



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDATIVE PROPERTIES AND NUTRITIONAL CONTENTS OF
SELECTED INDIGENOUS MICROALGAE**

NATRAH FATIN MOHD IKHSAN

IB 2006 9

Dedicated to

**All of my supervisors
Who inspired me the most**



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

**ANTIOXIDATIVE PROPERTIES AND NUTRITIONAL CONTENTS OF
SELECTED INDIGENOUS MICROALGAE**

By

NATRAH FATIN MOHD IKHSAN

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Chairman : Professor Fatimah Md. Yusoff, PhD

Institute : Bioscience

Microalgae are known to contain various beneficial pigments and are high in nutritional contents. The present studies were done to identify and explore the potential of indigenous microalgae as new natural sources for antioxidants with superior proximate and biochemical values.

Fourteen microalgae were isolated, purified and cultured from fresh and brackish waters. The ability of microalgae as natural resources of antioxidants were studied through screening test using three antioxidative chemical assays (ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1,1'-diphenyl-2-picrylhydrazyl (DPPH)). Preliminary screening results showed that methanol extracts from six microalgae (*Isochrysis galbana*, *Chaetoceros calcitrans*, *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Nannochloropsis oculata* and *Tetraselmis tetraethele*) were active in inhibiting the lipid peroxidation of linoleic acid. Among all the microalgae, *I. galbana* and *C. calcitrans*

showed highest antioxidative activities (>90%) in FTC and TBA assays indicating possibility of active constituents for the protection from lipid peroxidation.

Selected microalgae with high antioxidant values (*I. galbana* and *C. calcitrans*) were partitioned into different solvent fractions (hexane, dichloromethane, ethyl acetate, butanol and aqueous extracts) to separate components of wide mixture into group of compounds based on polarity and were re-tested with the antioxidative chemical assays. Dichloromethane extracts of *I. galbana* and *C. calcitrans* showed highest level of antioxidative activities with $97.1 \pm 0.1\%$ and $97.4 \pm 0.1\%$ linoleic acid peroxidation (LAP) inhibition in FTC assays respectively. Similar trends of high level antioxidative activities in these two microalgae were obtained in TBA assays.

Effects of selected microalgal extracts on up-regulation of antioxidant enzymes gene expression in murine monocytic macrophage (RAW 264.7) cell lines were studied. Both dichloromethane extracts of *I. galbana* and *C. calcitrans* showed high up-regulation in the expression of all antioxidant enzymes genes tested in RAW 264.7. Liquid chromatography-mass spectrometry was employed to identify the constituents which were responsible for all of the antioxidative activities. Fucoxanthin and its isomers were identified as the major constituents in both microalgal species.

Finally, nutritional analysis (proximate and biochemical) were done on microalgae with high antioxidative activities (*I. galbana* and *C. calcitrans*) in order to investigate their nutritive values. Both microalgae were found to be rich in nutrients with protein:

carbohydrate: lipid percentage composition of 47.9 ± 2.5 : 26.8 ± 0.2 : 14.5 ± 1.4 for *I. galbana* and 36.4 ± 1.7 : 27.4 ± 3.0 : 15.5 ± 0.9 for *C. calcitrans* respectively. Both species contained high level of n-3 HUFA ($28.0\%\pm 0.7$: *I. galbana* and $28.5\%\pm 1.4$: *C. calcitrans*) and n-6 HUFA ($6.5\%\pm 1.8$: *I. galbana* and $23.0\%\pm 2.5$: *C. calcitrans*). Amino acid analyses also showed both microalgae contained high composition of essential amino acids. This study illustrated that some microalgae such as *I. galbana* and *C. calcitrans* have the potential for effective natural sources of antioxidants with high nutritional values.

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

**CIRI ANTIOKSIDASI DAN KANDUNGAN NUTRISI MIKROALGA
TEMPATAN YANG TERPILIH**

Oleh

NATRAH FATIN MOHD IKHSAN

Disember 2006

Pengerusi : Profesor Fatimah Md. Yusoff, PhD

Institut : Biosains

Mikroalga dikenali sebagai organisma yang mengandungi pelbagai pigmen berguna dan mempunyai kandungan nutrisi yang tinggi. Penyelidikan ini dilakukan untuk mengenalpasti potensi mikroalga setempat sebagai sumber baru antioksidasi yang mempunyai nilai proksimat dan biokimia yang tinggi.

Empat belas mikroalga telah dipisahkan, dipencil dan dikultur dari air tawar dan air payau. Kemampuan mikroalga sebagai sumber asli antioksidasi telah dikaji melalui ujian saringan menggunakan tiga jenis asai kimia antioksidasi (ferric thiocyanate (FTC), thiobarbituric acid (TBA) dan 1,1'-diphenyl-2-picrylhydrazyl (DPPH)). Keputusan ujian awal mendapati bahawa ekstrak methanol dari enam jenis mikroalga, (*Isochrysis galbana*, *Chaetoceros calcitrans*, *Scenedesmus quadricauda*, *Chlorella vulgaris*, *Nannochloropsis oculata* dan *Tetraselmis tetraethele*) adalah aktif di dalam perencatan oksidasi lipid jenis asid linoleik. Antara keseluruhan mikroalga yang dikaji, *I. galbana*

dan *C. calcitrans* menunjukkan aktiviti antioksidasi yang tertinggi (>90%) di dalam asai FTC dan TBA yang menunjukkan terdapat kemungkinan kewujudan komponen aktif di dalam mikroalga untuk perlindungan dari oksidasi lipid.

Mikroalga yang mempunyai kandungan antioksida yang tertinggi (*I. galbana* dan *C. calcitrans*) telah dipisahkan kepada beberapa fraksi pelarut yang berbeza (hexane, dichloromethane, ethyl acetate, butanol dan ekstrak akueus) untuk memisahkan kumpulan komponen campuran yang luas kepada kumpulan komponen yang lebih kecil bergantung kepada keterlarutan komponen kepada pelarut berkutub dan telah diuji semula dengan asai kimia antioksidasi. Ekstrak dichloromethane untuk sampel *I. galbana* dan *C. calcitrans* menunjukkan tahap antioksidasi yang tertinggi di dalam asai FTC berbanding ekstrak pelarut yang lain dengan aktiviti perencatan oksidasi lipid sebanyak $97.1 \pm 0.1\%$ dan $97.4 \pm 0.1\%$ setiap satu. Keputusan yang sama juga telah diperolehi melalui asai TBA untuk kedua-dua mikroalga.

Kebolehan mikroalga untuk menambah ekspresi gen enzim antioksidasi di dalam sel makrofaj (RAW 264.7) telah dikaji. Kedua-dua ekstrak dichloromethane *I. galbana* dan *C. calcitrans* menunjukkan pertambahan ekspresi gen enzim antioksidasi di RAW 264.7. Kajian cecair kromatografi telah digunakan untuk mengenalpasti komponen yang berkemungkinan bertanggungjawab ke atas semua aktiviti antioksidasi. Fucoxanthin dan isomernya telah dikenalpasti sebagai komponen yang utama di dalam kedua-dua spesies.

Akhir sekali, analisis nutrisi (proksimat dan biokimia) telah dilakukan ke atas mikroalga yang menunjukkan kandungan aktiviti antioksidasi yang tinggi (*I. galbana* dan *C. calcitrans*) dengan tujuan untuk menyelidiki nilai nutrisinya. Kedua-dua mikroalga didapati kaya dengan nutrisi dengan peratusan komposisi protein: karbohidrat: lipid pada 47.9 ± 2.5 : 26.8 ± 0.2 : 14.5 ± 1.4 untuk *I. galbana* dan 36.4 ± 1.7 : 27.4 ± 3.0 : 15.5 ± 0.9 untuk *C. calcitrans* setiap satu. Kedua-dua spesis juga didapati mengandungi tahap n-3 HUFA ($28.0 \pm 0.7\%$: *I. galbana* dan $28.5 \pm 1.4\%$: *C. calcitrans*) dan n-6 HUFA ($6.5 \pm 1.8\%$: *I. galbana* dan $23.0 \pm 2.5\%$: *C. calcitrans*) yang tinggi. Analisis asid amino juga menunjukkan kedua-dua mikroalga mengandungi komposisi asid amino yang tinggi di dalam jenis asid amino perlu. Penyelidikan ini menggambarkan bahawa sebahagian mikroalga seperti *I. galbana* dan *C. calcitrans* mempunyai potensi untuk sumber efektif antioksidasi yang semulajadi.

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I certify that an Examination Committee has met on _____ to conduct the final examination of Natrah Fatin Mohd Ikhsan on her degree thesis entitled “Chemical Analysis of Antioxidative Fractions and Nutritional Contents of Selected Microalgae” 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 Examination Committee are as follows:

Aziz Arshad, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Khozirah Shaari, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal examiner)

Mohd Salleh Kamarudin, PhD

Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Internal examiner)

Michael Armin Borowitzka, PhD

Professor
Murdoch University
Western Australia
(External examiner)

HASANAH MOHD GHAZALI, PhD

Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



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

Fatimah Md. Yusoff, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Md. Nordin Haji Lajis, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Mohamed Shariff Mohamed Din, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Mariana Nor Shamsudin, PhD

Associate Professor
Faculty of Medical and Health Sciences
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD

Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 May 2007



DECLARATION

I hereby 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 or concurrently submitted for any other degree at UPM or other institutions.

NATRAH FATIN MOHD IKHSAN

Date:



TABLE OF CONTENTS

	PAGE
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	vi
ACKNOWLEDGEMENTS	ix
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xx
 CHAPTERS	
1 GENERAL INTRODUCTION	
1.1 Background of Study	1
1.2 Statement of Problems	5
 2 LITERATURE REVIEW	
2.1 General description of microalgae	9
2.2 History of microalgae	11
2.3 Isolation and culture of microalgae	12
2.4 Microalgal applications	15
2.4.1 Bioactive compounds and drugs	16
2.4.2 Free radical, reactive oxygen species and antioxidant	19
2.4.3 Microalgal pigments	36
2.4.4 Nutritional supplements for human	43
2.4.5 Feed and wastewater treatment	45
2.5 Potential market of microalgae	49
 3 GENERAL METHODOLOGY	
3.1 Collection of microalgae	52
3.2 Isolation and purification of microalgae	54
3.3 Culture of microalgae	56
3.4 Microalgal cell harvesting	58
3.5 Solvents	59
 4 SCREENING OF MICROALGAL CRUDE EXTRACTS AND SELECTED MICROALGAL FRACTIONS FOR ANTIOXIDANT PROPERTIES	
4.1 Introduction	60
4.2 Materials and method	63
4.2.1 Preparation of samples extracts	63
4.2.2 Lipid peroxidation method	63
4.2.3 Free radical scavenging activity method	65



4.2.4	Extraction and fractionation of microalgal extract	66
4.2.5	Statistical analyses	68
4.3	Results	70
4.3.1	Antioxidant activity from microalgal crude extracts	70
4.3.2	Antioxidant activity from microalgal fractions extracts	75
4.4	Discussions	78
5	EFFECTS OF SELECTED MICROALGAL FRACTIONS ON VIABILITY ANTIOXIDANT ENZYMES GENE EXPRESSION IN RAW 264.7 CELLS	
5.1	Introduction	85
5.2	Materials and method	87
5.2.1	Cell culture maintenance	87
5.2.2	Treatment of samples on cell lines for mRNA expression	88
5.2.3	Harvest of the treated cells for mRNA expression	88
5.2.4	Messenger ribonucleic acid (mRNA) extraction	89
5.2.5	Quantitation of ribonucleic acid (RNA)	90
5.2.6	Reverse transcriptase polymerase chain reaction	91
5.2.7	Multiplex polymerase chain reaction	92
5.2.8	Electrophoresis of PCR product	92
5.2.9	Relative quantitative of RT-PCR product	94
5.2.10	DNA sequencing	94
5.2.11	Statistical analysis	94
5.3	Results	95
5.3.1	Ribonucleic acid (RNA) purification from RAW 264.7 cells	95
5.3.2	Effects of microalgal fractions on antioxidant enzymes mRNA expression in RAW 264.7 cells	98
5.3.3	DNA sequencing	102
5.4	Discussions	106
6	IDENTIFICATION OF CHEMICAL CONSTITUENTS IN THE ACTIVE MICROALGAL FRACTIONS	
6.1	Introduction	111
6.2	Materials and methods	113
6.2.1	Sample and standard preparation	113
6.2.2	Instrumentation	114
6.2.3	HPLC-ESI-MS and MS-MS	114
6.3	Results	115
6.3.1	HPLC-DAD and TIC profiles of microalgal samples and standards	115
6.3.2	Mass spectrometry of microalgal fractions and standards	119
6.3.3	Possible fragmentation patterns	121
6.4	Discussions	122
7	NUTRITIONAL VALUES OF SELECTED MICROALGAE	
7.1	Introduction	126
7.2	Materials and method	128



7.2.1 Production cultures and harvest of microalgae	128
7.2.2 Proximate chemical analyses	128
7.2.3 Biochemical analyses	132
7.2.4 Statistical analysis	134
7.3 Results	134
7.3.1 Proximate analyses of selected microalgae	134
7.3.2 Biochemical analyses of selected microalgae	135
7.4 Discussions	137
8 GENERAL DISCUSSIONS	146
REFERENCES	150
APPENDICES	177
BIODATA OF THE AUTHOR	192



LIST OF TABLES

Table		Page
1	Applications of microalgae (adapted from Pulz and Gross, 2004)	16
2	Active oxygen and related species (Papas, 1999)	23
3	Types of antioxidant action (Benzie, 2000)	28
4	Properties of solvent used in the fractionation procedure	67
5	Classification of microalgal samples screened for antioxidant activity	71
6	Comparison of absorbance values and percent inhibition of linoleic acid peroxidation by microalgal methanolic crude extracts as measured by FTC and TBA antioxidant assays.	72
7	Comparison of absorbance values and percent inhibition of linoleic acid peroxidation by inactive microalgal methanolic crude extracts as measured by FTC and TBA antioxidant assays.	73
8	Comparison of radical scavenging activities in the DPPH assay of microalgal methanolic crude extracts.	74
9	Comparison of absorbance values and percent inhibition of linoleic acid peroxidation by fractions of <i>Isochrysis galbana</i> as measured by FTC and TBA antioxidant assays.	76
10	Comparison of absorbance values and percent inhibition of linoleic acid peroxidation by fractions of <i>Chaetoceros calcitrans</i> as measured by FTC and TBA antioxidant assays.	76
11	Radical scavenging activity from fractions of microalgae and standard (Vitamin E) by DPPH radical scavenging method.	77
12	Major pigments of all tested microalgal divisions	79
13	The sequences of primers used for detection and amplification of antioxidant enzymes in RAW 264.7 cell lines.	93
14	Quantitation of RNA from treated and untreated RAW 264.7 cells	97



15	Gradient profile of mobile phase	115
16	Components identified in different microalgal samples and standards using LC-DAD-ESI-MS/MS.	120
17	Proximate analyses (% dry weight) of selected microalgae.	135
18	Fatty acid composition (% of total fatty acid) of selected microalgae.	136
19	Amino acid analysis (% of total amino acids) of selected microalgae.	137



LIST OF FIGURES

Figure		Page
1	The characteristic patterns of growth shown by unicellular microalgae in a culture of limited volume (Fogg, 1975)	13
2	Overview formation of reactive oxygen species (Stahl and Sies, 2002)	23
3	Reactive oxygen species increase risk of disease through damage to key biological structures (Adapted from Strain and Benzie, 1999)	24
4	Enzymatic pathways to control superoxide and hydrogen peroxide (Fridovich, 1978)	28
5	Steps in lipid autoxidation (Shahidi, 1997).	31
6	Inhibition of lipid oxidation by antioxidants.	32
7	Radical-scavenging of stable 1,1-diphenyl-2 picrylhydrazyl radical	32
8	DNA amplification by the polymerase chain reaction (www.flmnh.ufl.edu.)	34
9	Structure of some common natural carotenoids.(Adapted from Kiokias and Gordon, 2004; Li <i>et al.</i> , 2005).	42
10	Contribution of microalgae in biological food web (Modified from Kumar and Singh, 1976; Salleh, 1996).	47
11	Applications of microalgae in various field (Adapted from Dufossé <i>et al.</i> , 2005)	51
12	Map of Peninsular Malaysia and Selangor State	53
13	Microalgal colonies in petri plates incubated in incubator.	56
14	Cultures of purified microalgae in batch and production cultures.	57
15	Fractionation procedure from 5 g methanolic microalgal extracts for both microalgal species.	69

16	Some of the microalgae tested for the assays	71
17	Extracted RNA from RAW 264.7 with or without treatments.	96
18	Effect of microalgal fractions on copper-zinc superoxide dismutase (CuZnSOD) expression in LPS stimulated RAW 264.7 macrophages.	99
19	Effect of microalgal fractions on manganese superoxide dismutase (MnSOD) expression in LPS stimulated RAW 264.7 macrophages.	100
20	Effect of microalgal fractions on phospholipid hydroperoxide glutathione peroxidase (PHGPx) expression in LPS stimulated RAW 264.7 macrophages.	101
21	Comparison between amplified copper-zinc superoxide dismutase with published sequences (El Moutassim <i>et al.</i> , 1999; accession number: NM_011434.1)	103
22	Comparison between amplified phospholipid glutathione peroxidase with published sequences (Pang <i>et al.</i> , 2000); accession number: NM_008612.2).	104
23	Comparison between amplified manganese superoxide dismutase with published sequences (El Moutassim <i>et al.</i> , 1999; accession number: NM_013671.3)	105
24	High performance liquid chromatography (HPLC)-diode array detector (DAD) and total ion chromatogram (TIC) by positive ion mode in electrospray/mass spectrometry (ESI/MS) for microalgal DCM fraction.	117
25	High performance liquid chromatography (HPLC)-diode array detector (DAD) and total ion chromatogram (TIC) by positive ion mode in electrospray/mass spectrometry (ESI/MS) for reference compounds.	119
26	Structure of 3'-acetoxo-6',7-didehydro-5,6-epoxy-5,5',6,6',7,8-hexahydro-3,5' dihydroxy- β - β -caroten-8-one (fucoxanthin). Adapted from Haugan and Liaaen-Jensen, 1994.	121
27	Proposed fragmentation pathways of fucoxanthin	122

LIST OF ABBREVIATIONS

AABA	alpha amino butyric acid
ABTS ⁺	2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate)
AH	antioxidant
ANOVA	analysis of variance
APCI	atmospheric pressure chemical ionization
API	atmospheric pressure ionization
ASP	amnesic shellfish poisoning
B-actin	beta actin
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
BSA	bovine serum albumin
BuOH	butanol
ca.	about
CAT	catalase
cDNA	complementary deoxyribonucleic acid
CH ₄	methane
COX-2	cyclooxygenase
CuZnSOD	copper-zinc superoxide dismutase
DAD	diode array detector
DCM	dichloromethane
DHA	docosahexaenoic acid



DMEM	dulbecco's modified eagle's medium
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotides
DPPH	1,1'-diphenyl-2-picrylhydrazyl
DSP	diarrhetic shellfish poisoning
EPA	eicosapentaenoic acid
ESI	electrospray
EtoAc	ethyl acetate
FAME	fatty acid methyl esters
Fe ²⁺	ferrous ion
Fe ³⁺	ferric ion
FeCl ₂	ferrous chloride
FCS	fetal calf serum
FR	free radical
FRAP	ferric reducing/ antioxidant power
FTC	ferric thiocyanate assay
GAPDH	glyceraldehyde-3-phosphate
GPS	global positioning system
GPX	glutathione peroxidase
GSH	glutathione
GSSG	oxidized glutathione
H ⁺	hydrogen
H ₂ O	water



H ₂ O ₂	hydrogen peroxide
HPLC-DAD-MS	high performance liquid chromatography-diode array detector-mass spectrometry
IFN- γ	interferon-gamma
iNOS	nitric oxide synthase
L \cdot	resultant alkyl free radical
LAP	linoleic acid peroxidation
LC-MS	liquid chromatography-mass spectrometry
LCPUFAs	long-chain polyunsaturated fatty acids
LH	fatty acid/lipid
LOO \cdot	unstable peroxy free radical
LOOH	hydroperoxide
LOOL	non radical products
LP	lipid peroxidation
MA	moisture and ash
MeOH	methanol
MgCl ₂	magnesium chloride
MnSOD	manganese superoxide dismutase
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MUFA	monounsaturated fatty acids
MW	molecular weight
m/z	mass-to-charge ratio
NaCl	sodium chloride



NaOH	sodium hydroxide
NaSO ₄	sodium sulfate
NCBI	National Center for Biotechnology Information
NH ₃	ammonia
NH ₄ SCN	ammonium thiocyanate
NO	nitric oxide
NO ₂	nitrogen dioxide
Non SeGPX	non-selenium dependent glutathione peroxidase
OD	absorbance
O ₂	oxygen
O ₂ ^{•-}	superoxide anion
OH [•]	hydroxyl radical
OONO [•]	peroxynitrite
PCR	polymerase chain reaction
PDA	photo diode array detector
PG	propyl gallate
PGE ₂	prostaglandin E ₂
PHGPX	phospholipid hydroperoxide glutathione peroxidase
PITC	phenyl isothiocyanate
PLGPX	plasma glutathione peroxidase
PSP	paralytic shellfish poisoning
PTC-amino acids	phenylthiocarbonyl amino acids
RAW 264.7	murine monocytic macrophage



RNA	ribonucleic acid
rRNA	ribosomal ribonucleic acid
RNS	reactive nitrogen species
ROH	alcohol
ROS	reactive oxygen species
RT	retention time
RT-PCR	polymerase chain reaction
SCP	single cell protein
SeGPX	selenium-dependent glutathione peroxidase
SF	sulfated polysaccharides
SFA	saturated fatty acids
SOD	superoxide dismutase
SPSS	statistical package for the social sciences
TBA	thiobarbituric acid
TBHQ	tert-butylhydroquinone
TEAC	trolox equivalent antioxidant capacity
TIC	total ion chromatogram
TNF- α , IL-1, IL-6	cytokines
TPTZ	(Fe ²⁺ /2,4,6-tripridyl-s-traizine
UV	ultraviolet
XDH	xanthine dehydrogenase
XO	xanthine oxidase

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of study

As a tropical country with ample sunlight and rain throughout the year, Malaysia is blessed with a myriad of natural resources that can be sustainably utilized. These resources which range from terrestrial to aquatic organisms (plant, bacteria, fungi and animals) provide an indispensable value for various industrial applications which include medicine, pharmaceutical, nutraceutical, cosmetics and aquaculture (Borowitzka and Borowitzka, 1988; Young, 1999). The value of natural products become higher due to the inability to find the cure of certain diseases such as cancer, AIDS, Alzheimer's disease and arthritis (to name a few). Since natural products offer a vast source of chemical diversity with unusual chemical structures, they could provide the solution alleviating these diseases. From 1960 to 1982, over 180,000 microbial-derived, some 16,000 marine organism-derived, and over 114,000 plant-derived extracts have been screened for anti-tumor activity which resulted in the discovery of a number of clinically effective chemotherapeutics agents (Cragg *et al.*, 1999).

Over the millennia, research on natural products has mainly focused on plants which have been proven as an excellent source for the treatment of several diseases. Plants are more fully studied than animals in terms of their pharmacological and therapeutical

