



UNIVERSITI PUTRA MALAYSIA

**THE CHEMISTRY AND BIOLOGICAL ACTIVITIES OF
ANTHRAQUINONES FROM THE CELL SUSPENSION CULTURE OF
MORINDA ELLIPTICA AND FLAVONOIDS FROM
HEDYCHIUM THYRSIFORME**

JASRIL

FP 2002 9

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By

JASRIL

**Thesis Submitted in Fulfilment of the Requirement for the Degree of
Doctor of Philosophy in the Graduate School
Universiti Putra Malaysia**

January 2002



DEDICATION

This thesis is dedicated in the loving memory to my dearest father, Karim. It is also dedicated to my dearest mother, Rosna and my loving wife, Yetti Nelmayeni, whose prayer has inspired me to achieve my academic goal

Abstract of 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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Chairman : Prof. Dr. Md. Nordin Hj. Lajis

Faculty : Science and Environmental Studies

Peninsular Malaysia is one of the mega diverse countries of the world. The Malaysian people have used these plants for medicinal purpose long before the development of modern medicine to treat various diseases. As part of intensive studies on medicinal plants particularly in Universiti Putra Malaysia (UPM), this research has been conducted to study the chemistry and biological activities of several selected plants as well as compounds isolated from the cell suspension culture of *Morinda elliptica* and the rhizome parts of *Hedychium thyriforme*. Based on literature search there have not been any reports on this particular research subjects.



Eight anthraquinones including nordamnacanthal (A-1), alizarin-1-methyl ether (A-2), anthragallol-1,2-dimethyl ether (A-3), purpurin-1-methyl ether (A-4), rubiadin (A-5), soranjidiol (A-6), lucidin- ω -methyl ether (A-7) and morindone (A-8) were isolated from the cell suspension culture of *Morinda elliptica*. Five flavonoids including 3,7,4'-trimethoxy-5-hydroxyflavone (F-1), 3,4'-dimethoxy-5,7-dihydroxyflavone (F-2), 5,7,4'-trimethoxy-3-hydroxyflavone (F-3), 3,5,7,4'-tetramethoxyflavone (F-4) and 7,4'-dimethoxy-3,5-dihydroxyflavone (F-5) were also isolated from the rhizome parts of *Hedychium thyrsoforme*. The structures of all the isolated compounds were established based on spectral data including ultraviolet-visible, infrared, mass and nuclear magnetic resonance spectra.

The study on biological activities of the isolated compounds was conducted using some *in vitro* bioassay procedures. In antitumor promoting assay, it is interesting to note that nordamnacanthal and 5,7,4'-trimethoxy-3-hydroxyflavone in concentration of 0.4 $\mu\text{g/ml}$ showed strong inhibition activity towards Epstein-Barr virus activation in Raji cells. Nordamnacanthal and 5,7,4'-trimethoxy-3-hydroxyflavone as well as morindone, 3,4'-dimethoxy-5,7-dihydroxyflavone and 7,4'-dimethoxy-3,5-dihydroxyflavone also showed strong antioxidant activity.



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

**KAJIAN KIMIA DAN KEAKTIFAN BIOLOGI ANTRAKUINON
DARIPADA KULTUR AMPAIAN SEL *MORINDA ELLIPTICA*
DAN FLAVONOID DARIPADA *HEDYCHIUM THYRSIFORME***

Oleh

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Semenanjung Malaysia merupakan salah satu kawasan hutan hujan tropika yang dikenali mempunyai berbagai-bagai spesies tumbuhan perubatan. Penduduk Malaysia telah menggunakan tumbuhan tropika dalam perubatan tradisional sebelum berkembangnya ubat-ubat modern untuk merawat berbagai penyakit. Kajian intensif telah dijalankan keatas tumbuhan ubatan terutamanya di Universiti Putra Malaysia (UPM). Penyelidikan ini telah dibuat untuk mengkaji kimia dan keaktifan biologi sebatian-sebatian yang diasingkan daripada kultur ampaian sel *Morinda elliptica* dan bahagian rizom *Hedychium thyriforme*. Berdasarkan rujukan lepas, belum ada laporan mengenai kajian ini.

Lapan antrakuinon iaitu nordamnakantal (A-1), alizarin-1-metil eter (A-2), antragallo-1,2-dimetil eter (A-3), purpurin-1-metil eter (A-4), rubiadin (A-5),



soranjidiol (A-6), lusidin- ω -metil eter (A-7) dan morindon (A-8); dan lima flavonoid iaitu 3,7,4'-trimetoksi-5-hidroksiflavon (F-1), 3,4'-dimetoksi-5,7-dihidroksiflavon (F-2), 5,7,4'-trimetoksi-3-hidroksiflavon (F-3), 3,5,7,4'-tetrametoksiflavon (F-4) dan 7,4'-dimetoksi-3,5-dihidroksiflavon (F-5) telah diasingkan daripada kultur ampai sel *Morinda elliptica* dan bahagian rizom *Hedychium thyrsoideum*. Struktur-struktur semua sebatian tersebut telah dikenal pasti berdasarkan kajian spektra termasuk spektra ultralembayung-nampak, inframerah, jisim dan resonan magnetik nuklear.

Kajian keaktifan biologi keatas semua sebatian telah dibuat menggunakan beberapa kaedah biocerakanin *in vitro*. Keputusan ujian-ujian ini mendapati bahawa banyak diantara sebatian yang diasingkan mempunyai keaktifan biologi yang menarik. Keputusan sangat menarik didapati dari ujian promotor antitumor iaitu nordamnakantal dan 5,7,4'-trimetoksi-3-hidroksiflavon pada kepekatan 0.4 $\mu\text{g/ml}$ didapati sangat kuat merencat keaktifan virus Epstein-Barr pada sel-sel Raji. Nordamnakantal and 5,7,4'-trimetoksi-3-hidroksiflavon bersama morindon, 3,4'-dimetoksi-5,7-dihidroksiflavon dan 7,4'-dimetoksi-3,5-dihidroksiflavon didapati juga mempunyai keaktifan antioksidan yang kuat.

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LIST OF ABBREVIATIONS

δ	chemical shift
λ	wavelength
μ	micro
ν	vibration
cm	centimeter
$^{\circ}\text{C}$	degree in celcius
^{13}C	carbon-13
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
EI-MS	Electron Impact-Mass Spectroscopy
FGHMBC	Field Gradient Heteronuclear Multiple Bond Correlation
FGMQC	Field Gradient Heteronuclear Multiple Quantum Correlation
g	gram
^1H	proton
Hz	Hertz
IC	Inhibition Concentration
i.d.	internal diameter
J	coupling constant
LC	Lethal Concentration
lit.	literature
m	multiplet
<i>m</i>	meta
max	maximum
nm	nanometer
<i>o</i>	ortho
ppm	parts per million
s	singlet
t	triplet



CHAPTER 1

INTRODUCTION

Medicinal Plants

Humankind is dependent on plants as the sources of carbohydrates, proteins, and fats for basic nutrition. Other useful materials such as wood, cellulose, gums, rubber, and several other products are also obtained from plant source. Plants have been known as a storehouse of various secondary metabolites that served humankind as source of medicinal agents. Many bioactive secondary compounds from plants have proven useful as model compounds for drug syntheses and semi-syntheses as well as target compounds for plant cell culture production. Thus the medicinally important plant-derived natural products have been instrumental and essential in the era of modern medicine and therapeutics. Today plants continue to be important sources of drugs for the treatment of diseases.

The world trade in plant-derived natural products has increased with time. For example, the European market for pharmaceuticals in 1995 was US \$1098 million and this has steadily increased throughout the 1990s (Fasihi, 1996). It is predicted that this trend will continue and that the market will be worth US \$1375 million by 2001. Among the most popular herbal extracts used in Europe are garlic (*Allium sativum* for antimicrobial, blood cholesterol lowering), ginkgo (*Ginkgo biloba* for circulatory insufficiency), saw palmetto (*Serenoa repens* for diuretic, reduction of enlarged prostate), milk thistle (*Silybum marianum* for treatment of liver disorders), bilberry (*Vaccinium myrtillus* for inflammation of mucous membranes,



diarrhoea) and grape seeds (*Vitis vinifera* for antioxidant, treatment of CVS diseases) (Phillipson, 1999).

Many medicinal plants are distributed in tropical area. Soejarto *et al.* (1991) estimated that one in every twelve drugs prescribed in the United States contains ingredients derived from tropical rain forest plants. Worldwide, one in three of plant-derived drugs come from tropical rain forest plants. Some clinically useful drugs from tropical rain forest plants are presented in Table 1.1. In view of the fact that 65% of flowering plants growing on our planet are found in the tropical belt, in which only a small fraction has been investigated for medical purpose, it is believed that further investigation of tropical rain forest plants will yield important drugs to treat diseases in which we still have no satisfactory cures. Although relatively few species of tropical plants have been investigated for possible medicinal effectiveness, those few that have entered the world's pharmacopeia have already had a major impact on developed-world health care (Gentry, 1993).

Table 1.1: Clinically Useful Drugs from Tropical Rain Forest Plants

Drug Name	Plant Source	Clinical Use
Ajmalicine	<i>Rauwolfia serpentina</i>	Circulatory stimulant
Andrographolide	<i>Andrographis paniculata</i>	Antibacterial
Atropine	<i>Duboisia myoporoides</i>	Anticholinergic
Camphor	<i>Cinnamomum camphora</i>	Rubefacient
Cocaine	<i>Erythroxylum coca</i>	Local anesthetic
Diserpidine	<i>Rauwolfia tetraphylla</i>	Antihypertensive
L-Dopa	<i>Mucuna deeringia</i>	Antiparkinsonism
Emetine	<i>Cephaelis ipecacuanha</i>	Emetic
Glaucarubin	<i>Simarouba glauca</i>	Amebicide
Glaziovine	<i>Ocotea glaziovii</i>	Antidepressant
Kawain	<i>Piper methysticum</i>	Tranquilizer
Monocrotaline	<i>Crotalaria spectabilis</i>	Antitumor
Ouabain	<i>Strophanthus gratus</i>	Cardiotonic
Physostigmine	<i>Physostigma venenosum</i>	Anticholinesterase
Picrotoxin	<i>Anamirta cocculus</i>	Analeptic
Pilocarpine	<i>Pilocarpus joborandi</i>	Parasympathomimetic
Quinidine	<i>Cinchona ledgeriana</i>	Antiarrhythmic
Quinine	<i>Cinchona ledgeriana</i>	Antimalarial
Quisqualic acid	<i>Quisqualis indica</i>	Anthelmintic
Rescinnamine	<i>Rauwolfia serpentina</i>	Antihypertensive
Reserpine	<i>Rauwolfia serpentina</i>	tranquilizer
Rorifone	<i>Rorippa indica</i>	Antitussive
Rotenone	<i>Lonchocarpus nicou</i>	Piscicide
Stevioside	<i>Stevia rebaudiana</i>	Sweetener
Theobromine	<i>Theobroma cacao</i>	Diuretic
Vasicine	<i>Adhatoda vasica</i>	Oxytocic
Vincristine	<i>Catharanthus roseus</i>	Antitumor
Yohimbine	<i>Pausinystalia yohimba</i>	Adrenergic blocker

Source: Soejarto *et al.* (1991)

Tropical rain forest of Southeast Asia in general and that of Malaysia in particular are widely acknowledged as one of the most species-rich terrestrial ecosystems in the world (Soepadmo, 1992). In this area, about 25,000 – 30,000 species of flowering plants have been recorded. In Peninsular Malaysia and its neighboring islands, there are about 6,000 – 7,000 species of higher plants that have been reported to have therapeutic or medicinal properties. The plants have been used