksik yang tinggi diikuti oleh ekstrak akar biji benih dan kulit batang.

CHAPTER 1

INTRODUCTION

Melaleuca cajuputi, previously referred to as *M. leucadendron,* is locally called gelam. It is also known as punk tree and paper bark tree (Hui and Moon Li, 1976), which means white stem. As it belongs to the family of *Myrtaceae,* subfamily *Leptospermoideae,* it is therefore closely related to the *Eucalyptus.* The family comprises 80 genera and more than 2500 species (Lymann, 1976). The plants are either shrubs or trees. In Australian and American tropics *Melaleucas* are usually found in damp soil and stagnant water.

One species of *Melaleuca* is found along the coastal regions in large parts of Peninsular Malaysia and Indonesia. It is a medium-sized single stem tree which can grow up to 25 m in height. Occasionally it may grow up to 40 m in height and 1.2 m in diameter or may be reduced to a shrub (Stoker, 1972). The bark is layered and papery. The leaves are straight or curved and often hairy. They are about 5-10 cm long, 1-4 cm wide, and 3-5 nerved. The inflorescence consists of 1-3 spikes up to 9 cm long with white, greenish white or cream coloured flowers. Fruits are capsules about 3 mm long and

4 mm wide with thinner valves than most *Melaleuca sp.* It flowers in whorls with numerous silky rachis. It has been cultivated from early times and is indigenous throughout its presentrange (Ridley, 1992). Most stands are found along drainagelines or on low, swampy coastal plains (Stoker, 1972). The timber of this tree is reddish brown or violet brown and, mottled or veined. It is a durable wood particularly for use in wet ground and sea water. Thus it is used for posts, pile shade shelter and in ship making industries. It is also used as firematerial, specially when mangrove is not available. In boat making industries, its papery bark is placed between two planks, because it has the capacity to swell thus sealing the seams. The soft bark is a promising source of fire board and packing material (Keating and Bolza , 1982).

In East Malaysia after mastication the softened bark is used on suppurating wounds to draw out the pus. The major use of *M. leucadendron (M. cajuputi)* is as a source of cajuput oil, obtained by steam distillation of the leaves and terminal branchlets. The active constituents of the oil are cineol (60%) and a-terpineol. This oil contains other monoterpenes such as a-pinene camphene, limonene, b-pinene, myrcyrene, terpenolene and linalool as minor components. It makes a good antiseptic and can be used as an insect repellent (Lassak and Carthy, 1983).

In Peninsular Malaysia, cajuputi oil is used for headache, toothache, ear pain, rheumatism, cramps and applied on fresh wounds to hasten healing. *Melaleuca sp* produces a large number of very fine seeds which do not need pretreatment but require a wet soil for germination. Dried fruits of this tree are sold in the market as *mericha bolong* which means black pepper. The fruits have medicinal value as they are used in tonics, and in a great variety of other drugs. Seeds from the fruit *sari bolong* are also used in medicine .

M. cajuputi is resistant to fire and can tolerate exposure to wind. It has an extensive root system and develops aerial adventitious roots on prolonged water logging soils. These roots can form buttresses on the lower trunk. A process for the regeneration of forests of *Melaleuca sp* is practised in Java. In the dry months, full grown trees are hewed and branches bearing seeds are cut and left to dry and then set on fire. It is believed that burning makes the *Melaleuca* grow more luxuriantly.

The objective of this study is to extract and isolate the constituents from the leaves, bark, roots, flowers and seeds of *M. cajuputi* and to identify these constituents using spectroscopic methods. It was felt that it would be of interest to carry out a bioassay of the extracts against mosquito larvae as it would allow for the tracing of biologically active constituents.

The tracing of biologically active compounds depends upon the reliability, convenience and effectiveness of the bioassay. The term itself means the measurement of the potency of any stimulus-physical, chemical or biological, physiological or psychological by means of the reactions that it produces in living matter (Mayer, 1976).

Larvicidal tests are conducted to estimate the toxicity of extracts of pure compounds before screening for biologically active compounds by cytotoxic or anti microbial tests. In terms of efficacy the larvicidal test lies between insecticides and cytotoxic agents. Its advantage is that, depending upon the results the testing material can be placed either into cytotoxic or insecticidal category. Moreover the eggs of *Aedes albapictus* can be collected, dried easily and hatched in ordinary tap water. Moreover, larvicidal tests are economical, less time consuming and do not require ethical considerations. The *Aedes aegypti* and *aedes albopictus* are responsible for the yellow fever and dengue (break bone fever). Therefore *Aedes* was selected for tests in order to develop a larvicide for the control of the vector.

CHAPTER 2

LITERATURE REVIEW

Heat of combustion which is a measure of the basic thermal property of an organic material is defined as the calories per gram of a moisture free sample. This property is normally used for the comparison of fuels. Wood with low moisture content is a more valuable fuel than that containing appreciable amounts of moisture because wood moisture diminishes the unable heat produced by the fuel (Wang *et al.*, 1981). The heat of combustion of bark of *Melaleuca sp* is unique and comparable to that of coal i.e 6000 cal/gm, the highest possible for woody material. The average values of wood bark, terminal branches and foliage of *Melaleuca sp* are given in Table 1.

Table 1

Heat of combustion of different parts of *Melaleuca species*

Parts of Malaleuca Species	Heat of Combustion (cal/gm)
Wood	4000
Bark	6160
Terminal Branches	4610
Foliage	4810

(Source : Wang *et al.*, 1981)

In the case of essential oils isolated from the leaves of *M. cajuputi*, the predominance of a mixture of phenolic compounds has been reported, namely 3-methyl-4,6-dimethoxypholoroacetophenone, eugenetin and isoeugenetin. However, Zakaria *et al.* (1988) found phenolic compounds to be absent in another chemovariety of *M. cajuputi* collected off the shores of Terengganu. Instead, they found more monoterpenes in the chemical composition of this plant compared to other varieties. It is also worth noting that nerolidol [1], a feeding deterrent compound was isolated from the leaves of *M. cajuputi, M. smithii* and *M.viridiflora* (Doskotch *et al., 1979*).

The components of essential oils obtained from the leaves of different *Melaleuca sp* have been identified chromatographically (Shieh, 1978). Some of the components are listed in Table 2.

A putative precursor of flavones, 4,6-dimethoxy-3,5-dimethyl-2-hydroxy acetophenone [2], was also isolated from the volatile oil of *M. cajuputi* while an emulsifying agent ursolic acid [3], was isolated from the leaf wax of *M. leucadendron* (Courtney *et al., 1983*). Besides Hui *et al.* (1976) reported the isolation of terpenoid constituents from light petroleum extract of the leaves and stem of *M. leucadendron*. They reported the isolation of frideline [4], sitosterol [5], antitumour agent betulin [6] and uvaol [7] from leaves while the stem provided taraxastenone, sitosterol, epitaraxeryl acetate, fridelin and betulin and betulinic acid [8].

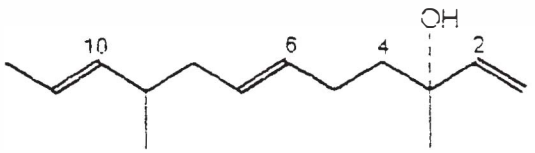
Table 2

Components of Essential Oils from Genus *Melaleuca*

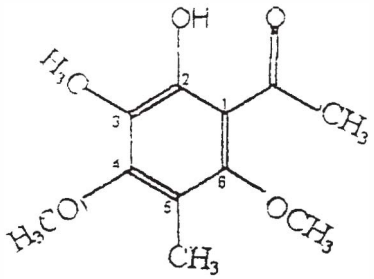
Components	*M.verdiflora*	*M.ericiflora*	M.*alternifolia*	*M.linarifolia*	*M.bracteata*	*M.cajuputi*
Aromadondrene				+		
E.lemicin					+	
Benzaldehyde	+					+
Eugenol	+				+	
Cinnmic acid					+	
Linalool	+	+				
1,8-cineol		+	+	+		+
Limonene	+	+				+
P.cymene			+	+		
Malalilol				+		
Methyl iso eugenol						
Methyl cinnamate	+					
a-Pinene	+	+	+	+		+
b-Pinene				+	+	
a-Bhellandrene					+	
a-Terpinene			+	+		
g-Terpinene			+	+		
a-Terpineol	+	+	+	+		+
Thujone				+		
Trans-b-ocimene	+					

Note: positive + means present
(Source: Shieh, 1978)

Wood species are naturally resistant to termite attack. Thus their bioactive extractives may be useful in the development of new naturally types of wood preservatives and *Melaleuca sp* is considered to be the source of such extractives. Carter and Hoffman (1982) reported that wooden block treated with extractives from *Melaleuca sp* exhibited toxicity towards termites such as *R. flavipes* and *C. formosanis*. The feeding response was poor and overall mean survival was 1% and 27%, respectively. They also reported that the extractability of anti-termite material from ground wood of *Melaleuca sp* depended on the polarity of the solvent used. Non-polar solvents such as pentane did not extract any anti-termite material, while acetone, acetone-hexane-water system (54: 44: 2) and 80% methanol were effective for extraction of anti-termite materials.



[1]



[2]