UNIVERSITI PUTRA MALAYSIA

CHEMICAL CONSTITUENTS OF *DYSOXYLUM ACUTANGULUM* Miq. AND THEIR BIOACTIVITIES

MOHD IZWAN MOHD LAZIM

IB 2012 11
CHEMICAL CONSTITUENTS OF *DYSOXYLUM ACUTANGULUM* Miq. AND THEIR BIOACTIVITIES

MOHD IZWAN MOHD LAZIM

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2012
CHEMICAL CONSTITUENTS OF *DYSOXYLUM ACUTANGULUM* Miq. AND THEIR BIOACTIVITIES

By

MOHD IZWAN MOHD LAZIM

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

March 2012
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CHEMICAL CONSTITUENTS OF *DYSOXYLUM ACUTANGULUM* Miq. AND THEIR BIOACTIVITIES

By

MOHD IZWAN MOHD LAZIM

March 2012

Chairman : Intan Safinar Ismail, PhD
Institute : Bioscience

A phytochemical studies on *Dysoxylum acutangulum* leaves have been carried out and resulted in the isolation of seven compounds including two new compounds assigned as chrotacumine E (62) and F (63), along with four known compounds; rohitukine (10), noreugenin (38), β-sitosterol (60), hexadecanoic acid (61) and one unsolved compound, Chrotacumine G (59). The structures of the compounds including their relative configurations were elucidated based on the spectroscopic data including Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), Fourier Transform Infrared (FT-IR), Circular Dichorism (CD) and also by comparison with the previous reported works.

*Dysoxylum* has been the subject of research interest in the field of natural products due to their unique chemistry and biological properties. *Dysoxylum acutangulum* are among those known to be used as traditional medicines. From the early
investigations on these plants, some interesting new compounds were discovered, some of which exhibited noteworthy biological properties such as anti-tumor promoting, anti-acetylcholinesterase and ichthyotoxic activities. Screening for biological activities is an important preliminary step in natural products research when aiming at discovering bioactive compounds from plant. In this research, two extraction methods have been done which is liquid-liquid partition and acid base extraction to obtain alkaloidal extract. All extract was subjected to column chromatography silica gel to fractionate and purify.

Based on a bioautographic Thin Layer Chromatography (TLC) profile of anti-acetylcholinesterase activity on the major fractions of *D. acutangulum* leaves, hexane fraction has showed a promising activity. The most active hexane subfraction was further fractionated and purified by a bioassay-guided fractionation wherein hexadecanoic acid (61) was obtained showing as a potent acetylcholinesterase (AChE) inhibitor. On the contrary, the AChE activity of 61 was not significant (1mg/mL) when tested by Ellman’s method. In the ichthyotoxic assay carried out on Zebra fish (*Denio rerio*), EtOAc fraction was found to possess significant ichthyotoxicity compared to other fractions; hexane, BuOH, CHCl₃ and aqueous. Meanwhile, BuOH fraction showed 70% inhibition in the tyrosinase assay. All of the isolated compounds were tested for their ichthyotoxicity, anti-acetylcholinesterase and anti-tyrosinase activity. However, none of them showed any significant activities.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KANDUNGAN KIMIA DYSOXYLUM ACUTANGULUM Miq. DAN AKTIVITINYA

Oleh

MOHD IZWAN MOHD LAZIM

Mac 2012

Pengerusi : Intan Safinar Ismail, PhD
Institut : Biosains

Kajian sebatian kimia terhadap daun Dysoxylum acutangulum telah menghasilkan tujuh sebatian termasuk dua sebatian baru yang dikenali sebagai chrotacumine E (62) dan F (63) bersama-sama dengan empat sebatian lain yang telah ditemui sebelum ini iaitu; rohitukine (10), noreugenin (38), β-sitosterol (60), asid heksadekanoik (61) dan satu sebatian yang belum dikenalpasti iaitu chrotacumine G (59). Struktur sebatian termasuk konfigurasi relatif telah dikenal pasti berdasarkan data spektroskopi seperti NMR, GC-MS, FTIR, CD dan perbandingan dengan penemuan yang terdahulu.

Dysoxylum merupakan salah satu genus yang telah menjadi subjek penyelidikan sebatian semulajadi yang utama disebabkan oleh kandungan sebatian kimia dan biologinya. Dysoxylum acutangulum telah dikenalpasti dan digunakan sebagai ubat tradisional. Beberapa sebatian baru yang menarik telah ditemui semasa proses
saringan dan penyelidikan awal ke atas tumbuhan ini, dimana terdapat sifat-sifat biologi yang perlu diberi perhatian seperti menggalakkan anti-tumor, anti acetylcholinesterase dan ichthyotoxic aktiviti. Saringan untuk aktiviti-aktiviti biologi adalah langkah awal yang penting dalam sesuatu penyelidikan yang bertujuan untuk menemui sebatian bioaktif dari sesuatu tumbuhan. Penyaringan awal akan membantu dalam memilih sampel tumbuhan untuk kajian yang selanjutnya berdasarkan aktiviti-aktiviti tertentu. Dalam kajian ini, dua kaedah pengekstrakan telah dijalankan iaitu cea-cair-ceair pemisah serta asid dan bes pengekstrakan untuk mendapatkan ekstrak alkaloidal. Semua ekstrak dipecahkan dan disaring sehingga mendapat satu sebatian tulen dengan menggunakan kromatografi silika gel.

Berdasarkan profil Lapisan Kromatografi Nipis autografi-bio, aktiviti perencatan acetylcholinesterase pada pecahan utama daun D. acutangulum, ekstrak heksana telah menunjukkan potensi sebagai aktif ekstrak. Pecahan heksana yang paling aktif dipisahkan dan diasingkan berpandu bioassay di mana asid heksadekanoik (61) telah dikenalpasti sebagai perencat acetylcholinesterase. Sebaliknya, aktiviti perencatan AChE (61) (1mg/mL) tidak ketara apabila diuji dengan kaedah Ellman. Dalam aktiviti ichthyotoxic yang telah dijalankan terhadap ikan zebra (Denio rerio), EtOAc telah menunjukkan aktiviti ichthyotoxic yang tinggi berbanding dengan pecahan lain; heksana, BuOH, CHCl$_3$ dan air. Manakala, pecahan BuOH menunjukkan perencatan 70% dalam ujian tyrosinase. Semua sebatian tulen yang diperolehi telah diuji untuk ichthyotoxicity, anti-acetylcholinesterase dan anti-tyrosinase aktiviti. Walau bagaimanapun, tiada satu sebatian pun menunjukkan apa-apa aktiviti yang penting.
ACKNOWLEDGEMENTS

Glory and praises be to God, the Most Gracious and Merciful, for guiding me and holding me steadfast in seeking knowledge and completing this research, despite the many challenges.

First, I would like to convey my deepest appreciation to my supervisor, Dr. Intan Safinar Ismail, for kindly accepting me as her student, and Professor Dr. Khozirah Shaari and Professor Dr. Hiroshi Morita for kindly being in my supervisory committee. Through the past two years, they not only gave me guidance in their respective field of expertise, encouragement and support, but also helped me become a better human being.

I would like to extend my warmest and best regards and appreciation to all former members of the Natural Products Laboratory, Institute of Bioscience for their help and friendship. I am particularly grateful to Dr. Ibrahim, Maulidiani, Nabil and Lam for the helpful discussions of this research. In addition, I am indebted to Shukri, Ilya, Aesya, Linda, Kok Wah and Rizal Fahmi for their kindness and support during my research. To friends I have found in the Bioscience Postgraduate Club (BPC), thank you for the cooperation and motivation.

I am also very grateful and like to express thousands of thanks to Science Officers, Mr. Salahudin, Mdm Mazina and Mdm Zurina in acquiring spectroscopic data and also special thanks go to Mr. Shamsul, Mr. Tajuddin and Mr. Fauzi in obtaining plant samples.
Last but not least, to my parents, who have always supported me to pursue my interests and encouraged me through challenging moments, I owe a debt that I can never repay. I wish to thank my siblings for their continuous support and understanding. The love that my family has provided transcends time and distance. To them, this thesis is dedicated.
I certify that a Thesis Examination Committee has met on 1st March 2012 to conduct the final examination of Mohd Izwan Bin Mohd Lazim on his thesis entitled ‘Chemical Constituents of Dysoxylum acutangulum (Meliaceae) and Their Bioactivity Studies’ in accordance with the Universities and University Colleges 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 degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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

**Mawardi Rahmani, PhD**
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

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

**Farediah Ahmad, PhD**
Associate Professor
Department of Chemistry
Faculty of Science
Universiti Teknologi Malaysia
Malaysia
(External Examiner)

________________________________
SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: ______________ 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)

**Khozirah Shaari, PhD**  
Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Member)

**Hiroshi Morita, PhD**  
Professor  
Faculty of Pharmaceutical Science  
Hoshi University, Japan  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**  
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.

________________________________________
MOHD IZWAN MOHD LAZIM

Date: 1 March 2012
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ABSTRACT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>vii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF CHARTS</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xix</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION

1.1 Natural products research in Malaysia  
1.2 Alzheimer’s Disease (AD)  
1.3 Tyrosinase inhibitors  
1.4 Ichthyotoxicity  
1.5 Research statements  
1.6 Research objectives

### 2 LITERATURE REVIEW

2.1 Meliaceae – a brief overview  
2.1.1 Taxonomy of Meliaceae  
2.1.2 Medicinal uses of Meliaceae plants  
2.2 Genus – *Dysoxylum*  
2.2.1 Use and commercial importance of *Dysoxylum*  
2.3 Phytochemical and bioactivity studies on *Dysoxylum*  
2.3.1 Alkaloids  
2.3.2 Terpenoids  
2.3.3 Limonoids (tetranortriterpenoid)  
2.4 *Dysoxylum acutangulum* Miq.  
2.4.1 General information and taxonomy  
2.4.2 Common names  
2.4.3 Ecology and distribution  
2.4.4 Functional and medicinal uses  
2.4.5 Phytochemical and pharmacological studies of *D. acutangulum*  
2.5 Bioactivity studies  
2.5.1 Anti-acetylcholinesterase activity  
2.5.2 Tyrosinase inhibitors  
2.5.3 Ichthyotoxicity
3 EXPERIMENTAL

3.1 General instrumentation

3.2 Chromatographic techniques

3.3 Solvents

3.4 Experimental methods

3.4.1 Plant materials

3.4.2 Preparation of crude extract

3.4.3 Liquid-liquid partition

3.5 Isolation of compounds from D.acutangulum leaves

3.5.1 Compounds from EtOAc extract

3.5.2 Compounds from CHCl₃ extract

3.5.3 Compounds from Hexane extract

3.5.4 Compounds from BuOH and Aqueous extract

3.6 Preparation of crude extract via acid base method

3.6.1 Isolation of compounds from the CHCl₃ 2 (K2) extract via acid base method

3.6.2 Isolation of compounds from the CHCl₃ 1 (K1) and aqueous 1 (Aq 1) extract via acid base method

3.7 Spectra data of isolated compounds

3.8 Bioassay procedures

3.8.1 Acetylcholinesterase (AChE) inhibitor

3.8.1.1 TLC bioautographic AChE assay

3.8.1.2 Ellman’s method

3.8.2 Tyrosinase inhibitor activity

3.8.2.1 96’s well microplate method

3.8.3 Ichthyotoxicity assay

4 RESULTS AND DISCUSSION

4.1 Studies of chemical constituents of D.acutangulum leaves

4.1.1 Chrotacumine E (62), a new chromone alkaloid analogues

4.1.2 Chrotacumine F (63), a new chromone alkaloid analogues

4.1.3 Chrotacumine G, (59)

4.1.4 HPLC analysis

4.1.5 Physicochemical properties of known compounds

4.2 Bioassay study

4.2.1 Preliminary study: ichthyotoxicity test

4.2.2 Anti-acetylcholinesterase

4.2.3 Anti-tyrosinase

5 CONCLUSION AND SUGGESTIONS
REFERENCES 135
APPENDIX (1) 141
APPENDIX (2) 143
APPENDIX (3) 146
BIODATA OF STUDENT 148
LIST OF PUBLICATIONS 149