UNIVERSITI PUTRA MALAYSIA

CHEMISTRY AND BIOLOGICAL ACTIVITY OF ANTHRAQUINONES FROM *MORINDA ELLIPTICA* (RUBIACEAE)

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CHEMISTRY AND BIOLOGICAL ACTIVITY OF ANTHRAQUINONES FROM *MORINDA ELLIPTICA* (RUBIACEAE)

By

NOR HADIANI ISMAIL

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This dissertation is dedicated,
In loving memory,
To my dearest father,

ISMAIL AZAD KHAN

A kind and gentle man
Whom I will always remember as a great teacher
He had taught me how to learn...

Al fatihah...

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So many good people have helped me along this journey. It is impossible to mention all of them, but let my thanks to a few apply equally well for the others.

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ABBREVIATIONS USED IN TEXT

δ	chemical shift in ppm
µg	microgram
°C	degree in celcius
bp	boiling point
br	broad
BuOH	butanol
cm	centimeter
COSY	Correlated Spectroscopy
^{13}C	carbon-13
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
dt	doublet of triplet
ED	effective dose
EIMS	electron impact mass spectroscopy
EtOH	ethanol
FGHMBC	Field Gradient Heteronuclear Bond Connectivity by 2D Multiple Quantum NMR
FGHMQC	Field Gradient ^{1}H-Detected Heteronuclear Multiple Quantum Coherence via Direct Coupling
GC-MS	Gas Chromatography-mass spectroscopy
^{1}H	proton
HETCOR	Heteronuclear Chemical Shift Correlation
HRMS	high resolution mass spectrum
HPLC	high performance liquid chromatography
Hz	Hertz
IC	inhibition concentration
id	internal diameter
IR	infrared
J	coupling in Hz
l	liter
LC	lethal concentration
LD	lethal dose
Lit.	literature
m	multiplet
m	meta
M	molar
MeOH	methanol
MHz	megaHertz
MIC	minimum inhibition concentration
Mol. wt.	molecular weight
mp	melting point
MS	mass spectrum / mass spectrometry
nm	nanometer
NMR	nuclear magnetic resonance
NOE	Nuclear Overhauser Enhancement
o	ortho
PE	petroleum ether
PLC	preparative layer chromatography
ppm	parts per million

q	quartet
s	singlet
sh	shoulder
t	triplet
td	triplets of doublet
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultraviolet

Abstract of the dissertation presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy.

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NOR HADIANI ISMAIL
February 1999

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

Faculty : Science and Environmental Studies

Morinda elliptica or locally known as "mengkudu kecil" is a medicinal plant commonly used by the Malays in Peninsular Malaysia. The plant parts are used in various ways to treat many health problems and ailments. Literature search did not reveal any reports on its chemical constituents or biological activity. So, it is believed that this is the first study on the chemical constituents and biological activity of *Morinda elliptica*.

Phytochemical studies on roots of this plant have resulted in the isolation of a new anthraquinone, 2-formyl-1-hydroxyanthraquinone, and ten known anthraquinones, 1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, soranjidiol, morindone, rubiadin-1-methyl ether, morindone-5-methyl ether and alizarin-1-methyl ether. The structures of the anthraquinones were established based on spectral studies using ultraviolet-visible spectroscopy, infrared spectroscopy, one and two dimensional nuclear magnetic resonance spectra, and mass spectrometry.

The new anthraquinone, 2-formyl-1-hydroxyanthraquinone, was synthesized through a four step synthesis from phthalide anhydride and 4-bromophenol. The steps involved a Friedel-Crafts condensation of the starting material to form the anthraquinone rings followed by debromination and methylation before an oxidation step to the targeted aldehyde.

Studies on the biological activity of the anthraquinones were conducted using some selected bioassay procedures. The anthraquinones were subjected to a battery of bioactivity testings, which included antimicrobial, antiviral, cytotoxicity and antioxidant assays. The results of these tests indicated that some of the compounds do possess interesting biological activity especially in the cytotoxicity and antioxidant assays. Damnacanthal and nordamnacanthal were found to be active against many cell lines tested and may have potential to be developed as anticancer agents.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi keperluan Ijazah Doktor Falsafah.

KAJIAN KIMIA DAN KEAKTIFAN BIOLOGI ANTRAKUINON DARIPADA *MORINDA ELLIPTICA* (RUBIACEAE)

oleh

NOR HADIANI ISMAIL
Februari 1999

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Morinda elliptica, atau lebih dikenali oleh penduduk tempatan sebagai mengkudu kecil, ialah sejenis tumbuhan ubatan yang kerap digunakan oleh orang-orang Melayu di Semenanjung Malaysia. Bahagian-bahagian pokok ini digunakan dalam pelbagai cara untuk merawat banyak masalah kesihatan dan penyakit. Kajian literatur tidak mendedahkan apa-apa laporan tentang kandungan kimia ataupun keaktifan biologi tumbuhan ini. Jadi, adalah dipercayai bahawa kajian kandungan kimia dan keaktifan biologi ini adalah yang pertama kali dilakukan ke atas *Morinda elliptica*.

Kajian fitokimia ke atas akar tumbuhan in telah berakhir dengan pemencilan satu antrakuinon baru, 2-fomil-1-hidroksiantrakuinon, serta sepuluh antrakuinon yang telah diketahui, 1-hidroksi-2-metilantrakuinon, nordamnakantal, damnakantal, lusidin-ω-metil eter, rubiadin, saranjidiol, morindon, rubiadin-1-metil eter, morindon-5-metil eter dan alizarin-1-metil eter. Struktur-struktur antrakuinon tersebut dikenal pasti berdasarkan kajian spektroskopi menggunakan spektroskopi ultra lembayung-nampak, spektroskopi inframerah, spektroskopi resonan magnetik nuklear satu dan dua dimensi, serta spektroskopi jisim.

Antrakuinon yang baru, 2-fomil-1-hidroksiantrakuinon, telah disintesiskan melalui sintesis empat langkah bermula dengan ftalik anhidrida dan 4-bromofenol. Langkah-langkah tersebut melibatkan kondensasi Fridel-Craft bahan permulaan untuk menghasilkan gelang anthrakuinon, diikuti dengan pendebrominan serta pemetilan sebelum satu langkah pengoksidaan untuk menghasilkan aldehid yang dirancang.

Kajian keaktifan biologi ke atas antrakuinon-antrakuinon yang diperolehi dilakukan menggunakan beberapa kaedah biocerakinan yang terpilih. Kaedah-kaedah tersebut termasuklah ujian antimikrob, ujian antivirus, ujian sitotoksik serta ujian antioksidaan. Keputusan ujian-ujian ini menunjukkan sebahagian daripada sebatian-sebatian ini mempunyai sifat keaktifan biologi yang menarik, terutamanya dalam ujian-ujian sitotoksik dan antioksidaan. Damnakantal dan nordamnakantal didapati aktif terhadap beberapa talian sel yang diuji serta berpotensi untuk dibangunkan sebagai agen-agen antikanser.

CHAPTER 1
INTRODUCTION

"Plant in a good soil grows by the will of its Lord"

[7:58] Surah Al 'araf

General

God given Nature provided humans with sources of many essentials of life; food, medicines, and raw materials for the manufacturing of clothing and shelters. Higher plants in particular, have been the source of medicinal agents since earliest times. Today, they continue to play a dominant role in the primary health care of about 80% of the world's population (Farnsworth *et al*., 1985). More than 50% of all drugs currently in use are of natural product origin (Balandrin *et al*., 1993). Of the world's 25 best selling pharmaceutical agents, 12 are natural product-derived (O'neil and Lewis, 1993). Research into the chemical and biological properties of natural products over the past two centuries has resulted in the discovery of many drugs for the treatment of human ailments. It has also provided the stimulus for the development of modern synthetic chemistry.

Pioneering studies on the active constituents of *Podophyllum peltatum* L. followed by discovery and development of the antileukemic agents, vinblastine and vincristine, from *Catharanthus roseus* L. provided convincing evidence that plants could be sources of novel, potential chemotherapeutic agents (Baker *et al*., 1995). Discovery of taxol and camptothecin was the result of NCI's systematic effort in

collecting and screening plants for antitumour activity (Cragg *et al.*, 1993). Taxol has now been approved to be used for the treatment of ovarian and breast cancer (Kingston, 1993). Camptothecin, on the other hand, has been converted to several analogues through semi-synthesis. Some of the analogues are showing promise in advanced clinical trials (Wall and Wani, 1993). Table 1.1 listed some of the important pharmaceutical agents that are of natural product origin.

Table 1.1 : Some Pharmaceuticals of Plant or Microorganism Origin

Pharmaceuticals	Sources
Steroids from diosgenin	*Dioscorea deltoides*
Codeine	*Papaver somniferum*
Atropine/Hyoscyamine	*Hyoscyamus niger*
Reserpine	*Rauwolfia serpentine*
Digoxin	*Digitalis lanata*
Digitoxin	*Digitalis purpurea*
Scopolamine	*Datura metel*
Pilocarpine	*Pilocarpus jabonandi*
Quinidine	*Cinchona ledgerina*
Vincristine and Vinblastine	*Catharanthus roseus*
Penicillin G	*Penicillium*
Cephalosporins	*Cephalosporium acrimonium*
Streptomycin	*Streptomyces griseus*
Tetracycline	*Streptomyces sp.*
Ephedrine	*Ephedra sinica*
Colchicine	*Colchicum autumnale*

Source: N. H. Lajis, 1993.

In spite of many successful developments in synthetic chemistry, the potential of higher plants as sources for new drugs is still not exhausted. Of the estimated 250 000 existing plant species world wide, only a small percentage has been investigated for the presence of bioactive compounds. Very little is known about the plants secondary metabolites; and this is especially true for tropical flora. Tropical flora constitute over 60% of the estimated 250 000 higher plant species. Given the rapid destruction of tropical habitats especially the rainforests, this

potential loss of knowledge and useful products is alarming. Considering that the 119 plant-derived drugs commonly in use in one or more countries were isolated from only about 90 plant species (Farnsworth, 1993), the potential for drug discovery from plants and other natural sources is enormous.

Besides its vast and diverse flora, the tropical rainforest with its humid climate, also store immense variety of insects, bacteria and fungi. Such co-existence creates interaction between living organisms occupying the same habitat. Thus it can be expected that plants through evolution are able to produce highly active, low molecular weight compounds as defence compounds. These compounds are of special interest in the development of new medicinal agents. The interests showed by the various organisations through out the world especially National Cancer Institute of USA illustrate the importance of the chemical constituents of higher plants from tropical rainforests. In September 1986, NCI initiated plant and marine invertebrate collections through contracts with several organisations. Currently the collections are being carried out in more than 25 countries, situated mainly in tropical and subtropical regions (Baker *et al*., 1995).

Natural Product Research in Malaysia

Malaysia being located in the tropical belts still maintains quite a substantial area of tropical rainforest despite the rapid developments that is taking place in the country. Its rainforest stores a large collection of plant species, which is important as the source of traditional medicine. It is estimated that there are about 10 000 species of higher plants and about 2000 species of lower plants available in Peninsular Malaysia with approximately sixteen percent of these claimed to be used

for medicinal purposes (Lattif, 1984) and has potential to be developed into various useful natural products.

The earliest records on the use of plants as medicine in Malaysia were given by Burkill and Haniff (1930), Burkill (1935) and Gimlett and Burkill (1930). However, chemical studies on Malaysian plants only began in the late fifties when Douglas and Kiang published their first phytochemical survey report (Douglas and Kiang, 1957). This was the beginning of natural product chemistry research in Malaysia. Since then, several other surveys were conducted and reported by Carrick (1968), Chan (1969), Chan (1972) and other groups (Rahmani, 1985). Nakanishi (1965) not only reported on phytochemical survey, but also included pharmacological screenings.

Currently, natural product chemistry is an active field of research in Malaysia. Phytochemical screenings are being conducted routinely by various research groups. The reasons are obvious. The country has a rich and diverse floral heritage, most of which has not been fully investigated and are waiting to be discovered. This is further enhanced by the blend of its multiracial societies, which stores broad traditional knowledge about the folkloric uses of many plants for health care and other purposes. It is expected that research in this area can generate new knowledge and information about the plants not found in other parts of the world. An example is the isolation of calanolide A, a compound that was shown to possess anti-HIV activity from *Calophyllum lenigerum* (Kashman *et al.*, 1992) found in Sarawak.

In the Natural Products Laboratory of UPM, the study on the alkaloid content of *Alseodaphne perakensis* (Lauraceae) (Lajis *et al.*, 1989; 1991; 1992), *Psychotria rostrata* (Rubiaceae) (Lajis *et al.*, 1993; Mahmud *et al.*, 1993), *Breynia coronata*

(Euphorbiaceae) (Lajis *et al.*, 1992; 1995), *Ophiorrhiza* [illegible] and *Opnorriza tomentosa* (Hamzah *et al.*, 1994; Arbain *et al.*, 1993), *Lerchea bracteata* (Arbain *et al.*, 1992) and a number of *Hedyotis* species (Hamzah *et al.*, 1994; 1997; Ahmad *et al.*, 1996) have revealed several interesting new compounds some of which showed rather interesting biological activities such as analgesic activity, cytotoxicity as well as antimicrobial and antiviral activities. Screening of local plants collected from various parts of the country for biological activities are done routinely as part of the natural products research program in the university. The species studied in this project was selected based on the results of one of the screening programs.

Isolation of Biologically Active Compounds from Higher Plants

Phytochemical investigation will provide data on the chemical constituents of the plant concerned. However, many new natural compounds were isolated, characterised and published without any biological testing (Meyer *et al.*, 1982) and their useful biological activities can remain unknown for a long time. The significance of the phytochemical work is greatly increased if the fine chemicals isolated from the plant possess certain biological activity, particularly if the activity is in line with the development of medicinal agents. So, the objective of most research in this area is now focused on obtaining biologically active compounds. This is made possible with the aid of simple bioassays that can be used in screening crude plant extracts for biological activity. Once the biological activity of a plant extract has been confirmed, fractionation and separation procedures that lead to the isolation of the active compounds can be performed.

So, if the aim of the work is to isolate bioactive compounds, the use of some form of bioassay in screening and fractionation of samples is crucial. A reliable general bioassay system that can detect a broad spectrum of pharmacological