STRUCTURAL DIVERSITY, CHEMOSYSTEMATICS, AND BIOLOGICAL POTENTIAL OF BORNEAN LIVERWORTS (ORDER JUNGERMANNIALES)

NG SHEAN YEAW

THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH
2017

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: **STRUCTURAL DIVERSITY, CHEMOSYSTEMATICS, AND BIOLOGICAL POTENTIAL OF BORNEAN LIVERWORTS (ORDER JUNGERMANNIALES)**

IJAZAH: **DOCTOR OF PHILOSOPHY (ADVANCEMENT OF BIODIVERSITY)**

Saya **NG SHEAN YEAW**, Sesi **2013/2014 – 2016/2017**, mengaku membenarkan tesis Doktor falsafah ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:

1. Tesis ini adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/):

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

Disahkan Oleh,
NURULAIN BINTI ISMAIL
LIBRARIAN
UNIVERSITI MALAYSIA SABAH

NG SHEAN YEAW
DX1311003T

(Tandatangan Pustakawan)

Tarikh: 07 Ogos 2017

(Prof. Dr. Charles S. Vairappan)
Penyelia

(Assoc. Prof. Dr. Monica Suleiman)
Penyelia bersama

(Dr. Takashi Kamada)
Penyelia bersama

DECLARATION

I declare that this dissertation is based on my original work, except for quotations and summaries, each of which has been fully acknowledged.

7th AUGUST 2017

NG SHEAN YEAW
DX1311003T

CERTIFICATION

NAME : NG SHEAN YEAW

MATRIC NO : DX1311003T

TITLE :STRUCTURAL DIVERSITY, CHEMOSYSTEMATICS, AND BIOLOGICAL POTENTIAL OF BORNEAN LIVERWORTS (ORDER JUNGERMANNIALES)

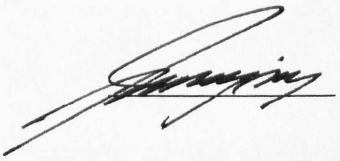
DEGREE : DOCTOR OF PHILOSOPHY (ADVANCEMENT OF BIODIVERSITY)

VIVA DATE : 19th July 2017

CERTIFIED BY

SIGNATURE

SUPERVISOR
Prof. Dr. Charles S. Vairappan



CO-SUPERVISOR
Assoc. Prof. Dr. Monica Suleiman

CO-SUPERVISOR
Dr. Takashi Kamada

ACKNOWLEDGEMENT

The completion of this research dissertation is to fulfill the requirement for Doctor of Philosophy degree in Advancement of Biodiversity. Several parties have involved in giving support, advice and guidance throughout the research's period.

First of all, I would like to express my heartfelt gratitude to my supervisor, Prof. Dr. Charles Santhanaraju Vairappan, my co-supervisors Assoc. Prof. Dr. Monica Suleiman and Dr. Takashi Kamada, who gave endless guidance and valuable advice throughout the research investigation and preparation of this dissertation. I sincerely appreciate all the opportunities and exposure given as well as time spent in nurturing me into a mature, independent and knowledgeable person.

Next, I wish to thank Sabah Parks and Sabah Forestry Department for giving the permission to collect the precious liverworts specimens. Thanks are also given to Prof. Dr. Tatsuwo Furuki (Natural History Museum and Institute, Chiba, Japan), Dr. Matt von Konrat (The Field Museum, Chicago, USA) and Miss Laura Briscoe (New York Botanical Garden, New York, USA) for the liverworts species identification. In addition, I would like to thanks Ministry of Higher Education for the MyBrains15 (MyPhD) scholarship for the past three years.

I would like to express my deepest appreciation towards all the staffs of Institute for Tropical and Biological Conservation (ITBC) who involved and helped me for the completion of this research. My appreciation is also extended to Prof. Dr. Jeon You-Jin from Jeju National University for his supervision in conducting cytotoxic assay. I would also convey my gratitude to Prof. Dr. Tatsufumi Okino from Hokkaido University, Japan for his assistance in high resolution mass spectroscopy analysis.

My appreciation also goes to Ms. Ang Mei Yan and Dr. Zhan Zhaoqi from Shimadzu in providing training for liquid chromatography mass spectroscopy and gas chromatography mass spectroscopy.

Finally, I would like to say thank you to my fellow labmates for always being there to give suggestions and share knowledge whenever I encountered problems.

Ng Shean Yeaw

21th July 2017

ABSTRACT

Natural products played a critical role in the development of modern drugs. Over the past 30 years, up to 50 percent of the approved drugs are derived from natural products and more than 75 percent of them are derived from plants. High incidence of cancer, rising rate of infection and inflammation due to antibiotic-resistant bacteria have admonished scientists to look for alternative ways to combat these major medical concerns. Marchantiophyta (liverworts), representing lower plant which are grouped under Bryophytes, has been reported to synthesize diverse array of chemical structures and show several interesting biological activities. Although there are many reports pertaining to liverworts chemistry, yet the information about Bornean liverworts is still scarce. Present study aimed **i)** to evaluate the structural diversity of selected Bornean liverworts **ii)** to investigate the chemosystematics of isolated secondary metabolites from collected populations **iii)** to determine the antibacterial property of pure compounds against human pathogenic bacteria and **iv)** to investigate the anti-proliferative effect of pure compounds on selected cancer cell lines. Secondary metabolites present in five species of liverworts (Order Jungermanniales) were isolated using chromatographic technique and their spectral data obtained via NMR, HRMS, FTIR and polarimeter. A total of 29 compounds were isolated; a total of 10 new compounds, with another 19 known metabolites. These compounds were sesquiterpenes and diterpenes with interesting chemical skeleton and functionalities. Some of these compounds showed excellent profiles as chemotaxonomical markers, particularly for *Mastigophora diclados*. Isolated compounds were also subjected to bioassay against antibiotic resistant clinical bacteria and cancer cell lines (HL-60, B16-F10, A549, and HT-29). Chandonanol (**CH-1**) isolated from *Chandonanthus hirtellus* exhibited bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* where the MIC/MBC ratio was less than four. In addition, herbertene-1,2-diol (**MD-5**) isolated from *Mastigophora diclados* showed inhibition against HL-60 cells in a dose-dependent manner through induction of apoptosis. The underlying mechanism of action was via intrinsic mitochondrial pathway by up-regulation of p53 and regulated the ratio of Bax/Bcl-xL in the cells. The compound *cis*-3,14-clerodadien-13-ol (**SA-2**) isolated from *Schistochila acuminata* displayed weak cytotoxic inhibition against B16-F10 cells and was not taken forward for other in-depth analysis. In conclusion, this study has provided valuable information pertaining to the diversity of secondary metabolites in the species studied. It is apparent that information obtained could be used for taxonomical interpretation and as reference in the formulation of lead pharmaceutical candidates.

ABSTRAK

Kepelbagaian Struktur, Kimosistematik, dan Potensi Aktiviti Biologi Metabolit Kedua daripada Lumut Hati di Borneo (Order Jungermanniales)

Produk semula jadi memainkan peranan penting dalam penghasilan ubat-ubtan moden. Sejak 30 tahun yang lalu, sebanyak 50 peratus ubat-ubtan yang diluluskan adalah diperolehi daripada produk semula jadi dan lebih daripada 75 peratus produk semula jadi tersebut adalah berasal daripada tumbuh-tumbuhan. Kejadian kes kanser yang tinggi, peningkatan kadar jangkitan dan keradangan yang disebabkan bakteria yang tahan antibiotik telah menggesa para saintis untuk mencari jalan alternatif untuk memerangi kebimbangan utama ini dalam bidang perubatan. Marchantiophyta (lumut hati), mewakili tumbuhan rendah dibawah kumpulan Briofit telah dilaporkan memiliki kepelbagaian struktur kimia yang tinggi dan menunjukkan beberapa aktiviti biologi yang menarik. Walaupun banyak kajian tentang lumut hati telah dijalankan, namun maklumat tentang lumut hati dari Borneo adalah masih terhad. Matlamat kajian ini adalah **i)** untuk menyiasat kepelbagaian struktur kimia lumut hati Borneo yang terpilih **ii)** untuk mengkaji kimosistematik daripada metabolit sekunder yang terpencil dari populasi yang dikumpulkan **iii)** untuk menentukan antibakteria aktiviti sebatian tulen terhadap bakteria patogenik manusia **iv)** untuk mengkaji kesan anti-kanser sebatian tulen terhadap sel kanser yang terpilih. Metabolit sekunder di dalam lima spesies lumut hati (Order Jungermanniales) telah dipencilkan melalui teknik kromatografi dan data spektrum tersebut diperolehi melalui NMR, HRMS, FTIR dan polarimeter. Sebanyak 29 sebatian tulen telah dipencilkan termasuk 10 sebatian baru dan 19 metabolit dikenali. Sebatian ini adalah sesquiterpenes dan diterpenes dengan rangka kimia dan fungsi yang menarik. Beberapa sebatian ini menunjukkan profil yang sangat baik sebagai penanda kimotaksonomi, terutamanya bagi *Mastigophora diclados*. Sebatian terpencil juga tertakluk kepada bioesei terhadap bakteria klinikal tahan antibiotic dan sel kanser (HL-60, B16-F10, A549 dan HT-29). Chandonanol (**CH-1**) yang dipencilkan daripada *Chandonanthus hirtellus* telah menunjukkan aktiviti pembunuhan bakteria terhadap *Staphylococcus aureus* dan *Escherichia coli* di mana nisbah MIC/MBC adalah kurang daripada empat. Selain itu, herbertene-1,2-diol (**MD-5**) yang dipencilkan dari *Mastigophora diclados* telah menunjukkan perencatan terhadap sel-sel HL-60 dengan cara yang bergantung kepada dos sebatian melalui induksi apoptosis. Mekanisme mendasari tindakan adalah melalui laluan mitokondria intrinsic dengan pertambahan p53 dan mengawal selia nisbah Bax/Bcl-xL dalam sel. Sebatian *cis*-3,14-clerodadien-13-ol (**SA-2**) yang dipencilkan dari *Schistochila acuminata* telah menunjukkan perencatan sitotoksik yang lemah terhadap sel-sel B16-F10, dan analisis yang mendalam tidak dijalankan. Kesimpulannya, siasatan telah memberikan maklumat berharga yang berkaitan dengan kepelbagaian metabolit sekunder dalam species yang dikaji. Maklumat ini boleh digunakan dalam tafsiran taksonomi dan sebagai rujukan calon farmaseutikal.

LIST OF CONTENTS

CHAPTER 6 CYTOTOXIC PROPERTIES OF SECONDARY METABOLITES FROM BORNEAN LIVERWORTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

δ	Delta
°C	Degree Celsius
%	Percentage
λ	Wavelength
v/v	Volume/volume
/	Or
g	Gram
mg	Milligram
µg	Microgram
mm	Millimeter
nm	Nanometer
ml	Milliliter
µl	Microliter
mM	Millimolar
µM	Micromolar
cm^{-1}	Reciprocal wavelength
rpm	Revolutions per minute
µg/ml	Microgram per millilitre
ml/min	Milliliter per minute
U/ml	Unit/milliliter
m/z	Mass per charge

LIST OF ABBREVIATIONS

Hz	Hertz
MHz	Megahertz
IC_{50}	Half maximal inhibitory concentration
$CHCl_3$	Chloroform
$CDCl_3$	Deuterated chloroform
C_6D_6	Deuterated benzene
dH_2O	Distilled water
D_2O	Deuterium oxide
DMSO	Dimethyl sulfoxide
EtOAc	Ethyl Acetate
Hex	Hexane
MeCN	Acetonitrile
MeOH	Methanol
Tol	Toluene
TMS	Tetramethylsilane
CO_2	Carbon dioxide
UV	Ultraviolet
NA_2SO_4	Anhydrous sodium sulphate
SiO_2	Silicon dioxide
MIC	Minimum inhibitory concentration
MID	Minimum inhibitory dose
MBC	Minimum bactericidal concentration
CC	Column Chromatography
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
HPTLC	High Performance Thin Layer Chromatography
HPLC	High Performance Liquid Chromatography
NMR	Nuclear magnetic resonance
1D-NMR	One-dimensional nuclear magnetic resonance
2D-NMR	Two-dimensional nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
^{13}-NMR	Carbon-13 nuclear magnetic resonance
DEPT	Distortionless enhancement by polarization transfer

HSQC	Heteronuclear single-quantum correlation spectroscopy
HMBC	Heteronuclear multiple-bond correlation spectroscopy
^{1}H-^{1}H COSY	Proton-proton correlation spectroscopy
NOESY	Nuclear Overhauser effect spectroscopy
HRMS	High resolution mass spectroscopy
FTIR	Fourier Transform Infra Red
LC-MS-IT-TOF	Liquid chromatography mass spectroscopy-ion trap-time-of-flight
LC-SPE-NMR-MS	Liquid chromatography-solid phase extraction-nuclear magnetic resonance-mass spectroscopy
BORH	BORNEENSIS Herbarium
DPPH	1,1-Diphenyl-2-picryl-hydrazyl
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-dephenyltetrazolium bromide
CAPE	Phenethyl ester
DMEM	Dulbecco's modified eagle's medium
DPBS	Dulbecco's phosphate buffer saline
FBS	Fetal bovine serum
NO	Nitric oxide
iNOS	Nitric oxide synthase
LPS	Lipopolysaccharide
HDL	High density lipoprotein
KB	Human pharyngeal squamous carcinoma
HL-60	Human promyelocytic leukimia
MCF-7	Human breast adenocarcinoma
A549	Human lung carcinoma
B16-F10	Mus musculus skin melanoma
HL-60	Human promyelocytic leukemia
HT-29	Human colorectal adenocarcinoma
HIV	Human immunodeficiency virus

LIST OF APPENDICES