ANTIOXIDANTS FROM MALAYSIAN AGARWOOD (AQUILARIA SPP.) LEAF EXTRACTS AND THEIR APPLICATIONS IN IN VITRO MODEL AND FOOD SYSTEM

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By

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Agriculture by-products are undervalued substrates that are normally removed and disposed from the food production line. In this study, young leaves from three commercial species of Malaysian agarwood (*Aquilaria malaccensis*, *Aquilaria subintegra* and *Aquilaria crassna*) were examined. These are by-products from agarwood plantations and are usually discarded during the cultivation of agarwood trees. The first part of the present study assessed the effects of the ethanol concentration (0-100%) (v/v), solid-to-solvent ratio (1:10-1:60) (w/v) and extraction time (30-180 min) on the extraction of polyphenols from young agarwood leaves. The total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical-scavenging capacity were used for the quantification of polyphenols, and assessment of antioxidant capacity. The appropriate ranges of the extraction parameters were determined through single-factor experiments, and the optimal conditions for polyphenol extraction were established through response surface methodology (RSM). Among the three species of young agarwood leaves, the extract from *A. subintegra* young leaves (ASE) obtained through extraction with 64.64% (v/v) ethanol and a solid-to-solvent ratio of 1:70 (w/v) for 120 min was selected for the second part of the study. The antioxidative interactions (synergy, additive and antagonism) between ethanolic ASE and α-tocopherol were evaluated through assays of the DPPH radical-scavenging activity, the β-carotene bleaching system and liposome peroxidation. Among all of the combinations of ASE and α-tocopherol, only fraction 1/3 showed a synergistic interaction, which agrees well with the results from three different assays. In the third part of this study, ASE was incorporated into soybean oil-based mayonnaise at a concentration of 1/3 relative to the tocopherol content to prevent its deterioration during storage. The peroxide value (POV), total oxidation value (TOTOX) and conjugated trienes (CT) of ASE-enriched mayonnaise were significantly ($p < 0.05$) lower than the negative control mayonnaise samples at the end of the storage period. The addition of ASE to mayonnaise presented comparable ability to protect against deterioration compared with the addition of the commercially available synthetic ethylenediaminetetraacetic acid (EDTA). ASE was further tested for its toxicity *via* brine shrimp lethality and hemolytic analyses. Low brine shrimp lethality and low

hemolytic activity with IC_{50} values of 96.6 µg/mL and 1864.7 µg/mL against brine shrimp and human erythrocytes, respectively, were observed. In addition, the major bioactive compound in ASE, namely iriflophenone 3-C-*β*-glucoside (4.1%, w/w) with an IC_{50} of 54.5 mg/L for scavenging DPPH radicals was identified and quantified based on 1D NMR, 2D-NMR, LC-MS and UV-vis spectral data. In summary, this study may serve as a reference for future natural polyphenol applications in the food industry.

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

MENOROKAI ANTIOKSIDAN DARIPADA PRODUK SAMPINGAN TIGA SPESIS KOMERSIL GAHARU MALAYSIA DAN APLIKASI MEREKA DI DALAM MODEL IN VITRO DAN SISTEM MAKANAN

Oleh

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Produk sampingan pertanian adalah substrat bernilai rendah yang biasanya dikeluarkan dan dibuang daripada proses pengeluaran makanan. Dalam kajian ini, daun muda daripada tiga spesis komersial gaharu Malaysia (*Aquilaria malaccensis*, *Aquilaria subintegra* and *Aquilaria crassna*) telah dikaji. Ia adalah produk sampingan daripada ladang gaharu dan biasanya dibuang semasa penanaman pokok gaharu. Bahagian pertama kajian ini adalah mengenai pengaruh kepekatan etanol (0-100%) (v/v), nisbah pepejal-pelarut (1:10-1:60) (w/v) dan masa pengekstrakan (30-180 min) kepada pengekstrakan polifenol daripada daun muda gaharu. Hasil fenolik (jumlah kandungan fenolik; TPC dan jumlah kandugan flavonoid; TFC) telah digunakan untuk penentuan dan kuantifikasi polifenol, dan kapasiti pengikatan radikal 2,2-diphenyl-1-picrilhydrazil (DPPH) telah dinilai untuk menetukan kapasiti antioksidan. Julat parameter pengekstrakan yang sesuai telah ditentukan dengan menggunakan eksperimen-faktor tunggal dan keadaan pengekstrakan yang optimum untuk pengekstrakan polifenol telah diperolehi dengan cara pengoptimuman melalui metodologi respon permukaan (RSM). Antara tiga spesis daun muda gaharu, ekstrak daripada daun muda *A. subintegra* (ASE) yang diperolehi dengan menggunakan 64.64% (v/v) etanol, 1:70 (w/v) pepejal-pelarut nisbah dan 120 min masa pengekstrakan telah dipilih untuk bahagian kedua kajian. Interaksi (sinergim tambahan dan antagonistik) antara ASE dan α-tokoferol telah dinilai dengan menggunakan menggunakan kapasiti pengikatan radikal DPPH, sistem peluntturan β-karotena dan peroksidaan liposom. Di antara semua kombinasi ASE dan α-tokoferol, hanya pecahan 1/3 menunjukkan interaksi sinergim yang sesuai bagi tiga kaedah yang berbeza. Dalam bahagian ketiga kajian ini, ASE telah dimasukkan ke dalam mayones berasaskan minyak soya pada kepekatan 1/3 berbanding dengan kandungan tokoferol dalam minyak soya untuk mengelakkan kerosakan semasa penyimpanan. POV, TOTOX dan CT dari mayonis disediakan dengan menggunakan ASE lebih rendah daripada sampel kawalan negatif pada akhir tempoh penyimpanan. Mayones yang disediakan dengan menggunakan ASE mempunyai kemampuan yang standing dengan asid atelindiamintetrasetik (EDTA) sintetik dalam melindungi mayonis daripada kemerosotan. Seterusnya ketoksikan ASE telah diuji melalui analisis kadar kemautan

udang brin dan hemolitik. Kadar kemautan udang brin dan aktiviti hemolitik yang rendah dengan nilai IC_{50} sebanyak 96.6 µg/mL dan 1864.7 µg/mL terhadap udang brin dan eritrosit manusia telah diperhatikan. Akhirnya, sebatian bioaktif yang utama dalam ASE telah dipencilkan dan diperolehi dengan menggunakan kromatografi turus (CC) and kromatografi lapisan nipis (TLC). Iriflophenone 3-C-β-glucoside telah dikenal pasti berdasarkan resonans magnet nukleus dan spektrometri jisim. Ia telah dikira sebagai polifenol utama (4.1%, w/w) di dalam ASE dengan nilai IC_{50} sebanyak 54.5 mg/L dalam pengikatan radikal DPPH. Kesimpulannya, kajian ini boleh digunakan sebagai rujukan dalam aplikasi ekstrak polifenol semulajadi daripada gaharu dalam industri makanan.

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

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LIST OF ABBREVIATIONS

A	
a*	redness
AAPH	2,2´ azobis (2-aminopropane) dihydrochloride
ABTS	2,2´-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)
A. crassna	*Aquilaria crassna*
ACE	*Aquilaria crassna* extract
A. malaccensis	*Aquilaria malacensis*
AME	*Aquilaria malacensis* extract
ANOVA	analysis of variance
AR	analytical reagent
ArO•	aroxyl radical
ArOH	antioxidant
ASE	*Aquilaria subintegra* extract
AOCS	american oil chemists' society
APCI	atmospheric pressure chemical ionization
B	
b*	yellowness
BCB	β-carotene bleaching
BHA	butylated hydroxyanisole
BHT	butylated hydroxytoluene
C	
^{13}C	carbon-13
CAT	catalase
CCC	conventional column chromatography
CCD	central composite design
CD	conjugated dienes
CD_3OD	deuterated methanol
COSY	correlation spectroscopy
CT	conjugated trienes
CTC	condensed tannins content
CV	coefficient variation
D	
d	doublet
dd	doublet doublet
1D	one dimensional
2D	two dimensional
DAD	diode array detector
DMSO	dimethyl sulfoxide
DPPH	2,2´-diphenyl-1-picrylhydrazyl
DPPH-H	1,1-diphenyl-2-picryl hydrazine
DW	dry weight

E

E°	reduction potential
EDTA	ethylenediaminetetra-acetic acid
ESI	electrospray ion source
Expt.	experimental data

F

FAME	fatty acid methyl esters
FC	folin-Ciocalteu
FIC	ferrous iron-chelating
FID	flame ionization detector
FRAP	ferric ion reducing antioxidant power
FTIR	fourier transform infrared
F-value	statistical significance

G

GAE	gallic acid equivalent
GC	gas chromatography
GC-MS	gas chromatography mass spectrometry
GPx	glutathione peroxidase
GR	glutathione reductase
GRAS	generally recognized as safe
GSH	glutathione

H

^{1}H	proton
H_2O	water
H_2SO_4	sulphuric acid
HAT	hydrogen atom transfer
HMBC	heteronuclear multiple bond correlation
HO•	hydroxyl radical
H_2O_2	hydrogen peroxide
$HO_2•^-$	perhydroxyl radical
HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum coherence

I

IC_{50}	50% inhibitory concentration

J

J	coupling constant

L

L^*	lightness
LC	liquid chromatography
LC_{50}	50% lethality concentration
LC-MS	liquid chromatography mass spectrometry
LDL	low density lipoproteins

M

m	multiplet
MeOH	methanol
Meq	milliequivalents of peroxide per kilogram
MPOB	malaysia palm oil board
MS	mass spectrometry
MUFA	monounsaturated fatty acids
N	
NMR	nuclear magnetic resonance
NO•	nitric oxide
O	
$O_2•^-$	superoxide anion
-OH	hydroxyl group
ORAC	oxygen radical absorbance capacity
O/W	oil-in-water
P	
PBS	phosphate-buffered saline
PCA	plate count agar
PDA	photodiode array/ potato dextrose agar
PG	propyl gallate
PORIM	palm oil research institute of malaysia
POV	peroxide values
PPO	enzyme polyphenol oxidase
Pred.	predicted value
PUFA	polyunsaturated fatty acid
Q	
QE	quercetin equivalent
R	
R•	free radical
R^2	coefficient of determination
R_f	retention factor
RNS	reactive nitrogen species
RO•	alkoxyl radicals
ROO•	peroxyl radical
ROOH	lipid hydroperoxide
ROS	reactive oxygen species
RSM	response surface methodology
S	
s	singlet
SD	standard deviation
SDS	sodium dodecyl sulfate
SET	single electron transfer
SOD	dismutase superoxide
T	
TAC	total anthocyanin content

TBA	thiobarbituric acid
TBHQ	tertbutyl hydroquinone
TFA	total flavanol assay
TFC	total flavonoid content
TLC	thin layer chromatography
TMS	tetramethylsilane
TO•	α-tocopheroxyl radical
TOTOX	total oxidation values
TPC	total phenolic content
TRAP	total radical trapping antioxidant parameter
TVC	total viable counts

U

UV-Vis	ultraviolet-visible

X

X_1	linear coefficients
X_2	linear coefficients
X_1^2	quadratic coefficients
X_2^2	quadratic coefficients
X_1X_2	interaction coefficient

Y

Y_n	response variables

CHAPTER I

INTRODUCTION

Background

The health deterioration information is currently widespread, and the awareness of health problems is increasing. One of the crucial substances that cause problems to human health is free radicals. Free radicals are essential for human immune system responses and are produced in normal cell metabolism or physiological processes that utilize oxygen (Kalaivani & Mathew, 2010). However, the uncontrolled production of oxygen-derived free radicals leads to diseases, such as cancer, rheumatoid arthritis, cirrhosis and degenerative processes associated with aging (Tachakittirungrod, Ikegami, & Okonogi, 2007). Therefore, free radicals have been justified as harmful when they react with important cellular components, such as proteins, DNA and the cell membrane (Mantena, et al., 2008).

Other than health deterioration, free radicals have negative impacts in the food industry, including the induction of lipid oxidation by reacting with food lipids (Chan, Lee, Yap, Wan Aida, & Ho, 2009). Hence, to prolong the storage life of foods and to diminish damage to the human body, synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are added to fresh or processed foods (Soong & Barlow, 2004). Nevertheless, a decline in the use of synthetic antioxidants has been observed due to their possible toxic and carcinogenic effects (Arabshahi-Delouee & Urooj, 2007). In some countries, such as Japan, Canada and Europe, BHA and tert-butylhydroquinone (TBHQ) are prohibited. Thus, the use of natural antioxidants that not only are low in toxicity but also possess pronounced antioxidant activity is encouraged in this health-conscious society.

Today, there are increased lines of evidence suggesting that phytochemicals from natural plant sources possess antioxidant properties associated with a lower risk of mortality from many diseases (Dixon, Xie, & Sharma, 2005; Rice-Evans, 2004). The first marketed natural antioxidant was rosemary extract, which was found to have antioxidant activity as effective as that of synthetic antioxidants (Martínez, Penci, Ixtaina, Ribotta, & Maestri, 2013). In addition, bamboo leaf extracts have been approved as a natural food additive by the Ministry of Health in China (Zhang, Jiao, Liu, Wu, & Zhang, 2008). The antioxidant properties of plant extracts are mainly attributed to the presence of polyphenols. Polyphenols of plants are hydroxylated derivatives of benzoic acid and cinnamic acids and have been reported to exert antioxidative and anticarcinogenic effects (Muanda, Kone, Dicko, Soulimani, & Younos, 2011).

Agarwood was recently categorized as one of the seven types of herbs that obtained approval from Terengganu to be developed into a high-value herbal plantation on 461 hectares of land in Pasir Raja, Terengganu (ETP 2011). In the last ten years, 1000 hectares of agarwood have been planted in Malaysia, as reported by Barden, Noordainie, Mullihen, and Song (2000). Malaysia is the third largest exporter in the world after India and Indonesia, and more than 200 products that contain *Aquilaria* are currently registered with the Ministry of Health (Lim & Noorainie, 2010). As a consequence, the widely available agarwood assures the availability of easy accessible and sustainable sources of its by-product, namely young leaves. Agarwood leaves were prepared as tea and used traditionally for the treatment of trauma-related illness. It has also been reported to have analgesic, anti-inflammatory, laxative and anti-diabetics activities (Zhou, Wang, Suolangjiba, Kou, & Yu, 2008). As a result, a comprehensive exploration of the young agarwood leaf would be an interesting research topic.

Solvent extraction is a common and popular approach used to obtain desired nutrients and nutraceuticals from plant materials (Gupta, Naraniwal, & Kothari, 2012). However, the extraction of bioactive compounds is complicated by the complexity of bioactive compounds and plant matrices. An effective solvent extraction system is a good way to achieve cost efficiency and economic feasibility in an industrial process. Various novel extraction techniques and parameters have been developed; however, there is no universal standardized optimal extraction condition for achieving maximal yields of antioxidant compounds and activity in different plants. Consequently, a systematic study of the efficiency of solvent extraction from young agarwood leaves to obtain polyphenols with high antioxidant activity at a reduced production cost is essential.

The application of natural antioxidants has not been widely adopted by the food industries due to their undesirable flavor, color, aroma, and, most importantly, higher production cost, which may not be affordable compared with conventional food processing. The addition of exogenous antioxidants from extracts to a food system may interact with each other or with the endogenous antioxidants present in the food, resulting in different overall effects as expected. Therefore, it is of special interest to search for synergistic combinations of natural antioxidants that would enhance the expected pronounced antioxidant effects and eventually reduce the production cost in the food industry. In general, the antioxidants present in plant extracts in various combinations and their interactions are found to be important for their overall effect. The interactions between these bioactive compounds have been categorized as synergistic, additive and antagonistic interactions. Synergism is defined as an interaction that exerts a larger overall effect compared with the effect expected from the simple addition of the effects of the individual bioactive compounds (Uri, 1961). To achieve synergism, the types of compounds, their concentration and the ratios at which they are mixed play important roles (Schwarz, Frankel, & German, 1996). For example, Thoo, et al. (2013) reported that polyphenols work synergistically with α-tocopherol when mixes at a 2:1 ratio *via* the regeneration of α-tocopherol by polyphenols to provide long-lasting tocopherol.

In food processing, lipid oxidation is the major cause of food quality deterioration, including flavor, texture, aroma and nutritional value (Li, Kim, Li, Lee, & Rhee, 2014). Lipid oxidation is initiated in unsaturated fatty acids and causes the formation of hydroperoxides, which are susceptible to decomposition and results in the formation of secondary reaction products, such as aldehydes, ketones, acids and alcohols (Kolakowska, 2003). These compounds are responsible for changes in the overall food quality. Mayonnaise is a semi-solid oil-in-water (O/W) emulsion with a high content of polyunsaturated fatty acids (PUFAs), which are very susceptible to oxidation (Alamed, McClements, & Decker, 2006). The oxidation in mayonnaise can be reduced by applying antioxidant agents to retard spoilage, extent shelf life and maintain quality and safety (Devatkal & Naveena, 2010). However, the study and use of natural herb extracts as natural food additives in mayonnaise has been limited. Therefore, the development of natural antioxidant-enriched mayonnaise is encouraged.

Over the last few decades, the methodologies used in the research of natural products have evolved significantly. Older methodologies or strategies, such as chemotaxonomic investigation, focused on the chemistry of compounds instead of their activity, and hence, chemical compounds with no activity were always identified from natural sources (Sarker, Latif, & Gray, 2006). In recent years, the isolation and identification of natural products were mainly based on biological activity testing. It is known that young agarwood leaf is an abundant source of antioxidants, and thus, it was interesting to isolate and identify the major constituents that contribute to its antioxidant activity using a bioassay-guided isolation technique. Moreover, an adequate understanding of the chemical components, chemical natures, solubility and glycosylation positions may provide consistent functional food applications (Ito, et al., 2012).

Scope and Objectives

The first part of present study undertakes a systematic optimization of the solvent extraction of polyphenols from young *A. malaccensis, A. subintegra* and *A. crassna* leaves using single-factor experiments and response surface methodology (RSM) to gain a better understanding of the effects of the ethanol concentration, solid-to-solvent ratio and extraction time on the yield of polyphenols and the antioxidant capacity. After obtaining the maximal yields and antioxidant capacities of the extracts, the antioxidant properties of the extracts from three agarwood species were compared, and one species was selected for further study. The second part describes the effects of the interaction between agarwood extracts and α-tocopherol on the antioxidant properties in various *in vitro* model systems. The combination ratio of extracts and α-tocopherol that exhibited synergism in all of the model systems was used for the subsequent study described in Chapter V, which aimed to evaluate the capability of the extracts to inhibit lipid oxidation in a mayonnaise food system during eight weeks of storage. The toxicological risks of the extracts were evaluated before the incorporation of the extract into mayonnaise. The last part elucidates the structure of the potent antioxidant(s) in the extracts that were found to contribute to their antioxidant capacity using a detailed bioassay-guided isolation protocol.

The main objectives of this research study were:

1. to extract bioactive compounds (polyphenols) from young *A. malaccensis*, *A. subintegra* and *A. crassna* leaves (Chapter III);

2. to evaluate the synergism of the isolated antioxidant with α-tocopherol in *in vitro* model systems (Chapter IV);

3. to study their toxicological risks and ability to inhibit lipid oxidation in mayonnaise (Chapter V); and

4. to identify the major bioactive compound in the extracts (Chapter VI).

characterization of more bioactive compounds with pronounced biological activities present in young agarwood leaf extracts may be useful for their application in the nutraceutical and pharmaceutical industries.

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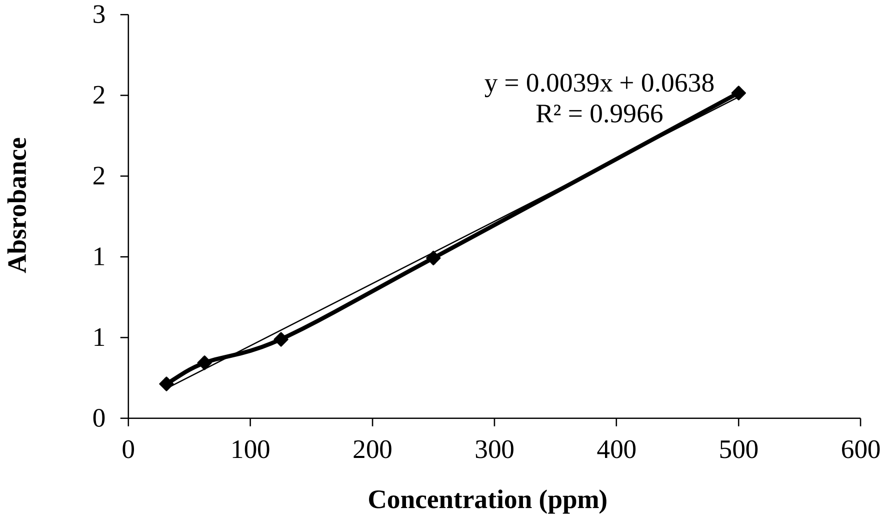
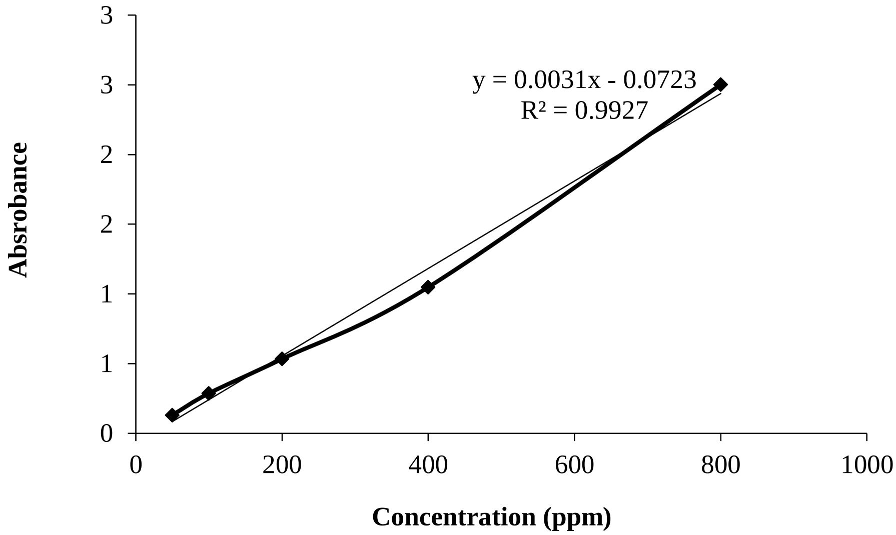
APPENDICES

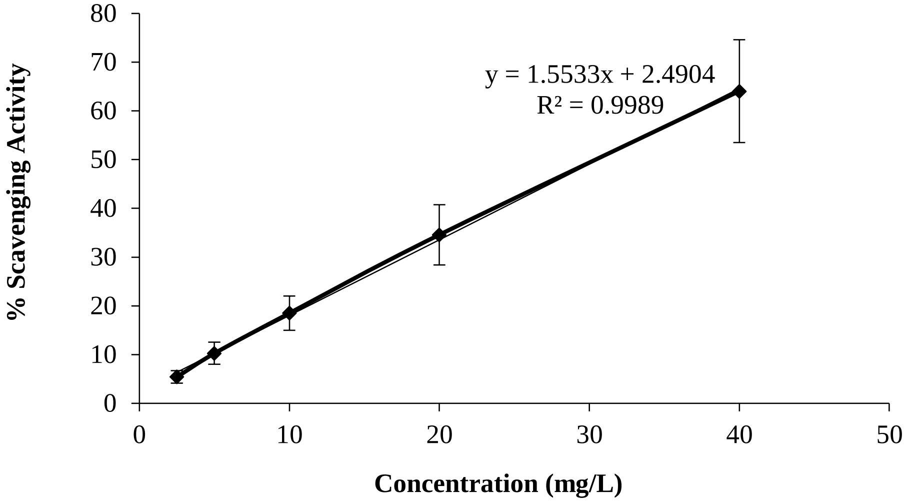
A1: Calibration curves for gallic acid for determination of TPC.



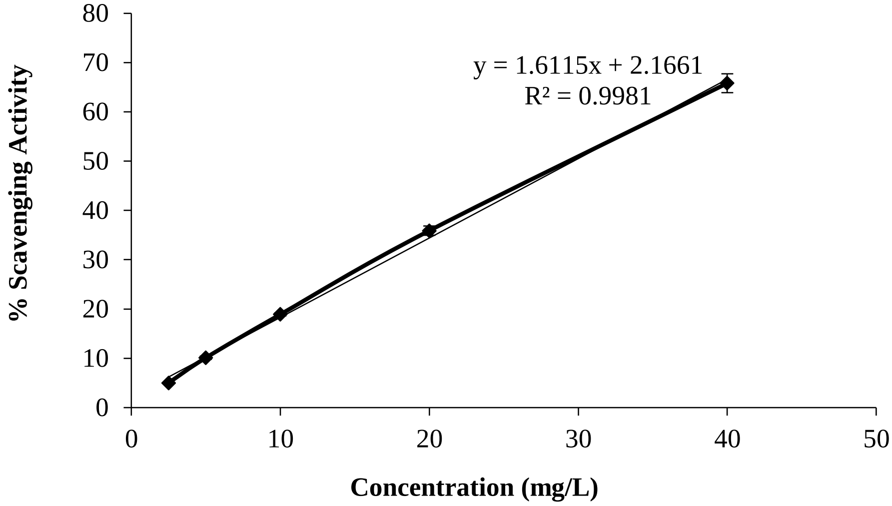
A2: Calibration curves for quercetin for determination of TFC.



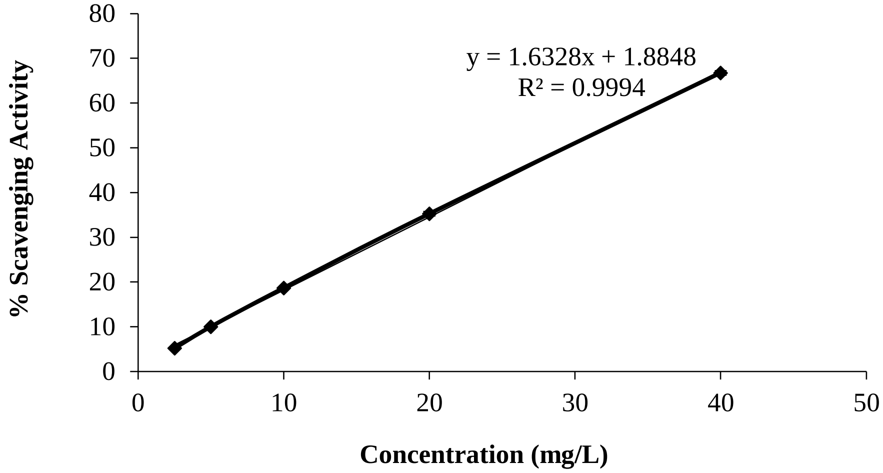
A3: IC_{50} curves of DPPH % scavenging activity versus concentration of AME obtained at optimum extraction condition by using single factor experiment.



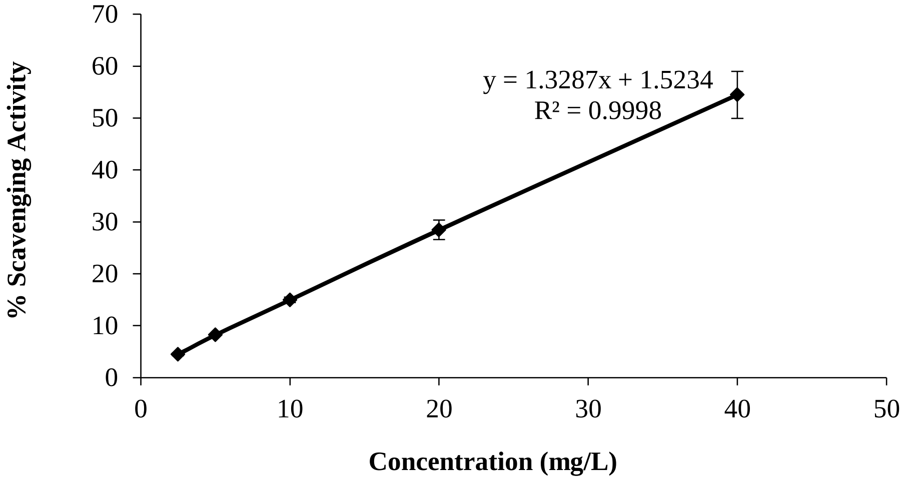
A4: IC_{50} curves of DPPH % scavenging activity versus concentration of ASE obtained at optimum extraction condition by using single factor experiment.



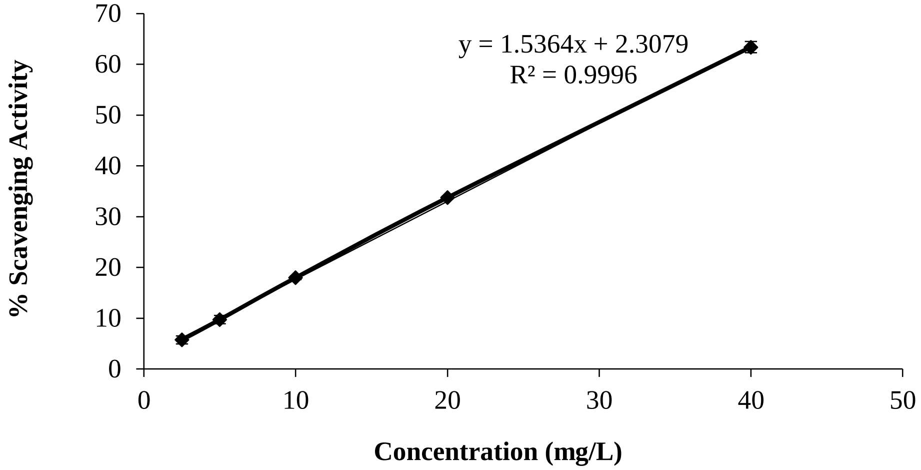
A5: IC_{50} curves of DPPH % scavenging activity versus concentration of ACE obtained at optimum extraction condition by using single factor experiment.



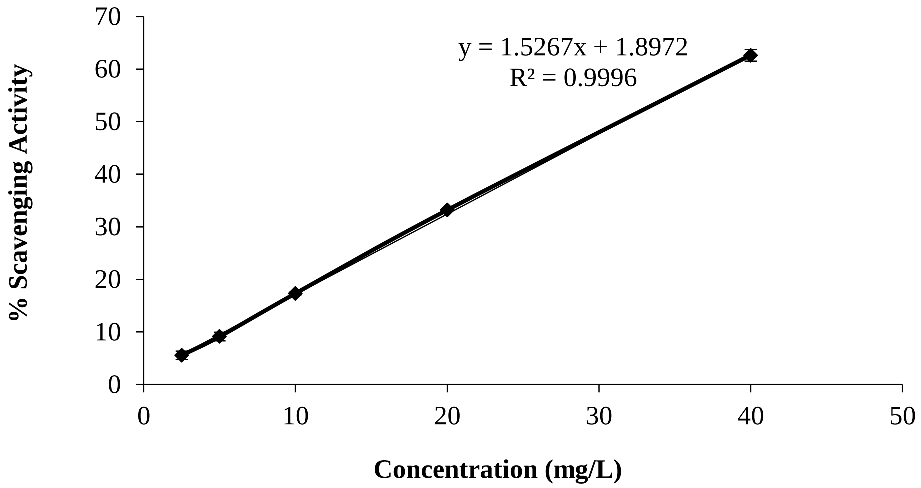
A6: IC_{50} curves of DPPH % scavenging activity versus concentration of AME obtained at optimum extraction condition by using RSM.



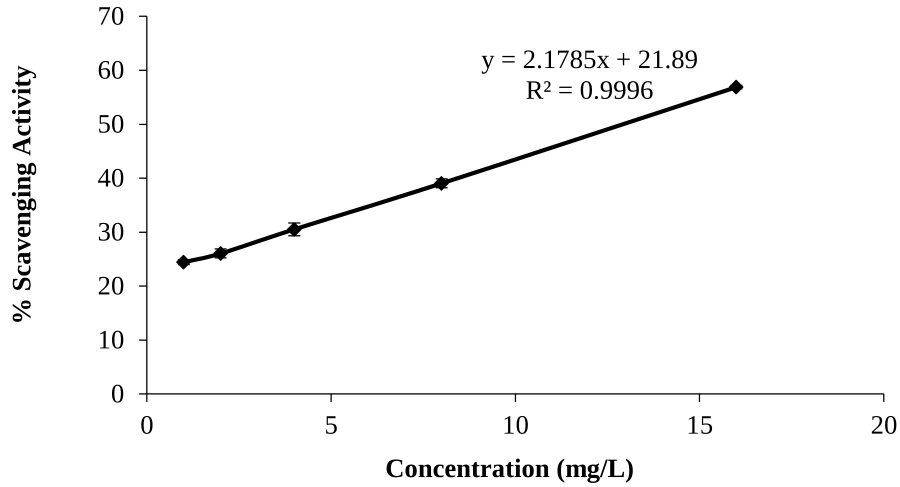
A7: IC_{50} curves of DPPH % scavenging activity versus concentration of ASE obtained at optimum extraction condition by using RSM.



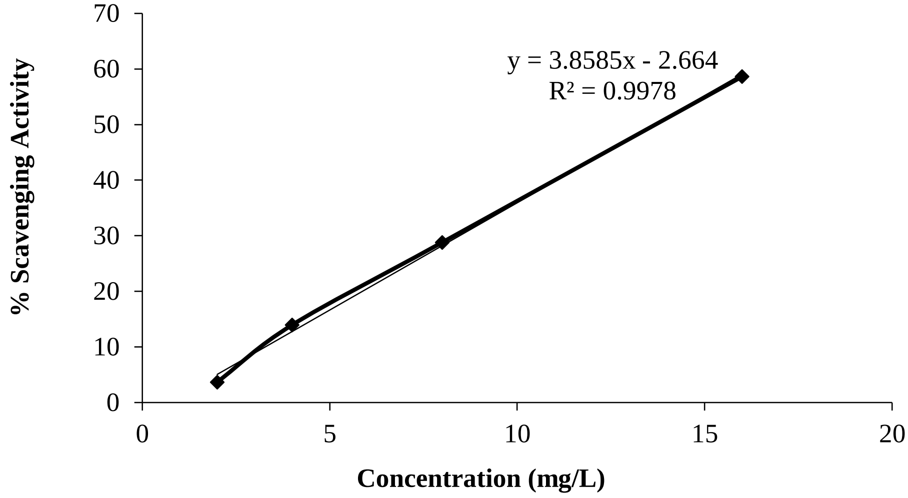
A8: IC_{50} curves of DPPH % scavenging activity versus concentration of ACE obtained at optimum extraction condition by using RSM.



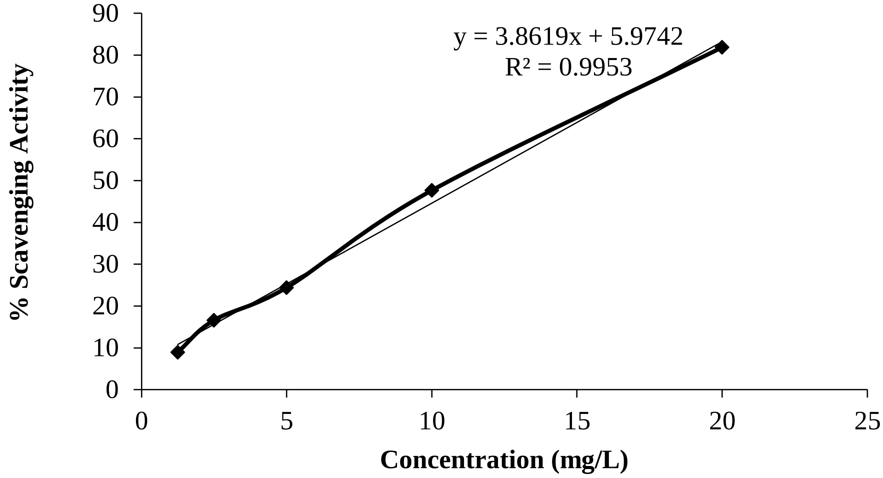
A9: IC_{50} curves of DPPH % scavenging activity versus concentration of ascorbic acid.



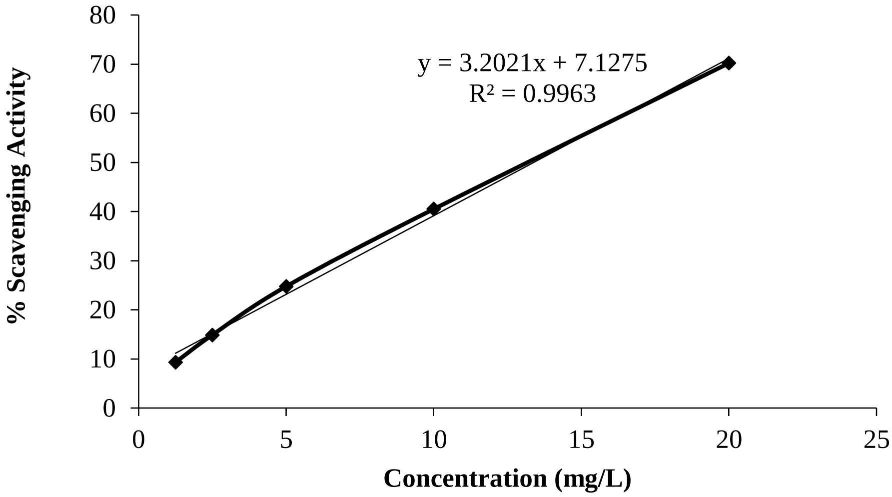
A10: IC_{50} curves of DPPH % scavenging activity versus concentration of α-tocopherol.



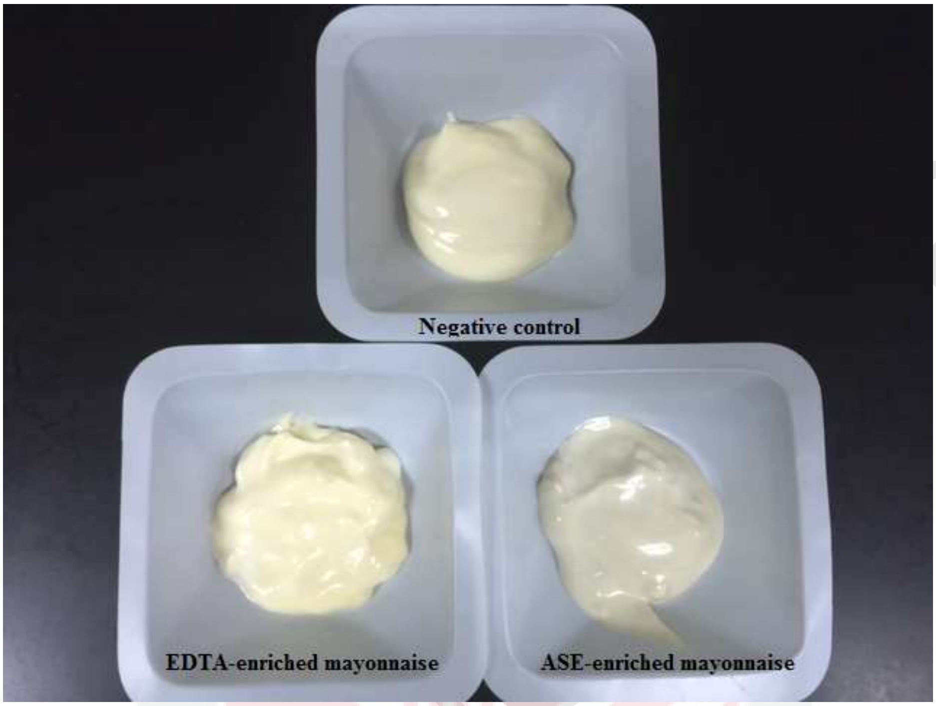
A11: IC_{50} curves of DPPH % scavenging activity versus concentration of (+)-catechin hydrate.



A12: IC_{50} curves of DPPH % scavenging activity versus concentration of BHA.



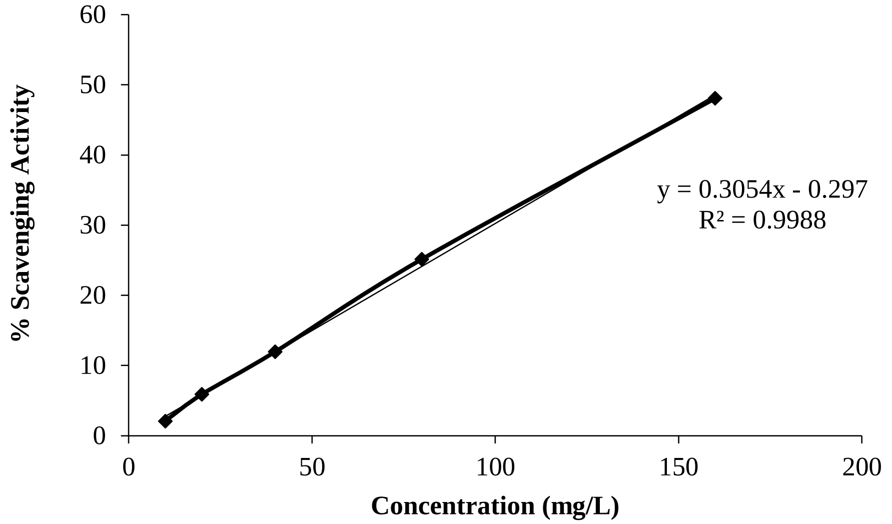
A13: Color of mayonnaises



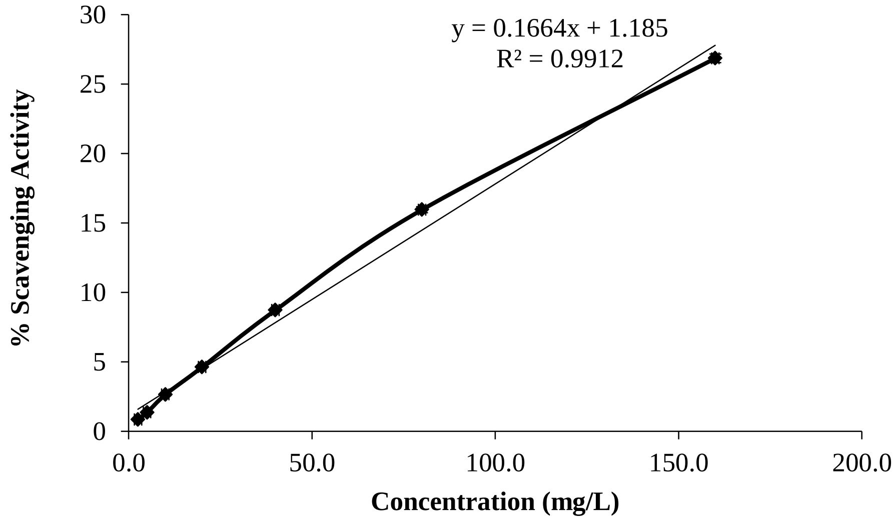
A14: Yield of *Aquilaria subintegra* young leaves extracted from different solvent fractions.

Fractions	Yield of samples (g)	Recovery rate (%)
Hexane	1.7822	2.31
Chloroform	3.7520	4.86
Ethyl acetate	7.0238	9.09
Water	59.3425	76.82
Total	71.9005	93.07
Total weight of the sample before liquid-liquid extraction = 77.2529 g		

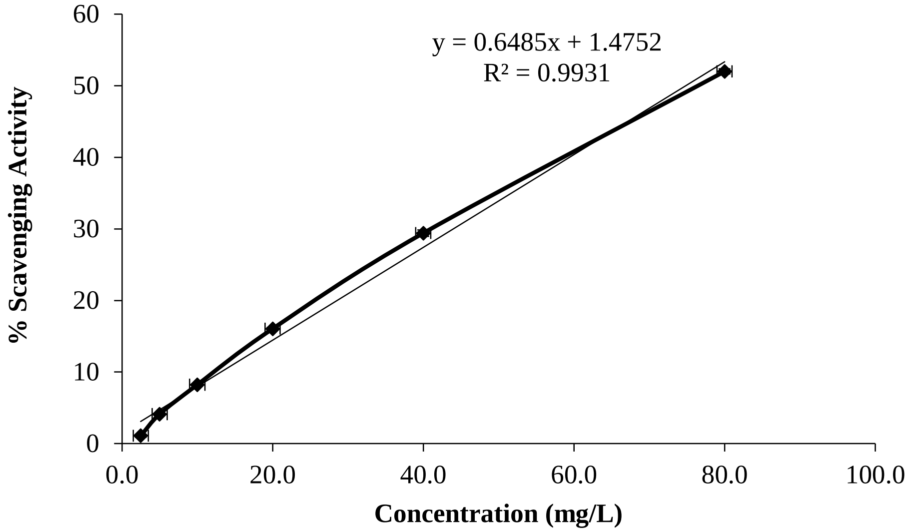
A15: IC_{50} curves of DPPH % scavenging activity versus concentration of hexane fraction.



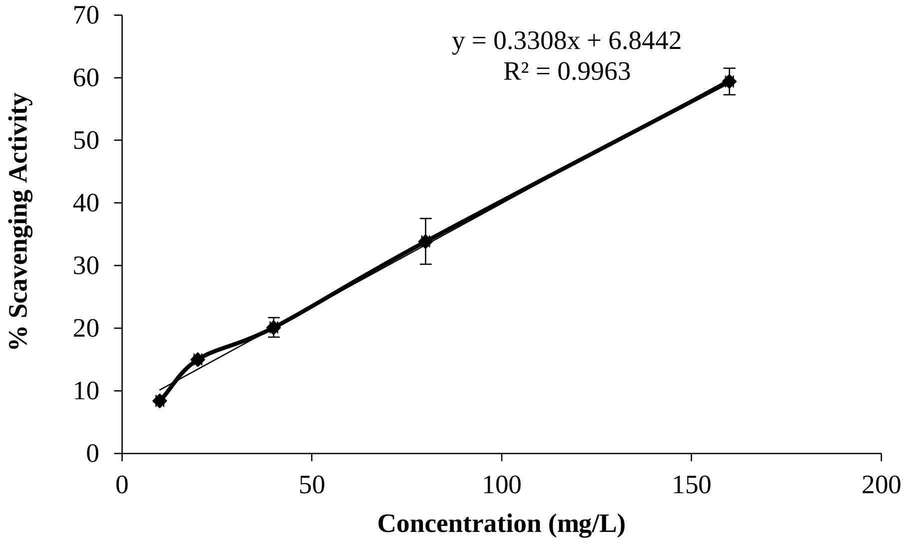
A16: IC_{50} curves of DPPH % scavenging activity versus concentration of chloroform fraction.



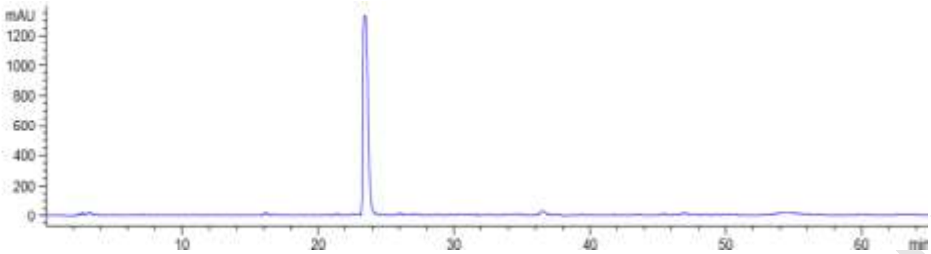
A17: IC_{50} curves of DPPH % scavenging activity versus concentration of ethyl acetate fraction.



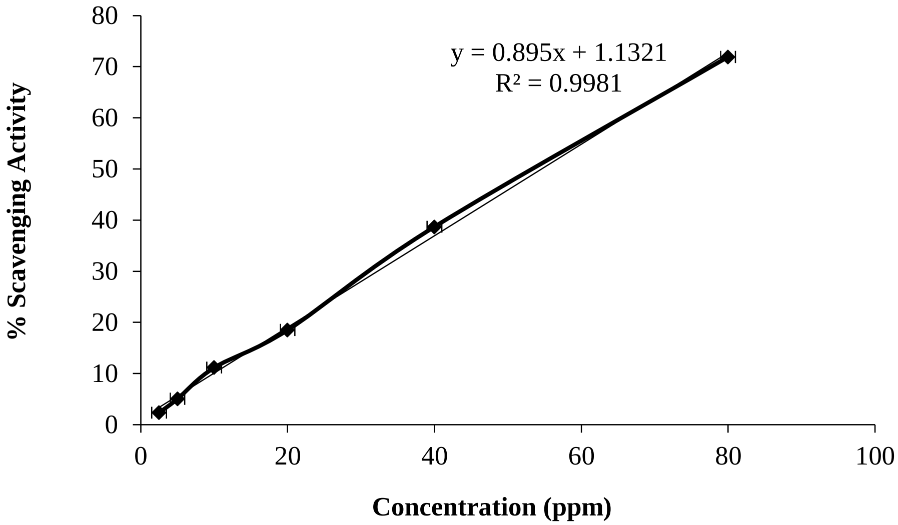
A18: IC_{50} curves of DPPH % scavenging activity versus concentration of water fraction.



A19: HPLC chromatogram of iriflopheone-3-*C*-β-glucoside.



A20: IC_{50} curves of DPPH % scavenging activity versus concentration of Compound 1.



BIODATA OF STUDENT

Tay Pei Yin was born on 17th February 1989 and raised in a small town, Muar in a state of Johor. She comes from a family of six and is the second daughter to her parents. She completed her primary and secondary school education at SJK(C) Chung Hwa Presbyterian and Chung Hwa High School, respectively.

After graduating from secondary school, she enrolled UCSI University in the subsequent year and successfully completed with First Class Honours in Bachelor of Science in Food Science and Technology in 2011. Her honour thesis entitled "Effects of ethanol concentration, solid-to-solvent ratio and extraction time on antioxidant properties of gaharu (*Aquilaria crassna*) young shoots (3.0mm)" was presented in August 2011 under the supervision of Assoc. Prof. Dr. Ho Chun Wai.

After the graduation, she had worked as a research assistant at the Faculty of Applied Sciences, UCSI for one year. In September 2012, she entered Universiti Putra Malaysia (UPM) as a PhD candidate under the supervision of Prof. Dr. Tan Chin Ping. As indicated in the present thesis, the principle objectives of her project were to extract the polyphenols from three commercial species of Malaysian agarwood by-product, followed by the evaluation of antioxidant synergism with α-tocopherol in various *in vitro* model system and its ability to inhibit lipid peroxidation in mayonnaise, as well as the identification and quantification of the major polyphenol constituent presented in agarwood by-product.

LIST OF PUBLICATIONS

1. Tay, P. Y., Tan, C. P., Abas, F., Yim, H. S., and Ho, C. W. (2014). Assessment of extraction parameters on antioxidant capacity, polyphenol content, epigallocatechin gallate (EGCG), ppicatechin gallate (ECG) and iriflophenone 3-C-β-glucoside of agarwood (*Aquilaria crassna*) young leaves. Molecules, 19 (8), 12304-12319.

2. Tay, P. Y., Tan, C. P., Abas, F., Yim, H. S.,and Ho, C. W. (In preparation). Effect of processing parameters on antioxidant capacity and polyphenol content of *Aquilaria malaccensis* leaves.

3. Tay, P. Y., Tan, C. P., Abas, F., Yim, H. S., and Ho, C. W. (In preparation). Effects of binary solvent extraction system, solid-to-solvent ratio, and extraction time on polyphenol content, antioxidant capacity, epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and iriflophenone 3-C-*β*-glucoside from agarwood (*Aquilaria subintegra*) young leaves.

4. Tay, P. Y., Abas, F., Ho, C. W., Thoo, Y. Y., and Tan, C. P. (In preparation). Antioxidant activity of an ethanolic extract from young agarwood (*Aquilaria subintegra*) leaves and its synergism with added α-tocopherol in model systems.

5. Tay, P. Y., Abas, F., Ho, C. W., and Tan, C. P. (In preparation). Antioxidative activity and chemical safety of agarwood (*Aquilaria subintegra*) young leaves extract during storage of mayonnaise.

6. Tay, P.Y., Tan, C. P., Abas, F., and Ho, C. W. Influence of processing parameters on antioxidant capacity and polyphenol content of agarwood (*Aquilaria malaccensis*) young leaves, Proceedings of 6th International Conference and Exhibition on Nutraceuticals & Functional Foods, Taipei, Taiwan, Nov. 5-9, 2013.