

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

NG SHEAN YEAW



UIMS

**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

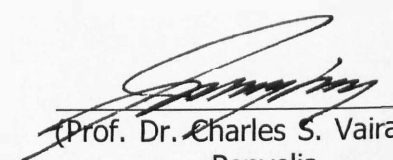


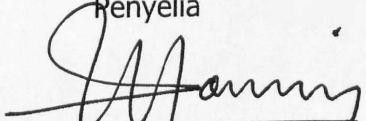
NG SHEAN YEAW
DX1311003T

Tarikh: 07 Ogos 2017

Disahkan Oleh,
NURULAIN BINTI ISMAIL
LIBRARIAN


(Tandatangan Pustakawan)


(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



UMS
UNIVERSITI MALAYSIA SABAH

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

UMS
UNIVERSITI MALAYSIA SABAH

SIGNATURE

SUPERVISOR

Prof. Dr. Charles S. Vairappan

A handwritten signature in black ink, appearing to read 'Charles S. Vairappan', written over a horizontal line.

CO-SUPERVISOR

Assoc. Prof. Dr. Monica Suleiman

A handwritten signature in black ink, appearing to read 'Monica Suleiman', written over a horizontal line.

CO-SUPERVISOR

Dr. Takashi Kamada

A handwritten signature in black ink, appearing to read 'Takashi Kamada', written over a horizontal line.

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 thank 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



UMS
UNIVERSITI MALAYSIA SABAH

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

Produk semula jadi memainkan peranan penting dalam penghasilan ubat-ubatan moden. Sejak 30 tahun yang lalu, sebanyak 50 peratus ubat-ubatan 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 ketidakseimbangan 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 kimosistemik 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 antibiotik 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

	Page
TITLE	i
DECLARATION	ii
CERTIFICATION	iii
ACKNOWLEDGEMENT	iv
ABSTRACT	vi
ABSTRAK	vii
LIST OF CONTENT	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF SYMBOLS	xx
LIST OF ABBREVIATIONS	xxi
LIST OF APPENDICES	xxiii

CHAPTER 1 INTRODUCTION

1.1	Natural Products Chemistry	1
1.2	Natural Products in Drugs Discovery	2
1.3	Sources of Natural Product-Derived Compounds	3
1.4	Liverwort Biology	4
1.5	Objectives	7

CHAPTER 2 LITERATURE REVIEW

2.1	Liverwort Botany	8
2.2	Secondary Metabolites in Liverworts	9
2.2.1	Sesquiterpenoids; Aromadendranes	10
2.2.2	Sesquiterpenoids; Bisabolanes	12
2.2.3	Sesquiterpenoids; Eudesmanes	14
2.2.4	Sesquiterpenoids; Herbertanes	16
2.2.5	Diterpenoids; Cembranes	18
2.2.6	Diterpenoids; Clerodanes	20
2.2.7	Diterpenoids; Dolabellanes	22
2.2.8	Diterpenoids; Pimaranes	24
2.2.9	Triterpenoids	26
2.2.10	Aromatic Compounds; Bibenzyls	28
2.2.11	Aromatic Compounds; Bis-bibenzyls	30
2.2.12	Flavonoids; Flavones, Flavanones, and Anthocyanins	32
2.2.13	Acetogenins	34

2.3	Chemosystematics Significance of Liverworts	36
2.3.1	Chemosystematics of Porellaceae	36
2.3.2	Chemosystematics of Radulaceae	38
2.3.3	Chemosystematics of Schistochilaceae	39
2.4	Biological Activities of Secondary Metabolites from Liverworts	41
2.4.1	Antioxidant Activity	41
2.4.2	Antimicrobial Activity	42
2.4.3	Cytotoxic Activity	43
2.4.4	Neuroprotective Activity	44
2.4.5	Muscle Relaxant Activity	44
2.4.6	Inhibition of Nitric Oxide Production	44
2.4.7	Liver X-Receptor (LXR) α Agonist and LXR β Antagonist Activity	45
2.5	Liverwort Lead Pharmaceutical Resource	45

CHAPTER 3 STRUCTURAL DIVERSITY OF FIVE SPECIES OF BORNEAN LIVERWORTS

3.1	Introduction	47
3.2	Methodology	49
3.2.1	Collection of Specimens	49
3.2.2	Chemical Extraction	51
3.2.3	Chemical Profiling	52
	3.2.3(i) High Performance Thin Layer Chromatography (HPTLC) Profiling	52
	3.2.3(ii) High Performance Liquid Chromatography (HPLC) Profiling	53
3.2.4	Isolation and Purification of Compounds	53
	3.2.4(i) Preparative Thin Layer Chromatography (PTLC)	54
	3.2.4(ii) High Performance Liquid Chromatography (HPLC)	54
3.2.5	Spectroscopic Data Measurement and Chemical Structure Elucidation	54
3.3	Results and Discussion	55
3.3.1	Chemical Profiling of Bornean Liverworts	55
3.3.2	Structural Diversity of Bornean Liverworts	58
	3.3.2(i) Secondary Metabolites Composition in <i>Mastigophora diclados</i>	59
	3.3.2(ii) Secondary Metabolites Composition in <i>Chandonanthus hirtellus</i>	73
	3.3.2(iii) Secondary Metabolites Composition in <i>Gottscheia schizopleura</i>	82

3.3.2(iv)	Secondary Metabolites Composition in <i>Schistochila acuminata</i>	94
3.3.2(v)	Secondary Metabolites Composition in <i>Acrobolbus saccatus</i>	103
3.4	Conclusion	116

CHAPTER 4 CHEMOTYPE DIFFERENCES OF BORNEAN LIVERWORTS *MASTIGOPHORA DICLADOS*

4.1	Introduction	118
4.2	Methodology	120
4.2.1	Collection of Specimens, Chemical Extraction and Chemical Profiling	120
4.2.2	Isolation and Structural Elucidation	120
	4.2.2(i) Preparative Thin Layer Chromatography (PTLC)	121
4.3	Results and Discussion	121
4.4	Conclusion	127

CHAPTER 5 ANTIBACTERIAL PROPERTIES OF SECONDARY METABOLITES FROM BORNEAN LIVERWORTS

5.1	Introduction	128
5.2	Methodology	131
5.2.1	Collection of Specimens, Extraction, Isolation and Structure Elucidation	131
5.2.2	Antibacterial Assay	131
	5.2.2(i) Minimum Inhibitory Concentration (MIC) Determination	132
	5.2.2(ii) Minimum Bactericidal Concentration (MBC) Determination	133
5.3	Results and Discussion	133
5.3.1	Antibacterial Activity of Bornean Liverwort <i>Mastigophora diclados</i> Derived Compounds	133
5.3.2	Antibacterial Activity of Bornean Liverwort <i>Chandonanthus hirtellus</i> Derived Compounds	135
5.3.3	Antibacterial Activity of Bornean Liverwort <i>Gottschelia schizopleura</i> Derived Compounds	137
5.3.4	Antibacterial Activity of Bornean Liverwort <i>Schistochila acuminata</i> Derived Compounds	138
5.3.5	Antibacterial Activity of Bornean Liverwort <i>Acrobolbus saccatus</i> Derived Compounds	139
5.3.6	Mechanism of Action	140
5.4	Conclusion	141

CHAPTER 6 CYTOTOXIC PROPERTIES OF SECONDARY METABOLITES FROM BORNEAN LIVERWORTS

6.1	Introduction	142
6.2	Methodology	144
6.2.1	Collection of Specimens, Extraction, Isolation and Structure Elucidation	144
6.2.2	Cytotoxic Assay	144
6.2.2(i)	Chemical and Cancer Cell Lines	144
6.2.2(ii)	Cell Culture	145
6.2.2(iii)	Cell Growth Inhibition Assay	145
6.2.2(iv)	Nuclear Staining with Hoechst 33342	146
6.2.2(v)	Cell Cycle Analysis	146
6.2.2(vi)	Western Blot Analysis	147
6.2.2(vii)	Statistical Analysis	148
6.3	Results and Discussion	148
6.3.1	Cytotoxic Potential of <i>Mastigophora dicladus</i> Derived Secondary Metabolites	148
6.3.2	Cytotoxic Potential of <i>Chandonanthus hirtellus</i> Derived Secondary Metabolites	150
6.3.3	Cytotoxic Potential of <i>Gottschelia schizopleura</i> Derived Secondary Metabolites	150
6.3.4	Cytotoxic Potential of <i>Schistochila acuminata</i> Derived Secondary Metabolites	151
6.3.5	Cytotoxic Potential of <i>Acrobolbus saccatus</i> Derived Secondary Metabolites	152
6.3.6	Cytotoxicity of Herbertane-1,2-diol (MD-5) Against HL-60 Cell Line	152
6.4	Conclusion	157

CHAPTER 7 CONCLUSION 159

REFERENCES 163

APPENDICES 177

LIST OF TABLES

	Page
Table 3.1: Biomass of the crude extracts obtained from liverwort specimens	57
Table 3.2: ^1H - and ^{13}C -NMR data for dicladoic acid (MD-1) at 600 and 150 MHz in CDCl_3 .	65
Table 3.3: ^1H - and ^{13}C -NMR data for <i>ent</i> -chlorantene G (MD-2) at 600 and 150 MHz in C_6D_6 .	67
Table 3.4: ^1H - and ^{13}C -NMR data for chandonanol (CH-1) at 600 and 150 MHz in CDCl_3 .	78
Table 3.5: ^1H - and ^{13}C -NMR data for (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-ol (GS-1) at 600 and 150 MHz in CDCl_3 .	86
Table 3.6: ^1H - and ^{13}C -NMR data for (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-al (GS-2) at 600 and 150 MHz in CDCl_3 .	89
Table 3.7: ^1H - and ^{13}C -NMR data for schistochilic acid D (SA-1) at 600 and 150 MHz in CDCl_3 .	98
Table 3.8: ^1H - and ^{13}C -NMR data for saccatene A (AS-1) at 600 and 150 MHz in CDCl_3 .	108
Table 3.9: ^1H - and ^{13}C -NMR data for saccatene B (AS-3) at 600 and 150 MHz in CDCl_3 .	110
Table 3.10: ^1H - and ^{13}C -NMR data for saccatene C (AS-4) at 600 and 150 MHz in CDCl_3 .	112
Table 3.11: ^1H - and ^{13}C -NMR data for saccatene D (AS-5) at 600 and 150 MHz in CDCl_3 .	114
Table 4.1: Chemotype Variations of <i>Mastigophora diclados</i> from Borneo Island.	126

Table 5.1:	Antibacterial activity of compounds from <i>Mastigophora dicladus</i> .	134
Table 5.2:	Antibacterial activity of compounds from <i>Chandonanthus hirtellus</i> .	135
Table 5.3:	Antibacterial activity of compounds from <i>Gottschelia schizopleura</i> .	137
Table 5.4:	Antibacterial activity of compounds from <i>Schistochila acuminata</i> .	138
Table 5.5:	Antibacterial activity of compounds from <i>Acrobolbus saccatus</i> .	140
Table 6.1:	Cytotoxic activities of compounds from <i>Mastigophora dicladus</i> .	149
Table 6.2:	Cytotoxic activities of compounds from <i>Schistochila acuminata</i> .	151



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF FIGURES

	Page
Figure 1.1: Compound structures of approved drugs derived from natural products.	4
Figure 1.2: Taxonomy classification of studied liverworts species.	6
Figure 2.1: Oil bodies of the liverwort, <i>Bazzania harpago</i> (De Not.) Schiffn.	9
Figure 2.2: Chemical structure of lunularic acid.	10
Figure 2.3: Aromadendrane-type sesquiterpenoids from liverworts.	11
Figure 2.4: Bisabolane-type sesquiterpenoids from liverworts.	13
Figure 2.5: Eudesmane-type sesquiterpenoids from liverworts.	15
Figure 2.6: Herbertane-type sesquiterpenoids from liverworts.	17
Figure 2.7: Cembrane-type diterpenoids from liverworts.	19
Figure 2.8: Clerodane-type diterpenoids from liverworts.	21
Figure 2.9: Dolabellane-type diterpenoids from liverworts.	23
Figure 2.10: Pimarane-type diterpenoids from liverworts.	25
Figure 2.11: Several triterpenoids from liverworts.	27
Figure 2.12: Several bibenzyls from liverworts.	29
Figure 2.13: Several bis-bibenzyls from liverworts.	31
Figure 2.14: Several flavonoids from liverworts.	33
Figure 2.15: Several acetogenins from liverworts.	35

Figure 2.16:	Several chemical types from <i>Porella</i> species.	37
Figure 2.17:	Several chemical structures from <i>Radula</i> species.	38
Figure 2.18:	Several chemical types from <i>Schistochila</i> species.	40
Figure 3.1:	Photographs of the collected liverworts species. A: <i>Mastigophora diclados</i> , B: <i>Chandonanthus hirtellus</i> , C: <i>Gottscheia schizopleura</i> , D: <i>Schistochila acuminata</i> , E: <i>Acrobolbus saccatus</i>	50
Figure 3.2:	Map of sampling sites.	51
Figure 3.3:	Photographs, oil bodies, HPLC and HPTLC chromatogram crude extract profiles of the collected liverwort specimens (Left; $\lambda = 254\text{nm}$, Right; $\lambda = 366\text{ nm}$); A: <i>Mastigophora diclados</i> , B: <i>Chandonanthus hirtellus</i> , C: <i>Gottscheia schizopleura</i> , D: <i>Schistochila acuminata</i> , E: <i>Acrobolbus saccatus</i> .	56
Figure 3.4:	Chemical structures of the isolated secondary metabolites from <i>Mastigophora diclados</i> .	60
Figure 3.5:	Isolation scheme of <i>Mastigophora diclados</i> (Mount Kinabalu).	61
Figure 3.6:	Isolation scheme of <i>Mastigophora diclados</i> (Mount Alab).	62
Figure 3.7:	Isolation scheme of <i>Mastigophora diclados</i> (Mount Trus Madi).	62
Figure 3.8:	Key COSY and HMBC correlations of dicladoic acid (MD-1).	64
Figure 3.9:	Chemical structures of dicladoic acid (MD-1) with assigned chemical shifts.	65
Figure 3.10:	Key COSY and HMBC correlations of <i>ent</i> -chlorantene G (MD-2).	67
Figure 3.11:	Chemical structures of <i>ent</i> -chlorantene G (MD-2) with assigned chemical shifts.	67
Figure 3.12:	Chemical structures of herbertene (MD-3) with assigned chemical shifts.	69

Figure 3.13:	Chemical structures of α -herbertenol (MD-4) with assigned chemical shifts.	70
Figure 3.14:	Chemical structures of herbertene-1,2-diol (MD-5) with assigned chemical shifts.	70
Figure 3.15:	Chemical structures of <i>ent</i> -7-hydroxyeudesm-4-en-6-one (MD-6) with assigned chemical shifts.	71
Figure 3.16:	Chemical structures of 4- <i>epi</i> -sandaracopimaric acid (MD-7) with assigned chemical shifts.	72
Figure 3.17:	Chemical structures of rosa-1(10),15-dien-18-säure (MD-8) with assigned chemical shifts.	73
Figure 3.18:	Chemical structures of the isolated secondary metabolites from <i>Chandonanthus hirtellus</i> .	74
Figure 3.19:	Isolation scheme of <i>Chandonanthus hirtellus</i> .	75
Figure 3.20:	Key COSY and HMBC correlations of chandonanol (CH-1).	77
Figure 3.21:	Chemical structures of chandonanol (CH-1) with assigned chemical shifts.	77
Figure 3.22:	Chemical structures of anastreptene (CH-2) with assigned chemical shifts.	79
Figure 3.23:	Chemical structures (6 <i>R</i> , 7 <i>S</i>)-sesquiphellandrene (CH-3) with assigned chemical shifts.	80
Figure 3.24:	Chemical structures of chandonanthone (CH-4) with assigned chemical shifts.	81
Figure 3.25:	Chemical structures of iso-chandonanthone (CH-5) with assigned chemical shifts.	82
Figure 3.26:	Chemical structures of the isolated secondary metabolites from <i>Gottschelia schizopleura</i> .	83
Figure 3.27:	Isolation scheme of <i>Gottschelia schizopleura</i> .	84

Figure 3.28:	Key COSY and HMBC correlations of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-ol (GS-1).	86
Figure 3.29:	Chemical structures of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-ol (GS-1) with assigned chemical shifts.	86
Figure 3.30:	Key COSY and HMBC correlations of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-al (GS-2).	89
Figure 3.31:	Chemical structures of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)- <i>cis</i> -cleroda-3,13-dien-15-methoxy-16-oic acid-16,15-olide-18-al (GS-2) with assigned chemical shifts.	89
Figure 3.32:	Chemical structures of (-)-5- <i>epi</i> -hardwickiic acid (GS-3) with assigned chemical shifts.	91
Figure 3.33:	Chemical structures of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)-15,16-epoxy- <i>cis</i> -cleroda-3,13(16),14-trien-18-al (GS-4) with assigned chemical shifts.	92
Figure 3.34:	Chemical structures of (-)-(5 <i>R</i> ,8 <i>S</i> ,9 <i>R</i> ,10 <i>S</i>)-15,16-epoxy- <i>cis</i> -cleroda-3,13(16),14-trien-ol (GS-5) with assigned chemical shifts.	93
Figure 3.35:	Chemical structures of (-)-(15-hydroxy- <i>cis-ent</i> -cleroda-3,(13 <i>E</i>)-diene)(GS-6) with assigned chemical shifts.	94
Figure 3.36:	Chemical structures of the isolated secondary metabolites from <i>Schistochila acuminata</i> .	95
Figure 3.37:	Isolation scheme of <i>Schistochila acuminata</i> .	96
Figure 3.38:	Key COSY and HMBC correlations of schistochilic acid D (SA-1).	98
Figure 3.39:	Chemical structures of schistochilic acid D (SA-1) with assigned chemical shifts.	98
Figure 3.40:	Chemical structures of <i>cis</i> -clerod-3,13(16),14-trien-19-oic acid (SA-2) with assigned chemical shifts.	100

Figure 3.41:	Chemical structures of (-)-3,12 <i>E</i> ,14- <i>cis</i> -clerodatrien-18-oic acid (SA-3) with assigned chemical shifts.	101
Figure 3.42:	Chemical structures of <i>cis</i> -3,14-clerodadien-13-ol (SA-4) with assigned chemical shifts.	102
Figure 3.43:	Chemical structures of 12-hydroxydolabella-(3 <i>E</i> ,7 <i>E</i>)-diene (SA-5) with assigned chemical shifts.	103
Figure 3.44:	Chemical structures of the isolated secondary metabolites from <i>Acrobolbus saccatus</i> .	104
Figure 3.45:	Isolation scheme of <i>Acrobolbus saccatus</i> .	105
Figure 3.46:	Key COSY and HMBC correlations of saccatene A (AS-1).	107
Figure 3.47:	Chemical structures of saccatene A (AS-1) with assigned chemical shifts.	108
Figure 3.48:	Key COSY and HMBC correlations of saccatene B (AS-3).	109
Figure 3.49:	Chemical structures of saccatene B (AS-3) with assigned chemical shifts.	110
Figure 3.50:	Key COSY and HMBC correlations of saccatene C (AS-4).	112
Figure 3.51:	Chemical structures of saccatene C (AS-4) with assigned chemical shifts.	112
Figure 3.52:	Key COSY and HMBC correlations of saccatene D (AS-5).	114
Figure 3.53:	Chemical structures of saccatene D (AS-5) with assigned chemical shifts.	114
Figure 3.54:	Chemical structures of radulanin A-5-one (AS-2) with assigned chemical shifts.	115
Figure 3.55:	Chemical structures of isolated new secondary metabolites.	117
Figure 4.1:	Chemical structures of isolated secondary metabolites from <i>Mastigophora dicladus</i> collected from different countries.	119

Figure 4.2:	HPTLC profiling of <i>Mastigophora diclados</i> crude extracts (Top λ = 254 nm, Bottom λ = 366 nm; A1 = Mount Kinabalu, A2 = Mount Alab, A3 = Mount Trus Madi)	124
Figure 4.3:	HPLC profiling of <i>Mastigophora diclados</i> crude extracts (A1 = Mount Kinabalu, A2 = Mount Alab, A3 = Mount TrusMadi)	125
Figure 4.4:	Chemical structures of the isolated secondary metabolites from <i>Mastigophora diclados</i> .	126
Figure 4.5:	Morphological differences of leaves from <i>Mastigophora diclados</i> .	127
Figure 5.1:	Structures of compounds with positive inhibition against antibiotic-resistance microbes.	130
Figure 6.1:	Chemical structure of compound herbertene-1,2-diol (MD-5).	153
Figure 6.2:	Cell viability of HL-60 upon compound treatment.	153
Figure 6.3:	Microscopic image of apoptotic bodies upon compound treatment.	154
Figure 6.4:	Percentage of apoptotic sub-G ₁ DNA content.	155
Figure 6.5:	Apoptosis-related protein expressions in HL-60 cells upon treatment.	156
Figure 6.6:	A proposed model for isolated compound-induced apoptosis in HL-60 cells.	157
Figure 7.1:	Chemical structures of isolated new secondary metabolites.	160

LIST OF SYMBOLS

δ	Delta
$^{\circ}\text{C}$	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
$\mu\text{g/ml}$	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 ₆ D ₆	Deuterated benzene
dH ₂ O	Distilled water
D ₂ O	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 ₂ SO 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 C-NMR	Carbon-13 nuclear magnetic resonance
DEPT	Distortionless enhancement by polarization transfer

HSQC	Heteronuclear single-quantum correlation spectroscopy
HMBC	Heteronuclear multiple-bond correlation spectroscopy
^1H - ^1H 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

	Page
Appendix 1: ^1H -NMR Spectrum of MD-1 .	177
Appendix 2: ^{13}C -NMR Spectrum of MD-1 .	178
Appendix 3: HSQC Spectrum of MD-1 .	179
Appendix 4: HMBC Spectrum of MD-1 .	180
Appendix 5: COSY Spectrum of MD-1 .	181
Appendix 6: NOESY Spectrum of MD-1 .	182
Appendix 7: ^1H -NMR Spectrum of MD-2 .	183
Appendix 8: ^{13}C -NMR Spectrum of MD-2 .	184
Appendix 9: HSQC Spectrum of MD-2 .	185
Appendix 10: HMBC Spectrum of MD-2 .	186
Appendix 11: COSY Spectrum of MD-2 .	187
Appendix 12: NOESY Spectrum of MD-2 .	188
Appendix 13: ^1H -NMR Spectrum of CH-1 .	189
Appendix 14: ^{13}C -NMR Spectrum of CH-1 .	190
Appendix 15: HSQC Spectrum of CH-1 .	191
Appendix 16: HMBC Spectrum of CH-1 .	192