



UNIVERSITI PUTRA MALAYSIA

***PHYTOCHEMICAL STUDIES OF GARCINIA EUGENIFOLIA WALL., G.
NITIDA PIERRE., G. MANGOSTANA L., AND MORINDA CITRIFOLIA L.
AND THEIR BIOLOGICAL ACTIVITIES***

VIVIEN JONG YI MIAN

FS 2012 63

**PHYTOCHEMICAL STUDIES OF *GARCINIA EUGENIFOLIA* WALL., *G. NITIDA* PIERRE., *G. MANGOSTANA* L., AND *MORINDA CITRIFOLIA* L.
AND THEIR BIOLOGICAL ACTIVITIES**

By

VIVIEN JONG YI MIAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

June 2012

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

PHYTOCHEMICAL STUDIES OF *GARCINIA EUGENIFOLIA* WALL., *G. NITIDA* PIERRE., *G. MANGOSTANA* L., AND *MORINDA CITRIFOLIA* L. AND THEIR BIOLOGICAL ACTIVITIES

By

VIVIEN JONG YI MIAN

June 2012

Chairman : Professor Gwendoline Ee Cheng Lian, PhD

Faculty : Science

Phytochemical studies were carried out on four plants, *Garcinia eugenifolia*, *Garcinia nitida*, *Garcinia mangostana* and *Morinda citrifolia*. The chemical investigation has resulted in the isolation of 22 compounds which covered xanthones, triterpenoids and quinones. These compounds were isolated using common chromatographic techniques and were identified using spectroscopic experiments such as NMR, MS, IR and UV.

The roots of *Garcinia eugenifolia* afforded six compounds comprising three triterpenoids, β -sitosterol (**145**), magniferolic acid (**147**) and euphadienol (**148**); two methanone, (3'-hydroxyphenyl)(2,4,6-trihydroxyphenyl)methanone (**149**) and (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)methanone (**150**); and a xanthone, 1,6-dihydroxy-7-methoxy-6',6'-dimethyl-2H-pyrano[2',3':3,2]-xanthone (**146**). (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)methanone (**150**) was isolated as a new compound. Besides this, the major compound, magniferolic acid (**147**) was

isolated for the first time from *Garcinia eugenifolia*. Meanwhile, the roots of *Garcinia nitida* afforded a new compound 1,6-dihydroxy-5-methoxy-6,6-dimethylpyrano[2',3':2,3]-xanthone (**151**), together with three known xanthenes and two common triterpenoids, inophyllin B (**152**), caloxanthone A (**154**), rubraxanthone (**156**) stigmasterol (**153**) and friedelin (**155**).

Garcinia mangostana (roots) provided three pure compounds, α -mangostin (**157**), β -mangostin (**158**) and cowagarcinone B (**160**). β -mangostin was isolated as the major compound from the roots of *Garcinia mangostana*. Acetylation of β -mangostin (**158**) successfully yielded 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one-6-acetate (**159**) as the product. Meanwhile, purification of the roots of *Morinda citrifolia* using chromatotron gave seven anthraquinones, damnacanthal (**161**), nordamnacanthal (**162**), sorandidiol (**163**), rubiadin (**164**), 1-hydroxy-2-methylanthraquinone (**165**), 2-ethoxy-1-hydroxyanthraquinone (**166**) and rubiadin-1-methyl ether (**167**).

Cytotoxic tests were carried out using HeLa, MCF-7 and HL-60 cell lines on the crude extracts of *Garcinia eugenifolia* and *Garcinia nitida*. The pure compounds from all the four plants were assayed against HT-29 (Human Colorectal Cancer) and A549 (Human Lung Cancer). *Garcinia eugenifolia* were found to show moderate cytotoxicity with IC₅₀ values ranging from 11 to 77 μ g/mL for HeLa and MCF-7 cell lines. Meanwhile, for the HL-60 cell line, the hexane and ethyl acetate extracts of *Garcinia eugenifolia* indicated strong cytotoxicities with IC₅₀ values of 1.9 μ g/mL and 2.5 μ g/mL respectively. For *Garcinia nitida*, the leaf extracts showed strong cytotoxic activity with IC₅₀ values of 4 and 7 μ g/mL respectively towards the HeLa

and MCF-7 cell lines. However, the crude extract of the roots and twigs of *Garcinia nitida* was found to be inactive to MCF-7 activity test and weak cytotoxic activity against the HeLa cell line.

Twelve pure compounds were subjected to cytotoxic assays using HT-29 and A549 cell lines. Magniferolic acid (**147**) gave IC_{50} values of 4.8 and 4.7 $\mu\text{g/mL}$ respectively towards HT-29 and A549 cell lines while euphadienol (**148**) gave an IC_{50} value of 2.7 $\mu\text{g/mL}$ towards the A549 cell line but showed moderate inhibition to HT-29 cell line. Meanwhile, 2-ethoxy-1-hydroxyanthraquinone (**166**) gave strong cytotoxicities ($IC_{50} = 4.5 \mu\text{g/mL}$) towards HT-29 but moderate activities (11.2 $\mu\text{g/mL}$) against A549. Both damnacanthal (**161**) and nordamnacanthal (**162**) showed moderate inhibitory activities ($IC_{50} < 10 \mu\text{g/mL}$) towards HT-29 cell line.

The antimicrobial activity test was also carried out using five pathogenic bacteria, namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Streptococcus pyogenes* and *Escherichia coli*. It was observed that most of the crude extracts from both *Garcinia* species exhibited strong inhibitory activities against the microbes. The only weak activity observed was for the *Staphylococcus aureus* microbe for both methanol and ethyl acetate extracts of *Garcinia nitida* with an inhibitory diameter ranging from 4 to 8 mm.

The DPPH antioxidant assay was carried out for both *Garcinia eugenifolia* and *Garcinia nitida*. Of all the tested extracts of *Garcinia eugenifolia* and *Garcinia nitida*, only the methanol extract from *Garcinia eugenifolia* stem bark and leaves exhibited moderate antioxidant activity ($EC_{50} = 21.1$ and $29.5 \mu\text{g/L}$) when compared

to ascorbic acid with EC_{50} values of 15.32 $\mu\text{g/L}$. As for total phenolic content, the twigs of *Garcinia eugenifolia* had the highest total phenolic content of 0.2322 ± 0.059 $\mu\text{g GAE/mg extract}$, followed by the roots (0.2274 ± 0.067 $\mu\text{g GAE/mg extract}$) and stem (0.2138 ± 0.013 $\mu\text{g GAE/mg extract}$).



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KAJIAN FITOKIMIA DAN AKTIVITI-AKTIVITI BIOLOGI DARIPADA
GARCINIA EUGENIFOLIA WALL., *G. NITIDA* PIERRE., *G. MANGOSTANA*
L., DAN *MORINDA CITRIFOLIA* L.**

Oleh

VIVIEN JONG YI MIAN

Jun 2012

Pengerusi : Profesor Gwendoline Ee Cheng Lian, PhD

Fakulti : Sains

Kajian fitokimia telah dijalankan ke atas empat tumbuh-tumbuhan, *Garcinia eugenifolia*, *Garcinia nitida*, *Garcinia mangostana* dan *Morinda citrifolia*. Kajian kimia terperinci menghasilkan 22 sebatian semulajadi yang merangkumi xanthon, triterpenoid dan kuinon. Sebatian-sebatian ini telah diasingkan dengan menggunakan teknik-teknik kromatografi biasa dan telah dikenal pasti dengan menggunakan eksperimen spektroskopi seperti NMR, MS, IR dan UV.

Akar daripada *Garcinia eugenifolia* telah memberikan enam sebatian iaitu tiga triterpenoid, β -sitosterol (**145**), asid magniferolik (**147**) dan euphadienol (**148**); dua metanon, (3'-hidroksifenil)(2,4,6-trihidroksifenil)metanon (**149**) dan (3,4-dihidroksifenil)(3-hidroksi-5-metoksifenil)metanon (**150**); dan satu xanthon, 1,6-dihidroksi-7-metoksi-6',6'-dimetil-2H-pyrano[2',3':3,2]-xanthon (**146**). (3,4-dihidroksifenil)(3-hidroksi-5-metoksifenil)metanon (**150**) telah diasingkan sebagai sebatian yang baru. Selain itu, sebatian utama, asid magniferolik (**147**) juga dihasilkan untuk kali yang pertama daripada *Garcinia eugenifolia*. Sementara itu,

akar *Garcinia nitida* memberikan satu sebatian baru iaitu 1,6-dihidroksi-7-metoksi-6',6'-dimetil-2H-pyrano[2',3':3,2]-xanthon (**151**), bersama dengan tiga xanthon yang diketahui dan dua triterpenoid biasa, inophyllin B (**152**), caloxanthone A (**154**), rubraxanthone (**156**) stigmasterol (**153**) dan friedelin (**155**).

Garcinia mangostana (akar) menghasilkan tiga sebatian tulen, α -mangostin (**157**), β -mangostin (**158**) dan cowagarcinone B (**160**). β -mangostin diasingkan sebagai sebatian utama daripada akar *Garcinia mangostana*. Pengasetilan β -mangostin (**158**) berjaya menghasilkan 1-hidroksi-3,7-dimetoksi-2,8-bis(3-metil-2-butenyl)-9H-xanthen-9-one-6-asetat (**159**) sebagai produk. Manakala penulenan akar *Morinda citrifolia* menggunakan chromatotron memberi tujuh antrakuinon, damnacanthal (**161**), nordamnacanthal (**162**), sorandidiol (**163**), rubiadin (**164**), 1-hidroksi-2-metil-antrakuinon (**165**), 2-etoksi-1-hidroksiantrakuinon (**166**) dan rubiadin-1-metil eter (**167**).

Ujian sitotoksik telah dijalankan menggunakan sel HeLa, MCF-7 dan HL-60 untuk ekstrak mentah *Garcinia eugenifolia* dan *Garcinia nitida*. Manakala sebatian tulen daripada semua empat tumbuhan dijalankan terhadap sel HT-29 (Kanser Kolorektal Manusia) dan A549 (Kanser paru-paru Manusia). *Garcinia eugenifolia* telah didapati menunjukkan sitotoksik yang sederhana dengan nilai IC_{50} antara 11 hingga 77 $\mu\text{g/mL}$ untuk sel HeLa dan MCF-7. Sementara itu, bagi sel HL-60, ekstrak heksana dan etil asetat *Garcinia eugenifolia* menunjukkan aktiviti sitotoksik yang baik dengan nilai IC_{50} 1.9 $\mu\text{g/mL}$ dan 2.5 $\mu\text{g/mL}$ masing-masing. Bagi *Garcinia nitida*, ekstrak daun menunjukkan aktiviti sitotoksik yang baik dengan nilai IC_{50} 4 dan 7 $\mu\text{g/mL}$ masing-masing terhadap sel HeLa dan MCF-7. Walau bagaimanapun, ekstrak

mentah akar dan ranting *Garcinia nitida* didapati tidak aktif untuk ujian aktiviti sel MCF-7 dan sitotoksik yang lemah terhadap sel HeLa.

Dua belas sebatian-sebatian tulen telah diuji sitotoksik menggunakan sel HT-29 dan A549. Asid magniferolik (**147**) memberi nilai IC_{50} sebanyak 4.8 dan 4.7 $\mu\text{g/mL}$ masing-masing pada sel HT-29 dan A549 manakala euphadienol (**148**) memberi nilai IC_{50} sebanyak 2.7 $\mu\text{g/mL}$ terhadap sel A549 tetapi menunjukkan perencatan yang sederhana pada sel HT-29. Sementara itu, 2-etoksi-1-hidroksiantrakuinon (**166**) memberi sitotoksik yang kuat ($IC_{50} = 4.5 \mu\text{g/mL}$) terhadap sel HT-29 tetapi aktiviti yang sederhana (11.2 $\mu\text{g/mL}$) terhadap sel A549. Kedua-dua damnacanthal (**161**) dan nordamnacanthal (**162**) menunjukkan aktiviti perencatan yang sederhana ($IC_{50} < 10 \mu\text{g/mL}$) pada sel HT-29.

Ujian aktiviti antimikrob juga dijalankan dengan menggunakan lima bakteria patogenik, iaitu, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium difficile*, *Streptococcus pyogenes* dan *Escherichia coli*. Adalah diperhatikan bahawa sebahagian besar daripada ekstrak mentah dari kedua-dua spesies *Garcinia* mempamerkan aktiviti perencatan yang tinggi terhadap mikrob. Satu-satunya aktiviti lemah diperhatikan untuk mikrob *Staphylococcus aureus* bagi kedua-dua ekstrak metanol dan etil asetat *Garcinia nitida* dengan diameter perencatan antara 4 hingga 8 mm.

Ujian antioksidan DPPH telah dijalankan untuk kedua-dua *Garcinia eugenifolia* dan *Garcinia nitida* untuk ujian DPPH. Daripada kesemua ekstrak yang diuji, hanya ekstrak metanol daripada batang kulit dan daun pokok *Garcinia eugenifolia*

menunjukkan aktiviti antioksidasi yang sederhana ($EC_{50} = 21.1$ dan $29.5 \mu\text{g/L}$) berbanding dengan asid askorbik dengan nilai EC_{50} $15.32 \mu\text{g/L}$. Bagi jumlah kandungan fenolik, ranting *Garcinia eugenifolia* mempunyai jumlah kandungan fenolik tertinggi sebanyak $0.2322 \pm 0.059 \mu\text{g GAE/mg}$ ekstrak, diikuti oleh akar ($0.2274 \pm 0.067 \mu\text{g GAE/mg}$ ekstrak) dan batang ($0.2138 \pm 0.013 \mu\text{g GAE/mg}$ ekstrak).



ACKNOWLEDGEMENTS

I would hereby like to acknowledge and express my most sincere thanks and appreciation to the following people and institutions for the significant role they each played in enabling me to complete my PhD degree:

My supervisor, Prof. Dr. Gwendoline Ee Cheng Lian, for providing me with the thoughtful advice, constant encouragement and for her guidance throughout this study.

My sincere and deepest gratitude are also extended to my supervisory committee member Prof. Dr. Aspollah Hj. Sukari and Prof. Dr. Taufiq Yap Yun Hin for their support.

My labmates Sim Wei Chung, Wen Yin Ping, Shaari Daud, Teh Sook Sin and Mah Siau Hui for their help and encouragement during this research.

The academic and technical staff of Chemistry Department of UPM, for their advice and encouragement. A special thanks to Mr. Zainal Abidin Kassim for mass spectral measurement, Mr. Johardi Iskandar and Miss Shareena for NMR spectral analysis and Mrs. Rusnani Amirudin for providing the IR data.

Ministry of Higher Education, FRGS for their financial assistance.

My beloved family and friends for always being there for me.

My best friend, Stephanie Oh Siaw Wee for her invaluable moral support. Thank you for always believing in me and your constant encouragement.

My parents for the abundant love and unselfish support shown towards me throughout my life.

Last but not least, my wonderful husband, Joshua Pui, who was a pillar of strength through the tough times. Thank you for your patience, love, understanding and endless support.

All praise and glory be to God – through Him all things are possible.

I certify that a Thesis Examination Committee has met on 6 June 2012 to conduct the final examination of Vivien Jong Yi Mian on her thesis entitled "**Phytochemical Studies of *Garcinia eugenifolia* Wall., *G. nitida* Pierre., *G. mangostana* L., and *Morinda citrifolia* L. 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Mohd Zobir Husseinn, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mawardi Rahmani, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Dr Siti Mariam Mohd Nor, PhD

Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Yoshinori Asawaka, PhD

Professor
Faculty of Pharmaceutical Sciences
Tokushima Bunri University
Japan
(External Examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Gwendoline Ee Cheng Lian, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohd. Aspollah Sukari, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Taufiq Yap Yun Hin, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotation and citations which have been duly acknowledged. I also declare that it has been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

VIVIEN JONG YI MIAN

Date: 6 June 2012

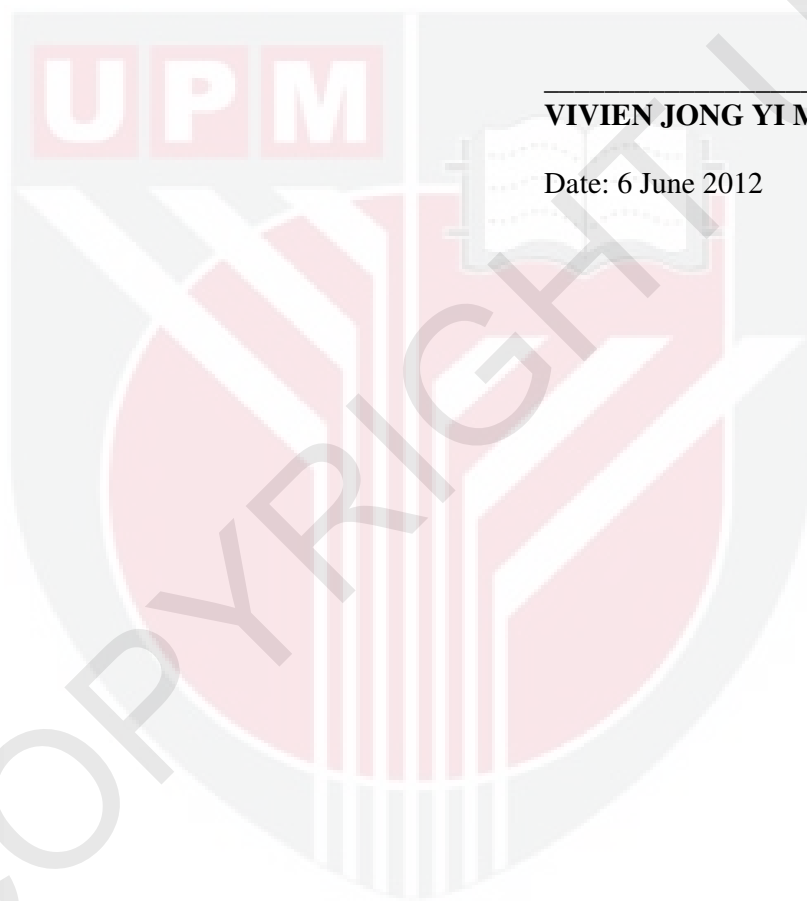


TABLE OF CONTENTS

ABSTRACT	Page ii
ABSTRAK	vi
ACKNOWLEDGEMENTS	x
APPROVAL	xiii
DECLARATION	xiv
LIST OF TABLES	xix
LIST OF FIGURES	xxi
LIST OF ABBREVIATIONS	xxxi
CHAPTER	
1 INTRODUCTION	1
1.1 General Introduction	1
1.2 The Genus <i>Garcinia</i>	3
1.3 The Genus <i>Morinda</i>	6
1.4 Purpose of Present Study	8
2 LITERATURE REVIEW	10
2.1 Plant Secondary Metabolites	10
2.2 Chemistry of <i>Garcinia</i> species	10
2.2.1 Xanthones	10
2.2.2 Benzophenones	20
2.2.3 Flavonoids	25
2.2.4 Triterpenoids	27
2.3 Biological Activities of <i>Garcinia</i> Species	30
2.4 Chemistry of <i>Morinda</i> species	33
2.4.1 Anthraquinones	33
2.4.2 Flavonoids	35
2.4.3 Coumarins	36
2.4.4 Iridoids	37
2.4.5 Fatty Acids	37
2.5 Biological Activities of <i>Morinda</i> Species	38
3 EXPERIMENTAL	41
3.1 Plant Materials	41
3.2 Phytochemical Screening	41
3.3 General Instrumentation	42
3.4 Chromatographic Method	42
3.4.1 Column Chromatography	42
3.4.2 Thin Layer Chromatography (TLC)	43
3.4.3 Preparative Thin Layer Chromatography (PTLC)	43
3.4.4 Centrifugal Thin Layer Chromatography (Chromatotron)	44

3.5 Extraction and Isolation of Compounds from	
<i>Garcinia eugenifolia</i> L., <i>Garcinia nitida</i> L., <i>Garcinia mangostana</i> L. and <i>Morinda citrifolia</i> L.	45
3.5.1 <i>Garcinia eugenifolia</i> L.	45
3.5.1.1 Isolation of Compounds from <i>Garcinia eugenifolia</i>	46
3.5.1.2 Physical and Spectral Data of Compounds from <i>Garcinia eugenifolia</i>	47
3.5.1.2.1 1,6-dihydroxy-7-methoxy-6',6'-dimethyl-2H-pyrano[2',3':3,2]-xanthone (146)	47
3.5.1.2.2 magniferolic acid (147)	48
3.5.1.2.3 euphadienol (148)	49
3.5.1.2.4 (3'-hydroxyphenyl)(2,4,6-trihydroxyphenyl)-methanone (149)	50
3.5.1.2.5 (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)-methanone (150)	51
3.5.2 <i>Garcinia nitida</i> L.	52
3.5.2.1 Isolation of Compounds from <i>Garcinia nitida</i>	52
3.5.2.2 Physical and Spectral Data of Compounds from <i>Garcinia nitida</i>	53
3.5.2.2.1 1,6-dihydroxy-5-methoxy-6,6-dimethyl-pyrano[2',3':2,3]-xanthone (151)	53
3.5.2.2.2 inophyllin B (152)	54
3.5.2.2.3 caloxanthone A (154)	55
3.5.2.2.4 rubraxanthone (156)	56
3.5.3 <i>Garcinia mangostana</i> L.	57
3.5.3.1 Isolation of Compounds from <i>Garcinia mangostana</i>	58
3.5.3.2 Physical and Spectral Data of Compounds from <i>Garcinia mangostana</i>	59
3.5.3.2.1 α -mangostin (157)	59
3.5.3.2.2 β -mangostin (158)	60
3.5.3.2.3 cowagarcinone B (160)	61
3.5.3.3 Acetylation of β -mangostin (158) to 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one-6-acetate (159)	62
3.5.3.3.1 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one-6-acetate (159)	62
3.5.4 <i>Morinda citrifolia</i>	63
3.5.4.1 Isolation of Compounds from <i>Morinda citrifolia</i>	63
3.5.4.2 Physical and Spectral Data of Compounds from <i>Morinda citrifolia</i>	64
3.5.4.2.1 damnacanthol (161)	64

3.5.4.2.2	nordamnacanthal (162)	65
3.5.4.2.3	sorandidiol (163)	66
3.5.4.2.4	rubiadin (164)	66
3.5.4.2.5	1-hydroxy-2-methylanthraquinone (165)	67
3.5.4.2.6	2-ethoxy-1-hydroxyanthraquinone (166)	68
3.5.4.2.7	rubiadin-1-methyl ether (167)	69
3.6	Bioassay	70
3.6.1	Cytotoxic Assay	70
3.6.1.1	Cell Culture	70
3.6.1.2	Cell Proliferation Assay	70
3.6.2	Antimicrobial Assay	71
3.6.3	Antioxidant Assay	73
3.6.3.1	DPPH assay	73
3.6.3.2	Determination of Total Phenolic Content	74
3.6.3.2.1	Principle of assay	74
3.6.3.2.2	Preparation of standard curve	74
3.6.3.2.3	Determination of Total Phenolic Content in Samples	75
4	RESULTS AND DISCUSSION	77
4.1	Isolation of Chemical Constituents from <i>Garcinia eugenifolia</i>	77
4.1.1	Characterization of 1,6-dihydroxy-7-methoxy-6',6'-dimethyl-2H-pyrano[2',3':3,2]-xanthone (146)	79
4.1.2	Characterization of magniferolic acid (147)	89
4.1.3	Characterization of euphadienol (148)	101
4.1.4	Characterization of (3'-hydroxyphenyl)(2,4,6-trihydroxyphenyl)-methanone (149)	116
4.1.5	Characterization of (3,4-dihydroxyphenyl)(3-hydroxy-5-methoxyphenyl)methanone (150)	126
4.2	Isolation of chemical constituents from <i>Garcinia nitida</i>	137
4.2.1	Characterization of 1,6-dihydroxy-5-methoxy-6,6-dimethyl-pyrano[2',3':2,3]-xanthone (151)	139
4.2.2	Characterization of inophylin B (152)	152
4.2.3	Characterization of caloxanthone A (154)	162
4.2.4	Characterization of rubraxanthone (156)	172
4.3	Isolation of chemical constituents from <i>Garcinia mangostana</i>	189
4.3.1	Characterization of α -mangostin (157)	191
4.3.2	Characterization of β -mangostin (158)	201
4.3.3	Characterization of 1-hydroxy-3,7-dimethoxy-2,8-bis(3-methyl-2-butenyl)-9H-xanthen-9-one-6-acetate (159)	212
4.3.4	Characterization of cowagarcinone B (160)	221
4.4	Isolation of chemical constituents from <i>Morinda citrifolia</i>	230
4.4.1	Characterization of damnacanthol (161)	231

4.4.2	Characterization of nordamnacanthal (162)	242
4.4.3	Characterization of sorandidiol (163)	255
4.4.4	Characterization of rubiadin (164)	268
4.4.5	Characterization of 1-hydroxy-2-methyl anthraquinone (165)	278
4.4.6	Characterization of 2-ethoxy-1-hydroxy anthraquinone (166)	293
4.4.7	Characterization of rubiadin-1-methylether (167)	306
4.5	Bioassay Results	316
4.5.1	Cytotoxic Activity	316
4.5.2	Antimicrobial Assay	321
4.5.3	Antioxidant Activity	322
4.5.3.1	DPPH Assay	322
4.5.3.2	Total Phenolic Content	323
5	CONCLUSIONS	325
	REFERENCES	329
	APPENDICES	339
	BIODATA OF STUDENT	360
	LIST OF PUBLICATIONS	361