



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS FROM *CALOPHYLLUM INOPHYLLUM*
AND *CRATOXYLUM ARBORESCENS* AND THEIR BIOLOGICAL
ACTIVITIES**

VIVIEN JONG YI MIAN

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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of Science**

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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May 2007

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Chemical and biological studies were carried out on two plants, *Calophyllum inophyllum* (Guttiferae) and *Cratoxylum arborescens* (Guttiferae). The chemical investigation covered triterpenoids, quinones and xanthenes. These compounds were isolated using common chromatographic techniques and were identified using spectroscopic experiments such as NMR, MS, IR and UV.

Calophyllum inophyllum (root bark) afforded sitosterol (**71**) and six other known xanthenes which are brasilixanthone (**72**), inophyllin A (**26**), inophyllin B (**27**), 1,3,5-trihydroxy-2-methoxy xanthone (**73**), caloxanthone A (**74**) and pyranojacareubin (**75**). Meanwhile, studies on *Cratoxylum arborescens* (stem bark) provided one triterpenoid, friedelin (**76**) together with three others known quinones. These are vismione (**77**), vismiaquinone (**78**), and 1,8-dihydroxy-3-methoxy-6-methyl anthraquinone (**79**).



Cytotoxic tests were carried out using CEM-SS cell line and HL-60 cell line. The crude hexane extract of *Cratoxylum arborescens* B. was found to be inactive to cytotoxic activity. The crude chloroform and methanol extracts of *Cratoxylum arborescens* B. showed good cytotoxic activity with IC₅₀ values of 16 and 18 µg/ml, respectively. Meanwhile, the crude chloroform extract of the stem bark of *Calophyllum inophyllum* L. also showed a good cytotoxic activity with an IC₅₀ value of 17 µg/ml. Two pure compounds obtained from *Calophyllum inophyllum* L., inophylin B (**26**) and caloxanthone A (**74**) also showed good activities against the HL-60 cell line with IC₅₀ values of 15 and 29 µg/ml respectively.

The antimicrobial activity test was also carried out using four pathogenic bacteria, namely, Methicilin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus typhimurium* and *Bacillus subtilis*. However, most of the crude extracts gave only moderate or weak activity.

The larvicidal tests were performed against the larvae of *Aedes aegypti* using the WHO (1981) standard procedures with slight modifications. The crude hexane, chloroform and methanol extracts of *Calophyllum inophyllum* and *Cratoxylum arborescens* were inactive against the larvae of *Aedes aegypti*.

No activity was recorded for the antifungal activity test.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA BINTANGOR
(*CALOPHYLLUM INOPHYLLUM*) DAN GERONGGANG (*CRATOXYLUM
ARBORESCENS*)**

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Kajian Kimia dan aktiviti biologi telah dijalankan terhadap dua tumbuhan iaitu *Calophyllum inophyllum* (Guttiferae) dan *Cratoxylum arborescens* (Guttiferae). Kajian kimia terperinci merangkumi jenis sebatian seperti triterpenoid, kuinon dan xanthon. Sebatian- sebatian ini diasingkan dengan menggunakan pelbagai teknik kromatografi dan dikenalpasti dengan menggunakan eksperimen spektroskopi seperti NMR, MS, IR dan UV.

Calophyllum inophyllum (akar batang) telah menghasilkan satu sitosterol (**71**) dan enam xanthon yang lain iaitu brasilixanthon (**72**), inophyllin A (**26**), inophyllin B (**27**), 1,3,5-trihydroxy-2-methoxy xanthon (**73**), caloxanthon A (**74**) dan pyranojacareubin (**75**). Sementara itu, *Cratoxylum arborescens* (kulit batang) telah memberikan satu triterpenoid, friedelin (**76**) bersama dengan tiga kuinon yang lain, vismion (**77**), vismiakuinon (**78**), dan 1,8-dihydroxy-3-methoxy-6-methyl anthrakuinon (**79**).



Ujian Sitotoksik telah dijalankan dengan menggunakan sel CEM-SS dan HL-60. Ekstrak mentah heksana dari *Cratoxylum arborescens* tidak aktif terhadap aktiviti sitotoksik. Manakala ekstrak mentah klorofom dan metanol menunjukkan aktiviti yang baik dengan nilai IC_{50} 16 dan 18 $\mu\text{g/ml}$ masing-masing. Ekstrak mentah klorofom daripada *Calophyllum inophyllum* juga menunjukkan aktiviti yang baik dengan nilai IC_{50} 17 $\mu\text{g/ml}$. Selain daripada itu, dua sebatian tulen iaitu inophyllin B (27) dan caloxanthon A (74) juga menunjukkan aktiviti yang baik dengan nilai IC_{50} 15 dan 29 $\mu\text{g/ml}$.

Aktiviti antimikrob dijalankan dengan menggunakan empat jenis bakteria iaitu Methicilin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus typhimurium* dan *Bacillus subtilis*. Bagaimanapun, kebanyakan ekstrak mentah yang diuji dengan bakteria-bakteria ini hanya memberikan keaktifan yang sederhana dan lemah.

Ujian larva telah dijalankan dengan menggunakan larva jenis *Aedes aegyti* mengikut prosedur-prosedur piawai WHO (1981) dengan sedikit perubahsuaian. Ekstrak mentah heksana, klorofom dan metanol daripada kedua-dua tumbuhan *Calophyllum inophyllum* and *Cratoxylum arborescens* menunjukkan ketidakaktifan terhadap larva *Aedes aegyti*.

Tiada sebarang aktiviti yang direkodkan untuk ujian antifungal.

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations which have been duly acknowledged. I also declare that it has been previously or concurrently submitted for any other degree at UPM or other institutions.

VIVIEN JONG YI MIAN

Date: 29 MAY 2007



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LIST OF ABBREVIATIONS

α	alpha
β	beta
γ	gamma
δ	chemical shift in ppm
λ_{\max}	wavelength maxima in nm
μg	micro gram
brs	broad singlet
$^{\circ}\text{C}$	degree celcius
^{13}C	carbon-13
Ac_2O	acetic anhydride
CC	column chromatography
CD_3OD	deuterated methanol
CDCl_3	deuterated chloroform
COSY	Correlated Spectroscopy
cm	centimeter
cm^{-1}	per centimeter
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
$\text{DMSO-}d_6$	deuterated dimethylsulfoxide
dt	doublet of triplet
EIMS	Electron ionization mass spectrometry
EtOAc	ethyl acetate
FeCl_3	Ferric Chloride



FTIR	Fourier Transform Infra Red
g	gram
GC	Gas Chromatography
GC-MS	Gas Chromatography- mass spectroscopy
^1H	proton
HETCOR	Heteronuclear Chemical Shift-correlation
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HMQC	Heteronuclear Multiple Quantum Coherence
HPLC	High Performance Liquid Chromatography
HR-EIMS	High Resolution- Electron ionization mass spectrometry
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz
IR	Infra Red
J	coupling constant in Hz
KBr	Kalium Bromide
kg	kilogram
l	litre
LC	Lethal Concentration
LD	Lethal Dose
Lit.	Literature
m	multiplet
M^+	Molecular ion
mg	milligram
ml	mililiter
mm	millimeter



Me ₂ CO	acetone
MeOH	methanol
MHz	megahertz
mp	melting point
MS	Mass Spectrum/ Spectra/ Spectrometer/ Spectrometry
m/z	mass per charge
nm	nanometer
NMR	Nuclear Magnetic Resonance
PLC	Preparative Layer Chromatography
ppm	part per million
R _f	migration distance of the sample divided by migration distance of solvent front
s	singlet
t	triplet
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
ν _{max}	Wavenumber maxima in cm ⁻¹
UV	Ultra Violet
WHO	World Health Organization



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CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural product chemistry is a branch of chemistry that deals with the isolation, identification, structure elucidation, and study of the chemical characteristics of chemical substances produced by living organisms. Natural products are defined as chemical compounds that are derived from the living organisms such as plants, animals, insects and other organisms. Natural product is a term used commonly in reference to chemical substances found in nature that have distinctive pharmacological effects.

Natural products have been used as medicinal agents for many years. Before the availability of synthetic drugs, mankind was completely dependent on medicinal herbs for the prevention and treatment of diseases. This is particularly true in Chinese medicine. Chinese herbs have been used as tonics (they increase energy, mood, motivation, alertness, etc.) apart from being said as having an influence on erectile function and immune system (Zhu *et al.*, 2004). Some of the early records are the Shen Nung Pen Tsao (2700 B.C.) which indicates the usefulness of plants for treating diseases in China, Eber papyrus (1550 B.C.) which records the plants used in Egyptian medicine and Ayurveda (1000 B. C.), the ancient healing system of India (Snedden, 2004).



Plants continue to be used worldwide for the treatment of diseases and novel drugs continue to be developed through research. The World Health Organization notes that of the 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by nature cultures. Today approximately 25 percent of all prescription drugs are still derived from trees, shrubs, or herbs (Farnsworth *et al.*, 1985).

Located in Southeast Asia, Malaysia is blessed with natural biodiversity that has yet to be utilized fully. Realizing the vast medicinal potential that lies in the tropical forest of Malaysia, much effort have been put into research and development to make full use of nature's gift. The bio resources are known to be important sources of bioactive components with health, nutritional and pharmacological properties. Malaysia is one of the 12 mega diverse resources of natural products in the world, thus the area of natural products is of great interest to Malaysia. However, the research on higher plant as a natural source of drugs is still largely unexplored. There are 12,000 species of flowering plants of which about 1,300 of these species have been reported to be used as traditional medicine by various ethnic population and only about a hundred have been fully investigated for their potential use as novel therapeutic agents (Burkill, 1935).



1.2 The Genus *Calophyllum*

The genus *Calophyllum* belongs to the Guttiferaceae family. It is also sometimes categorized under the family of Hypericaceae. *Calophyllum* is an endemic tree which grows in the lowland, evergreen and wet zone forest (Dharmaratne *et al.*, 1997).

The genus *Calophyllum* consists of 187 species worldwide. In Peninsular Malaysia, the genus *Calophyllum* is represented by 45 species, that is *Calophyllum inophyllum*, *Calophyllum teysmannii*, *Calophyllum moonii*, *Calophyllum calaba*, *Calophyllum lanigerum*, *Calophyllum mucigerum* etc.

Calophyllum species have been often used in traditional medicine. The balsam from the bark of *Calophyllum inophyllum* ‘Alexandrian Laurel’ is called an ‘oleoresin’ and used as a cicatrisant, whereas an infusion or decoction of the leaves has been used as an eye remedy in Asian medicine (Iinuma *et al.*, 1993).

Calophyllum inophyllum is locally known as ‘Bintangor’ and is in a genus of about 110 species that are pantropical in distribution but most common in tropical Asia (Shalan *et al.*, 1971). Native from east Africa to Australia and Malesia, *Calophyllum inophyllum* has been widely planted throughout the tropics, including many south and central Pacific islands, the Hawaiian Islands, and the Caribbean islands.

Calophyllum inophyllum is a low-branching evergreen tree with a broad, spreading crown of irregular, gnarled branches. It typically attains 8 to 20 m in height. It is generally described as slow-growing. *Calophyllum inophyllum* is primarily a tree of the seashore and adjacent lowland forests, although it occasionally grows at higher