

**EVALUATION AND COMPARISON ON
CHEMICAL COMPONENTS, ANTIOXIDANT,
ANTIMICROBIAL, AND ANTIDIABETES
ACTIVITIES FROM SELECTED
TROPICAL PLANTS**

LILY ANG ZUIN PING

UNIVERSITI SAINS MALAYSIA

2016

**EVALUATION AND COMPARISON ON
CHEMICAL COMPONENTS, ANTIOXIDANT,
ANTIMICROBIAL, AND ANTIDIABETES
ACTIVITIES FROM SELECTED
TROPICAL PLANTS**

by

LILY ANG ZUIN PING

**Thesis submitted in fulfillment of the requirements
for the degree of
Doctor of Philosophy**

JANUARY 2016

ACKNOWLEDGEMENT

When I was in my final semester of Bachelor's degree, doing a final year project, I saw one of my classmates taking some flowers to her laboratory. I was curious and I asked her why because we have never studied botany in our degree. She told me they are flowers of Cannonball trees and she shared with me about her project which is natural products from plants. The project is very interesting and it caught my eyes. Then, I have decided I would like to take this project if I ever wanted to embark on a Ph.D. program.

I would like take this opportunity to thank my supervisor Professor Dr. Rokiah Hashim and co-supervisor Professor Dr. Shaida Fariza Sulaiman for taking me on board as their student. They have deeply guided and encouraged me throughout the journey to complete my Ph.D. with their patience, wisdom, humor and self-sacrificial characteristic. From them, I gain a lot of knowledge and experiences.

I want to thank those who at different time-lapse helping me at the laboratory: Dr Ahmed Yacouba Coulibaly, Dr Ooi Kheng Leong and Nurul Shafiqah. My project will not be possible without their support and teachings especially in thesis writing. My Ph.D. life would have been harder if I do not have friends who accompanied me and gave me moral supports. They are friends I shared a study room with when we were in school. Even though the postgraduate room we shared is small but I could see their big hearts in accepting shortcoming of each other. I treasure these friendships very much and they will be forever to me.

I would like to thank my family, especially my parents and my partner for their patience, cares, motivations and supports given to me throughout my life. Everything is from them and for their hard works I dedicate this to them. Last but not least, I would like to thank Universiti Sains Malaysia for the financial support (RU-PRGS grant: *1001/PTEKIND/814160*) and Mybrain15 MyPhD scholarship funded by the Ministry of Higher Education (MOHE).

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iv
LIST OF TABLES	xii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xix
ABSTRAK	xx
ABSTRACT	xxii
CHAPTER 1 GENERAL INTRODUCTION	1
1.1. Justification	4
1.2. Objectives	7
1.3. Research hypothesis	7
CHAPTER 2 LITERATURE REVIEW	8
2.1 Family of selected tropical plants	8
2.1.1 Anacardiaceae	8
2.1.2 Araucariaceae	11
2.1.3 Myrtaceae	13
2.1.4 Podocarpaceae	17
2.2 Preparation of extracts	19
2.3 Concept of free radicals	20

2.3.1	Free radicals formation	21
2.4	Antioxidant	24
2.4.1	Mechanism of antioxidant defense system	24
2.4.2	Metal chelating assay	26
2.4.3	Diphenylpicryl-hydrazyl (DPPH) radical scavenging assay	27
2.4.4	Ferric reducing antioxidant power (FRAP)	28
2.4.5	2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging assay	28
2.4.6	Phenolic compounds as antioxidants	29
2.5	Antidiabetes	33
2.5.1	Complications of Diabetes Mellitus	34
2.5.2	Phenolic compounds as enzyme inhibitors	36
2.6	Antimicrobial	38
2.6.1	Bacteria and fungi	38
2.6.2	Phenolic compounds as antimicrobial	41
	CHAPTER 3 MATERIALS AND METHODS	43
3.1	Chemicals	43
3.2	Plant materials	43
3.3	Extraction	45
3.4	Determination of total phenolic content	45
3.5	Determination of total flavonoid content	46
3.6	Antioxidant assay	47
3.6.1	Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay	47
3.6.2	Ferric reducing antioxidant potential (FRAP) assay	49

3.6.3	2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging assay	50
3.6.4	Metal chelating assay	51
3.7	Determination of α -amylase and α -glucosidase inhibitory activities	51
3.7.1	Determination of α -amylase inhibitory activities	51
3.7.2	Determination of α -glucosidase inhibitory activities	52
3.8	Determination of antimicrobial activity	53
3.8.1	Microorganism	53
3.8.2	Preparation of inoculum	54
3.8.3	Determination of antibacterial activity	55
	(a) Disc application	55
	(b) Minimal inhibitory concentration (MIC)	56
3.8.4	Determination of antifungal activity	57
3.9	Fractionation of crude extracts using liquid - liquid fractionation	57
3.10	Thin Layer Chromatography (TLC) fractionation	58
3.11	Bioautography	58
3.12	Phenolic compounds identification	59
3.13	Statistical analysis	59
	CHAPTER 4 ANTIOXIDANT ACTIVITIES OF 19 CRUDE EXTRACTS	60
4.1	Introduction	60
4.2	Materials and Methods	61
4.3	Results	61
4.3.1	Yield of 19 crude extracts	61
4.3.2	Total phenolic content (TPC) of 19 crude extracts	63
4.3.3	Total flavonoid content (TFC) of 19 crude extracts	65

4.3.4	Comparison of TPC and TFC of each part of plants	67
4.3.5	DPPH radical scavenging assay of 19 crude extracts	68
	(a) Percentage of radical scavenging activity	68
	(b) EC ₅₀ values	70
4.3.6	Ferric reducing antioxidant power assay of 19 crude extracts	74
	(a) Percentage of reducing power	74
	(b) EC ₅₀ values	74
4.3.7	ABTS radical scavenging assay of 19 crude extracts	79
	(a) Percentage of radical scavenging activity	79
	(b) EC ₅₀ values	79
4.3.8	Metal chelating assay of 19 crude extracts	84
	(a) Percentage of metal chelating activity	84
4.3.9	Correlation between antioxidant activities, total phenolic content (TPC) and total flavonoid content (TFC)	86
	(a) Correlations between TPC and antioxidant activities	86
	(b) Correlations between TFC and antioxidant activities	89
4.3.10	Correlations between antioxidant assays	91
4.4	Discussion	93
4.4.1	Yield of extracts	93
4.4.2	Analysis of total phenolic content (TPC) and total flavonoid content (TFC)	94
4.4.3	Analysis of DPPH radical scavenging activity	96
4.4.4	Analysis of ferric reducing antioxidant power (FRAP) activity	97
4.4.5	Analysis of ABTS radical scavenging activity	98
4.4.6	Analysis of metal chelating activity	100

4.4.7	Analysis of correlations between antioxidant assays	101
4.5	Conclusion	102
CHAPTER 5 BIOASSAY GUIDED FRACTIONATIONS AND IDENTIFICATION OF PHENOLIC COMPOUNDS IN <i>G. TORQUATA</i> SAPWOOD		
		103
5.1	Introduction	103
5.2	Materials and Methods	104
5.3	Results	104
5.3.1	Liquid-liquid fractionations of <i>G. torquata</i> sapwood	104
(a)	DPPH radical scavenging assay of the fractions	106
(b)	Ferric reducing antioxidant power (FRAP) assay of the fractions	108
(c)	ABTS radical scavenging activity of the fractions	110
5.3.2	Thin layer chromatography (TLC) of the fraction	112
(a)	DPPH radical scavenging assay of the fraction	114
(b)	Ferric reducing antioxidant power (FRAP) assay of the fraction	116
(c)	ABTS radical scavenging activity of the fraction	116
5.3.3	Ultra Performance Liquid Chromatography (UPLC) analysis of phenolic compounds in <i>G. torquata</i> sapwood	118
5.4	Discussion	120
5.4.1	Bioassay guided fractionations	120
5.4.2	Identification of phenolic compounds in <i>G. torquata</i> sapwood	121
5.5	Conclusion	123

CHAPTER 6	α- AMYLASE AND α- GLUCOSIDASE INHIBITORY	
	ACTIVITIES OF CRUDE EXTRACTS AND BIOASSAY	
	GUIDED FRACTIONATIONS OF <i>G. TORQUATA</i>	
	SAPWOOD	124
6.1	Introduction	124
6.2	Materials and Methods	126
6.3	Results	127
6.3.1	α -Amylase inhibition assay of 19 crude extracts	127
	(a) Percentage of α - amylase inhibition activity	127
	(b) EC ₅₀ values	127
6.3.2	α -Glucosidase inhibition assay of 19 crude extracts	130
	(c) Percentage of α -glucosidase inhibition activity	130
	(d) EC ₅₀ values	130
6.3.3	α -Amylase inhibition assay of the fractions	134
	(a) Percentage of α -amylase inhibition activity	134
	(b) EC ₅₀ values	136
6.3.4	α -Glucosidase inhibition assay of the fractions	137
	(a) Percentage of α -glucosidase inhibition activity	137
	(b) EC ₅₀ values	137
6.3.5	Correlation between enzymes (α -amylase and α -glucosidase) inhibition activities, total phenolic content (TPC) and total flavonoid content (TFC)	139
6.3.6	Ultra Performance Liquid Chromatography (UPLC) analysis of phenolic compounds in <i>G. torquata</i> sapwood	142

6.4	Discussion	145
6.4.1	<i>In vitro</i> α -amylase and α -glucosidase inhibition assessment	145
6.4.2	Analysis of α -amylase and α -glucosidase inhibition activities	146
6.4.3	Correlation between enzymes (α -amylase and α -glucosidase) inhibition activities, total phenolic content (TPC) and total flavonoid content (TFC)	147
6.4.4	Identification of phenolic compounds in <i>G. torquata</i> sapwood	148
6.5	Conclusion	150

CHAPTER 7 ANTIMICROBIAL ACTIVITIES OF CRUDE EXTRACTS, BIOASSAY GUIDED FRACTIONATION OF *G. TORQUATA* LEAF EXTRACT AND *L. FLAVESCENS* LEAF EXTRACT 151

7.1	Introduction	151
7.2	Materials and Methods	152
7.3	Results	153
7.3.1	Antimicrobial assay of 19 crude extracts	153
7.3.2	Correlation between antibacterial activities, total phenolic content (TPC) and total flavonoid content (TFC)	158
7.3.3	Thin Layer Chromatography (TLC) of <i>G. torquata</i> leaf	161
7.3.4	Liquid-liquid fractionation of <i>L. flavescens</i> leaf	163
7.3.5	Minimum inhibitory concentration (MIC) of fractions	164
7.3.6	Ultra Performance Liquid Chromatography (UPLC) analysis of phenolic compounds	166

7.4	Discussion	170
7.4.1	Analysis of antimicrobial activity	170
7.4.2	Correlation between antibacterial activities, total phenolic content (TPC) and total flavonoid content (TFC)	171
7.4.3	Identification of phenolic compounds in <i>G. torquata</i> leaf and <i>L. flavescens</i> leaf	172
7.5	Conclusion	174
	CHAPTER 8 CONCLUSION AND RECOMMENDATIONS	175
8.1	Conclusion	175
8.2	Recommendations	176
	REFERENCES	178
	LIST OF PUBLICATIONS	195

LIST OF TABLES

Table	Page
2.1	Scientific classification of <i>Gluta torquata</i> 10
2.2	Scientific classification of <i>Agathis borneensis</i> 12
2.3	Scientific classification of <i>Eugenia chlorantha</i> 14
2.4	Scientific classification of <i>Leptospermum flavescens</i> 16
2.5	Scientific classification of <i>Podocarpus neriifolius</i> 18
2.6	Main classes of phenolic compounds 30
4.1	Percentage of yield of 19 extracts 62
4.2	Total phenolic content of 19 extracts 64
4.3	Total flavonoid content of 19 extracts 66
4.4	DPPH radical scavenging activity (%) and EC ₅₀ values of 19 extracts together with positive control 69
4.5	Reducing power (%) and EC ₅₀ values of 19 extracts together with positive control 75
4.6	ABTS radical scavenging activity (%) and EC ₅₀ values of 19 extracts together with positive control 80
4.7	Metal chelating power (%) of 19 extracts together with positive control 85
5.1	Yield and antioxidant activities of 4 fractions from <i>G. torquata</i> sapwood liquid-liquid fractionations and crude extract 105
5.2	Properties of TLC subfractions positions 113
5.3	Yield and antioxidant activities of 4 subfractions from TLC of <i>G. torquata</i> sapwood ethyl acetate fraction and its L-L fraction 115
5.4	Spectral of phenolic compounds 118

6.1	α -Amylase inhibition activity of extracts obtained from 19 extracts and positive control	128
6.2	α -Glucosidase inhibition activities of extracts obtained from 19 extracts and positive control	131
6.3	α -Amylase and α -glucosidase inhibition activities of crude extract and fractions	135
7.1	Antimicrobial activity of 19 extracts along with positive controls	154
7.2	Properties of fraction position of <i>Gluta torquata</i> leaf TLC	162
7.3	Minimum inhibitory concentration (MIC) of fractions	165

LIST OF FIGURES

Figure	Page
2.1 <i>Gluta torquata</i> (a) tree (b) leaf	10
2.2 <i>Agathis borneensis</i> (a) tree (b) leaf	12
2.3 <i>Eugenia chlorantha</i> (a) tree (b) leaf	14
2.4 <i>Leptospermum flavescents</i> (a) tree (b) leaf	16
2.5 <i>Podocarpus neriifolius</i> (a) tree (b) leaf	18
2.6 Formation of free radicals	22
2.7 Lipid peroxidation mechanisms	23
2.8 Oxidative stress complications	23
2.9 <i>General classifications of defence mechanisms against free radicals</i> 24	
2.10 Formation of ferrous-ferrozine complex	26
2.11 Mechanism of DPPH• accepting hydrogen from an antioxidant	27
2.12 Reduction of ferric tripyridyl triazine (Fe ³⁺ -TPTZ) complex to ferrous form (Fe ²⁺ -TPTZ) complex by antioxidant	28
2.13 Mechanism of ABTS•+ accepting hydrogen from an antioxidant	29
2.14 Main classes of phenolic compounds	31
2.15 Chemical structure of kaempferol	32
2.16 The mechanisms of oxidative stress in diabetes which lead to diabetes-relation complications	35
2.17 Basic skeletons of flavonoids	37
2.18 The mechanisms of fungal attack on lignocellulose	41
3.1 Flow chart of the study	44
3.2 DPPH assay in a 96-well plate	48
3.3 FRAP assay in a 96-well plate	49

4.1	Gallic acid calibration curve for determination of total phenolic content using Folin-Ciocalteu colorimetric method	63
4.2	Quercetin calibration curve for determination of total flavonoid content using aluminium chloride colorimetric method	65
4.3	Percentages of yields, TPC and TFC in each part of plants	67
4.4	Percentage of radical scavenging activity versus log concentration of methanolic extracts of <i>G. torquata</i> (leaf, bark and sapwood) and <i>E. chlorantha</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	71
4.5	Percentage of radical scavenging activity versus log concentration of methanolic extracts of <i>A. borneensis</i> (leaf, bark and sapwood) and <i>L. flavescens</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	72
4.6	Percentage of radical scavenging activity versus log concentration of methanolic extracts of <i>P. neriifolius</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	73
4.7	Percentage of reducing power versus log concentration of methanolic extracts of <i>G. torquata</i> (leaf, bark and sapwood) and <i>E. chlorantha</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	76
4.8	Percentage of reducing power versus log concentration of methanolic extracts of <i>A. borneensis</i> (leaf, bark, heartwood and sapwood) and <i>L. flavescens</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	77
4.9	Percentage of reducing power versus log concentration of methanolic extracts of <i>P. neriifolius</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	78
4.10	Percentage of radical scavenging activity versus log concentration of extracts methanolic extracts of <i>G. torquata</i> (leaf, bark and sapwood) and <i>E. chlorantha</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin.	81
4.11	Percentage of radical scavenging activity versus log concentration of methanolic extracts of <i>A. borneensis</i> (leaf, bark heartwood and sapwood) and <i>L. flavescens</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin.	82
4.12	Percentage of radical scavenging activity versus log concentration of methanolic extracts of <i>P. neriifolius</i> (leaf, bark, heartwood and sapwood), together with positive control, quercetin	83

4.13	Correlation between DPPH radical scavenging activity and TPC of the extracts	87
4.14	Correlation between reducing power and TPC of the extracts	87
4.15	Correlation between ABTS radical scavenging activity and TPC of the extracts	88
4.16	Correlation between metal chelating and TPC of the extracts	88
4.17	Correlation between DPPH radical scavenging activity and TFC of the extracts	89
4.18	Correlation between reducing power and TFC of the extracts	90
4.19	Correlation between ABTS radical scavenging activity and TFC of the extracts	90
4.20	Correlation between metal chelating and TFC of the extracts	91
4.21	Correlation between DPPH radical scavenging activity and reducing power	92
4.22	Correlation between DPPH radical scavenging activity and ABTS radical scavenging activity	92
4.23	Correlation between reducing power and ABTS radical scavenging activity	93
5.1	Percentage of DPPH radical scavenging activity versus log concentration of fraction F1, F2, F3 and F4, together with positive control, quercetin	107
5.2	Percentage of reducing powers versus log concentration of fraction F1, F2, F3 and F4, together with positive controls, quercetin	109
5.3	Percentage of ABTS radical scavenging activity versus log concentration of extracts fraction F1, F2, F3 and F4, together with positive control, quercetin	111
5.4	Thin Layer Chromatography (a) Investigation positions of fraction F2 (b) Bioautography on TLC (c) Colours of the bands under long wave of UV light	113
5.5	Percentage of DPPH radical scavenging activities versus log concentration of subfraction F2d, together with positive control, quercetin	114

5.6	Percentage of ABTS radical scavenging activity versus log concentration of subfraction F2a, b and d together with positive controls, quercetin	117
5.7	UPLC chromatograms of F2d subfraction (at 280 nm) and the UV spectra of peaks 1 assessed by photo-diode array detector	119
5.8	Chemical structure of protocatechuic acid	<u>122</u>
6.1	Percentages of α -amylase inhibition activities versus log concentration of <i>G. torquata</i> (bark and sapwood), <i>E. chlorantha</i> (bark, heartwood and sapwood), <i>L. flavescens</i> (heartwood and sapwood), <i>P. neriifolius</i> (leaf) and acarbose (positive control)	129
6.2	Percentages of α -glucosidase inhibition activities versus log concentration of <i>G. torquata</i> (leaf, bark and sapwood), <i>E. chlorantha</i> (leaf, bark, heartwood and sapwood) and acarbose (positive control)	132
6.3	Percentages of α -glucosidase inhibition activities versus log concentration of <i>L. flavescens</i> (leaf, bark, heartwood and sapwood), <i>P. neriifolius</i> (leaf, bark, heartwood and sapwood) and acarbose (positive control)	133
6.4	Percentages of α -amylase inhibition activities versus log concentration fractions F1, F2, F3, F4 and acarbose (positive control)	136
6.5	Percentages of α -glucosidase inhibition activities versus log concentration fractions F1, F2, F3, F4 and acarbose (positive control)	138
6.6	Correlations between TPC and α -amylase inhibition activity of 19 extracts	139
6.7	Correlations between TPC and α -glucosidase inhibition activities of 19 extracts	140
6.8	Correlations between TFC and α -amylase inhibition activity of 19 extracts	141
6.9	Correlations between TFC and α -glucosidase inhibition activities of 19 extracts	141
6.10	UPLC chromatograms of fraction F2 (at 350 nm) and the UV spectra of peaks 1-6 assessed by photo-diode array detector	144
6.11	Examples chemical structure of (a) caffeic acid, (b) sulfuretin, (c) quercetin and (d) kaempferol	148
7.1	Correlation between inhibition zone diameter and TPC of the extracts	158
7.2	Correlation between inhibition zone diameter and TPC of the extracts	159

7.3	Correlation between inhibition zone diameter and TFC of the extracts	160
7.4	Correlation between inhibition zone diameter and TFC of the extracts	160
7.5	Thin Layer Chromatography. (a) Investigation of fraction position of <i>G. torquata</i> leaf extract. (b) Bioautography on TLC. (c) Colours of the bands under long wave of UV light	162
7.6	The clear inhibition zones. (a) ethyl acetate fraction (B2) on brown rot fungi <i>Gloeophyllum trabeum</i> . (b) Ethyl acetate fraction (B2) on white rot fungi <i>Pycnoporus sanguineus</i> at 1000 µg	163
7.7	UPLC chromatograms of fraction A (at 350 nm) and the UV spectra of peak 1 assessed by photo-diode array detector	167
7.8	UPLC chromatograms of fraction B2 (at 350 nm) and the UV spectra of peaks 1 and 2 assessed by photo-diode array detector	169
7.9	Chemical structures of a) kaempferol and b) quercetin	174

LIST OF ABBREVIATIONS

ABTS	2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
ATCC	American Type Culture Collection
BUT	Butanol
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide
DPPH	Diphenylpicryl-hydrazyl
EA	Ethyl acetate
FRAP	Ferric reducing antioxidant potential
GAE	Gallic acid equivalent
HEX	Hexane
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
mM	Milimolar
NA	Nutrient agar
NB	Nutrient broth
PDA	Potato dextrose agar
PDB	Potato dextrose broth
Rf	Relative frequency
SD	Standard deviation
TPC	Total phenolic content
TFC	Total flavonoid content
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet

PENILAIAN DAN PERBANDINGAN KOMPONEN KIMIA, ANTIOKSIDAN, ANTIMIKROB DAN ANTIDIABETES DARI TUMBUHAN TROPIKA TERPILIH

ABSTRAK

Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens*) dan Podo Bukit (*Podocarpus neriifolius*) adalah spesies pokok yang tersebar luas yang boleh didapati di hutan tropika Malaysia. Sampel pokok ini telah dipilih dalam kajian ini kerana kekurangan bukti saintifik dan pengenalan fitokimia. Kajian ini adalah untuk menyiasat jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) daripada 19 ekstrak metanol daripada 4 bahagian tumbuhan (daun, kulit kayu, kayu teras dan kayu gubal) sebagai antioksidan untuk perencatan α -amilase dan α -glucosidase dan antimikrobial. Untuk *Gluta torquata* hanya daun, kulit kayu dan kayu gubal dipilih. Sifat-sifat antioksidan ekstrak metanol telah dianalisis dengan menggunakan asai penyingkiran radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH), potensi antioksida mengurangkan ferik (FRAP), penyingkiran radikal bebas 2'-azinobis (2-etilbenzothiazolin-6-sulfonik asid) (ABTS), dan penglekat logam. Menggunakan hasil daripada kajian ini, semua ekstrak daun yang menghasilkan lebih banyak ekstrak mengandungi kandungan fenolik yang lebih tinggi dalam peratusan daripada bahagian-bahagian lain tumbuhan seperti kulit kayu, kayu teras dan kayu gubal. Jumlah kandungan fenolik (TPC) mempunyai korelasi positif dengan asai DPPH, FRAP dan ABTS. Menariknya, kedua-dua DPPH dan FRAP asai lebih berkait rapat berbanding dengan asai antioksidan lain. Kayu gubal *Gluta torquata*

menghasilkan jumlah kandungan tertinggi flavonoid dengan nilai 39.6 (0.1) μg GAE / mg ekstrak, kedua tertinggi jumlah kandungan fenolik dengan nilai 79 (2) μg GAE / mg ekstrak dan menunjukkan aktiviti antioksidan yang tertinggi. Kayu gubal *Gluta torquata* dipilih untuk proses fraksinasi. Dengan menggunakan Kromatografi Cecair Prestasi Ultra (UPLC), kayu gubal *Gluta torquata* telah dikesan mengandungi sebatian fenolik seperti asid protokatekuik yang merupakan penyumbang kepada aktiviti antioksidan yang baik. Aktiviti perencatan α -amylase and α -glucosidase untuk semua ekstrak sampel telah diuji untuk mengenali keupayaan sampel dalam melambatkan pencernaan karbohidrat yang membantu untuk mengawal hiperglisemia setelah makan. Kayu gubal *Gluta torquata* menunjukkan perencatan α -amylase and α -glucosidase tertinggi masing masing dengan nilai EC_{50} 122 (1) mg / mL dan 6.5 (0.1) mg / mL. Kayu gubal *Gluta torquata* dipilih untuk proses fraksinasi dan keputusan analisis UPLC menunjukkan kandungan asid kafeik, sulfuretin, kuersetin dan kaempferol. Aktiviti-aktiviti yang telah disumbangkan oleh sebatian fenolik tetapi tidak bergantung kepada dos kandungan sebatian fenolik. Dalam kajian ini, bakteria lebih mudah terdedah kepada semua sampel berbanding dengan kulat. Daun *Gluta torquata* dikesan mengandungi kuersetin manakala daun *Leptospermum flavescens* dikesan mengandungi kuersetin dan kaempferol yang menyumbang kepada aktiviti antimikrob.

EVALUATION AND COMPARISON ON CHEMICAL COMPONENTS, ANTIOXIDANT, ANTIMICROBIAL, AND ANTIDIABETES ACTIVITIES FROM SELECTED TROPICAL PLANTS

ABSTRACT

Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens*), and Podo Bukit (*Podocarpus neriifolius*) are widespread distributed plant species which can be found in Malaysia tropical forests. The plant species were selected in this study due to their lack of scientific evidence and phytochemical identification. This study is to investigate the total phenolic (TPC) and total flavonoid contents (TFC) of the 19 methanolic crude extracts from 4 parts of plants (leaf, bark, heartwood and sapwood) as antioxidants for α -amylase and α -glucosidase inhibitory and antimicrobial. For *Gluta torquata*, only leaf, bark and sapwood of were chosen. The antioxidant properties were analyzed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, the ferric reducing antioxidant power (FRAP), 2, 2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging, and metal chelating assays. Using the results obtained from this study, all leaf extracts which yield more extracts contain higher phenolic compounds in percentages than other parts of plants such as bark, heartwood and sapwood. The total phenolic content (TPC) have positive correlations with DPPH, FRAP and ABTS assays. Interestingly, both DPPH and FRAP assays are correlated strongly compare with other antioxidant assays. *Gluta torquata* sapwood yielded the highest total flavonoid content which was 39.6 (0.1) μg QE/ mg extract, the highest of total phenolic content which was 79 (2) μg

GAE/ mg extract and exhibited outstanding antioxidant activities. *Gluta torquata* sapwood was chosen for further fractionation. By using the Ultra Performance Liquid Chromatography (UPLC), *Gluta torquata* sapwood was detected to contain phenolic compounds such as protocatechuic acid which was contributor to the good antioxidant activity. The inhibitory effects of all samples on α -amylase and α -glucosidase were tested *in vitro* to investigate the ability in delaying the breakdowns of carbohydrates which helps to control postprandial hyperglycaemia. *Gluta torquata* sapwood showed excellent α -amylase and α -glucosidase enzyme inhibitions with EC₅₀ values of 121 (1) μ g/mL and 6.5 (0.1) μ g/mL, respectively. *Gluta torquata* sapwood was further fractionated and the UPLC analysis identified the presence of caffeic acid, sulfuretin, quercetin and kaempferol. The activities were contributed by these phenolic compounds but not dose-dependent on the content of phenolic compounds. In this study, bacteria were more susceptible to all the samples compared to fungi. *Gluta torquata* leaf was detected to contain quercetin while *Leptospermum flavescens* leaf was detected to contain quercetin and kaempferol which contributed to the antimicrobial activities.

Chapter 1 General introduction

There are many species of Malaysian plants are being left without knowing their functions and compositions, for example, Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens*) and Podo Bukit (*Podocarpus neriifolius*). They are highly available because they are widespread species of tropical plants.

There are incomplete studies on antioxidant, antimicrobial and antidiabetic activities of these widespread species of tropical plants as mentioned before. Furthermore, each part of plants has different properties because the chemical compositions of each part are different. In this study, the five species of Malaysia tropical plant are separated into 4 different parts to be investigated on their antioxidant, antimicrobial and antidiabetic activities except for *Gluta torquata* where only 3 parts are chosen.

Plants have been a great source of bioactive compounds. Investigations in the past proven that bioactive compounds from plants have been a potential source of natural antioxidant, antifungal and other biological activities. They are present in the form of extractives in trees and soluble in organic solvents (Bernhoft, 2010). These compounds are also known as secondary metabolites (Lee *et al.*, 2013). The most widespread class of secondary metabolites are phenolic compounds. Their redox potential enables them to act excellently in different mechanisms of antioxidant, antifungal and antidiabetes (Pereira *et al.*, 2009). Phenolic compounds can be further divided into two groups which are phenolic acids and flavonoids. The evaluations of