UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL STUDIES AND BIOACTIVITY TESTS OF *MURRAYA PANICULATA JACK, AEGLE MARMELOS* CORREA AND *ZINGIBER AMARICANS* BLUME

SUGENG RIYANTO

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By

SUGENG RIYANTO

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

January 2003

DEDICATION

*This thesis is dedicated to: my son, Ikhsan Adi Nugroho (**IAN**)*

*and my daughter, Asefin Nurul Ikhtiarini (**ANI**)*

and my beloved wife Naniek Suharyani

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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January 2003

Chairman: Associate Professor Mohd. Aspollah Hj. Sukari, Ph.D.

Faculty: Science and Environmental Studies

South East Asian countries are very rich in biodiversity with thousands of the plants species of which only a small proportion of the species have been investigated in detail. This research was carried out to investigate the chemical constituents and bioactivities of three well known medicinal plants *Murraya paniculata* Jack, *Aegle marmelos* Correa (Rutaceae) and *Zingiber amaricans* Blume (Zingiberaceae).

Leaves and stem bark of *M. paniculata;* leaves, bark and root of *A. marmelos* and rhizomes of *Z. amaricans* were separately extracted with various solvents. The extracts and isolated pure compounds were examined for their bioactivity against microbes and cancer cell line. Cytotoxic assay was performed

against CEM-SS (human T-lymphoblastic leukaemia) cell line with MTT reagent, while antimicrobial tests were conducted by diffusion method against a list of the pathogenic bacteria and fungi. The structures of the isolated compounds were elucidated by using spectroscopic methods.

Eight compounds were isolated from *M. paniculata* and identified as auraptene, stigmasterol, β-sitosterol, sucrose and methoxylated flavones including 3,3',4',5,5',6,7,8-octamethoxyflavone, 3,3',4',5,5',6,7-heptamethoxyflavone, 3,3', 4',5,5',7-hexamethoxyflavone and 3',4',5,5',7,-pentamethoxyflavone. The study on *A. marmelos* afforded eleven compounds; β-sitosterol, stigmasterol and hopane triterpenes including zeorin and dustanin; alkaloids aegeline and skimmianin; epilupeol, lupenone and coumarin derivates; auraptene, epoxyauraptene and marmin. By employing the same procedures, the extracts of *Z. amaricans* afforded zerumbone as the major constituent, together with cholesterol, campesterol, stigmasterol and β-sitosterol.

The results of antimicrobial and cytotoxic tests revealed that some of the extracts showed strong activities. Nevertheless, most of the isolated compounds showed only weak activity. On the other hand, the cytotoxic test results on dustanin and epilupeol showed moderate activity with IC_{50} values of $1.19\ 10^{-2}$ μmol/ml and $1.43\ 10^{-2}$ μmol/ml, respectively. The results obtained for other compounds revealed weak cytotoxic activity as indicated by their IC_{50} values; zerumbone ($6.88\ 10^{-2}$ μmol/ml), zeorin ($3.76\ 10^{-2}$ μmol/ml), lupenone ($4.08\ 10^{-2}$ μ mol/ml), marmin ($6.66\ 10^{-2}$ μmol/ml) and aegeline ($7.58\ 10^{-2}$ μmol/ml).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan ijazah Doktor Falsafah

KAJIAN FITOKIMIA DAN UJIAN KEAKTIFAN BIOLOGI DARIPADA *MURRAYA PANICULATA* JACK, *AEGLE MARMELOS* CORREA AND *ZINGIBER AMARICANS* BLUME

Oleh

SUGENG RIYANTO

Januari 2003

Pengerusi: Profesor Madya Mohd. Aspollah Hj. Sukari, Ph.D.

Fakulti: Sains dan Pengajian Alam Sekitar

Negara-negara di Asia tenggara mempunyai kepelbagaian biologi yang kaya, ribuan spesies pokok tumbuh-tumbuhan wujud di daerah ini, tetapi hanya sebahagian kecil spesies dikaji secara mendalam. Kajian ini telah dijalankan untuk menyelidiki kandungan kimia dan keaktifan biologi daripada tiga tanaman ubatan yang terkenal iaitu : *Murraya paniculata* Jack, *Aegle marmelos* Correa dan *Zingiber amaricans* Blume.

Daun dan kulit batang daripada *M. paniculata*; daun, kulit batang dan akar daripada *A. marmelos* dan rizom daripada *Z. amaricans* diekstrak secara berasingan dengan berbagai pelarut. Ekstrak dan sebatian yang berjaya dipencilkan diperiksa keaktifan biologinya terhadap sel kanser dan mikrob. Ujian sitotoksik dilakukan ke atas sel leukaemia T-lymfoblast manusia (CEM-SS)

dengan menggunakan reagen MTT, sedangkan untuk mengesan keaktifan antimikrob dilakukan dengan kaedah peresapan cakera ke atas senarai bakteria patogen dan fungi. Struktur molekul daripada sebatian yang telah dipencilkan dikenal pasti dengan kaedah spektroskopi.

Lapan sebatian yang telah berjaya dipencilkan dari *M. paniculata* dikenal pasti sebagai: auraptene, stigmasterol, β-sitosterol, sukros and flavon termetoksi iaitu 3,3',4',5,5',6,7,8-oktametoksiflavon, 3,3',4',5,5',6,7-hepta-metoksiflavon, 3,3',4',5,-5',7-heksametoksiflavon and 3',4',5,5',7-pentametoksi-flavon. Penyelidikan ke atas *A. marmelos* memperolehi sebelas sebatian, yakni: β-sitosterol, stigmasterol dan triterpena hopana iaitu zeorin dan dustanin; alkaloid, aegeline and skimmianin; epilupeol, lupenon dan terbitan koumarin; auraptene, epoxyauraptene and marmin. *Z. amaricans* diselidiki menggunakan kaedah yang sama menghasilkan zerumbone sebagai kandungan utama, bersama dengan cholesterol, campesterol, stigmasterol and β-sitosterol.

Keputusan ujian antimikrob dan sitotoksik menunjukkan bahawa beberapa ekstrak sangat berkesan. Namun begitu, kebanyakan sebatian yang telah dipencilkan didapati mempunyai keaktifan lemah. Manakala keputusan ujian sitotoksik mendapati bahawa dustanin dan epilupeol menunjukkan kesan sederhana dengan nilai IC_{50} masing-masing 1.19 10^{-2} μmol/ml dan 1.43 10^{-2} μ mol/ml,. Keputusan yang diperolehi daripada sebatian lain menunjukkan kesan sitotoksik lemah dengan nilai IC_{50}, yaitu: zerumbon (6.88 10^{-2} μmol/ml), zeorin

(3.76 10^{-2} μmol/ml), lupenon (4.08 10^{-2} μmol/ml), marmin (6.66 110^{-2} μmol/ml) dan aegelin (7.58 10^{-2} μmol/ml).

ACKNOWLEDGEMENTS

This work was carried out with a hope to contribute towards the expansion of our currently limited knowledge on compounds isolated from medicinal plants and its bioactivities. The completion of this thesis would have been impossible without the assistance and direct involvement of so many kindhearted individual. Thus, I am very much indebted to my previous mentors and I have no way of repaying such a debt except to express my sincerest gratitude.

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I certify that an Examination Committee met on 2nd January 2003 to conduct the final examination of Sugeng Riyanto on his Doctor of Philosophy thesis entitled "Phytochemical Studies and Bioactivity Tests of *Murraya paniculata* Jack, *Aegle marmelos* Correa and *Zingiber amaricans* Blume" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements to the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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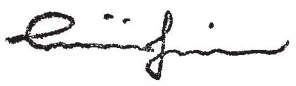

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Date : 13 MAR 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SUGENG RIYANTO
Date : 24-1-2003

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δ	chemical shift
ε	molar absorption
λ	wavelength
λ_{max}	maximum wavelength
μ	micro
μmol	micro molar
AcONa	sodium acetate
$AlCl_3$	aluminum chloride
^oC	degree in Celcius
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
cm^{-1}	per centi meter
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
DMSO	dimethyl sulphoxide
ED_{50}	Effective Dose (50%mortality)
EtOAc	ethyl acetate
FAB	Fast Atom Bombardment
g	gram
^1H	proton
HMBC	Heteronuclear Multiple Bond Connectivity
HMQC	Heteronuclear Multiple Quantum Coherent
HPLC	High Performance Liquid Chromatography
Hz	hertz
IC_{50}	Inhibition Concentration (50%mortality)
IR	Infrared
iu	international unit
J	coupling constant
kg	kilogram
Lit.	literature
m	multiplet
M^+	molecular ion
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
m.p.	melting point
MeOH	methanol
MeO	methoxy(l)
MeONa	sodium methoxide
ml	mililitre
MS	(Electron Impact) Mass Spectroscopy
m/z	mass per zarah
NCI	Negative Chemical Ionisation

n.hexane	normal hexane
nm	nanometer
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser and Exchange Spectroscopy
ppm	parts per million
s	singlet
t	triplet
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet

CHAPTER I

INTRODUCTION

History

The use of plants as medicines started since the early time. Literature evidence of man utilizing plants for the treatment of various diseases have been documented by the great civilization of the ancient China, India and Egypt. The prominent scholar in ancient Greece, named Theophratus (372-278 BC) has been described by some as the father of botany who studied over five hundred plants from the Mediterranean valley and also introduced the virtues of medicinal plants. At that time the scholars classified and gave the description of the plants, which aided the identification process. In the 19th century man started to isolate the active substances from medicinal plants and it was marked by the discovery of an alkaloid, quinine from *Cinchona* bark. Such discovery led scientists to search and collect the plants from jungles for sources of medicines from the natural resources. Some of natural products isolated from plants are still in use to day, such as quinine from *Cinchona* bark, morphine and codeine from the *Papaver somniverum*, digoxin from *Digitalis purpurea* leaves, atropine and hyoscine from *Atropa belladonna.*

Traditional Medicinal Plants

Tropical rainforest of southeast Asia, particularly in Malaysia stores large collection of plant species which provide an important source of traditional medicine. There are over 20,000 species of tropical plants in the country and about 500 genera containing 15,000 species in Peninsula Malaysia, of which 16% are claimed to have medicinal properties (Zakaria *et al.*,1994). The plants have been used for generations in various traditional medicine practices in Malaysia, Indonesia and its surrounding areas. Traditional medicine is well known for its high nutritional value, as well as its ability to cure various ailments. There are vast similarities between local Malay traditional medicine practices compared to the Indonesian traditional medicines. Probably a more scientific and modern approach may earn traditional medicine a more respectable place in the community. Traditional medicine is part of our national heritage and must be accorded its proper place. As it has been realized in some countries which introduced and promoted their traditional medicinal plant such as in Korea with *Panax ginseng*, India with *Rauwolfia serpentina* (Apocynaceae) and China with *Rheum palmatum* (Rhamnaceae). Nowadays, generally public has accepted these plants as one of the sources of modern medicines. Malaysia for example has the resource and capacity to investigate and promote certain well-known medicinal plants such as *Eurycoma longifolia* (Tongkat Ali) (Simarubaceae) and the other promising herbs for commercial exploitation. In Indonesia, traditional medicine has become a lucrative commercial industry bringing in large profits to entrepreneurs and farmers. Some medicine manufacturers treat the traditional medicinal plants as