



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
GARCINIA MANGOSTANA L. AND
*PIPER BETLE***

YEAP SOO FONG

FS 2009 31



**CHEMICAL CONSTITUENTS AND BIOLOGICAL
ACTIVITIES OF *GARCINIA MANGOSTANA* L. AND
*PIPER BETLE***

YEAP SOO FONG

**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

2009



**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
GARCINIA MANGOSTANA L. AND *PIPER BETLE***

By

YEAP SOO FONG

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Master of Science**

October 2009



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of requirement for the degree of Master in Science

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF
GARCINIA MANGOSTANA L. AND *PIPER BETLE***

By

YEAP SOO FONG

October 2009

Chairman : Mawardi bin Rahmani, PhD

Faculty : Science

Young fruits of *Garcinia mangostana* L. from Guttiferae family and leaves of *Piper betle* from Piperaceae family were phytochemically studied and screened for their biological activities. The young fruits of *Garcinia mangostana* L. were collected from Negeri Sembilan while the leaves of *Piper betle* were collected from Sabah. The phytochemical works involved extraction of the plant materials with organic solvents of different polarity and chromatographic separation of the extracts with several techniques to obtain pure compounds. The structures of the compounds were determined by using spectroscopic techniques such as IR, MS, NMR and UV. The crude extracts from both plants were screened for antimicrobial (against four pathogenic bacteria and 3 pathogenic fungi), cytotoxic activities and antioxidant using disc diffusion method, Tetrazolium Salt (MTT) assays and 1,2-Diphenyl-2-picrylhydrazyl (DPPH) respectively. Three isolated compounds, epicatechin (**39**), 4-hydroxybenzoic acid (**114**) (both from *mangostana* L.) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**) (from *P. betle*) were tested for antioxidant by using DPPH.



Separation of the extracts of young fruits of *Garcinia mangostana* L. afforded seven chemical compounds identified as methylparaben (**110**), methyl 3,4,5-trihydroxybenzoate (**111**), parvifoliol A1 (**112**), methyl 2,3-dihydroxybenzoate (**113**), 4-hydroxybenzoic acid (**114**), epicatechin (**39**) and a xanthone, mangostanin (**20**) after extensive various chromatographic techniques. Two compounds, methylparaben (**110**) and methyl 3,4,5-trihydroxybenzoate (**111**) have not been previously reported to occur in *Garcinia mangostana*. This is the first report on the occurrence of these compounds in *Garcinia mangostana* and the proper technical name for methylparaben (**110**) is methyl 4-hydroxybenzoate. On isolation and purification of the leaves extracts of *Piper betle* led to the isolation of four compounds chavibetol (**77**), 2-hydroxychavicol (**80**), β -sitosterol (**47**) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**).

The antimicrobial activity test for both plant extracts was carried out using seven microbes namely, *methicilin resistant Staphylococcus aureus* (MRSA), *Bacillus substili*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus ochraceus* and *Saccharomyces cerevisiae*. However, no activity was observed in the crude extracts of both *Garcinia mangostana* L. and *Piper betle*. The same results were obtained for the cytotoxic activity using Tetrazolium Salt (MTT) assay. When tested for antioxidant by using 1,2-Diphenyl-2-picrylhydrazyl (DPPH), all the crude extracts failed to exhibit any activity. However two of the isolated compounds, epicatechin (**39**) and 2-allyl-3,4-dihydroxybenzaldehyde (**115**) showed strong activity with $IC_{50} < 7.81 \mu\text{g/mL}$ in comparison with the standard, ascorbic acid ($IC_{50} < 11.70 \mu\text{g/mL}$).



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

KANDUNGAN KIMIA DAN AKTIVITI BIOLOGI DARIPADA *GARCINIA MANGOSTANA L.* DAN *PIPER BETLE*

Oleh

YEAP SOO FONG

Oktober 2009

Pengerusi : Mawardi bin Rahmani, PhD

Fakulti : Sains

Buah muda *Garcinia mangostana* L. daripada famili Guttiferae dan daun *Piper betle* daripada famili Piperaceae telah dikaji secara fitokimia dan diuji aktiviti biologi. Buah muda *Garcinia mangostana* L. diperolehi dari Negeri Sembilan manakala daun *Piper betle* pula dibawa dari negeri Sabah. Kajian fitokimia ini melibatkan pengekstrakan sebatian daripada tumbuhan dengan menggunakan pelarut organik yang berbeza kekutubannya serta pemisahan dengan pelbagai teknik kromatografi ke atas ekstrak untuk memperolehi sebatian tulen. Struktur sebatian kemudiannya dikenalpasti melalui teknik spektroskopi seperti IR, MS, NMR and UV. Ekstrak mentah daripada kedua-dua tumbuhan diuji aktiviti antimikrob (terhadap empat bakteria patogen dan tiga fungi patogen), sitotoksik, dan antioksidan masing-masing dengan menggunakan kaedah peresapan cakera, garam Mikrokultur Tetrazolium (MTT) dan 1,2-difenil-2-pikrilhidrazil (DPPH). Tiga sebatian tulen yang diperolehi, epikatekin (**39**), asid hidroksi benzoik (**114**) (kedua-duanya daripada *G. mangostana* L.) dan 2-alil-3,4-dihidroksibenzaldehid (**115**) (daripada *P. betle*) telah diuji aktiviti antioksidan.



Pemisahan berterusan dengan pelbagai teknik kromatografi ke atas ekstrak buah muda *Garcinia mangostana* L. telah menghasilkan tujuh sebatian kimia yang dikenalpasti sebagai metilparaben (**110**), metil 3,4,5-trihidroksibenzoat (**111**), parvifoliol A1 (**112**), metil 2,3-dihidroksibenzoat (**113**), asid hidroksi benzoik (**114**), epikatekin (**39**) dan juga xanthone iaitu mangostanin (**20**). Dua sebatian, metilparaben (**110**) dan metil 3,4,5-trihidroksibenzoat (**111**) belum pernah dilaporkan berlaku dalam *Garcinia mangostana*. Ini merupakan laporan pertama di mana dua sebatian ini dipisahkan daripada *Garcinia mangostana* dan nama teknikal yang betul untuk metilparaben (**110**) ialah metil 4-hydroxybenzoat. Pemisahan dan penulenan ekstrak daun *Piper betle* telah mendorong kepada pemisahan dan pengenalpastian empat sebatian iaitu kavibetol (**77**), 2-hidroksikavikol (**80**), β -sitosterol (**47**) dan 2-alil-3,4-dihidroksibenzaldehid (**115**).

Ujian aktiviti antimikrob ke atas ekstrak mentah kedua-dua tumbuhan dijalankan dengan menggunakan tujuh mikrob iaitu *methicilin resistant Staphylococcus aureus* (MRSA), *Bacillus substili*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Candida albicans*, *Aspergillus ochraceus* and *Saccharomyces cerevisiae*. Walau bagaimanapun, tiada aktiviti diperhatikan ke atas ekstrak mentah kedua-dua spesies kajian. Keputusan yang sama juga diperoleh untuk ujian aktiviti sitotoksik dengan garam Mikrokultur Tetrazolium (MTT). Apabila diuji aktiviti antioksidan dengan 1,2-difenil-2-pikrilhidrazil (DPPH), didapati semua ekstrak mentah gagal menunjukkan sebarang aktiviti manakala dua sebatian tulen yang diuji, epikatekin (**39**) dan 2-alil-3,4-dihidroksibenzaldehid (**115**) menghasilkan aktiviti yang kuat dengan $IC_{50} < 7.81 \mu\text{g/mL}$ berbanding dengan standard, asid askorbik ($IC_{50} < 11.70 \mu\text{g/mL}$).

ACKNOWLEDGEMENTS

I wish to express my sincere, deepest appreciation and gratitude to those involved either direct or indirectly in completing my thesis as well as the challenging research that lies behind. To God, the Lord Almighty where the strength and encouragement were always seek, only by His grace and merciful that kept me going in completing this thesis.

I am indebted to my supervisor, Prof. Dr. Mawardi Rahmani for introducing natural product as well as for his great understanding, advice, and assistance throughout the research and thesis preparation. My sincere and deepest gratitude are also extended to my supervisory committee members Dr. Intan Safinar Ismail and Assoc. Prof. Dr. Radzali Muse. Financial support from Kementerian Pelajaran Malaysia, cooperation and moral support from staffs of SMK Putrajaya Presint 8 (1) are also greatly appreciated.

Thanks also go to instrument officers: En. Johadi (NMR), Pn. Ros (FTIR), En. Zainal (GCMS) and all the staffs of chemistry department. My special and warmest thanks to my labmates: Najihah, Shireen, Parimah, Winda, Rose W., Rufaidah, Maisarah and juniors; friends, Yin Pin, Paw, Ratiah, Mrs Soon, Siew Eng, Wai Ching for their valuable support, understanding and the friendship that will be treasured.

To my beloved husband, Liang Tin Pin and beloved sons (John L.Q.S., Joshua L.Q.Y. and Joseph L.Q.Z), my deepest love for their prayers, understanding, patience, and the hardship that they have to bear with me. Not to forget, my deepest gratitude to my mum, siblings, and Liang's family members for their moral support and encouragement.



I certify that an Examination Committee has met on 21 October 2009 to conduct the final examination of Yeap Soo Fong on her Master of Science thesis entitled “Chemical Constituents and Biological Activities Of *Garcinia Mangostana* L. and *Piper Betle*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the Candidate be awarded the relevant degree.

Members of the Examination Committee are as follows:

Gwendoline Ee Cheng Lian, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairperson)

Faujan Bin Hj Ahmad, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mohd Aspollah Sukari, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Rafediah Ahmad, PhD

Associate Professor
Faculty of Science
Universiti Teknologi Malaysia
(External Examiner)

BUJANG BIN KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



This thesis was submitted to the Senate of Universities Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mawardi Rahmani, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Intan Safinar Ismail, PhD

Faculty of Science
Universiti Putra Malaysia
(Member)

Radzali Muse, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 January 2010



DECLARATION

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

YEAP SOO FONG

Date: 1 February 2010



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENTS	vi
APPROVAL	vii
DECLARATION	ix
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xvii
CHAPTER	
1 INTRODUCTION	
1.1 General Introduction	1
1.2 Objectives of Study	3
2 LITERATURE REVIEW	
2.1 Botanical Aspects of The Plants	4
2.1.1 The Family Guttiferae	4
2.1.2 <i>Garcinia</i>	4
2.1.3 <i>Garcinia mangostana</i> L.	5
2.1.4 The Family Piperaceae	7
2.1.5 <i>Piper</i>	7
2.1.6 <i>Piper betle</i>	8
2.2 Chemical Constituents	
2.2.1 Chemical Constituents of Genus <i>Garcinia</i>	9
2.2.2 Chemical Constituents of <i>Garcinia mangostana</i> L.	14
2.2.3 Chemical Constituents of Genus <i>Piper</i>	16
2.2.4 Chemical Constituents of <i>Piper betle</i>	22
2.3 Biological Activities	
2.3.1 Biological Activities of Genus <i>Garcinia</i>	24
2.3.2 Biological Activities of <i>Garcinia mangostana</i> Linn	26
2.3.3 Biological Activities of Genus <i>Piper</i>	27
2.3.4 Biological Activities of <i>Piper betle</i>	29
3 METHODOLOGY	
3.1 Plant Materials	31
3.2 Instruments	
3.2.1 Infrared (IR)	31
3.2.2 Mass Spectra (MS)	31
3.2.3 Melting Point	32
3.2.4 Nuclear Magnetic Resonance (NMR)	32
3.2.5 Ultraviolet (UV)	32
3.3 Chromatographic Methods	32
3.3.1 Column Chromatography	32



3.3.2	Chromatotron	33
3.3.3	Preparative Layer Chromatography	33
3.3.4	Thin Layer Chromatography (TLC)	34
3.4	Extraction and Isolation of Compounds from <i>Garcinia mangostana</i> L.	
3.4.1	Extraction of The Young Fruits	34
3.4.2	Fractionation of the Chloroform Extract (CC 1)	35
3.4.3	Fractionation of the Ethyl acetate Extract (CC 2)	38
3.5	Extraction and Isolation of Compounds of <i>Piper betle</i>	
3.5.1	Extraction of the Leaves	41
3.5.2	Fractionation of Chloroform Extract (CC 3)	41
3.5.3	Fractionation of Methanol Extract (CC 4)	44
3.6	Biological Activities	46
3.6.1	Microorganisms	46
3.6.2	Antimicrobial Activity Assay	46
3.6.3	Cytotoxic Assay	47
3.6.4	DPPH Free Radical Scavenging Activity	49
4	RESULTS AND DISCUSSION	
4.1	Isolation of Chemical Constituents from <i>Garcinia mangostana</i> L. and <i>Piper betle</i>	50
4.2	Chemical Constituents from <i>Garcinia mangostana</i> L.	51
4.2.1	Characterization of Methylparaben (110)	52
4.2.2	Characterization of Methyl 3,4,5-trihydroxybenzoate (111)	61
4.2.3	Characterization of Mangostanin (20)	70
4.2.4	Characterization of Parvifoliol A1 (112)	81
4.2.5	Characterization of Epicatechin (39)	92
4.2.6	Characterization of Methyl 3,4-dihydroxybenzoate (113)	102
4.2.7	Characterization of 4-Hydroxybenzoic acid (114)	111
4.3	Chemical Constituents from of <i>Piper betle</i>	119
4.3.1	Characterization of Chavibetol (77)	119
4.3.2	Characterization of β -Sitosterol (47)	129
4.3.3	Characterization of 2-Hydroxychavicol (80)	134
4.3.4	Characterization of 2-allyl-3,4-dihydroxybenzaldehyde (115)	144
4.4	Bioassay Results	
4.4.1	Antimicrobial Assay	153
4.4.2	Cytotoxic Assay	154
4.4.3	DPPH Free Radical Scavenging Activity	155
5	CONCLUSIONS	157
	BIBLIOGRAPHY	160
	APPENDIX	167
	BIODATA OF STUDENT	174



LIST OF TABLES

Table		Page
1	Phytochemical from eleven <i>Piper</i> species	17
2	Compounds isolated from <i>Garcinia mangostana</i> L. and <i>Piper betle</i>	50
3	NMR spectral data of methylparaben (110)	53
4	NMR spectral data of methyl 3, 4, 5-trihydroxybenzoate (111)	62
5	NMR spectral data of mangostanin (20)	73
6	NMR spectral data of parvifoliol A1 (112)	84
7	NMR spectral data of epicatechin (39)	94
8	NMR spectral data of methyl 3,4-dihydroxybenzoate (113)	104
9	NMR spectral data of 4-hydroxybenzoic acid (114)	112
10	NMR spectral data of chavibetol (77)	121
11	NMR spectral data of β -sitosterol (47)	130
12	NMR spectral data of 2-hydroxychavicol (80)	136
13	NMR spectral data of 2-allyl-3,4-dihydroxybenzaldehyde (115)	146
14	The diameter of inhibition zone (mm) of pathogenic bacteria	153
15	The diameter of inhibition zone (mm) of pathogenic fungi	154
16	Cytotoxicity of crude extracts towards HL-60 (human promyelocytic leukemia cells)	155
17	The IC ₅₀ value of crude extracts of <i>Garcinia mangostana</i> L. and <i>Piper betle</i> towards DPPH free radical	156



LIST OF FIGURES

Figure		Page
1	IR spectrum of methylparaben (110)	54
2	EI mass spectrum of methylparaben (110)	54
3	¹ H-NMR spectrum of methylparaben (110)	55
4	¹³ C-NMR spectrum of methylparaben (110)	56
5	DEPT spectra of methylparaben (110)	57
6	COSY spectrum of methylparaben (110)	58
7	HMQC spectrum of methylparaben (110)	59
8	HMBC spectrum of methylparaben (110)	60
9	IR spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	63
10	EI mass spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	63
11	¹ H-NMR spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	64
12	¹³ C-NMR spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	65
13	DEPT spectra of methyl 3, 4, 5-trihydroxybenzoate (111)	66
14	COSY spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	67
15	HMQC spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	68
16	HMBC spectrum of methyl 3, 4, 5-trihydroxybenzoate (111)	69
17	IR spectrum of mangostanin (20)	73
18	EI mass spectrum of mangostanin (20)	74
19	Mass fragmentation patterns of mangostanin (20)	74
20	¹ H-NMR spectrum of mangostanin (20)	75
21	¹³ C-NMR spectrum of mangostanin (20)	76



22	DEPT spectra of mangostanin (20)	77
23	COSY spectrum of mangostanin (20)	78
24	HMQC spectrum of mangostanin (20)	79
25	HMBC spectrum of mangostanin (20)	80
26	Parvifoliol A	83
27	IR spectrum of parvifoliol A1 (112)	84
28	EI mass spectrum of compound (112)	85
29	¹ H-NMR spectrum of parvifoliol A1 (112)	86
30	¹³ C-NMR spectrum of parvifoliol A1 (112)	87
31	DEPT spectra of parvifoliol A1 (112)	88
32	COSY spectrum of parvifoliol A1 (112)	89
33	HMQC spectrum of parvifoliol A1 (112)	90
34	HMBC spectrum of parvifoliol A1 (112)	91
35	IR spectrum of epicatechin (39)	94
36	UV spectrum of epicatechin (39)	95
37	EI mass spectrum of epicatechin (39)	95
38	Mass fragmentation patterns of epicatechin (39)	95
39	¹ H-NMR spectrum of epicatechin (39)	96
40	¹³ C-NMR spectrum of epicatechin (39)	97
41	DEPT spectra of epicatechin (39)	98
42	COSY spectrum of epicatechin (39)	99
43	HMQC spectrum of epicatechin (39)	100
44	HMBC spectrum of epicatechin (39)	101
45	IR spectrum of methyl 3,4-dihydroxybenzoate (113)	104



46	EI mass spectrum of methyl 3,4-dihydroxybenzoate (113)	104
47	¹ H-NMR spectrum of methyl 3,4-dihydroxybenzoate (113)	105
48	¹³ C-NMR spectrum of methyl 3,4-dihydroxybenzoate (113)	106
49	DEPT spectra of methyl 3,4-dihydroxybenzoate (113)	107
50	COSY spectrum of methyl 3,4-dihydroxybenzoate (113)	108
51	HMQC spectrum of methyl 3,4-dihydroxybenzoate (113)	109
52	HMBC spectrum of methyl 3,4-dihydroxybenzoate (113)	110
53	IR spectrum of 4-hydroxybenzoic acid (114)	113
54	EI mass spectrum of 4-hydroxybenzoic acid (114)	113
55	¹ H-NMR spectrum of 4-hydroxybenzoic acid (114)	114
56	¹³ C-NMR spectrum of 4-hydroxybenzoic acid (114)	115
57	COSY spectrum of 4-hydroxybenzoic acid (114)	116
58	HMQC spectrum of 4-hydroxybenzoic acid (114)	117
59	HMBC spectrum of 4-hydroxybenzoic acid (114)	118
60	IR spectrum of chavibetol (77)	122
61	EI mass spectrum of chavibetol (77)	122
62	¹ H-NMR spectrum of chavibetol (77)	123
63	¹³ C-NMR spectrum of chavibetol (77)	124
64	DEPT spectra of chavibetol (77)	125
65	COSY spectrum of chavibetol (77)	126
66	HMQC spectrum of chavibetol (77)	127
67	HMBC spectrum of chavibetol (77)	128
68	IR spectrum of β -sitosterol (47)	131
69	EI mass spectrum of β -sitosterol (47)	131



70	¹ H-NMR spectrum of β-sitosterol (47)	132
71	¹³ C-NMR spectrum of β-sitosterol (47)	133
72	IR spectrum of 2-hydroxylchavicol (80)	137
73	EI mass spectrum of 2-hydroxylchavicol (80)	137
74	¹ H-NMR spectrum of 2-hydroxylchavicol (80)	138
75	¹³ C-NMR spectrum of 2-hydroxylchavicol (80)	139
76	DEPT spectra of 2-hydroxylchavicol (80)	140
77	COSY spectrum of 2-hydroxylchavicol (80)	141
78	HMQC spectrum of 2-hydroxylchavicol (80)	142
79	HMBC spectrum of 2-hydroxylchavicol (80)	143
80	IR spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	146
81	EI mass spectrum of compound (115)	146
82	¹ H-NMR spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	147
83	¹³ C-NMR spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	148
84	DEPT spectra of 2-allyl-3,4-dihydroxybenzaldehyde (115)	149
85	COSY spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	150
86	HMQC spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	151
87	HMBC spectrum of 2-allyl-3,4-dihydroxybenzaldehyde (115)	152
88	Mechanism of DPPH free radical scavenging of antioxidant	156



LIST OF ABBREVIATIONS

α	alpha
β	beta
δ	delta
δ	chemical shift in ppm
λ_{\max}	maximum wavelength in nm
μ	microgram
μL	microliter
^{13}C	carbon-13
$^{\circ}\text{C}$	degree celcius
CDCl_3	deuterated chloroform
CHCl_3	chloroform
cm^{-1}	per centimeter
COSY	Correlated Spectroscopy
<i>d</i>	doublet
<i>dd</i>	doublet of doublet
DEPT	Distortionless Enhancement by Polarization Transfer
DMSO	dimethyl sulphoxide
EtOAc	ethyl acetate
EIMS	Electron Impact Mass Spectrometry
g	gram
GC-MS	Gas Chromatography-mass spectroscopy
^1H	proton



hex	hexane
HMBC	Heteronuclear Multiple Bond Connectivity by 2D Multiple
HMQC	Heteronuclear Multiple Quantum Coherence
IC ₅₀	Inhibition Concentration at 50 percent
Id	Inhibition diameter
<i>br</i>	broad
<i>t</i>	triplet
<i>s</i>	singlet
<i>m</i>	multiplet
MeOH	methanol
m.p	melting point
MS	mass spectrum
<i>m/z</i>	mass per charge
NMR	Nuclear Magnetic Resonance
TLC	Thin Layer Chromatography
IR	Infrared
UV	Ultraviolet
ϵ	molar absorptivity



CHAPTER I

INTRODUCTION

1.1 General Introduction

Focus on plant research has recently increased all over the world. It is well known that plants synthesize poisonous chemicals to defend themselves against hostile environment and various predators. Some of these chemical are very dangerous to human, but some may be very useful and can be used to treat diseases. Research on medicinal plants, especially in tropical areas of the Ancient World, is of special importance from a therapeutic point of view. Nature, both flora and fauna give us some interesting model compounds, providing chemists with lead compounds for the design and synthesis of more pharmacologically viable derivatives.

Natural product research plays a significant role in drug discovery especially in nutraceuticals, agrochemicals and traditional medicines research. Two of the challenges in natural products research are the unknown nature and complexity of natural products extracts and the detection of minor active compounds in biological assay.

Nature probably provides unlimited sources of valuable secondary metabolites, which might be of high biological importance for several kinds of applications. The World Health Organization has listed over 21,000 plant species used around the world for medicinal purposes. Asian countries are enormously rich in still widely unexplored medicinal plants and natural products of unknown biological activities. Malaysia is identified as one of 12 mega-diversity countries in the world. It is estimated that 1,200



plants species in Peninsular Malaysia and 2000 species in Sabah and Sarawak have been harvested for medicinal or herbal purposes (Perry, 1980). The rainforest of Malaysia is rich with many species of herbal and medicinal plants and offers great opportunities for chemical investigation. However, the research on higher plant as a natural source of drugs is still largely unexplored. According to Perry (1980), there are 12000 species of flowering plants found in Malaysia. Unfortunately, only about 100 of 1300 that are said to be medicinal have been investigated. Hence, there is huge potential in research on medicinal plants.

Garcinia mangostana L. is well studied plant of its different parts like leaves, heartwood, ripe fruits and especially fruit hull (pericarp or rind) which was reported to be a source of mangostin, xanthone, tannin, isoflavone and other bioactive substances (Deachathai *et al.*, 2005). However, phytochemical investigation of whole young fruit of *Garcinia mangostana* L. has never been reported. Hence, it was chosen to be studied further for its chemical constituent and biological activity. While *Piper betle* leaves were studied in order to identify the bioactive substance as it is widely use in traditional medicine in Malaysia.



1.2 Objectives of Study

The objectives of this study are:

1. To extract and isolate the chemical constituents of the young fruits of *Garcinia mangostana* L. and *Piper betle* leaves using chromatographic techniques.
2. To identify and elucidate the structure of the isolated pure compounds using various spectroscopic techniques (IR, UV, MS and NMR).
3. To test the bioactivity of the crude extracts and isolated compounds.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Aspects Of The Plants

2.1.1 The Family Guttiferae

The Guttiferae is widely distributed family of evergreen tropical trees with milky or colored sap, comprising of about 40 genera and 1000 species. It is also known as Clusiaceae and mainly found in humid and hot regions (Babu *et al.*, 1988). There are 4 genera and 121 species found spreading in all kind of habitats in Malaysia with the most common genera of *Garcinia*, *Calophyllum*, *Mesua* and *Mammea*. *Garcinia* and *Mesua* can be found in dry land forests; *Calophyllum* in swampy forests while *Mesua* in lowlands (Morton, 1987).

Guttiferae plants grow as trees, shrubs and herbs with yellow or brightly coloured resinous juice which can be used as timber and as a source of resins, gums, pigments, dyes, edible oil and fruits (Mabberly, 1987). The leaves are simple, entire, opposite and stipulate with the present of resin or oil gland. The flowers are regular and often bright in colour (Dale and Greenway, 1961).

2.1.2 *Garcinia*

Garcinia is the most numerous genus of the Guttiferae family with about 400 species widely distributed in tropical Asia, Africa, New Caledonia and Polynesia (Morton,



1987). Plants of this genus normally reach up to 20 metres in height; have green leaves, edible fruits and produce yellow latex or resins.

Garcinia is well known as a genus of fruit trees in Malaysia. The fruits of many species are edible. Mangosteen (*Garcinia mangostana* L.) in which the flesh is encased within an outer harden shell (rind) is eaten fresh; the acidic fruits like *Garcinia atroviridis*, *Garcinia cambogia* and *Garcinia planconi* serve as a substitute for tamarind in curries. Furthermore, the fruits can be preserved in a dry state, with or without the aid of salt (Burkill, 1993).

Garcinia is often used for traditional medicines to treat abdominal pain, dysentery, diarrhoea, infected wound and gonorrhoea (Jayaprakasha *et al.*, 2006). The fruits of *Garcinia xanthochymus* have been widely used for bilious condition, diarrhoea and dysentery in Thailand (Perry, 1980). Meanwhile the fruit hull of *Garcinia mangostana* L. used for healing skin infections and wound (Mahabusarakum *et al.*, 1987). In Indonesia, the leaves and seeds of *Garcinia dulcis* have been used for the treatment of lymphatitis, parotitis and struma (Kosela *et al.*, 2000).

2.1.3 *Garcinia mangostana* L.

The origin of *Garcinia mangostana* L. is in Southeast Asia and distributed in Northern Australia, Brazil, Central America, Hawaii, Southern India, Indonesia, Malaysia, Thailand and other tropical countries (Morton, 1987).