ISOLATION AND CHARACTERIZATION OF SELECTED ANTICANCER COMPOUNDS FROM ACANTHACEAE AND VERBENACEAE MEDICINAL PLANTS

ANGELINA CHENG YING FANG

PENFUSIAN

THESIS SUBMITTED IN FULFILLMENT FOR THE MASTER OF SCIENCE DEGREE

UNIVERSITI MALA

BIOTECHNOLOGY RESEARCH INSTITUTE UNIVERSITI MALAYSIA SABAH 2017

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL : THE ISOLATION AND CHARACTERIZATION OF SELECTED ANTICANCER ANTICANCER COMPOUNDS FROM ACANTHACEAE AND VERBENACEAE MEDICINAL PLANTS

IJAZAH : MASTER OF SCIENCE (BIOTECHNOLOGY)

Saya **ANGELINA CHENG YING FANG**, Sesi Pengajian <u>2013-2017</u>, mengaku membenarkan tesisi Ijazah Sarjana 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.
- Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
- 4. Sila tandakan (/)



(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA 1972)

TERHAD

TIDAK TERHAD

(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

RHAD NEINTI ISMAIL IBRARIAN TI MALAYSIA SABAH (Tandatangan Pustakawan)

(Dr. Teoh Peik Lin) Penyelia Bersama

ANGELINA CHENG YING FANG

Tarikh: 19 June 2017

(Mdm. Cheong Bo Eng) Penyelia Utama

CERTIFICATION

NAME	: ANGELINA CHENG YING FANG
MATRIC NO.	: MZ1321002T
TITLE	: ISOLATION AND CHARACTERIZATION OF SELECTED ANTICANCER COMPOUNDS FROM ACANTHACEAE AND VERBENACEAE MEDICINAL PLANTS
DEGREE	: MASTER OF SCIENCE (BIOTECHNOLOGY)
VIVA DATE	: 24 MARCH 2017

CERTIFIED BY



Signature

UNIVERSITI MALAYSIA SABAH

2) CO-SUPERVISOR Dr. Teoh Peik Lin



DECLARATION

I hereby declare that the material in this thesis is of my own effort, except for the quotations, excerpts, equations, summaries and references which have been duly acknowledged.

19 June 2017

Angelina Cheng Ying Fang MZ1321002T



ACKNOWLEDGEMENT

I would like to express my sincere gratitude and appreciation to my supervisors, Mdm. Cheong Bo Eng and Dr. Teoh Peik Lin for all of their valuable advices, guidance and continuous support in this research work that lead to the completion of this thesis. Besides that, I would like to convey my utmost appreciation to the Research Grant Scheme (Grant Code: SBK0070-SG-2013) awarded by Universiti Malaysia Sabah. In addition, I wish to express sincere gratitude to Associate Professor Dr. Berhaman Ahmad from the School of International Tropical Forestry, Universiti Malaysia Sabah for the botanical identification of the three medicinal plants used in this study.

For the correction of this thesis, I sincerely appreciate the advices and suggestions by Associate Professor Dr. Noumie Surugau from the Faculty of Science and Natural Resources from Universiti Malaysia Sabah and Professor Dr. Taufiq Yap Yun Hin from the Department of Chemistry, Faculty of Science from Universiti Putra Malaysia.

I also wish to express my appreciation to my seniors for their guidance in laboratory works. In addition, my sincere gratitude is to Mr. Zahid Yusoff for carrying out my plant samples for the NMR analysis at Universiti Kebangsaan Malaysia. Furthermore, I really appreciate the BRI's staffs for their efforts in helping my research works in the institute. Lastly, I would also like to thank my family and friends for their continuous advice and support.



ABSTRACT

Abundance in nature, plants are easy to obtain and comprehensively studied for their pharmacological activities in various health issues including complicated diseases such as cancers. Numerous reports on Ruellia tuberosa and Phyla nodiflora showed to have significance inhibition on human breast cancer, MCF-7 cells however, there is a lack of scientific report on the isolation of anti-cancer compounds from these plants. Therefore, this study was carried out to isolate and characterize targeted anticancer compounds from the leaves of Ruellia tuberosa and the stems of Phyla nodiflora which included the isolation of selected anti-cancer compounds from roots of *Clinacanthus nutans* for the first time. The preliminary MTT assay on MCF-7 cells exhibited the ethyl acetate extract of *Clinacanthus nutans* root with a significant inhibition with IC_{50} value of 20.590±2.318 µg/ml which was comparable to the Ruellia tuberosa leaf methanol extract with IC_{50} value of 20.563 ± 2.825 µg/ml. Besides that, the ethyl acetate extract of Phyla nodiflora stem showed the weakest anti-cancer effect on MCF-7 cells than the other two extracts with an IC₅₀ value of 35.597±3.276 µg/ml. These MTT assay results indicated the capabilities of the extracts for further study in isolation and characterization of selected anti-cancer compounds from the plants. The isolation of lupeol was carried out using maceration of the dried *Clinacanthus nutans* roots in ethyl acetate and successive recrystallization in methanol which yielded 1.138 g of white amorphous powder. The leaves of Ruellia tuberosa were extracted using soxhlet extraction in methanol and undergo repeated silica gel column chromatography using hexane and ethyl acetate as the solvents with gradient elution method which yielded 1.628 g of yellow translucent liquid. In addition, the Phyla nodiflora stems were extracted using soxhlet extraction in ethyl acetate and repeated silica gel column chromatography was also carried out using hexane and ethyl acetate as the solvents by gradient elution which resulted in 0.064 g of a white solid. From structural elucidation, the white amorphous powder of 1.138 g isolated from *Clinacanthus nutans* root ethyl acetate extract was suggested to be lupeol and furthermore, squalene was determined from the 1.628 g of vellow translucent from *Ruellia tuberosa* leaf methanol extract by comparison to the published literature data. The white solid obtained from Phyla nodiflora stem ethyl acetate extract with a yield of 0.064 g was assumed to consist a mixture of three main dietary phytosterols namely stigmasterol, campesterol and β -sitosterol as elucidated by spectroscopic analysis compared to the previous literature data. The MTT assay result revealed that squalene isolated from *Ruellia tuberosa* leaf methanol extract had the highest anti-cancer activity on MCF-7 cells with an IC₅₀ value of 4.225±0.590 µg/ml followed by lupeol isolated from *Clinacanthus nutans* root ethyl acetate extract with IC_{50} value of 16.813 ± 1.316 µg/ml. The phytosterols mixture isolated from Phyla nodiflora stem ethyl acetate extract had the lowest anti-cancer activity with IC_{50} value of 36.497±2.530 µg/ml in MTT assay. The findings indicate all of the extracts and their isolated compounds showed the potential in causing 50% growth inhibition of the MCF-7 cells in MTT assay. Thus, this study is important for future drug development in creating safer alternative plant-derived medicinal drug than the more adverse effects of chemotherapy.

ABSTRAK

PEMENCILAN DAN PENCIRIAN SEBATIAN-SEBATIAN ANTI-KANSER TERPILIH DARI TUMBUHAN UBATAN ACANTHACEAE DAN VERBENACEAE

Kelimpahan dalam alam semula jadi, tumbuhan adalah mudah diperolehi dan dikaji secara menyeluruh mengenai aktiviti farmakologi dalam pelbagai masalah kesihatan termasuk penvakit rumit seperti kanser, Banvak laporan pada Ruellia tuberosa dan Phyla nodiflora menuniukkan kepentingan dalam perencatan pada kanser payudara manusia, sel MCF-7 bagaimanapun, terdapat kekurangan laporan saintifik mengenai pemencilan sebatian anti-kanser dari tumbuhan tersebut. Oleh itu, kajian ini dijalankan untuk memencilkan dan mencirikan sebatian-sebatian anti-kanser daripada daun Ruellia tuberosa dan batang Phyla nodiflora yang termasuk pemencilan sebatian anti-kanser terpilih dari akar Clinacanthus nutans buat kali pertama. Dalam kajian ini, ekstrak etil asetat akar Clinacanthus nutans menunjukkan nilai IC₅₀ 20.590±2.318 µa/ml jaitu setanding dengan ekstrak metanol duan Ruellia tuberosa dengan nilai IC₅₀ 20.563±2.825 µg/ml. Selain itu, ekstrak etil asetat batang Phyla nodiflora menunjukkan aktiviti anti-proliferatif yang paling lemah pada MCF-7 sel berbanding dengan dua ekstrak yang lain dengan nilai IC₅₀ 35.597±3.276 µg/ml. Pemencilan lupeol dengan menggunakan kaedah pemaseratan daripada ekstrak etil asetat akar kering Clinacanthus nutans dan penghabluran semula secara berturutturut dalam metanol telah menghasilkan 1.138 g sebatian amorfus putih. Daun Ruellia tuberosa yang telah diekstrak dengan menggunakan pengekstrakan soxhlet dalam metanol dan menjalani berulang dengan heksana dan etil asetat mengikut kaedah kecerunan yang menghasilkan 1.628 g cecair lut kuning. Di samping itu, batang Phyla nodiflora diekstrak dengan menggunakan pengekstrakan soxhlet dalam etil asetat dan berulang kromatografi turus gel silika juga dijalankan dengan menggunakan heksana dan etil asetat juga dengan kaedah kecerunan vang menghasilkan 0.064 g pepejal putih. Dari struktur elusidasi, amorfus putih 1.138 g dipencilkan daripada ekstrak etil asetat akar Clinacanthus nutans disyorkan sebagai lupeol dan tambahan pula, squalene telah ditentukan daripada 1.628 g cecair lut kuning yang terpencil terutamanya daripada ekstrak metanol daun Ruellia tuberosa dibandingkan dengan data sastera sebelumnya. Pepejal putih yang diperolehi daripada ekstrak etil acetate batang Phyla nodiflora dengan hasil 0.064 g diandaikan terdiri daripada campuran tiga fitosterol utama iaitu stigmasterol, campesterol dan βsitosterol oleh analisis spektroskopi juga berbanding dengan data sastera sebelumnya. Keputusan asai MTT mendedahkan bahawa squalene yang dipencilkan daripada ekstrak methanol daun Ruellia tuberosa mempunyai aktiviti anti-kanser yang tertinggi pada sel MCF-7 dengan IC₅₀ 4.225±0.590 µg/ml diikuti oleh lupeol vang dipencilkan daripada ekstrak etil asetat akar Clinacanthus nutans dengan IC_{50} 16.813±1.316 µg/ml. Fitosterol campuran dipencilkan daripada ekstrak etil asetat batang Phyla nodiflora mempunyai aktiviti anti-kanser yang paling lemah dengan IC_{50} 36.497±2.530 µg/ml berbanding dengan squalene dan lupeol dalam MTT assai. Kajian ini menunjukkan semua daripada ekstrak dan sebatian terpencil mereka memaparkan potensi dalam menyebabkan 50% perencatan pertumbuhan pada sel MCF-7 dalam asai MTT. Oleh itu, kajian ini adalah penting dalam menghasilkan ubat perubatan alternatif dari tumbuhan yang lebih selamat berbanding dengan kesan kemoterapi.

LIST OF CONTENTS

		Page
TITLE		i
CERT	FICATION	ii
DECL	ARATION	iii
ACKN	OWLEDGEMENT	iv
ABSTI	RACT	v
ABST	RAK	vi
LIST	OF CONTENTS	vii
LIST	OF TABLES	xii
LIST	OF FIGURES	viii
LIST	DF PHOTOGRAPH	xiii
LIST	OF ABBREVIATIONS/SYMBOLS	xiv
LIST	DF APPENDIX	xviii
СНАР	TER 1 : INTRODUCTION	1
1.1	Research Background	1
1.2	Problem Statement and Significance of Study	3
1.3	Objectives of Study	6
CHAP	TER 2 : LITERATURE REVIEW	7
2.1	Acanthaceae	7
2.2	Botanical Description of Clinacanthus nutans	9
2.3	Phytochemicals Constituents of Clinacanthus nutans	10
2.4	Biological Activities of Clinacanthus nutans	15
2.5	Botanical Description of Ruellia tuberosa	18
2.6	Phytochemicals Constituents of Ruellia tuberosa	19

2.7	Biological Activities of Ruellia tuberosa	25
2.8	Verbenaceae	28
2.9	Botanical Description of Phyla nodiflora	28
2.10	Phytochemicals Constituents of Phyla nodiflora	30
2.11	Biological Activities of Phyla nodiflora	35
2.12	Basic of Cell Cycle	38
2.13	Breast Cancer Cell Origin	39
2.14	Common Genetic Alteration in Breast Cancer	42
2.15	Cancer Treatment, Medication and its Hazardous	43
	2.15.1 Surgery and Radiotherapy	43
	2.15.2 Endocrine Therapy	44
	2.15.3 HER2-Targeted Therapy	45
2.16	Medicinal Plants as Anticancer Drugs	47
2.17	Phytochemical Methods on Isolation and Characterization	48
	2.17.1 Extraction of Compounds	48
	2.17.2 Maceration UNIVERSITI MALAYSIA SABAH	48
	2.17.3 Soxhlet Extraction	50
	2.17.4 Fractionation and Further Purification Methods	51
2.18	Identification and Quantification Methods	52
	2.18.1 Gas Chromatography	52
	2.18.2 Mass spectrometry (MS)	55
2.19	Spectral Characterization of Compounds	56
	2.19.1 Introduction to Nuclear magnetic resonance (NMR)	56
	2.19.2 One-dimensional NMR Spectroscopy	58
	2.19.3 Two-Dimensional NMR Spectroscopy	59
2.20	Fourier-Transform Infrared Spectroscopy	60
2.21	UV-Vis Spectrophotometer	61

	2.22	Plant Secondary Metabolites	63
		2.22.1 Triterpenoids Classes	64
		2.22.2 Phytosterols Group	65
C	HAPI	TER 3 : MATERIALS AND METHODS	71
	3.1	Chemicals and Reagents	71
	3.2	Overview of Study	72
	3.3	Plant Materials	73
	3.4	Preparation of Plants Extracts	73
		3.4.1 Maceration	73
		3.4.2 Soxhlet Extraction	74
	3.5	Chromatographic Technique	75
		3.5.1 Silica Gel Column Chromatography	75
	3.6	Gas-Chromatography-Mass spectroscopy (GC-MS) analysis	76
	3.7	Method Validation of Compounds Isolated from Plants by GC-MS	77
	3,8	Recrystallization Method	77
	3.9	Phytochemicals Screening UNIVERSITI MALAYSIA SABAH	78
	3.10	Structural Elucidation	79
		3.10.1 UV-Visible Spectroscopy	79
		3.10.2 Fourier Transform Infrared (FTIR) Spectroscopy	80
		3.10.3 Melting Point (MP) Determination	80
		3.10.4 Nuclear Magnetic Resonance (NMR) Spectroscopy	80
	3.11	Cytotoxicity Assay of Crude Extracts	81
		3.11.1 Cell Lines	81
	3.12	Recovery of Frozen Cell Lines	81
	3.13	Sub-Culture of Adherent Cell Lines	82
	3.14	Cell Storage	82
	3.15	Cells Counting by Hemacytometer	83

	3.16	MTT assay and Statistical Analysis	84
(CHAP	TER 4 : RESULTS	86
	4.1	Preparation of Crude Plant Extracts	86
	4.2	Cytotoxicity Assay of Crude Plant Extracts	87
	4.3	GC-MS Analysis of the Plants' Extracts	89
	4.4	Isolation and Identification of Compounds	96
		4.4.1 Lupeol	96
		4.4.2 Squalene	97
	4.5	Characterization of the Isolated Compounds	98
		4.5.1 Lupeol	98
		4.5.2 Squalene	108
	4.6	Isolation and Characterization of Phytosterols	115
		4.6.1 Phytosterols	115
	4.7	Cytotoxicity Assay of Standards and Compounds	120
	4.8	Method Validation of Compounds Isolated from Plants by GC-MS	125
C	НАРТ	TER 5 : DISCUSSION UNIVERSITI MALAYSIA SABAH	126
	5.1	Plant Sample Preparation and Extraction Method	126
	5.2	Isolation and Structure Elucidation of anti-cancer compound	129
	5.3	Anticancer Activity of Lupeol isolated from <i>Clinacanthus nutans</i> root	131
	5.4	Isolation of Squalene from Ruellia tuberosa leaf	132
	5.5	Anticancer Activity of Squalene isolated from Ruellia tuberosa leaf	133
	5.6	Isolation of Phytosterols Mixture from Phyla nodiflora stem	134
	5.7	Anticancer Activity of Phytosterols Mixture isolated from Phyla nodiflora	135
	5.8	The Differences between Natural Drugs and Synthetic Drugs	137
	5.9	Method Validation of Compounds Isolated from Plants by GC-MS	138
	5.10	The Presence of Similar Compounds at Different Retention Times	140

CHAPTER 6 : CONCLUSION	146
REFERENCES	148
APPENDICES	184



LIST OF TABLES

	ł	Page
Table 2.1:	Taxonomic classification and nomenclature of the <i>Clinacanthus nutans</i>	8
Table 2.2:	Phytochemical compounds present in different parts of <i>Clinacanthus nutans</i> and their chemical structures	11
Table 2.3:	The reported pharmacological activities of the <i>Clinacanthus nutans</i>	17
Table 2.4:	Taxonomic classification and nomenclature of Ruellia tuberosa	19
Table 2.5:	Phytochemical compounds present in different parts of <i>Ruellia tuberosa</i> and their chemical structures	21
Table 2.6:	The reported pharmacological activities of Ruellia tuberosa	26
Table 2.7:	Taxonomic classification and nomenclature of Phyla nodiflora	28
Table 2.8:	Phytochemical compounds present in different parts of <i>Phyla</i> nodiflora and their chemical structures	31
Table 2.9:	The reported pharmacological activities of Phyla nodiflora	36
Table 2.10:	Triterpenoid classes and their pharmacological activities	65
Table 2.11:	Phytosterols and their detailed pharmacological activities	67
Table 3.1:	List of solvents used and their solvent ratio in silica gel column chromatography	76
Table 3.2:	Details of the type, origin and growth of MCF-7 cell	81
Table 4.1:	Different part of the plants, extraction and their extraction yields	88
Table 4.2:	Cytotoxicity effects of plant crude extracts against MCF-7 cells	89
Table 4.3:	The list of compounds detected in total ion chromatography (TIC) of <i>Clinacanthus nutans</i> root ethyl acetate extract	91
Table 4.4:	The list of compounds detected in total ion chromatography (TIC) of <i>Ruellia tuberosa</i> leaf methanol extract	93

Table 4.5:	The list of compounds detected in total ion chromatography (TIC) of <i>Phyla nodiflora</i> stem ethyl acetate extract	95
Table 4.6:	Parameters for method validation of the isolated compounds	125
Table 5.1:	The assay validation Sheet for squalene isolated from <i>Ruellia tuberosa</i> leaf MeOH extract	203



LIST OF FIGURES

		Page
Figure 2.1:	The overview of the cell cycle in a typical eukaryotic cell	39
Figure 2.2:	Schematic illustration of the normal breast with the ducts and TDLUs (Terminal Duct Lobular Units)	40
Figure 2.3:	The schematic diagram of four stages of the breast cancer	42
Figure 2.4:	Schematic illustration of GC-MS and its functional parts	53
Figure 2.5:	Schematic illustration of separation of compounds in gas- chromatography	54
Figure 2.6:	Schematic illustration of mass-spectrometry and its compartments	55
Figure 2.7:	FT-NMR workstation from sample processing and data analysis	57
Figure 2.8:	Proton ¹ H-NMR spectrum of vanillin	58
Figure 2.9:	Two-dimensional correlation spectroscopy (COSY) spectrum of ibuprofen	59
Figure 2.10:	The electromagnetic spectrum represented the complete range of electromagnetic radiation	60
Figure 2.11:	Schematic illustration of the energy state in electromagnetic region	62
Figure 2.12:	Chemical structure of stigmasterol ($C_{29}H_{48}O$)	66
Figure 2.13:	Chemical structure of β -sitosterol (C ₂₉ H ₅₀ O)	66
Figure 2.14:	Chemical structure of campesterol ($C_{28}H_{50}O_{48}$)	67
Figure 3.1:	Flowchart of the isolation and characterization methods for the extracts and their targeted anti-cancer compounds	72
Figure 4.1:	Cytotoxicity effects of extracts of the extracts against MCF-7 cells with concentration range from 0 to 50 μ g/ml and 0 μ g/ml as the control (DMSO)	88
Figure 4.2:	Total Ion Chromatography (TIC) of <i>Clinacanthus nutans</i> root ethyl acetate extract at 8000 ppm	90
Figure 4.3:	Total Ion Chromatography (TIC) of <i>Ruellia tuberosa</i> leaf methanol extract at 8000ppm	92

Figure 4.4:	Total Ion Chromatography (TIC) of <i>Phyla nodiflora</i> stem ethyl acetate extract at 8000ppm	94
Figure 4.5:	Total Ion Chromatography (TIC) of standard lupeol in GC-MS at 200ppm	96
Figure 4.6:	Total Ion Chromatography (TIC) of lupeol isolated from the <i>Clinacanthus nutans</i> roots ethyl acetate extract in GC-MS at 100ppm	96
Figure 4.7:	Total Ion Chromatography (TIC) mixture of standard and lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract in GC-MS	97
Figure 4.8:	Total Ion Chromatography (TIC) of standard squalene in GC-MS at 200ppm	97
Figure 4.9:	Total Ion Chromatography (TIC) of squalene isolated from <i>Ruellia tuberosa</i> leaf methanol extract in GC-MS at 100ppm	98
Figure 4.10:	Total Ion Chromatography (TIC) mixture of standard and squalene isolated from <i>Ruellia tuberosa</i> leaf methanol extract in GC-MS	98
Figure 4.11:	FTIR spectrum of lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract	101
Figure 4.12:	EI-MS of lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract	102
Figure 4.13:	UV-Vis spectrum of standard lupeol in chloroform (CHCl ₃) at 1000ppm	103
Figure 4.14:	UV-Vis spectrum of lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract in chloroform (CHCl ₃) at 1000ppm	104
Figure 4.15:	The ¹ H-NMR of lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract	105
Figure 4.16:	The ¹³ C-NMR of lupeol isolated from <i>Clinacanthus nutans</i> root ethyl acetate extract	107
Figure 4.17:	FTIR spectrum of squalene isolated from <i>Ruellia tuberosa</i> leaf methanol extract	110
Figure 4.18:	EI-MS of squalene isolated from <i>Ruellia tuberosa</i> leaf methanol extract	111

Figure 4.19:	The ¹ H-NMR spectrum of squalene isolated from <i>Ruellia</i> <i>tuberosa</i> leaf methanol extract	112
Figure 4.20:	The ¹³ C-NMR spectrum of squalene isolated from <i>Ruellia</i> <i>tuberosa</i> leaf methanol extract	114
Figure 4.21:	FTIR spectrum of isolated phytosterols mixture from <i>Phyla</i> nodiflora stem ethyl acetate extract	117
Figure 4.22:	The ¹ H-NMR spectrum of phytosterols mixture from <i>Phyla</i> nodiflora stem ethyl acetate extract	118
Figure 4.23:	The ¹³ C-NMR spectrum of phytosterols mixture from <i>Phyla</i> nodiflora stem ethyl acetate extract	119
Figure 4.24:	The cytotoxicity effect of standard lupeol, isolated lupeol from <i>Clinacanthus nutans</i> root EtOAc and lupeol isolated from <i>Ruellia tuberosa</i> leaf MeOH extracts on MCF-7 cells with 0 µg/ml as the control (DMSO)	120
Figure 4.25:	The cytotoxicity effect of standard squalene, isolated squalene from <i>Ruellia tuberosa</i> leaf MeOH and <i>Phyla nodiflora</i> stem EtOAc extracts against MCF-7 cells with the 0 µg/ml as the control (DMSO)	121
Figure 4.26:	The cytotoxicity effect of phytosterols mixture isolated from the <i>Phyla nodiflora</i> stem EtOAC extract on MCF-7 cells with the 0 µg/ml as the control (DMSO)	122
Figure 4.27 :	The cytotoxicity effect standard stigmasterol on MCF-7 cells with the 0 μ g/ml as the control (DMSO)	123
Figure 4.28:	The cytotoxicity effect standard β -sitosterol on MCF-7 cells with the 0 $\mu g/ml$ as the control (DMSO)	123
Figure 4.29:	The cytotoxicity effect standard campesterol on MCF-7 cells with the 0 $\mu g/ml$ as the control (DMSO)	124
Figure 5.1:	The mass spectrum of eicosane identified from <i>Clinacanthus</i> <i>nutans</i> root EtOAc extract against NIST11 library at 14.208 minutes	141
Figure 5.2:	The mass spectrum of eicosane identified from <i>Clinacanthus</i> <i>nutans</i> root EtOAc extract against NIST11 library at 19.693 minutes	141
Figure 5.3:	The mass spectrum of eicosane identified from <i>Clinacanthus</i> <i>nutans</i> root EtOAc extract against NIST11 library at 21.226 minutes	142

Figure 5.4:	The mass spectrum of hexadecane identified from <i>Clinacanthus nutans</i> root EtOAc extract against NIST11 library at 11.841 minutes	143
Figure 5.5:	The mass spectrum of hexacosane identified from <i>Clinacanthus nutans</i> root EtOAc extract against NIST11 library at 16.207 minutes	143
Figure 5.6:	The mass spectrum of tetracosane identified from <i>Clinacanthus</i> <i>nutans</i> root EtOAc extract against NIST 11 library at 18.026 minutes	144
Figure 5.7:	The mass spectrum of eicosane identified from the <i>Phyla</i> <i>nodiflora</i> stem EtOAc extract against the NIST 11 library at 21.226 minutes	144
Figure 5.8:	The mass spectrum of eicosane identified from the <i>Phyla</i> <i>nodiflora</i> stem EtOAc extract against the NIST 11 library at 26.868 minutes	145
Figure 6.1:	The cells viabilities (%) of the <i>Clinacanthus nutans</i> root EtOAc extract on MCF-7 cells	189
Figure 6.2:	The concentrations used (X-axis) values were transformed to \log_{10} (X-axis) values	189
Figure 6.3:	The result of the log ₁₀ (X-axis) values for the three independent experiments (n=3)	190
Figure 6.4:	The parameter of the non-linear regression (curve fit) and log (inhibitor) vs. normalized response was selected	190
Figure 6.5:	The result of the IC_{50} values from the three independent experiments (n=3)	191
Figure 6.6:	The average mean from the three IC_{50} values were calculated using row means with standard deviation (SD)	191
Figure 6.7:	The final result showed the IC50 value as $20.590\pm2.318 \ \mu\text{g/ml}$ of <i>Clinacanthus nutans</i> root EtOAC extract on MCF-7 cells was calculated	192
Figure 6.8:	Suitable data options were added into SPSS prior to statistics analysis	193
Figure 6.9:	The cells viabilites (%) from the three independent experiments (n=3) were added into SPSS version 22.0 analysis	194

Figure 6.10:	The variable and dependent factors were selected followed by <i>post hoc</i> test (Dunnett T3 test)	194
Figure 6.11:	The readings were calculated using one-way Anova followed by <i>post hoc</i> Dunnett T3 test in SPSS version 22.0 with the significance level set at 0.05 value	195
Figure 6.12:	The result revealed the level of significance (* p <0.05, ** p <0.01 and *** p <0.001) of the <i>Clinacanthus nutans</i> root EtOAc extract by comparison to the control	195
Figure 6.13:	The concentration of squalene (ppm) from <i>Ruellia tuberosa</i> leaf MeOH extract and their analyte peak area (count) were added into Microsoft Excel	197
Figure 6.14:	The data were plotted as concentration (ppm) of squalene against analyte peak area (count)	197
Figure 6.15:	The equation of the straight line (Y= mx+c) was displayed on the chart along with R-squared (R^2) value	198
Figure 6.16:	The found concentrations (µg/ml) of the squalene isolated from <i>Ruellia tuberosa</i> leaf MeOH extract were determined	198
Figure 6.17:	The recovery (%) of the squalene isolated from <i>Ruellia</i> tuberosa leaf MeOH extract were calculated	199
Figure 6.18:	The average of the recovery (%) value was determined	199
Figure 6.19:	The standard deviation of the recovery value was calculated	200
Figure 6.20:	The standard error (SE) of the intercept was calculated using regression analysis	200
Figure 6.21:	A table of data potrayed the standard error (SE) was calculated in Microsoft Excel	201
Figure 6.22:	The standard deviation (SD) of the intercept was calculated using the formula	201
Figure 6.23:	The limit of detection (LOD) for the squalene isolated from <i>Ruellia tuberosa</i> leaf MeOH extract was calculated	202
Figure 6.24:	The limit of quantification (LOQ) for the squalene isolated from <i>Ruellia tuberosa</i> leaf MeOH extract was calculated	202

LIST OF PHOTOGRAPH

		Page
Photograph 2.1:	The picture of <i>Clinacanthus nutans</i>	9
Photograph 2.2:	The picture of Ruellia tuberosa	19
Photograph 2.3:	The picture of Phyla nodiflora	30
Photograph 2.4:	The <i>Clinacanthus nutans</i> root extract soaked in ethyl acetate for a maceration proces	49
Photograph 2.5:	Soxhlet extraction of Ruellia tuberosa leaf in methanol	50
Photograph 2.6:	The ethyl acetate extract of <i>Phyla nodiflora</i> stem with a solvent ratio of hexane: ethyl acetate (93:7) in column chromatography	52
Photograph 4.1:	Lupeol isolated from Clinacanthus nutans root ethyl	
	acetate extract in qualitative phytochemical screening	99
Photograph 4.2:	Squalene isolated from <i>Ruellia tuberosa</i> leaf methanol extract for qualitative phytochemical screening	108
Photograph 4.3:	Phytosterols group isolated from <i>Phyla nodiflora</i> stem ethyl acetate extract in qualitative phytochemical screening	115

UNIVERSITI MALAYSIA SABAH

LIST OF ABBREVIATIONS/SYMBOLS

VZV	-	Varicella-Zoster Virus		
HSV	-	Herpes Simplex Virus		
HER2	-	Human Epidermal Growth Factor Receptor 2		
ILC	-	Invasive Lobule Carcinoma		
IDC	-	Invasive Ductal Carcinoma		
SERM	-	Selective Estrogen Receptor Modulator		
LCIS	-	Lobular Carcinoma In-Situ		
TGF-β	-	Transforming Growth Factor-β		
TDLUs	1 - M	Terminal Duct Lobular Units		
EAC	20	Erich's Ascites Carcinoma		
NK	-	Natural Killer Cell		
DCIS	and Lachard	Ductal Carcinoma In-Situ		
K-562	-	Human Erythroleukemia Cell Line		
GSH	-	Glutathione		
CAT	-	Catalase		
SOD	-	Superoxide Dismutase		
RAJI	-	Human Burkitt's Lymphoma cell line		
DPPH	-	2,2-diphenyl-1-picrylhydrazyl		
CSC	-	Cancer Stem Cell		
H460		Human Lung Carcinoma		
MDA-MB231	-	Human Breast Adenocarcinoma		
STZ		Streptozotocin		

LVEF	-	Left Ventricular Ejection Fraction	
MTT	-	3-(4,5-dimethyl2-thiazolyl)-2,5-diphenyltetrazolium bromide	
MCF-7	-	Human Breast Cancer Cell Line	
ATTC		American Type Culture Collection	
TLC	-	Thin Layer Chromatography	
VLC		Vacuum Liquid Chromatography	
GC	-	Gas Chromatography	
FTIR	_	Fourier-Transform Infrared	
МР	-	Melting Point	
NMR		Nuclear Magnetic Resonance	
MS		Mass Spectrometry	
CO2	-222	Carbon Dioxide	
ha gi		microgram	
ml		millilitre	
kg	ABA	kilogram	
δ	<u>.</u>	chemical shift	
MHz	-	Megahertz	
ppm	-	part per million	
J		coupling constant	
Hz	-	Hertz	
FBS	-	Ferum Bovine Serum	
rpm	-	Revolutions per minute	
PBS	-	Phosphate-Buffered Saline	
nm	_	nanometre	

OD	-	Optical Density
MCG-803	-	Human Stomach Cancer
SK-N-AS	-	Human Neuroblastoma Cell Line
HT-29	-	Human Colorectal Adenocarcinoma Cell Line
HMG-CoA	-	3-hydroxy-3 methylglutaryl-coenzyme A
CINV	-	Chemotherapy-Induced Nausea and Vomiting
FEC	-	Fluorouracil, Cyclophosphamide, Epirubicin
FAC	-	Fluorouracil, Doxorubin, Cyclophosphamide
СМГ	-	Cyclophosphamide, Methotrexate, Fluorouracil
Іо	-	Intensity of the light entering the sample
It	-	Intensity of the light exiting the sample
T STI		Transmittance
NF-кВ		Nuclear Factor kappa B
AIs		Aromatase Inhibitors
ER+	ALL A	Positive Oestrogen Receptor SIA SABAH
OS	-	Overall Survival
PFS	-	Progression-Free Survival
EGFR		Epidermal Growth Factor Receptor
HER1	-	Human Epidermal Growth Factor Receptor 2
EI	-	Electron Impact
COSY	•	Correlation Spectra
HSQC		Heteronuclear Single Quantum Coherence
НМВС	4	Heteronuclear Multiple Bond Correlation
hv	2	Photon's Energy

ε	-	Absorptivity
i.d.		internal diameter
±	-	plus minus
g	-	gram
μМ	-	micromolar
EtOAc	-	Ethyl Acetate
MeOH	-	Methanol

