CHARACTERISATION OF MALAYSIAN HONEYS AND ELECTROCHEMICAL DETECTION OF GALLOTANNIN FOR PURE HONEY IDENTIFICATION

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Biomedical Engineering)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

MARCH 2017

Specially for the persons so dear to my heart,
my love, my strength, my remedy.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim. *Alhamdulillahi rabbil ‘alamin*, all praises and gratitude be to Allah SWT for His bounty, mercy and blessings that inspire and give me strength to go through this journey of *‘ilm*. Along the way, I have gained many experiences, learned and rejoiced in a lot of things.

The success of this thesis would not have been possible without the support of so many people. First of all, I would like to express my sincerest thanks and appreciation to my thesis advisors Prof. Dato’ Ir. Dr. Mohammed Rafiq bin Dato’ Abdul Kadir, Dr. Razauden bin Mohamed Zulkifli, Assoc. Prof. Dr. Shafinaz binti Shahir, Assoc. Prof. Dr. Nasrul Humaimi bin Mahmood and Dr. Mahaneem binti Mohamed for their thoughtful consideration, support, encouragements, patience, critical opinions and invaluable guidance. With them besides me, I was able to stand and face the challenges till the end of this journey.

My special thanks go to Prof. Dr. Siti Amrah binti Sulaiman (Universiti Sains Malaysia), Dr. Dedy H.B. Wicaksono and Prof. Datin Dr. Zaharah binti Ibrahim for their ideas and sharing. I want to thank Mr. Afiezy, Mr. Hairol, Dr. Sindhu, Mrs. Norliza, Dr. Chua, Dr. Sornambikai and Mr. Madhan for their technical assistance and guidance in the instrumental part. I appreciate Mr. Om Prakash’s help in PCA analysis. I acknowledge Mr. Willy (KPD Sabah), Mr. Zakbah (Johor Department of Agriculture), Mr. Ibrahim (KOPEMAT), Mrs. Noriah (Min House Camp) and Mr. Rosli (Afnan Tech.) for supplying honey samples for this study. I would like to thank the laboratory and management staff of Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia (UTM) as well as School of Medical Sciences and School of Health Sciences, USM Kelantan for their kind assistance. I would like to extend my gratitude to UTM for funding my Ph.D. study.

I am very grateful for the eternal support and unconditional love that I received from my mother Hajah Subaidah Haleed, spouse Mohd. Faais, mother in-law Hajah Hindun Yahya, father in-law Haji Yusof Md Alias, dearest siblings, family and friends for the past several years. May Allah bless my late father, Haji Ismail Wagiyo for bringing me up the way I am today. Lastly, to my lab mates and best friends Atiqah, Helmi, Dayang, Mimi, Balqis, Yani, Ird, Aya, Tikah, Fareh, Jannah, Sarina, Sham, Syahiran, Azi, Hakim, Ammar, Wei Keng, Haslin, Zara and Munirah, thank you very much for the memories we had together.

ABSTRACT

Seventeen samples (n = 17) of Malaysian gelam, acacia, nanas, tualang and kelulut honeys were analysed for their physicochemical, biochemical and phytochemical properties to evaluate their influence on floral source and bee type. Comparisons were also made with synthetic honeys to determine a suitable measure for fast identification of pure honey from synthetic honey. Solid phase extraction (SPE) was utilised for isolation of phenolic compounds in honey samples. The phenolic compounds present in the samples were analysed using high performance liquid chromatography-diode array detector (HPLC-DAD) and liquid chromatography tandem mass spectrometry (LC-MS/MS). Three electrode systems were utilised for rapid identification of pure Malaysian honeys. Properties of honey were shown to be influenced by the floral source and bee type to the lesser extent. Kelulut honeys were observed of having lower pH, higher free acid, moisture and ash contents as well as higher electrical conductivity (EC), the properties that distinguish *Trigona* honey from the common *Apis* honey. Antioxidant properties were different for the five types of honey with *Trigona* honey dominating most of the antioxidant tests. Up to 16 phenolic compounds were identified using HPLC-DAD system. Similar dominant compounds were observed between tualang and acacia honeys, and between kelulut and gelam honeys, suggesting that the floral source of unifloral honey is an equally important food source for the analysed multifloral honey. More phenolic compounds were detected spectrometrically using full scan method and multiple reaction monitoring (MRM). Plant gallotannin, penta-*O*-galloyl-*β*-D-glucose (PGG) was successfully detected at low potential 0.173 V vs Ag/AgCl in pH 7 phosphate buffer solution using glassy carbon electrode (GCE) without any prior electrode activation, chemical modification and pre-concentration at the GCE. The PGG detection in blank pure honey and via standard addition approach in the Malaysian honeys revealed its presence only in the pure honeys. The present study suggested that electrochemical detection of PGG using GCE could be used as a tool for pure honey identification through a rapid and simple method rather than other conventional, highly-technical, expensive and time-consuming analytical techniques.

ABSTRAK

Analisa sifat-sifat fizikokimia, biokimia dan fitokimia telah dijalankan ke atas tujuh belas (n = 17) madu Malaysia yang terdiri daripada jenis gelam, akasia, nanas, tualang dan kelulut bagi menilai pengaruh sumber bunga dan jenis lebah ke atas sifat-sifat tersebut. Perbandingan dengan madu tiruan juga telah dijalankan bagi tujuan penetapan kaedah yang sesuai untuk mengenalpasti madu asli daripada madu tiruan dengan pantas. Kaedah pengekstrakan fasa pepejal (SPE) telah digunakan untuk pengasingan kompaun fenolik di dalam sampel madu. Campuran fenolik yang terdapat di dalam sampel telah dianalisa menggunakan kromatografi cecair berprestasi tinggi gabungan pengesan tatasusun diod (HPLC-DAD) dan kromatografi cecair gabungan spektroskopi jisim selaras (LC-MS/MS). Sistem tiga elektrod telah digunakan untuk mengenalpasti madu asli Malaysia dengan pantas. Sifat-sifat madu didapati dipengaruhi oleh sumber bunga dan sehingga tahap yang lebih rendah adalah jenis lebah. Pemerhatian terhadap madu kelulut menunjukkan ia mempunyai nilai pH yang rendah, komposisi asid bebas, kandungan air dan kandungan abu serta kekonduksian elektrik (EC) yang tinggi, yang merupakan sifat-sifat yang membezakan madu *Trigona* daripada kebanyakan madu *Apis*. Sifat-sifat antioksidan bagi kelima-lima jenis madu adalah berbeza dengan madu kelulut mendominasi kebanyakan daripada ujian-ujian antioksidan. Sebanyak 16 kompaun fenolik telah dikenalpasti menggunakan sistem HPLC-DAD. Sumber bunga untuk madu jenis satu bunga adalah merupakan sumber makanan yang sama untuk madu jenis banyak bunga berdasarkan kepada persamaan pada kompaun yang dominan yang telah dilihat antara madu tualang dan akasia, dan antara madu kelulut dan gelam. Lebih banyak kompaun fenolik telah dikesan secara spektrometri menggunakan kaedah imbasan menyeluruh dan pengawasan tindak balas berbilang (MRM). Gallotannin tumbuhan iaitu penta-*O*-galloil-*β*-D-glukosa (PGG) telah dikesan dengan jayanya pada keupayaan rendah 0.173 V melawan Ag/AgCl dalam cairan penampan fosfat dengan nilai pH 7 menggunakan elektrod karbon berkaca (GCE) tanpa sebarang pendahuluan pengaktifan elektrod, pengubahsuaian kimia dan kepekatan terdahulu yang dilakukan ke atas GCE. Pengesanan PGG dalam madu asli kosong dan menerusi kaedah penambahan piawai ke dalam madu Malaysia membuktikan kehadiran PGG hanya terdapat di dalam madu asli. Kajian ini mencadangkan bahawa pengesanan elektrokimia ke atas PGG dengan menggunakan kaedah GCE boleh digunakan sebagai instrumen untuk mengenalpasti madu asli menerusi kaedah yang cepat dan ringkas berbanding teknik-teknik analitikal yang lazim digunakan yang memerlukan kepakaran teknikal yang tinggi, mahal dan memakan masa.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xiv
	LIST OF FIGURES	xvi
	LIST OF ABBREVIATIONS	xxi
	LIST OF SYMBOLS	xxvi
	LIST OF APPENDICES	xxix
1	**INTRODUCTION**	**1**
	1.1 Background of research	1
	1.2 Problem statements	4
	1.3 Objectives of study	5
	1.4 Scope of study	5
	1.5 Significance of study	6
2	**LITERATURE REVIEW**	**8**
	2.1 Introduction	8
	2.2 Honey	8
	2.2.1 Foraging by bees	10
	2.2.1.1 Apis and Trigona *spp*.	12
	2.2.1.2 Foraging activities	17

2.2.2 Physicochemical properties of honey 19

2.3 The importance of honey in medicine and its threats 22

2.3.1 Traditional and modern use of honey in medical treatment 22

2.3.2 Nutritional values and health benefits of honey 23

2.3.3 Possible adverse health effects of synthetic honey 25

2.3.3.1 Hyperglycaemia, Diabetes mellitus and Cardiovascular disease 28

2.3.4 Role of honey in hyperglycaemia and diabetes mellitus 29

2.4 Free radicals, oxidative stress and defense mechanisms by antioxidants 32

2.4.1 Free radicals 32

2.4.1.1 Generation of free radicals 33

2.4.1.2 Beneficial and deleterious activities of free radicals 34

2.4.2 Oxidative stress 35

2.4.2.1 Oxidative damage to lipids, proteins and nucleic acids 36

2.4.2.2 Effects of oxidative stress 39

2.4.3 Defence mechanisms by antioxidants 40

2.5 Honey as a source of antioxidants and polyphenols 44

2.5.1 Antioxidants in honey 44

2.5.2 Main polyphenols in honey – structure, function and properties 45

2.5.2.1 Flavonoids 52

2.5.2.2 Phenolic acids 56

2.5.2.3 Tannins 59

	2.5.2.4 Limitations of polyphenols	60
	2.6 Analytical methods	62
	2.6.1 Spectrophotometric determination of total polyphenols in honey	62
	2.6.1.1 Folin-Ciocalteau assay	62
	2.6.2 Spectrophotometric determination of antioxidant activity of honey	62
	2.6.3 Chromatographic determination of individual polyphenols and phenolic profile of honey	63
	2.6.3.1 Sample preparation	63
	2.6.3.2 Separation and identification of honey polyphenols by liquid chromatography (LC)	65
	2.6.3.3 Identification of honey polyphenols by gas chromatography (GC)	67
	2.6.4 Electrochemical detection of polyphenols in foodstuffs	67
3	**MATERIALS AND METHODS**	**71**
	3.1 Introduction	71
	3.2 Overview of the research workflow	71
	3.3 Materials	73
	3.3.1 Honey samples	73
	3.3.2 Chemicals and reagents	75
	3.3.3 Electrodes	76
	3.3.4 Equipments	76
	3.4 Physicochemical analysis	77
	3.4.1 Measurement of pH	77
	3.4.2 Measurement of free acidity	77
	3.4.3 Measurement of total soluble solids (TSS)	78

3.4.4 Measurement of refractive index (RI) 78
3.4.5 Measurement of moisture content 78
3.4.6 Measurement of density 79
3.4.7 Measurement of hydroxymethylfurfural (HMF) 79
3.4.8 Measurement of ash content 80
3.4.9 Measurement of electrical conductivity (EC) 80
3.4.10 Measurement of colour 81
3.4.11 Statistical analysis 81
3.5 Spectrophotometric analysis 82
3.5.1 Determination of Total Phenolic Content (TPC) by Folin-Ciocalteau assay 82
3.5.2 Determination of Total Flavonoid Content (TFC) 82
3.5.3 Determination of total protein content by Bradford assay 83
3.5.4 Determination of radical-scavenging activity by DPPH assay 83
3.5.5 Determination of total antioxidant activity by FRAP assay 84
3.5.6 Statistical analysis 84
3.6 Chromatographic analysis 85
3.6.1 Determination of polyphenolic profile and possible floral markers using HPLC-DAD 85
3.6.1.1 Isolation of phenolic compounds using SPE 85
3.6.1.2 Sample, standard and mobile phase preparations for HPLC-DAD 85
3.6.1.3 Identification and quantification of phenolic

compounds using HPLC-DAD 86
3.6.1.4 Multivariate analysis 87
3.6.2 Determination of phenolic compounds using QTRAP LC-ESI-MS/MS 87
3.6.2.1 Isolation of phenolic compounds using SPE 87
3.6.2.2 Sample, standard and mobile phase preparations for LC-MS/MS 88
3.6.2.3 Screening and identification of polyphenols using QTRAP LC-ESI-MS/MS 88
3.7 Electrochemical (voltammetric) analysis 90
3.7.1 Determination of polyphenols using CV and DPV techniques 90
3.7.1.1 Working solution, sample and standard preparations 90
3.7.1.2 Cell system 90
3.7.1.3 GCE cleaning procedure 91
3.7.1.4 Analysis of redox properties of polyphenols using CV 91
3.7.1.5 Analysis of scan rate and pH effect to CV 92
3.7.1.6 Characterisation of polyphenols by DPV 92
3.7.1.7 Analysis of concentration effect by CV and DPV 93
3.7.1.8 Characterisation of pure honeys, synthetic honeys and main honey sugars by DPV 93
3.7.1.9 Data analysis 93

4	**RESULTS AND DISCUSSION**	**94**
	4.1 Introduction	94
	4.2 Physicochemical properties	94
	4.2.1 pH	97
	4.2.2 Free acidity	99
	4.2.3 Total soluble solids (TSS)	101
	4.2.4 Refractive index (RI)	102
	4.2.5 Moisture content	103
	4.2.6 Density	105
	4.2.7 Hydroxymethylfurfural (HMF)	106
	4.2.8 Ash content	109
	4.2.9 Electrical conductivity (EC)	110
	4.2.10 Colour	111
	4.2.11 Comparison between *Trigona* and *Apis* honeys, pure and synthetic honeys	113
	4.2.12 Correlation between physicochemical tests	114
	4.3 Antioxidant properties	116
	4.3.1 Total Phenolic Content (TPC)	118
	4.3.2 Total Flavonoid Content (TFC)	119
	4.3.3 Total protein content	121
	4.3.4 DPPH radical scavenging activity (RSA)	122
	4.3.5 Ferric Reducing Antioxidant Power (FRAP)	125
	4.3.6 Comparison between *Trigona* and *Apis* honeys, pure and synthetic honeys	127
	4.3.7 Correlation between antioxidant tests	128
	4.4 Phenolic compound analysis by liquid chromatography techniques	129
	4.4.1 Isolation of phenolic compounds	129
	4.4.2 Polyphenolic profile of honeys determined using HPLC-DAD	130
	4.4.2.1 Gelam honey	137

		4.4.2.2	Acacia honey	138
		4.4.2.3	Nanas honey	139
		4.4.2.4	Tualang honey	140
		4.4.2.5	Kelulut honey	141
		4.4.2.6	Comparison between unifloral and multifloral honeys	142
		4.4.2.7	Synthetic honeys	142
	4.4.3	Phenolic compounds as possible floral markers		144
	4.4.4	Relationship between species-specific foraging activity and phenolic composition of honeys		147
	4.4.5	Characterisation of phenolics in honeys using LC-ESI-MS/MS		151
		4.4.5.1	Screening of polyphenols	151
		4.4.5.2	Multiple reaction monitoring (MRM) analysis	153
4.5	Phenolic compound analysis by electrochemistry techniques			154
	4.5.1	Electrochemical behaviour of polyphenols on GCE		154
	4.5.2	Effects of scan rate and pH		164
	4.5.3	Effect of concentration		166
	4.5.4	Reproducibility		168
	4.5.5	Interference studies		168
	4.5.6	Analysis of polyphenols in real honey samples		170

5	**CONCLUSION AND RECOMMENDATIONS**	**175**
	5.1 Conclusions	175
	5.2 Recommendations	176

REFERENCES	**178**
APPENDICES A - E	206-235

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Descriptions of the four major *Apis* spp. in Malaysia. [a]	13
2.2	General composition criteria for honey (EU Council, 2002).	19
2.3	Hyperglycaemia-induced oxidative stress following glucose (dextrose) and/or carbohydrate intake.	27
2.4	Association between postprandial hyperglycaemia, T2D and CVD.	28
2.5	Free radicals.	32
2.6	Sources and functions of dietary antioxidants.	42
2.7	Classes, chemical structures and food sources of polyphenols.	46
2.8	Flavonoid subclasses and major food sources (Bhagwat *et al.*, 2013).	54
2.9	Structures of the prominent naturally occurring phenolic acids (Robbins, 2003).	57
2.10	Voltammetry methods used for ED of polyphenols in food. [a]	69
3.1	Malaysian unifloral and multifloral honeys used in this work.	74
3.2	Synthetic honeys used in this study.	75
3.3	USDA colour standard designation for honey (USDA, 1985).	81
4.1	Physicochemical properties of analysed honey samples. [1,2,3]	95

4.2	Physicochemical properties of analysed honey samples. [1,2]	97
4.3	Correlation matrix between physicochemical tests.	115
4.4	Antioxidant properties of analysed honey samples. [1]	117
4.5	Correlation matrix between antioxidant activity and biochemical component.	128
4.6	Classes and retention times (RTs) of standard phenolic compounds used in HPLC-DAD analysis.	130
4.7	Content of phenolic acids and flavonoids in unifloral Malaysian honeys. [a,b,c]	132
4.8	Content of phenolic acids and flavonoids in multifloral Malaysian honeys. [a,b,c]	134
4.9	Content of tannin (PGG) and total polyphenols in unifloral and multifloral Malaysian honeys. [a,b]	135
4.10	Principal component analysis loading matrix.	146
4.11	Phenolic compounds as determined by negative ion mode of EPI scan.	151
4.12	MRM data for polyphenols detection in Malaysian pure honey samples.	153
4.13	Electrochemical parameters of phenolic compounds on GCE as measured by CV. [a,b]	156
4.14	Percentage of recovery for the analyses.	174

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Worker bees of nine *Apis* species (Source from Koeniger *et al*., 2011).	11
2.2	Nests of: a) *A. dorsata*, b) *A. mellifera* and c) *A. cerana*. d) The hexagonal cells containing honey and pollen of *Apis* species. Nests of: (e) *T. thoracica* and its entrance in shape of mount (f); (g) *T. itama* and its round ringed entrance (h). BC (brood cells), HP (honey pot), PP (pollen pot). Source (e) from Saufi and Thevan (2015).	16
2.3	Plasma membrane of animal cell.	37
2.4	A simplified overview of flavonoid biosynthetic pathway. Note that specific branches of the pathway might be plant species-specific (Source from Weston and Mathesius, 2013).	53
2.5	(a) Reactive oxygen species ($R^{\cdot}$) scavenged by flavonoids. (b) An *ortho*-dihydroxy (catechol) structure in the B ring (A), 2,3-double bond in conjugation with a 4-oxo function in the C ring (B) and hydroxyl groups at positions 3 and 5 (C). (Adapted from Procházková *et al.*, 2011).	55
2.6	Principal steps in the biosynthesis of gallotannins and ellagitannins. HHDP = 3,4,5,3’,4’,5’-hexahydroxy diphenoyl (Source from Gross, 2008).	59
3.1	Overview of research workflow.	72
3.2	Pure Malaysian honey samples (top, from left: tualang,	

	kelulut, acacia, nanas and gelam). Synthetic honey samples (bottom, from left: S1, S2, S3 and S4).	73
4.1	pH of honey samples.	98
4.2	Free acidity of honey samples.	100
4.3	TSS of honey samples.	102
4.4	RI of honey samples.	102
4.5	Moisture content of honey samples.	103
4.6	Density of honey samples.	105
4.7	HMF of honey samples.	106
4.8	Ash content of honey samples.	109
4.9	EC of honey samples.	111
4.10	Colour of honey samples.	112
4.11	Mean TPC of honey samples. In each column, values with same letters (superscripts) indicate no significant differences ($p < 0.05$).	118
4.12	Mean TFC of honey samples. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).	120
4.13	Mean total protein content of honey samples. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).	121
4.14	DPPH radical scavenging activity of honeys at different concentrations: a) acacia (A), gelam (G) and nanas (N); b) kelulut (K), tualang (T) and synthetic (S).	123
4.15	a) DPPH radical scavenging activity of ascorbic acid (AA); b) Mean IC_{50} of honey samples. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).	124
4.16	Mean FRAP of honey samples. In each column, values with same letters (superscripts) indicate no significant differences ($p < 0.05$).	126
4.17	HPLC chromatograms of unifloral (a) gelam honey, (b) acacia honey and (c) nanas honey at 290 nm.	

	Polyphenolic compounds are: U1 (unknown compound 1), U2 (unknown compound 2), 1 (syringic acid), 2 (*p*-coumaric acid), 3 (ferulic acid), 4 (1,2,3,4,6-Pentagalloyl-*β*-D-glucose), 5 (benzoic acid), U3 (unknown compound 3), 6 (rutin hydrate), 7 (ellagic acid), U4 (unknown compound 4), U5 (unknown compound 5), 8 (*trans*-cinnamic acid), 9 (quercetin), 10 (naringenin), 11 (luteolin), 12 (hesperetin), 13 (kaempferol), 14 (apigenin), and 15 (chrysin).	136
4.18	HPLC chromatograms of multifloral (a) tualang honey and (b) kelulut honey at 290 nm. Polyphenolic compounds are: U1 (unknown compound 1), 1 (syringic acid), 2 (*p*-coumaric acid), 3 (ferulic acid), 4 (1,2,3,4,6-Pentagalloyl-*β*-D-glucose), 5 (benzoic acid), U3 (unknown compound 3), 7 (ellagic acid), U4 (unknown compound 4), 10 (naringenin), 12 (hesperetin), 13 (kaempferol), and 14 (apigenin).	137
4.19	HPLC chromatograms of synthetic (a) S1 honey, (b) S2 honey and (c) S3 honey at 290 nm. Phenolic compound: U1 (unknown compound 1).	143
4.20	(a) Percentage of dominant polyphenols, and (b) principal component analysis showing plot of PC1 versus PC2 for the classification of the five types of honey. G = gelam; A = acacia; N = nanas; K = kelulut; T = tualang.	144
4.21	Sources of unifloral honey involved in this study: (a) *Melaleuca cajuputi* (gelam), (b) *Acacia mangium* (acacia), and (c) *Ananas comosus* (nanas). (d) Mean phenolic contents of honeys from different bee species. AM = *A. mellifera*; AC = *A. cerana indica*; AD = *A. dorsata*; TI = *T. itama*.	148
4.22	Mass spectra of ferulic acid in the positive ion mode of the EPI scan (Source from Chua *et al*., 2013).	152

4.23	Cyclic voltammograms of standard phenolic compounds 1 mM (a-p) in comparison to 17.3 µM PGG and supporting electrolyte of 0.1 M PBS pH 7.	157-159
4.24	((a) Electroactive groups: C (catechol); R (resorcinol), P (phenol) and (G) gallic. (b) Redox reaction for catechol. (c) Chemical structure of quercetin with the circles indicates sequence of oxidation (Information is retrieved from Kilmartin *et al*., 2001; Makhotkina and Kilmartin, 2010; Gil and Couto, 2013).	160
4.25	Cyclic voltammogram and reaction mechanism of hesperetin at the GCE (Adapted from Wu *et al*., 2016).	163
4.26	Proposed reaction mechanism of PGG at the GCE.	163
4.27	(a) Cyclic voltammograms of GCE in the presence of 15 µM PGG in 0.1 M PBS (pH 7) at scan rate varies from 5-500 mV s^{-1} and (b) calibration plot of log i_{pa} vs. log v for P1a. (c) Cyclic voltammograms of GCE in the presence of 15 µM PGG in 0.1 M PBS at pH varies from pH 2 to 8, v = 10 mV s^{-1} and (d) calibration plot of E_{pa} vs. pH for P1a.	165
4.28	(a) Cyclic voltammograms of GCE in the presence of various PGG concentrations in 0.1 M PBS (pH 7) and (b) differential pulse voltammograms of GCE in the presence of various PGG concentrations in 0.1 M PBS (pH 7). Plots of i_{pa} vs [analyte] are shown in the respective insets. v = 10 mV s^{-1} and cycle 1.	167
4.29	Cyclic voltammograms for: (a) bare GCE in the absence and presence of 15 µM PGG and (b) bare GCE with 15 µM PGG for 5 cycles, v = 10 mV s^{-1}.	168
4.30	Cyclic voltammograms of: (a-d) single standard phenolic compounds (15 µM) in comparison to 15 µM PGG and supporting electrolyte of 0.1 M PBS pH 7; (e-f) mixture of standard phenolic compounds (15 µM).	169
4.31	DP voltammograms of pure Malaysian honey samples	171

(a-e), synthetic honeys and main sugars (f) in 0.1 M PBS pH 7 at scan rate 10 mV s^{-1}. All voltammograms are presented after subtracting away the DPV obtained for a blank run. G (gelam), A (acacia), N (nanas), K (kelulut), Tualang (T), S (synthetic), P (peak), FRU (fructose), GLU (glucose) and SUC (sucrose).

4.32 DP voltammograms for the real sample analyses (by standard addition approach) of pure Malaysian honey and synthetic honey samples on GCE. (a) Kelulut 3, (b) Tualang 3, (c) Nanas 2, (d) Synthetic 1, (e) Synthetic 3. R (real sample), 1 (1 µM PGG), 2 (3 µM PGG) and 3 (5 µM PGG). 173

LIST OF ABBREVIATIONS

A	-	Acacia
AA	-	Ascorbic Acid
ABTS	-	2,2’-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
ANOVA	-	Analysis Of Variance
APCI	-	Atmospheric Pressure Chemical Ionization
API	-	Apigenin
AOAC	-	Association of Official Analytical Chemists
ATP	-	Adenosine Triphosphate
Aug	-	August
BEN	-	Benzoic acid
CAF	-	Caffeic acid
CAT	-	Catalase/ Catechin
CE	-	Catechin Equivalents
CHOL	-	Catechol
CHR	-	Chrysin
CID	-	Collision-Induced Dissociation
CIN	-	*Trans*-cinnamic acid
COU	-	*p*-Coumaric acid
CRP	-	C-reactive protein
CV	-	Cyclic Voltammetry
CVD	-	Cardiovascular Disease
DAD	-	Diode Array Detector
Dec	-	December
DM	-	*Diabetes mellitus*
DNA	-	Deoxyribonucleic Acid
DPPH	-	2,2-Diphenyl-1-picrylhydrazyl

DPV	-	Differential Pulse Voltammetry
e-nose	-	Electric nose
e-tongue	-	Electric tongue
e.g.	-	For example
et al.	-	And others
etc.	-	And other things
eqn	-	Equation
EC	-	Electrical Conductivity
ED	-	Electrochemical Detection
E_{pa}	-	Anodic peak potential
E_{pc}	-	Cathodic peak potential
ESI	-	Electrospray Ionization
FAB	-	Fast Atom Bombardment
Feb	-	February
FER	-	Ferulic acid
$FeSO_4.7H_2O$	-	Iron (II) sulfate heptahydrate
FFA	-	Free Fatty Acid
FIA	-	Flow Injection Analysis
FRAP	-	Ferric Reducing Antioxidant Power
FRU	-	Fructose
FTIR	-	Fourier Transform Infrared Spectroscopy
G	-	Gelam/ Electrical conductance
GAE	-	Gallic Acid Equivalents
GAL	-	Gallic acid
GCE	-	Glassy carbon electrode
GC-FID	-	Gas Chromatography-Flame Ionization Detection
GC-MS	-	Gas Chromatography-Mass Spectrometry
GI	-	Glycemic index
GLU	-	Glucose
GPx	-	Glutathione Peroxidase
H_2O_2	-	Hydrogen peroxide
HCl	-	Hydrochloric acid
HES	-	Hesperetin

HHDP	-	Hexahydroxydiphenoyl
HMF	-	Hyrdroxymethyfurfural
HPLC	-	High Performance Liquid Chromatography
HSD	-	Tukey's honestly significant difference
IC_{50}	-	Inhibitory concentration at 50%
ICAM-1	-	Intracellular Adhesion Molecule-1
IDDM	-	Insulin-Dependent *Diabetes Mellitus*
i.e.	-	That is
IGT	-	Impaired Glucose Tolerance
IHC	-	International Honey Commission
IL-6	-	Interleukin-6
IL-18	-	Interleukin-18
ISCIRA	-	Internal Standard Carbon Isotope Ratio Analysis
Jan	-	January
K	-	Cell constant
KAE	-	Kaempferol
KCl	-	Potassium chloride
LC-MS/MS	-	Liquid Chromatography tandem Mass Spectrometry
LLE	-	Liquid-Liquid Extraction
LUT	-	Luteolin
MAE	-	Microwave-Assisted Extraction
MDA	-	Malondialdehyde
meq/kg	-	milliequivalent of acid per kg of honey
MRM	-	Multiple Reaction Monitoring
MS^2	-	Mass spectrometry/mass spectrometry
MW	-	Molecular Weight
N	-	Nanas
NaOH	-	Sodium hydroxide
NAR	-	Naringenin
NIDDM	-	Non-Insulin-Dependent *Diabetes Mellitus*
NIR	-	Near Infrared Spectroscopy
NMR	-	Nucleus Magnetic Resonance
NOX	-	NADPH oxidase

Nov	-	November
Oct	-	October
OGTT	-	Oral Glucose Tolerance Test
o-quinone	-	*ortho*-quinone
ORAC	-	Oxygen Radical Absorbance Capacity
P	-	Peak
PBS	-	Phosphate Buffer Solution
PC	-	Principal Component
PCA	-	Principal Component Analysis
PGG	-	1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose
PII	-	Peak Incremental Index
PUFA	-	Polyunsaturated Fatty Acid
QE	-	Quercetin Equivalents
QUE	-	Quercetin
r	-	Pearson's correlation coefficient
RE	-	Rutin Equivalents
RI	-	Refractive Index
ROS	-	Reactive Oxygen Species
RNS	-	Reactive Nitrogen Species
RP	-	Reversed Phase
RSA	-	Radical Scavenging Activity
RT	-	Retention Time
RUT	-	Rutin hydrate
S	-	Synthetic
SD	-	Standard Deviation
SOD	-	Superoxide dismutase
SPE	-	Solid-phase extraction
SPSS	-	Statistical Package for Social Sciences
spp.	-	species
SUC	-	Sucrose
SYR	-	Syringic acid
T	-	Tualang
TAN	-	Tannic acid

T1D	-	Type 1 Diabetes
T2D	-	Type 2 Diabetes
TEAC	-	Trolox Equivalent Antioxidant Capacity
TFC	-	Total Flavonoid Content
TNF- α	-	Tumor Necrosis Factor-alpha
TP	-	Total Polyphenols
TPC	-	Total Phenolic Content
TPTZ	-	2,4,6-Tris(2-pyridyl)-*s*-triazine
TSS	-	Total Soluble Solids
TSP	-	Thermospray
UAE	-	Ultrasound-Assisted Extraction
UPLC	-	Ultra performance liquid chromatography
uq	-	Unquantified
USDA	-	United States Department of Agriculture
UV-Vis	-	Ultraviolet-Visible light
VCAM-1	-	Vascular Cell Adhesion Molecule
VCEAC	-	Vitamin C Equivalent Antioxidant Capacity
VFA	-	Visceral Fat Area
W_{Wed}	-	Water according to Wedmore
4-HNE	-	4-hydroxynonenal
8-OH-G	-	8-Hydroxyguanine

LIST OF SYMBOLS

amu	-	Atomic mass unit
α	-	Alpha
β	-	Beta
˚Brix	-	Percentage sugar
˚C	-	Degree Celcius
%	-	Percentage
min	-	Minute
kg	-	Kilogram
g	-	Gram
h	-	Hour
mg	-	Milligram
µg	-	Microgram
mL	-	Milliliter
µL	-	Microliter
M	-	Molar
mM	-	Millimolar
µM	-	Micromolar
nM	-	Nanomolar
µA	-	Microampere
nA	-	Nanoampere
m^2	-	Square meter
mm	-	Millimeter
µm	-	Micron/ micrometer
nm	-	Nanometer
ppm	-	Part per million
s	-	Second

mS	-	Millisecond
V	-	Volt
kV	-	Kilovolt
mV	-	Millivolt
m/z	-	Mass to charge ratio
w/v	-	Weight per volume
v/v	-	Volume per volume
vs	-	Versus
v	-	Scan rate
C18	-	Silica bonded with octadecyl chains
E	-	Potential
Fe^{2+}	-	Ferrous ion
Fe^{3+}	-	Ferric ion
i_{pa}	-	Anodic peak current
i_{pc}	-	Cathodic peak current
p value	-	Calculated probability
–COOH	-	Carboxyl group
HOCl	-	Hypochlorous acid
$HO_2^{\cdot}$	-	Hydroperoxyl radical
HOBr	-	Hypobromous acid
HNO_2	-	Nitrous acid
$NO^{\cdot}$	-	Nitric oxide
$NO_2^{\cdot}$	-	Nitrogen dioxide
N_2O_3	-	Dinitrogen trioxide
NO^+	-	Nitrosyl cation
NO^-	-	Nitrosyl anion
NO_2^+	-	Nitronium (nitryl) cation
O_2	-	Oxygen
O_3	-	Ozone
$O_2^{\cdot -}$	-	Superoxide anion radical
$^1O_2$	-	Singlet oxygen
OH	-	Hydroxyl group
$OH^{\cdot}$	-	Hydroxyl radical

OCH_3	-	Methoxy group
$ONOO^-$	-	Peroxynitrite
ONOOH	-	Peroxynitrous acid
$R^{\cdot}$	-	Carbon-centered radical
$RO^{\cdot}$	-	Alkoxyl radical
$ROO^{\cdot}$/ $LOO^{\cdot}$	-	Peroxyl radical
ROONO	-	Alkyl peroxynitrites
ROOH	-	Lipid hydroperoxide

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	ONE-Way ANOVA for the physicochemical parameters of analysed honeys.	206
B	ONE-Way ANOVA for the antioxidant parameters of analysed honeys.	225
C	HPLC chromatograms of a standard mixture of phenolic compounds (100µg/mL) at 290 nm (top) and 340 nm (bottom). The insert shows peak of gallic acid.	233
D	MS/MS spectra for derivative of syringic acid (top), and salicylic acid (bottom).	234
E	DP voltammograms of samples (R) and (R+5µM) of : (a) acacia 2, (b) acacia 3, (c) kelulut 4, (d) nenas 1, (e) synthetic 2 and (f) synthetic 4.	235

CHAPTER 1

INTRODUCTION

1.1. Background of research

Honey is produced in most of the countries in the world, therefore, as expected there are a variety of honeys available in the global market. Some of the honey constituents are derived from the plants and some of them are added by the bees (Anklam, 1998). Taking into account its contributing source of nectar and/or plants as well as location, honey composition is greatly influenced by its botanical and geographical origins (Anklam, 1998; Gheldof *et al.*, 2002; Kaškonienė and Venskutonis, 2010). Honey can be produced by honey bee (Apidae, Apini) and stingless bee (Apidae, Meliponini) which have also shown some influence on honey composition and properties (Vit *et al.*, 1994; Bogdanov *et al.*, 1996; Kaškonienė and Venskutonis, 2010). Other than carbohydrates and water, honey contains more than 180 other constituents such as enzymes, amino acids, organic acids, vitamins, minerals, carotenoids, polyphenols (phenolic compounds), and Maillard reaction products (Gheldof *et al.*, 2002; Al *et al.*, 2009).

Pure honey is highly acknowledged in these modern days for its valuable and extraordinary protective effects against oxidative stress in human body and oxidation of food products. Back to the ancient times of thousand years ago, honey has been found useful for the treatment of numerous human-related ailments (Molan, 1992; Al-Jabri, 2005; Cooper, 2011). It was believed that natural healing power of honey is attributed by its mere composition. With time, exploration on honey was made possible in favour of technological and medical advancements. In fact, research findings have discovered remarkable properties of honey and its previously undefined composition. Antioxidant is one of the distinguish properties of honey that works against the mess engaged by free radicals (Blasa *et al*., 2007; Mohamed *et al*., 2011). Free radicals reactions have been implicated in the aetiology of several human diseases including cancer, atherosclerosis, rheumatoid arthritis and neurodegenerative diseases as well as deterioration of food (Aruoma, 1998).

Interestingly, polyphenols including phenolic acids and flavonoids have been associated to honey antioxidant properties (Tenore *et al*., 2012). Polyphenols are plant secondary metabolites that present in honey when bees forage for food, normally from flowering plants. Polyphenols in plants play vital roles in growth and reproduction, protect against pathogens and predators as well as give colour and sensory characteristics of fruits and vegetables (Balasundram *et al*., 2006). Flavonoids constitute the largest group of plant phenolics and are the most abundant group of polyphenols in plant-based foods. Tannins, another subgroup of polyphenols, are having more complex structure than phenolic acids and flavonoids. Many research findings have reported the presence of flavonoids and phenolic acids in honey (Ferreres *et al.*, 1993; Andrade *et al*., 1997; Aljadi and Yusof, 2003; Yao *et al*., 2003; Kassim *et al*., 2010; Hussein *et al*., 2011; Khalil *et al*., 2011; Chua *et al*., 2013). However, findings reported the presence of tannins in honey is still scarce. Furthermore, analysis of polyphenols has been regarded as a very promising tool to determine the floral origin of honeys with phenolic acids and flavonoids as the potential markers (Anklam, 1998; Yao *et al*., 2003).

Nowadays, high cost, disparity in worldwide honey production as well as an intensifying demand of honey in the market leads to the phenomenon of the dishonest act of production of synthetic honey. Synthetic (artificial) honey is produced without involvement of bee feeding on nectar or tapping on living parts of plants or aphids. Its fraudulent production could be done chemically in such a way that mimicking pure natural honey to disguise consumers, for instance, by adding acidulent and honey flavouring (Molan, 1996). To enhance quality control of honey, European Union (EU), Food and Agriculture Organization (FAO), and International

Honey Commission (IHC) are among the regulatory bodies that help standardize the benchmarks for pure honey quality determination as per documented in Codex Alimentarius standard (Codex Alimentarius, 2001) and EU Directive (EU Council, 2002). However, these regulations focus more on physicochemical properties of Apini honey with no specific quality regulations is currently available for Meliponini honey indicating the raised needs for more information related to Meliponini honey through laboratory evidence (Chuttong *et al*., 2016).

Though it is well-known that honey consumption offers more health benefits, the major problem faced by Malaysians is in determining the authenticity of the honey sold. Lack of screening or strict quality assessments of small-scale natural products such as honey in Malaysia has opened the door for food product falsification (Zakaria *et al*., 2011). Some of the Malaysians tend to buy local honey from aboriginal people with trust that the honey is original in its content. Although this could be the safe and good choice they have, but it is not always the case. A finding by Yusoff and colleagues (2006) pointed out that 31 out of 40 honey samples of Malaysian origin tested for their sugars and hydroxymethylfurfural (HMF) contents were found either adulterated or synthetic honeys. Regrettably, some of those claimed as pure honey were also bought from aboriginal people. The false information about the product can be considered as a violation of consumer rights whereby products sold are contradictory to consumer interest and can cause physical harm or emotional distress.

Several studies have attempted to distinguish pure honey from adulterated and/or sugar solutions. Various techniques provided by a number of analytical instruments have been employed to observe any possible differences including fourier transform infrared spectroscopy (FTIR), near infrared spectroscopy (NIR), internal standard carbon isotope ratio analysis (ISCIRA), nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) (Bertelli *et al*., 2010; Zhu *et al*., 2010; Zakaria *et al*., 2011; Tosun, 2013; Wang *et al*., 2015). Despite their usefulness and high accuracy, these analytical techniques are time-consuming, require highly skilled operators, very expensive and some of the techniques require sample preparation prior to analysis

(Subari *et al*., 2012). Besides, the use of bio-mimicking sensors namely electronic nose (e-nose) and electronic tongue (e-tongue) systems that perceive distinct smell (aroma) and taste (flavour) of samples, respectively, was reported useful but differentiation of the three groups was hardly achieved without data fusion of both systems and multivariate analysis (Zakaria *et al*., 2011). Therefore, development of simple, rapid screening methods to evaluate honey authenticity are of utmost concern.

1.2. Problem statements

Susceptibility of honey to falsification masks the beneficial health effects offered by honey. Aside from adulterated honey, the emergence of synthetic honey imposes another threat to honey safety and authenticity due to its relatively high sugar content (Yusoff *et al*., 2006). Consumption of high-sugar foods leads to hyperglycemia, the primary causal factor associated with diabetes mellitus (Miyazaki *et al*., 2007) and its complications (Ceriello, 2005; O'Keefe and Bell, 2007). The rise in blood glucose after consumption of carbohydrate-rich meal subsequently evokes production of reactive oxygen species and may result in oxidative stress (Ceriello, 1997). Under hyperglycemic conditions, body's antioxidants are depleted (Kashiwagi, 2001). Consequently, regular and long-term intake of synthetic honey may pose detrimental health effects in young, healthy individuals as well as aggravation of pre-existing metabolic conditions in prediabetic and diabetic patients (Mohanty *et al*., 2000; Ceriello *et al*., 2002a; Miyazaki *et al*., 2007; Schindhelm *et al*., 2007). On the contrary, honey has antioxidant properties attributed mainly by its phenolic compounds (Tenore *et al*., 2012). Al-Waili (2004) observed that honey intake lowers plasma glucose level in both healthy and diabetic individuals. In order to be able to protect consumers from honey fraud, it is important that an experimental study be conducted to characterise and differentiate between pure Malaysian honey and synthetic honey as well as propose a new method for fast screening of polyphenol that could help to distinguish pure Malaysian honey from the synthetic ones.

1.3. Objectives of study

The objectives of this study are:

1) To investigate the physicochemical properties of Malaysian honeys of different floral origin and bee species with respect to international standards and compare with synthetic honey.
2) To evaluate and compare the phytochemical and protein contents as well as antioxidant activities of pure Malaysian honeys and synthetic honey.
3) To isolate, identify and quantify the polyphenols in Malaysian honeys and generate chromatographic fingerprints of SPE honey extract.
4) To identify possible floral markers in unifloral honey and discover relationship between bee species and honey polyphenolic content.
5) To develop rapid sensing methods for differentiation between pure and synthetic honeys by identifying polyphenols using electrochemistry techniques.

1.4. Scope of study

This research focus is directed only on pure honey and synthetic honey. The five types of 17 pure Malaysian honeys investigated in this study are gathered from both *Apis* spp. and *Trigona* spp. as well as from single and multiple floral sources in between August 2012 and February 2013. The synthetic honeys are either made experimentally comprising of fructose, glucose, maltose and sucrose or bought in the market in the form of syrup. The physicochemical properties were assessed according to the standardized IHC methods as well as reported methods. The tests include pH, free acidity, HMF, moisture content, refractive index, total soluble solids, ash, electrical conductivity, colour, and density. Total phenolic content (TPC) and total flavonoid content (TFC) were the phytochemical contents analysed spectrophotometrically whereas DPPH and ferric reducing antioxidant power (FRAP) measuring the antioxidant activity. The protein content was also determined spectrophotometrically. Isolation of polyphenols was achieved using solid phase extraction (SPE). Identification and quantification were performed using high-performance liquid chromatography (HPLC). Liquid chromatography tandem mass spectrometry (LC-MS/MS) was utilized to screen for more polyphenols in the SPE honey extracts and to confirm the presence of the HPLC-detected phenolic compounds. Floral markers identification and bee-plant relationship was shown and confirmed using principal component analysis (PCA). Electrochemical behaviour of polyphenols at the unmodified glassy carbon electrode (GCE) was examined using cyclic voltammetry (CV). Rapid sensing of 1,2,3,4,6-pentagalloyl-β-D-glucose (PGG) in honey samples was achieved using electrochemistry techniques of differential pulse voltammetry (DPV) and the data obtained was compared to synthetic honeys. PGG was proposed as a potential biomarker for differentiation between pure and synthetic honeys.

1.5. Significance of study

The trend of increased consumption of natural foods and health products in sustaining a good health and curbing disease progression is the reason for the renewed interest in bee products such as honey. Considering the fact that honey varies in its composition and properties depending on botanical origin, geographic location, seasonal, climatic conditions, bee species, and several other factors, the results of this study are useful to differentiate between:1) multifloral and monofloral Malaysian honeys, 2) *Apis* and *Trigona* honeys, and 3) pure and synthetic honeys. Validation against the international standards helps to determine the quality of Malaysian *Apis* honey. The information on the physicochemical properties of Malaysian *Trigona* honey provided from this work is useful to develop future quality regulations for stingless bee honey.

The study of polyphenols in the current work helps to justify the relationship between species-specific foraging activities and phenolic composition of honeys, distinguish polyphenolic profile of each type of honey studied and identify possible floral markers. The presence of these phytochemicals further supports the antioxidant and other biological activities of Malaysian honeys as demonstrated by this and other studies. In vivo studies, human clinical trials and epidemiological studies have shown that prolong consumption of pure honey exerts a wide range of therapeutic and valuable health effects (Al-Waili, 2004; Yaghoobi *et al*., 2008; Erejuwa *et al*., 2011a; Erejuwa *et al*., 2011b), while high-sugar foods (Miwa *et al*., 2000; Esposito *et al.*, 2002; Fisher-Wellman and Bloomer, 2010) and possibly synthetic honey in the opposite way may impose harmful effects. Thus, the current work emphasizes the need of a rapid polyphenol screening as a potential marker for authentication of Malaysian honey that can partly be achieved through the proposed electrochemistry methods.

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