



UNIVERSITI PUTRA MALAYSIA

**SECONDARY METABOLITES FROM PEPPER (PIPER NIGRUM) AND
TAHITIAN NONI (MORINDA CITRIFOLIA) AND THEIR BIOLOGICAL
ACTIVITIES**

WEN YIN PING

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By

WEN YIN PING

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

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Faculty : Science

Piper nigrum from the Piperaceae family and *Morinda citrifolia* from the Rubiaceae family were researched in this study. Detailed phytochemical investigations on the roots of these plants afforded ten pure compounds, which consists mainly of alkaloids and anthraquinones, and an acid. The structures of these compounds were elucidated based on ^1H NMR, ^{13}C NMR, COSY, DEPT, 2D NMR (HSQC and HMBC), mass spectrometry (MS), and Infrared spectroscopy (FTIR) analysis. Meanwhile, mixtures of compounds were identified through GCMS (gas chromatography-mass spectrometry).

From the roots of *Piper nigrum*, the alkaloids obtained are piperine, pellitorine and aristolactam AII. Besides that, the acid, 3,4-methylenedioxy benzoic acid was also present in the roots of this plant. Meanwhile, anthraquinones and their derivatives such as 1-hydroxy-2-methylanthraquinone, damnacanthal, nordamnacanthal, 2-formyl-1-hydroxyanthraquinone, 2-ethoxy-1-hydroxyanthraquinone and morindone-6-methylether were obtained from the roots of *Morinda citrifolia*. Among these

anthraquinones, 2-ethoxy-1-hydroxyanthraquinone is a new anthraquinone, while morindone-6-methylether is reported for the first time from the plant.

In the larvicidal test against the larvae of *Aedes aegypti*, the ethyl acetate extract of the roots of *Piper nigrum* showed good activity against the larvae. Pellitorine, on the other hand, gave a significant activity followed by piperine which only gave a moderate activity. The acid and aristolactam AII gave negative results which indicated that they are not biologically active towards the larvae of *Aedes aegypti*. For *Morinda citrifolia*, only the chloroform extract and the two anthraquinones, 1-hydroxy-2-methylantraquinone and damnacanthal are strongly active.

From the cytotoxic activity, where tests were conducted using the HL-60 (Acute Promyelocytic Leukaemia) and MCF7 cell lines (Human Breast Adenocarcinoma), the ethyl acetate extract of *Piper nigrum* gave weak activity with an IC_{50} value of more than 30 $\mu\text{g/ml}$ against the HL-60 cell line. However, in the tests against the same cell line using piperine and pellitorine, IC_{50} value of 7.5 $\mu\text{g/ml}$ and 1.5 $\mu\text{g/ml}$, respectively, were obtained indicating the individual compounds to be strongly cytotoxic. Aristolactam AII gave weak activity with an IC_{50} value of more than 30 $\mu\text{g/ml}$ when tested on the MCF-7.

Compared to the extract and alkaloids from *Piper nigrum*, the extracts and anthraquinones from *Morinda citrifolia* gave better results against both HL-60 and MCF7 cell lines. Three extracts amongst the five tested showed significant bioactivity against the HL-60 cell. These are the hexane extract, chloroform extract and petroleum ether extract, with IC_{50} values of 1.70 $\mu\text{g/ml}$, 9.3 $\mu\text{g/ml}$ and 11.0

µg/ml, respectively. The methanol and acetone extracts showed moderate activities against the same cell line (HL-60). Damnacanthal, nordamnacanthal and 1-hydroxy-2-methylantraquinone which were from the hexane and chloroform extracts gave IC₅₀ values of 1.6 µg/mL, 4.4 µg/mL and 15.0 µg/mL, respectively, against the same cell line (HL-60). A comparison of the IC₅₀ values of different extracts against the two cell lines indicated that these extracts did not give satisfying results against the MCF7 cell lines with only the chloroform and petroleum ether extracts giving a moderate activity.

The antifungal and antibacterial activities of the extracts and some compounds from the two plants were also evaluated. Fungi such as *Aspergillus ochraceus* and *Saccharomyces cerevisiae* were used in the antifungal screening. Meanwhile, MRSA, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Bacillus subtilis* were the microbes used in the antibacterial screenings. None of the extracts or alkaloids from *Piper nigrum* showed any inhibition in both screening.

As for *Morinda citrifolia*, only the chloroform and methanol extracts, and damnacanthal exhibited medium inhibition for *Bacillus subtilis* in the antibacterial screening. However, in the screening against *Salmonella choleraesuis*, only the chloroform extract and damnacanthal showed weak inhibition. Meanwhile, nordamnacanthal is the only sample tested that showed a very weak inhibition. In the antifungal screening, the two microbes were shown to be lightly susceptible to the chloroform extract and damnacanthal.

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

METABOLIT SEKUNDER DARIPADA *PIPER NIGRUM* DAN *MORINDA CITRIFOLIA* SERTA AKTIVITI BIOLOGINYA

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Piper nigrum dari famili Piperaceae dan *Morinda citrifolia* yang dari famili Rubiaceae telah diselidiki dalam kajian ini. Kajian fitokimia mendalam terhadap akar kedua-dua jenis tumbuhan ini telah menghasilkan sebanyak 10 sebatian tulen kebanyakannya adalah terdiri daripada alkaloid dan anthrakuinon, dan satu asid organik. Sebatian-sebatian ini telah dikenalpastikan berdasarkan analisis ¹H NMR, ¹³C NMR, COSY, DEPT, 2D NMR (HSQC and HMBC), spektrometri jisim (MS), dan spektroskopi inframerah (IR). Manakala untuk sebatian-sebatian dalam campuran telah dikenalpasti melalui kaedah GCMS (kromatografi gas-spektrometri jisim).

Dari akar *Piper nigrum*, alkaloid yang diperolehi ialah piperin, pellitorin dan aristolaktam AII. Di samping itu, asid 3,4-metilenedioksi benzoik juga terkandung dalam akar tumbuhan ini. Manakala anthrakuinon dan juga sebatian-sebatian terbitannya seperti 1-hidroksi-2-metilanthrakuinon, damnacanthal, nordamnacanthal, 2-formil-1-hidroksianthrakuinon, 2-etoksi-1-hidroksianthrakuinon dan 6-metileter-morindon telah diperoleh daripada *Morinda citrifolia*. Antara anthrakuinon-

anthrakuinon ini, 2-etoksi-1-hidroksiantrakuinon merupakan satu anthrakuinon yang baru sedangkan 6-metileter-morindon dilaporkan buat pertama kalinya daripada *Morinda citrifolia*.

Dalam ujian larvisidal terhadap nyamuk *Aedes aegypti*, ekstrak mentah etil asetat daripada akar *Piper nigrum* telah menunjukkan aktiviti yang baik. Manakala pellitorin mempamerkan keputusan yang nyata baik, diikuti oleh piperin yang hanya memberi keputusan yang sederhana. Asid dan aristolaktam AII pula memberikan keputusan negatif dalam ujian ini yang membawa makna bahawa mereka tidak aktif terhadap larva *Aedes aegypti*. Untuk *Morinda citrifolia*, hanya ekstrak mentah klorofom serta dua anthrakuinon, 1-hidroksi-2-metilanthrakuinon dan damnacanthal sahaja yang menunjukkan kebioaktifan yang kuat.

Daripada aktiviti sitotoksik yang dijalankan menggunakan sel HL-60 (Acute Promyelocytic Leukaemia) dan sel MCF7 (Human Breast Adenocarcinoma), ekstrak etil asetat *Piper nigrum* menunjukkan aktiviti yang lemah terhadap sel HL-60 dengan nilai IC_{50} yang melebihi 30 $\mu\text{g/mL}$. Akan tetapi dalam ujian terhadap sel yang sama, dua sebatian yang diekstrak daripada ekstrak ini, piperin dan pellitorin, menunjukkan keputusan yang memuaskan dengan nilai IC_{50} masing-masing 7.5 $\mu\text{g/mL}$ dan 1.5 $\mu\text{g/mL}$. Aristolaktam AII pula menunjukkan keputusan negatif terhadap sel MCF-7 dengan nilai IC_{50} yang melebihi 30 $\mu\text{g/mL}$.

Jika dibandingkan dengan ekstrak dan alkaloid daripada *Piper nigrum*, ekstrak dan anthrakuinon daripada *Morinda citrifolia* telah memberikan keputusan yang lebih positif terhadap kedua-dua sel tersebut. Daripada lima ekstrak yang dikaji, tiga

daripadanya menunjukkan bioaktiviti yang jelas terhadap sel HL-60. Tiga ekstrak ini ialah ekstrak heksana, ekstrak klorofom dan ekstrak petroleum-eter dengan nilai IC₅₀ masing-masing, 1.70 µg/mL, 9.3 µg/mL dan 11.0 µg/mL. Manakala, ekstrak metanol dan aseton menunjukkan keaktifan yang sederhana terhadap sel yang sama (HL-60). Damnacanthal, nordamnacanthal dan 1-hidroksi-2-metilanthrakuinon yang diasingkan daripada ekstrak heksana dan klorofom memberi nilai IC₅₀ masing-masing, 1.6 µg/mL, 4.4 µg/mL dan 15.0 µg/mL terhadap jenis sel-sel yang sama. Perbandingan antara kesan sitotoksik ekstrak-ekstrak terhadap kedua-dua jenis sel menunjukkan bahawa ekstrak-ekstrak ini tidak memberikan keputusan yang bagus terhadap sel MCF-7. Hanya ekstrak klorofom dan petroleum-eter yang memberikan aktiviti sederhana terhadap sel ini.

Aktiviti antifungal dan antibakteria untuk ekstrak dan sebatian-sebatian juga telah diuji. Fungi seperti *Aspergillus ochraceus* dan *Saccharomyces cerevisiae* telah digunakan dalam penyaringan antifungal. Manakala, MRSA, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* dan *Bacillus subtilis* adalah mikrob yang digunakan dalam penyaringan antibakteria. Tiada sebarang tanda rencatan yang ditunjukkan oleh ekstrak mahupun alkaloid daripada *Piper nigrum* dalam kedua-dua penyaringan ini.

Manakala untuk *Morinda citrifolia*, hanya ekstrak klorofom dan metanol dan damnacanthal menunjukkan rencatan yang sederhana terhadap *Bacillus subtilis* dalam penyaringan antibakteria. Akan tetapi, dalam penyaringan terhadap *Salmonella choleraesuis*, hanya ekstrak klorofom dan damnacanthal menunjukkan aktiviti rencatan yang sederhana. Manakala nordamnacanthal merupakan satu-satu

sampel yang menunjukkan rencatan yang lemah. Dalam penyaringan antifungal, kedua-dua jenis mikrob telah menunjukkan sensitiviti yang lemah terhadap ekstrak klorofom dan damnacanthal.



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I certify that a Thesis Examination Committee has met on 6th March 2009 to conduct the final examination of Wen Yin Ping on her thesis entitled “Secondary Metabolites from Pepper (*Piper nigrum*) and Tahitiam Noni (*Morinda citrifolia*) and Their Biological Activities” 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.

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TABLE OF CONTENTS

ABSTRACT
ABSTRAK
ACKNOWLEDGEMENTS
APPROVAL SHEETS
DECLARATION FORM
LIST OF TABLES
LIST OF FIGURES
LIST OF APPENDICES
LIST OF ABBREVIATIONS

CHAPTER

1	INTRODUCTION	1
1.1	General Introduction	
1.2	The Plant – <i>Piper nigrum</i>	3
1.3	The Plant – <i>Morinda citrifolia</i>	6
1.4	Remarks on Plant Selection	10
1.5	Objectives of studies	11
2	LITERATURE REVIEW	12
2.1	Chemistry of the <i>Piper</i> genera	12
2.1.1	Alkaloids	12
2.1.2	Terpenes	24
2.2	Biological Activities Reported from <i>Piper nigrum</i>	24
2.3	Chemistry of the <i>Morinda</i> genera	28
2.3.1	Anthraquinones and the Derivatives	28
2.3.2	Other Constituents from the Plant	36
2.4	Biological Activities Reported from <i>Morinda citrifolia</i>	38
3	EXPERIMENTAL	39
3.1	Plant Materials	39
3.2	Instruments	39
3.2.1	Infrared Spectroscopy (IR)	39
3.2.2	Mass Spectra (MS)	39
3.2.3	Melting Point	40
3.2.4	Nuclear Magnetic Resonance (NMR)	40
3.2.5	Ultra Violet (UV)	40
3.3	Chromatographic Method	40
3.3.1	Column Chromatography	40
3.3.2	Thin Layer Chromatographic (TLC)	41
3.4	Dyeing Reagents for TLC	42
3.4.1	Vanillin-Sulfuric Acid Staining Reagent	42
3.4.2	Iron(III) Chloride Staining reagent	42
3.4.3	Copper(II) Sulfate – Sodium Citrate	43
3.4.4	Dragendorff's Reagent (Munier and Macheboef Modification)	43
3.5	Extraction and Isolation of Compounds	44
3.5.1	<i>Piper nigrum</i>	44
3.5.2	<i>Morinda citrifolia</i>	51

3.6	Cytotoxic Assay	60
3.7	Antimicrobial Activity	61
3.8	Larvicidal Assay	63
4	RESULTS AND DISCUSSIONS	64
4.1	Isolation of Secondary Metabolites from <i>Piper nigrum</i>	64
4.1.1	Characterization of 3,4-Methylenedioxy benzoic acid (143)	66
4.1.2	Characterization of Piperine (1)	77
4.1.3	Characterization of Pellitorine (69)	90
4.1.4	Characterization of Aristolactam AII (144)	101
4.2	Isolation of Secondary Metabolites from <i>Morinda citrifolia</i>	111
4.2.1	Characterization of 2-(Ethoxy-ethyl)-1-hydroxy-anthraquinone (145)	114
4.2.2	Characterization of 1-Hydroxy-2-methyl-anthraquinone (125)	129
4.2.3	Characterization of Damnacanthal (101)	143
4.2.4	Characterization of Nordamnacanthal (99)	156
4.2.5	Characterization of 2-Formyl-1-hydroxy-anthraquinone (146)	168
4.2.6	Characterization of Morindone-6-methylether (147)	179
5	BIOLOGICAL ACTIVITIES	195
5.1	Larvicidal Activity	195
5.1.1	The Larvicidal Results for <i>Piper nigrum</i>	195
5.1.2	The Larvicidal Activity Results for <i>Morinda citrifolia</i>	197
5.2	Cytotoxic Activity	
5.2.1	Cytotoxicity of <i>Piper nigrum</i>	200
5.2.2	Cytotoxicity of <i>Morinda citrifolia</i>	203
5.3	Antimicrobial Activity	
5.3.1	Antibacterial Screening	206
5.3.2	Antifungal Screening	208
6	CONCLUSIONS	210
	BIBLIOGRAPHY	214
	APPENDICES	220
	BIODATA OF STUDENT	233
	LIST OF PUBLICATIONS	234

LIST OF TABLES

Table		Page
2.1	Structures of alkaloids / amides from <i>Piper nigrum</i>	14
2.2	Structures of compounds studied for biological activities	26
2.3	Structures of anthraquinones and derivatives found in <i>Morinda citrifolia</i>	31
4.1	¹ H NMR (400MHz, Acetone-D ₆), ¹³ C NMR (100 MHz, Acetone-D ₆), DEPT, HMBC, and COSY assignments for 3,4-methylenedioxy benzoic acid (143)	68
4.2	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for piperine (1)	80
4.3	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for pellitorine (69)	92
4.4	¹ H NMR (400MHz, Acetone-D ₆), ¹³ C NMR (100 MHz, Acetone-D ₆), DEPT, HMBC, and COSY assignments for Aristolactam AII (144)	103
4.5	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	117
4.6	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for 1-hydroxy-2-methyl-anthraquinone (125)	131
4.7	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for damnacanthal (101)	146
4.8	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for nordamnacanthal (99)	159
4.9	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for 2-formyl-1-hydroxy-anthraquinone (146)	170
4.10	¹ H NMR (400MHz, CDCl ₃), ¹³ C NMR (100 MHz, CDCl ₃), DEPT, HMBC, and COSY assignments for morindone-6-methylether (147)	183
5.1	Larvicidal activities of crude extract and pure compounds from <i>Piper nigrum</i>	196
5.2	Larvicidal activities of crude extracts from <i>Morinda citrifolia</i>	197

5.3	Larvicidal activities of pure compounds from <i>Morinda citrifolia</i>	198
5.4	Cytotoxicity (IC ₅₀ µg/mL) of extract and pure compounds from <i>Piper nigrum</i>	200
5.5	Cytotoxicity (IC ₅₀ µg/mL) of extract from <i>Morinda citrifolia</i>	203
5.6	Cytotoxicity (IC ₅₀ µg/mL) of pure compounds from <i>Morinda citrifolia</i>	204
5.7	Antibacterial activity of extracts and pure compounds from <i>Piper nigrum</i> and <i>Morinda citrifolia</i>	207
5.8	Antifungal activity of extracts and pure compounds from <i>Piper nigrum</i> and <i>Morinda citrifolia</i>	208



LIST OF FIGURES

Figure	Pages
1.1 Trees of <i>Piper nigrum</i>	5
1.2 Leaves and berries of <i>Piper nigrum</i>	5
1.3 <i>Morinda citrifolia</i>	9
1.4 The flower of <i>Morinda citrifolia</i>	9
1.5 Fruit of <i>Morinda citrifolia</i>	9
1.6 Ripened fruit of <i>Morinda citrifolia</i>	9
4.1 Acid and alkaloids isolated from the roots <i>Piper nigrum</i>	65
4.2 EI mass spectrum of 3,4-methylenedioxy benzoic acid (143)	69
4.3 Fragmentation of 3,4-methylenedioxy benzoic acid (143)	69
4.4 IR spectrum of 3,4-methylenedioxy benzoic acid (143)	70
4.5 ¹ H NMR (400 MHz, Acetone-D ₆) spectrum of 3,4-methylenedioxy benzoic acid (143)	71
4.6 ¹³ C NMR (100 MHz, Acetone-D ₆) spectrum of 3,4-methylenedioxy benzoic acid (143)	72
4.7 DEPT spectrum of 3,4-methylenedioxy benzoic acid (143)	73
4.8 COSY spectrum of 3,4-methylenedioxy benzoic acid (143)	74
4.9 HMQC spectrum of 3,4-methylenedioxy benzoic acid (143)	75
4.10 HMBC spectrum of 3,4-methylenedioxy benzoic acid (143)	76
4.11 EI mass spectrum of piperine (1)	81
4.12 IR spectrum of piperine (1)	81
4.13 ¹ H NMR (400 MHz, CDCl ₃) spectrum of piperine (1)	82
4.14 ¹³ C NMR (100 MHz, CDCl ₃) spectrum piperine (1)	83
4.15 DEPT spectrum of piperine (1)	84
4.16 COSY spectrum of piperine (1)	85

4.17	Expansion of COSY spectrum of piperine (1)	86
4.18	HMQC spectrum of piperine (1)	87
4.19	HMBC spectrum of piperine (1)	88
4.20	Expansion of HMBC spectrum of piperine (1)	89
4.21	EI mass spectrum of pellitorine (69)	93
4.22	Fragmentation of pellitorine (69)	93
4.23	IR spectrum of pellitorine (69)	94
4.24	¹ H NMR (400 MHz, CDCl ₃) spectrum of pellitorine (69)	95
4.25	¹³ C NMR (100 MHz, CDCl ₃) spectrum of pellitorine (69)	96
4.26	DEPT spectrum of pellitorine (69)	97
4.27	COSY spectrum of pellitorine (69)	98
4.28	HMQC spectrum of pellitorine (69)	99
4.29	HMBC spectrum of pellitorine (69)	100
4.30	EI mass spectrum of aristolactam AII (144)	104
4.31	IR spectrum of aristolactam AII (144)	105
4.32	¹ H NMR (400 MHz, Acetone-D ₆) spectrum of aristolactam AII (144)	106
4.33	¹³ C NMR (100 MHz, Acetone-D ₆) spectrum of aristolactam AII (144)	107
4.34	DEPT spectrum of aristolactam AII (144)	108
4.35	COSY spectrum of aristolactam AII (144)	109
4.36	HMQC spectrum of aristolactam AII (144)	110
4.37	HMBC spectrum of aristolactam AII (144)	111
4.38	Anthraquinones isolated from <i>Morinda citrifolia</i>	113
4.39	EI mass spectrum of 2-(ethoxy-ethyl)-1-hydroxy anthraquinone (145)	118
4.40	Fragmentation of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	119
4.41	¹ H NMR (400 MHz, CDCl ₃) spectrum of 2-(ethoxy-ethyl)-1-	

	hydroxyanthraquinone (145)	120
4.42	Expansion of ¹ H NMR (400 MHz, CDCl ₃) spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	121
4.43	¹³ C NMR (100 MHz, CDCl ₃) spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	122
4.44	DEPT spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	123
4.45	Expansion of DEPT spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	124
4.46	COSY spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	125
4.47	HMQC spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	126
4.48	HMBC spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	127
4.49	Expansion of HMBC spectrum of 2-(ethoxy-ethyl)-1-hydroxyanthraquinone (145)	128
4.50	EI mass spectrum of 1-hydroxy-2-methylanthraquinone (125)	132
4.51	Fragmentation of 1-hydroxy-2-methylanthraquinone (125)	133
4.52	IR spectrum of 1-hydroxy-2-methylanthraquinone (125)	134
4.53	¹ H NMR (400 MHz, CDCl ₃) spectrum of 1-hydroxy-2-methylanthraquinone (125)	135
4.54	Expansion of ¹ H NMR (400 MHz, CDCl ₃) spectrum of 1-hydroxy-2-methylanthraquinone (125)	136
4.55	¹³ C NMR (100 MHz, CDCl ₃) spectrum of 1-hydroxy-2-methylanthraquinone (125)	137
4.56	DEPT spectrum of 1-hydroxy-2-methylanthraquinone (125)	138
4.57	Expansion of COSY spectrum of 1-hydroxy-2-methylanthraquinone (125)	139
4.58	HMQC spectrum of 1-hydroxy-2-methylanthraquinone (125)	140
4.59	Expansion of HMQC spectrum of 1-hydroxy-2-methylanthraquinone (125)	141
4.60	HMBC spectrum of 1-hydroxy-2-methylanthraquinone (125)	142
4.61	EI mass spectrum of damnacanthal (101)	147
4.62	Fragmentation of damnacanthal (101)	147

4.63	IR spectrum of damnacanthal (101)	148
4.64	¹ H NMR (400 MHz, CDCl ₃) spectrum damnacanthal (101)	149
4.65	Expansion of ¹ H NMR (400 MHz, CDCl ₃) spectrum of damnacanthal (101)	150
4.66	¹³ C NMR (100 MHz, CDCl ₃) spectrum of damnacanthal (101)	151
4.67	DEPT spectrum of damnacanthal (101)	152
4.68	COSY spectrum of damnacanthal (101)	153
4.69	HMQC spectrum of damnacanthal (101)	154
4.70	Expansion of HMBC spectrum of damnacanthal (101)	155
4.71	EI mass spectrum of nordamnacanthal (99)	160
4.72	Fragmentation of nordamnacanthal (99)	160
4.73	IR spectrum of nordamnacanthal (99)	161
4.74	¹ H NMR (400 MHz, CDCl ₃) spectrum nordamnacanthal (99)	162
4.75	¹³ C NMR (100 MHz, CDCl ₃) spectrum of nordamnacanthal (99)	163
4.76	DEPT spectrum of nordamnacanthal (99)	164
4.77	COSY spectrum of nordamnacanthal (99)	165
4.78	HMQC spectrum of nordamnacanthal (99)	166
4.79	Expansion of HMBC spectrum of nordamnacanthal (99)	167
4.80	EI mass spectrum of 2-formyl-1-hydroxyanthraquinone (146)	171
4.81	Fragmentation of 2-formyl-1-hydroxyanthraquinone (146)	171
4.82	IR spectrum of 2-formyl-1-hydroxyanthraquinone (146)	172
4.83	¹ H NMR (400 MHz, CDCl ₃) spectrum of 2-formyl-1-hydroxyanthraquinone (146)	173
4.84	¹³ C NMR (100 MHz, CDCl ₃) spectrum of 2-formyl-1-hydroxyanthraquinone (146)	174
4.85	DEPT spectrum of 2-formyl-1-hydroxyanthraquinone (146)	175
4.86	COSY spectrum of 2-formyl-1-hydroxyanthraquinone (146)	176
4.87	HMQC spectrum of 2-formyl-1-hydroxyanthraquinone (146)	177

4.88	Expansion of HMBC spectrum of 2-formyl-1-hydroxy-anthraquinone (146)	178
4.89	EI mass spectrum of morindone-6-methylether (147)	184
4.90	Fragmentation of morindone-6-methylether (147)	185
4.91	IR spectrum of morindone-6-methylether (147)	186
4.92	¹ H NMR (400 MHz, CDCl ₃) spectrum of morindone-6-methylether (147)	187
4.93	¹³ C NMR (100 MHz, CDCl ₃) spectrum of morindone-6-methylether (147)	188
4.94	Expansion of ¹³ C NMR (100 MHz, CDCl ₃) spectrum of morindone-6-methylether (147)	189
4.95	DEPT spectrum of morindone-6-methylether (147)	190
4.96	COSY spectrum of morindone-6-methylether (147)	191
4.97	HMQC spectrum of morindone-6-methylether (147)	192
4.98	HMBC spectrum of morindone-6-methylether (147)	193
4.99	Expansion of HMBC spectrum of morindone-6-methylether (147)	194

LIST OF APPENDICES

Appendix		Page
1	24 hours mortality count and LC values for the ethyl acetate extract of <i>Piper nigrum</i> against <i>Aedes aegypti</i> larvae	220
2	24 hours mortality count and LC values for piperine (1) against <i>Aedes aegypti</i> larvae	221
3	24 hours mortality count and LC values for pellitorine (69) against <i>Aedes aegypti</i> larvae	222
4	24 hours mortality count for 3,4-methylenedioxy benzoic acid (143) against <i>Aedes aegypti</i> larvae	223
5	24 hours mortality count for aristolactam AII (144) against <i>Aedes aegypti</i> larvae	223
6	24 hours mortality count for hexane extract from <i>Morinda citrifolia</i> against <i>Aedes aegypti</i> larvae	223
7	24 hours mortality count for methanol extract from <i>Morinda citrifolia</i> against <i>Aedes aegypti</i> larvae	223
8	24 hours mortality count and LC values for chloroform extract from <i>Morinda citrifolia</i> against <i>Aedes aegypti</i> larvae	224
9	24 hours mortality count and LC values for petroleum ether extract from <i>Morinda citrifolia</i> against <i>Aedes aegypti</i> larvae	225
10	24 hours mortality count and LC values for acetone extract from <i>Morinda citrifolia</i> against <i>Aedes aegypti</i> larvae	226
11	24 hours mortality count and LC values for 1-hydroxy-2-methylantraquinone (125) against <i>Aedes aegypti</i> larvae	227
12	24 hours mortality count and LC values for damnacanthal (101) against <i>Aedes aegypti</i> larvae	228
13	24 hours mortality count for nordamnacanthal (99) against <i>Aedes aegypti</i> larvae	229
14	24 hours mortality count for 2-formyl-1-hydroxyanthraquinone (146) against <i>Aedes aegypti</i> larvae	229
15	24 hours mortality count for morindone-6-methylether (147) against <i>Aedes aegypti</i> larvae	229
16	Cytotoxic results of ethyl acetate extract against MCF7 cell line	230

17	Cytotoxic results of aristolactam AII (143) against MCF7 cell line	230
18	Cytotoxic results of piperine (1) and pellitorine (69) against HL-60 cell line	231
19	Cytotoxic results of various crude extracts from <i>Morinda citrifolia</i> against HL-60 cell line	231
20	Cytotoxic results of various crude extracts from <i>Morinda citrifolia</i> against MCF7 cell line	232
21	Cytotoxic results of various compounds from <i>Morinda citrifolia</i> against HL-60 cell line	232



LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	chemical shift in ppm
λ_{\max}	wavelength maxima in nm
ν_{\max}	wavenumber maxima in cm^{-1}
μg	microgram
br	broad
^{13}C	carbon-13
CDCl_3	deuterated chloroform
CHCl_3	chloroform
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	deuterated dimethylsulfoxide
EIMS	Electron ionization mass-spectroscopy
EtOAc	ethyl acetate
FeCl_3	Ferric chloride
FTIR	Fourier Transform Infra Red
^1H	proton
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple Quantum
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	hertz
IC	Inhibition Concentration