Dysoxylum grande HIERN AS A POTENTIAL SOURCE OF BIOLOGICALLY ACTIVE CONSTITUENTS

LOW KOK WAH

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Dysoxylum grande HIERN AS A POTENTIAL SOURCE OF BIOLOGICALLY ACTIVE CONSTITUENTS

By

LOW KOK WAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, In Fulfilment of the Requirement for the Degree of Master of Science

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

Dysoxylum grande HIERN AS A POTENTIAL SOURCE OF BIOLOGICALLY ACTIVE CONSTITUENTS

By

LOW KOK WAH

June 2012

Chairman : Intan Safinar Ismail, PhD

Faculty : Science

Dysoxylum grande was chosen to be studied due to the lack of reported scientific investigations on the plant and interesting phytochemical findings on another plant of the same genus, *Dysoxylum acutangulum*. Two biological activities; anti-acetylcholinesterase and ichthyotoxicity were used as the bioassay in guiding the fractionation step. As a result, nine compounds were isolated from the ichthyotoxic EtOAc leaves fraction. Among them, eight were identified as new compounds named as 20*S*,24*R*-epoxy-7*β*,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic acid (**CP1**), grandol A (**CP2**), grandol B (**CP3**), grandol C (**CP4**), grandol D (**CP5**), grandol E (**CP8**), grandol F (**CP9**) and grandol G (**CP10**) and the known compound was identified as *β*-sitosteryl-*β*-D-glucopyranoside (**CP11**). All new compounds were identified based on thorough analysis of spectroscopic data including 1D and 2D NMR. The structure for the known compound was established by spectral data analyses and by comparison with reported investigations. In ichthyotoxic assay carried out on Zebra fish (*Danio rerio*), EtOAc fraction (leaves) was found to

possess significant ichthyotoxicity compared to other fractions. However, the isolated pure compounds did not provide any IC_{50} values for the ichtyotoxicity test due to their limited amounts. All compounds were tested for anti-acetylcholinesterase activity using Thin Layer Chromatography (TLC)-bioautography with fast blue B salt. Only grandol A (**CP2**) and grandol B (**CP3**) showed positive results. However, the obtained IC_{50} for both of these compounds, grandol A (**CP2**) and B (**CP3**), using Ellman's method was not significant (>200 µg/mL).

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

Dysoxylum grande HIERN SEBAGAI SUMBER BERPOTENSI BAGI KANDUNGAN SEBATIAN AKTIF BIOLOGI

Oleh

LOW KOK WAH

Jun 2012

Pengerusi : Intan Safinar Ismail, PhD

Fakulty : Sains

Dysoxylum grande telah dipilih untuk kajian penyelidikan memandangkan kurangnya penyelidikan yang dilaporkan secara saintifik ke atas tumbuhan ini dan penemuan fitokimia yang menarik ke atas tumbuhan lain dalam genus yang sama, iaitu *Dysoxylum acutangulum*. Dua bioaktiviti; 'anti-acetylcholinesterase' dan 'ichthytoxicity' telah digunakan sebagai bioassai dalam membantu langkah pemeringkatan. Hasilnya, sembilan sebatian telah diasingkan daripada fraksi daun EtOAc yang 'ichthyotoxic'.Antaranya, lapan sebatian baru telah dikenapasti yang dinamakan sebagai asid 20*S*,24*R*-epoxy-7β,25-dihydroxy-3,4-secodammar-4(28)-en-3-oic (**CP1**), grandol A (**CP2**), grandol B (**CP3**), grandol C (**CP4**), grandol D (**CP5**), grandol E (**CP8**), grandol F (**CP9**) dan grandol G (**CP10**) dan satu sebatian yang telah diketahui iaitu β-sitosteril-β-D-glukopiranosida (**CP11**). Semua sebatian baru telah dikenalpasti melalui analisis ke atas data spektroskopi termasuk 1D dan 2D NMR. Struktur bagi sebatian yang telah diketahui telah dikenalpasti oleh analisis data spektrum dan perbandingan dengan penyelidikan yang dilaporkan sebelum ini.

Dalam assai 'ichthyotoxic' yang dijalankan ke atas ikan Zebra (*Danio rerio*), fraksi EtOAc (daun) didapati mempunyai aktiviti yang ketara berbanding dengan fraksi yang lain. Namun demikian, sebatian tulen yang dipisahkan tidak dapat memperolehi nilai IC_{50} dalam 'ichtyotoxicity' disebabkan oleh kuantiti sebatian yang tidak mencukupi. Semua sebatian juga telah diuji dengan 'anti-acetylcholinesterase' menggunakan kromatografi lapisan nipis (TLC)-bioautografi dengan 'fast blue salt B'. Hanya grandol A (**CP2**) dan grandol B (**CP3**) menunjukkan hasil yang positif. Namun demikian, nilai IC_{50} yang diperolehi bagi kedua-dua sebatian dengan mengguanakan kaedah Ellman didapati tidak ketara (>200 µg/mL).

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Lastly, I wish to thank my entire extended family for providing a loving environment for me and supporting me spiritually throughout my life.

I certify that a Thesis Examination Committee has met on 26 June 2012 to conduct the final examination of Low Kok Wah on his thesis entitled "*Dysoxylum grade* Hiern as a Potential Source of Biologically Active Constituents" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Mohd Aspollah bin Hj Md Sukari, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Gwendoline Ee Cheng Lian, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Siti Mariam binti Mohd Nor, PhD
Doctor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Hasnah Mohd Sirat, PhD
Professor
Faculty of Science
Universiti Teknologi Malaysia
(External Examiner)

_____
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date: 27 Septermber 2012

This thesis was 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 were as follows:

Intan Safinar Ismail, PhD
Lecturer
Institute of Bioscience
Universiti Putra Malaysia
(Chairman)

Faridah Abas, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

_____

BUJANG BIN KIM HUAT
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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LOW KOK WAH

Date: 26 June 2012

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LIST OF ABBREVIATIONS

δ	Chemical shift
bp	Boiling point
br	Broad
COSY	Correlation spectroscopy
d	Doublet
dd	Doublet of doublets
dt	Doublet of triplets
EIMS	Electron Impact Mass Spectrum
HMBC	Heteronuclear Multiple Bond Correlation
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
J	Coupling constant
m	Multiplet
m/z	Mass per charge
MHz	MegaHertz
mp	Melting Point
nm	Nanometer
NOESY	Nuclear Overhauser Effect Spectroscopy
q	Quartet
R_f	Retention factor
s	Singlet
t	Triplet

CHAPTER 1

Introduction

1.1 Natural Products

Natural products have been an overwhelming success as a rich source of therapeutically effective medicines. Approximately 60% of the world's population is fully relies on plants for medication. Among 520 new drugs approved between 1983 and 1994, 60 – 80% of antibacterial and anticancer drugs are derived from natural products and 39% are natural products or derived from natural products. Natural products in the present are commonly used to cure diseases which include anticholesteremic agents (e.g. levostatin and derivatives), antibiotics (e.g. erythromycin and derivatives, such as clarithromycin), antitumor agents (e.g. paclitaxel and camptothecin derivatives such as topotecan and irinotecan) and immunosuppressive agents (e.g. cyclosporine A and FK 506) (Bindseil *et al*., 2001).

Current commercial evidence supports the use of natural products. A study in 1999 has shown twenty best-selling non-protein drugs were either derived from or developed as the result of substances generated by natural products such as simvastatin, levostatin, enalapril, pravastatin, atorvastatin, augmentin, ciprofloxacin, clarithromycin and cyclosporine. These drugs have combined annual sales of more than US$16 billion. Current developments based on natural products include the antimalarial drug

artemisinin and the anticancer agents such as taxol, docetaxel and camptothecin. Therefore, the use of natural products has been the most successful strategy for the discovery of new medicines (Harvey, 2000).

1.2 **Meliaceae**

The Meliaceae family is characterized by the presence of limonoid triterpenes, many of which are biologically active against insects. Two limonoids have been commercialized which are azadirachtin in the U.S. and toosendanin in China from the Asian species *Azadirachta indica* and *Melia azedarach*. Extracts of several species from the genera *Cedrela*, *Trichilia*, *Swietenia* and *Guarea* from the Neotropics have demonstrated moderate to good growth reducing activity to European corn borer (ECB), *Ostrinia nubilalis* Hubner. Consequently, some of these species have been the subject of chemical and biological investigations in order to isolate new insecticidal agents (Jimenez *et al*., 1998).

Limonoids are an important group of metabolically altered-triterpenes, which are limited in their distribution. In recent years a large number of pharmacological studies have been carried out to indicate their beneficial effects. The medicinal properties reported include anti-cancer, anti-malarial, antimicrobial, anti-HIV, anti-viral and several others. Post-study health evaluation has established no ill effects among study subjects consuming high doses of limonin glucoside. Some of them have also shown allelopathic potential. The citrus limonoids exhibit promising health benefits (anti-cancer, cardio protective, anti-oxidant etc.) but are the major causes of concern due to their extreme

bitterness. There is a need to develop an acceptable and versatile debittering method that can substantially remove or mitigate the bitterness of fruits and juice (Amit and Shailendra, 2005).

Folk medicines from the Meliaceae plants have been traditionally used to cure malarial disease in many tropical and sub-tropical countries. The elevated incidence of malaria in recent years has been attributed to the development of resistance of the malarial protozoa (*Plasmodium falciparum*) to chloroquine and to the development of resistance of the vector mosquitoes to insecticides. In Africa, all species of *Khaya* used in the German timber and furniture industry contribute to malarial diseases. These plants usually contain a variety of limonoids and they possess antimalarial activities. Limonoids are the most representative class of secondary metabolites in the order Rutales, which includes the Rutaceae, Meliaceae, and Simaroubaceae families. They are tetranortriterpenoids with 4,4,8-trimethyl-17-furanylsteroidal skeleton, with several oxygenated functions. Several limonoids of angolensates, khivorins, mexicanolides, and fissinolides have been isolated from different parts of *Khaya anthotheca*. The limonoids have several beneficial properties such as low mammalian toxicity, lack of neurotoxic mode of action, low persistence in the environment and biodegradability and are reported to be new sources of biologically active natural products (Lee *et al*., 2007).

In our study group, two plants from the Meliaceae family had been investigated from which two novel triterpenes, acutaxylines A, B and three-type limonoids, sandrapins A, B and C have been successfully isolated from the *Dysoxylum acutangulum* and

Sandoricum koetjape respectively. The methanol extract from the *Dysoxylum acutangulum* leaves showed cytotoxicity against human cancer cells (Ismail *et al*., 2009).

1.3 Ichthyotoxic Assay

Ichthyotoxic assay is a quick preliminary test carried out to determine the possible potential biological activities that may be possessed by the plant under study. Ichthyo itself means fish and this test is performed by using fish to exhibit the significant toxicity. Based on the previous findings, the ichthyotoxic compounds often exhibited various biological activities such as insecticidal, anti-tumor and antibacterial (Ismail *et al*., 2003). Therefore, this assay is a suitable method to preliminarily identify the active fraction/compound. Several researchers had performed ichthyotoxic assay as a preliminary screening for biologically active substance. Ismail *et al.* (2003) had used this assay as a preliminary test to determine the potential biological activities in *Sandoricum koetjape* bark against killie-fish. In this study, it was shown that the ichthyotoxic compounds also exhibited cytotoxicity.

1.4 Acetylcholinesterase (AChE) Assay

Studies have shown that the inhibition of brain acetylcholinesterase (AChE) plays an important role for the treatment of many degenerative diseases such as shoulder tendonitis, heart disease, inflammatory bowel disease, rheumatoid arthritis, asthma and gouty arthritis which are always associated with inflammatory process (Fawole *et al.,* 2010). Inhibition of brain acetylcholinesterase (AChE) has been the major therapeutic

target of cholinesterase inhibitor (ChEI) treatment strategies for Alzheimer's disease (AD), Parkinson's disease, myasthenia gravis, dementia, ataxia, and senile. However, existing anticholinesterase drugs such as tacrine, physostigmine and heptylphysostigmine, donepezil and galantamine were reported to have several side effect and dangerous (Sancheti *et al*., 2010). Due to this, more new anticholinesterase drugs are needed to replace them. In this study, two acetylcholinesterase (AChE) assay methods were used to screen the fraction/compounds, which are Thin Layer Chromatography (TLC)-bioautography with fast blue B salt and Ellman's method.

1.5 Problems Statement

The world's population relies on plants for medication since ancient. However, there are still diseases which are unable to be cured or the existing drugs are having several side and/or adverse effects which could be dangerous when used. Therefore, there is need to increase the volume of research to find out more potential drugs from natural products. Tapping on plant is one of the many ways to discover the potential bioactive compounds natural resources. As *Dysoxylum grande* is one of the least explored plants of Malaysia but being traditional claimed as having bioactivities, we decided to explore the possibilities of discovering the potential properties of it through some set objectives.

1.6 Research Objectives

This study focuses on the isolation and characterization of the chemical constituents from *Dysoxylum grande* leaves and the bioactivities of the isolated compounds. All the

compounds obtained were characterized using thorough spectroscopic data obtained including ^{1}H, ^{13}C Nuclear Magnetic Resonance (NMR), 2D-NMR, mass spectroscopy (MS) and infrared (IR) spectroscopy.

Due to the lack of scientific report on this Meliaceae plant species and interesting characteristics of compounds with various biological activities, *D. grande* was chosen to be studied based on the objectives of:

1. To discover pure compounds from the ichthyotoxic plant part.
2. To determine the possible bioactivity (ies) of the isolated compounds.

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BIODATA OF STUDENT

Low Kok Wah was born in Taiping, Perak, Malaysia on the 25th March 1984. He had his early education at Sekolah Jenis Kebangsaan Hua Lian, Taiping and secondary education at Sekolah Menengah Jenis Kebangsaan Hua Lian. He completed his Sijil Pelajaran Malaysia (SPM) and Sijil Tinggi Pelajaran Malaysia (STPM) at the same school. After that, he was offered to study in Universiti Putra Malaysia in Bachelor of Science (Honours) degree in Industrial Chemistry. Then he worked as a Quality Assurance Executive at Lion Dri Sdn Bhd for two years. He resigned to further study at Universiti Putra Malaysia for Master in Science (Natural Product). He was also offered as a Grand Research Fellow holder during his MSc study.