

PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *Aquilaria subintegra* Ding Hou

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**SULTAN IDRIS EDUCATION UNIVERSITY
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PHYTOCHEMICALS AND BIOLOGICAL ACTIVITIES OF *Aquilaria subintegra*

Ding Hou

MASTURA BINTI IBRAHIM

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ABSTRACT

This study aims to investigate the phytochemicals and biological activities from the bark and leaf of *Aquilaria subintegra* Ding Hou. The samples were air-dried, ground, and gradient extracted by cold extraction using *n*-hexane, dichloromethane, and methanol successively. The bark and leaf crude extracts were purified *via* chromatographic techniques to produce pure compounds. The chemical structure of compounds were determined using spectroscopic methods such as nuclear magnetic resonance, ultraviolet, infrared, mass spectrometry and comparison from the literature review. The crude extracts and isolated compounds were tested for their anti-lipase, antioxidant and anticancer activities through colorimetric test, DPPH free radical scavenging and cytotoxicity assay, respectively. This study has successfully afforded 22 compounds, including three new compounds, namely aquilene A, aquilene B, and subintegranol. Other compounds were 5-hydroxy-7,4'-dimethoxyflavone, luteolin-7,3',4'-trimethyl ether, 5,3'-dihydroxy-7,4'-dimethoxyflavone, 7,3'-dimethoxyluteolin, 5,7-dihydroxy-4'-methoxyflavone, β -sitosterol, β -sitostenone, stigmasterol, friedelin, epifriedelanol, phytol, and hentriacontane. Anti-lipase activity of *n*-hexane extracts of leaf and methanol extracts of bark showed the highest inhibition, 46% and 52% compared with the control, respectively. Meanwhile, through computational study of 5-hydroxy-7,4'-dimethoxyflavone exhibited the highest inhibition and predicted as a competitive lipase inhibitor. Besides, the leaf of methanolic extract showed the strongest DPPH radical scavenger with IC₅₀ value of $29.56 \pm 6.37 \mu\text{g/mL}$. However, the tested isolated compounds showed less ability in antioxidant activity. In cytotoxic activity, the leaf and bark of methanolic extracts exhibited highest anticancer activity with IC₅₀ values of 24.30 ± 0.07 and $17.46 \pm 0.08 \mu\text{g/mL}$, respectively. Besides, 5-hydroxy-7,4'-dimethoxyflavone showed the highest cytotoxicity activity with IC₅₀ value of $38.48 \pm 0.06 \mu\text{g/mL}$. In conclusion, this study successfully revealed the presence of various phytochemicals in *A. subintegra* and their potential as anti-lipase, antioxidant and anticancer agents. In implication, this study enhances knowledge on the diversity of chemical compounds and their potential in modern medicine.



FITOKIMIA DAN AKTIVITI BIOLOGI DARIPADA *Aquilaria subintegra* Ding Hou

ABSTRAK

Kajian bertujuan untuk mengenal pasti fitokimia dan aktiviti biologi daripada kulit batang dan daun *Aquilaria subintegra* Ding Hou. Sampel dikering, dikisar dan diekstrak secara pengekstrakan dingin menggunakan pelarut *n*-heksana, diklorometana dan metanol secara berturutan. Ekstrak mentah kulit batang dan daun dipisahkan melalui teknik kromatografi untuk mendapatkan sebatian tulen. Struktur kimia sebatian tulen dikenalpasti menggunakan kaedah spektroskopi seperti resonans magnet nukleus, ultra lembayung, infra merah dan spektrometri jisim dan perbandingan daripada kajian lepas. Ekstrak mentah dan sebatian tulen diuji terhadap aktiviti anti-lipase, antioksidan dan antikanser masing-masingnya menerusi ujian warna, DPPH radikal bebas dan assai kesitotoksikan. Kajian ini berjaya mengasingkan 22 sebatian termasuk tiga sebatian baharu iaitu aquilen A, aquilen B dan subintegranol. Sebatian lain adalah 5-hidroksi-7,4'-dimetoksiflavin, luteolin-7,3',4'-trimetil eter, 5,3'-dihidroksi-7,4'-dimetoksiflavin, 7,3'-dimetoksi luteolin, 5,7-dihidroksi-4'-metoksiflavin, β -sitosterol, β -sitostenon, stigmasterol, fridelin, epifridelanol, phytol dan hentriakontan. Aktiviti anti-lipase ke atas ekstrak *n*-heksana daun dan ekstrak metanol kulit batang masing-masingnya menunjukkan perencatan tertinggi, 46% dan 52% berbanding kawalan. Manakala, kajian simulasi berkomputer terhadap sebatian 5-hidroksi-7,4'-dimetoksiflavin mempamerkan perencatan tertinggi dan diramalkan sebagai perencat lipase yang kompetitif. Selain itu, ekstrak daun mentah metanol menunjukkan aktiviti perencatan radikal DPPH yang kuat dengan nilai IC_{50} 29.56 ± 6.37 $\mu\text{g/mL}$. Namun, sebatian tulen yang diuji mempamerkan kebolehan yang rendah bagi aktiviti antioksidan. Bagi aktiviti sitotoksik, ekstrak metanol daun dan kulit batang masing-masingnya menunjukkan aktiviti antikanser yang kuat dengan nilai IC_{50} , 24.30 ± 0.07 dan 17.46 ± 0.08 $\mu\text{g/mL}$. Selain itu, 5-hidroksi-7,4'-dimetoksiflavin menunjukkan kesan sitotoksik yang kuat dengan nilai IC_{50} 38.48 ± 0.06 $\mu\text{g/mL}$. Kesimpulannya, kajian ini berjaya mendedahkan kepelbagaian fitokimia daripada *A. subintegra* dan potensinya sebagai agen anti-lipase, antioksidan dan antikanser. Implikasinya, kajian dapat meningkatkan pengetahuan mengenai kepelbagaian sebatian kimia dalam tumbuhan serta potensinya dalam perubatan moden.



CONTENTS

	Page	
ACKNOWLEDGEMENT	iv	
ABSTRACT	v	
ABSTRAK	vi	
CONTENTS	vii	
LIST OF TABLES	xiii	
LIST OF FIGURES	xvi	
LIST OF SCHEMES	xxii	
LIST OF ABBREVIATIONS	xxiii	
LIST OF APPENDICES	xxvi	
CHAPTER 1	INTRODUCTION	1
1.1	General Introduction	1
1.2	Problem Statement	6
1.3	Significance of Study	7
1.4	Objectives of Study	7
CHAPTER 2	LITERATURE REVIEW	9
2.1	Thymelaeaceae Family	9
2.2	Genera <i>Aquilaria</i>	12

2.2.1	<i>Aquilaria subintegra</i>	17
2.3	Phytochemical Studies of <i>Aquilaria</i> species	19
2.3.1	Phenolic Compounds	19
2.3.1.1	Flavonoids	22
2.3.1.2	Reported Flavonoids in <i>Aquilaria</i> Species	26
2.3.2	Terpenes	30
2.3.2.1	Monoterpene	33
2.3.2.2	Sesquiterpenes	34
2.3.2.3	Diterpenes	35
2.3.2.4	Triterpenes and Steroids	37
2.3.2.5	Terpenes in <i>Aquilaria</i> Species	42
2.3.3	Miscellaneous Phytochemicals Reported in <i>Aquilaria</i> species	47
2.4.	Pharmacological Studies of Chronic Disease	55
2.4.1	Obesity	55
2.4.2	Oxidant or Oxidative Stress	57
2.4.3	Cancer	59
2.5	Pharmacological Properties of <i>Aquilaria</i> species	62
2.5.1	Antioxidant Property	62
2.5.2	Anti-Obesity Property	63
2.5.3	Anticancer Property	64
2.5.4	Other Biological Properties	64

CHAPTER 3 METHODOLOGY 68

3.1	General Procedures	68
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3.1.1	Chemical Reagents	70
3.2	Plant Materials	71
3.3	Extraction of <i>Aquilaria subintegra</i>	71
3.4	Isolation and Purification	75
3.5	Chemical Constituents from Bark of <i>A. subintegra</i>	76
3.5.1	Physical and Spectral Data of Isolated Chemical Constituents from Bark	78
3.5.1.1	ASBD1: 5-Hydroxy-7,4'-dimethoxyflavone (36)	78
3.5.1.2	ASBD2: Luteolin 7,3',4'-trimethylether (37)	78
3.5.1.3	ASBD3: 5,3'-Dihydroxy-7,4'-dimethoxy flavone (41)	79
3.5.1.4	ASBD4: 7,3'-Dimethoxy luteolin (185)	80
3.5.1.5	ASBD5: β -Sitosterol (101)	80
3.5.1.6	ASBD6: β -Sitostenone (139)	81
3.5.1.7	ASBD7: Saturated triglyceride fatty acid (186)	82
3.5.1.8	ASBD8: 2,6-Dimethoxyquinone (187)	82
3.6	Chemical Constituents from <i>A. subintegra</i> Leaf	83
3.6.1	Physical and Spectral Data of Isolated Chemical Constituents from Leaf	85
3.6.1.1	ASLD1: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol) (74)	85
3.6.1.2	ASLD2: Friedelin (188)	85
3.6.1.3	ASLD3: Epifriedelanol (189)	86
3.6.1.4	ASLD4: Stigmasterol (102)	87
3.6.1.5	ASLD5: Hentriacontane (190)	87
3.6.1.6	ASLD6: 5,7-Dihydroxy-4'-methoxyflavone (39)	88

3.6.1.7	ASLD7: Aquilene A (191)	88
3.6.1.8	ASLD8: Aquilene B (192)	89
3.6.1.9	ASLD9: Subintegranol (193)	89
3.6.1.10	ASLD10: Saturated triglyceride fatty acid (186)	90
3.6.1.11	ASLD11: β -sitosterol (101)	91
3.6.1.12	ASLD12: β -sitostenone (139)	91
3.6.1.13	ASLD13: 5-Hydroxy-7,4'-dimethoxyflavone (36)	91
3.6.1.14	ASLD14: 5,3'-Dihydroxy-7,4'-dimethoxy flavone (41)	92
3.7	Bioactivity Studies	92
3.7.1	Antioxidant Activity	92
3.7.1.1	Instruments and Materials	93
3.7.1.2	Chemicals	93
3.7.1.3	DPPH radical scavenging activity	93
3.7.2	Anti-lipase Activity	95
3.7.2.1	Instruments and Apparatus	95
3.7.2.2	Chemical Reagents	96
3.7.2.3	Standard Curves of Free Fatty Acid	96
3.7.2.4	Assay for inhibitory pancreatic lipase activity	97
3.7.3	Anticancer Activity	99
3.7.3.1	Materials and Apparatus	99
3.7.3.2	Cytotoxic Assay	99
3.8	Mechanism of inhibition of pancreatic lipase activity	100
3.8.1	Molecular Docking	100

CHAPTER 4	RESULTS AND DISCUSSION	103
4.1	Phytochemical Studies	103
4.2	Chemical Constituents Isolated from bark of <i>A. subinterga</i>	106
4.2.1	ASBD1: 5-Hydroxy-7,4'-dimethoxyflavone (36)	106
4.2.2	ASBD2: Luteolin-7,3',4'-trimethyl ether (37)	112
4.2.3	ASDB3: 5,3'-Dihydroxy-7,4'-dimethoxyflavone (41)	117
4.2.4	ASDB4: 7,3'-Dimethoxy luteolin (185)	123
4.2.5	ASDB5: β -sitosterol (101)	128
4.2.6	ASBD6: β -sitostenone (139)	134
4.2.7	ASBD7: Saturated triglyceride fatty acid (186)	139
4.2.8	ASBD8: 2,6-Dimethoxyquinone (187)	147
4.3	Chemical Constituents Isolated from leaf of <i>A. subinterga</i>	152
4.3.1	ASLD1: 3,7,11,15 – Tetramethyl – 2 – hexadecen – 1 – ol (phytol) (74)	152
4.3.2	ASLD2: Friedelin (188)	157
4.3.3	ASLD3: Epifriedelanol (189)	162
4.3.4	ASLD4: Stigmasterol (102)	167
4.3.5	ASLD5: Hentriacontane (190)	172
4.3.6	ASLD6: 5,7-Dihydroxy-4'-methoxyflavone (39)	176
4.3.7	ASLD7: Aquilene A (191)	181
4.3.8	ASLD8: Aquilene B (192)	194
4.3.9	ASLD9: Subintegranol (193)	204
4.3.10	ASLD10: Saturated triglyceride fatty acid (186)	217
4.3.11	ASLD11: β -Sitosterol (101)	217

4.3.12	ASLD12: β -Sitostenone (139)	218
4.3.13	ASLD13: 5-Hydroxy-7,4'-dimethoxyflavone (36)	218
4.3.14	ASLD14: 5,3'-Dihydroxy-7,4'-dimethoxyflavone (41)	218
4.4	Biological Activity	219
4.4.1	Anti-lipase activity	219
4.4.2	Molecular Docking Study	226
4.4.2.1	Docking Study of Isolated Compound	228
4.4.3	Antioxidant Activity	233
4.4.3.1	DPPH Free Radical Scavenging Assay	233
4.4.4	Anticancer Activity	239
CHAPTER 5 CONCLUSION AND RECOMMENDATIONS		242
5.1	Conclusion	242
5.2	Recommendations for Future Study	245
REFERENCES		247

LIST OF TABLES

Table No.		Page
1.1	Medicinal plants reported in Malaysia	3
2.1	The chronology of changes of taxonomy affinities in family Thymelaeaceae and genus <i>Aquilaria</i>	10
2.2	Distribution of <i>Aquilaria</i> species	13
2.3	Medicinal uses of various <i>Aquilaria</i> species	17
2.4	Classes of Polyphenolic Compounds	21
2.5	Chemical structures of flavonoid groups	24
2.6	The classification of terpenes based on the number of isoprene units in their structure	33
2.7	Examples of diterpenes in various species	35
2.8	Other phytochemicals isolated from <i>Aquilaria</i> species	47
2.9	Pharmacological activities of several phytochemicals from <i>Aquilaria</i> species	66
3.1	Percentage yield and physical property of the crude extracts of <i>A. subintegra</i>	72
4.1	Phytochemicals isolated from bark and leaf of <i>A. subintegra</i>	105
4.2	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of ASBD1 and 5-hydroxy-7,4'-dimethoxyflavone (36)	111
4.3	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD2 and luteolin-7,3',4'-trimethyl ether (37)	116
4.4	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD3 and 5,3'-dihydroxy-7,4'-dimethoxyflavone (41)	122

4.5	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD4 and 7,3'-dimethoxyluteolin (185)	127
4.6	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD5 and β-sitosterol (101)	132
4.7	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD6 and β-sitostenone (139)	137
4.8	¹ H, ¹³ C and 2D-NMR spectral data of compound ASBD7 and triglycerides (FA) (186)	144
4.9	¹ H-NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectral data of compound ASBD8 and 2,6-dimethoxyquinone (187)	151
4.10	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectra data of compound ASLD1 and 3,7,11,15 – tetramethyl – 2 – hexadecen – 1 – ol (phytol) (74)	155
4.11	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectra data of compound ASLD2 and friedelin (188)	161
4.12	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectra data of compound ASLD3 and epifriedelanol (189)	166
4.13	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectra data of compound ASLD4 and stigmasterol (102)	171
4.14	¹ H NMR (500 MHz, CDCl ₃) and ¹³ C (125 MHz, CDCl ₃) spectra data of compound ASLD5 and hentriacontane (190)	175
4.15	¹ H NMR (500 MHz, CDCl ₃ +CD ₃ OD) spectral data of compound ASLD6 and 5,7-dihydroxy-4'-methoxyflavone (39)	179
4.16	¹ H (500 MHz, CDCl ₃), ¹³ C (125 MHz, CDCl ₃), and 2D-NMR spectral data of compound ASLD7 (191)	192
4.17	¹ H (500 MHz, CDCl ₃), ¹³ C (125 MHz, CDCl ₃), and 2D-NMR spectral data of compound ASLD8 (192)	202
4.18	¹ H (500 MHz, CDCl ₃), ¹³ C (125 MHz, CDCl ₃), and 2D-NMR spectral data of compound ASLD9 (193)	215
4.19	Binding energies and residues involved in binding of the isolated compound with PPL	230
4.20	Hydrogen bonds between isolated compounds and PPL	230

4.21	The DPPH radical scavenging activity of crude extracts of <i>A. subintegra</i> in comparison with the ascorbic acid standard	236
4.22	The DPPH radical scavenging activity of isolated compounds of <i>A. subintegra</i> in comparison with the ascorbic acid standard	237
4.23	Cytotoxicity of the crude extract against MCF-7's breast cancer cell with an IC ₅₀ value	240
4.24	Cytotoxicity of the isolated compound against MCF-7's breast cancer cell with an IC ₅₀ value	241

LIST OF FIGURES

Figure No.		Page
2.1	Distribution of <i>Aquilaria</i> species in Indomalesia region	15
2.2	(a) Bark, (b) leaf and (c) herbarium specimen <i>A. subintegra</i>	18
2.3	Basic skeleton of flavonoid	23
2.4	Biosynthesis of terpenes and their inhibitors	32
2.5	Examples of monoterpene compounds	33
2.6	Skeleton of sesquiterpenes	34
2.7	Skeleton of triterpenes: lanostane (78), dammarane (79), tetranortriterpenoid (80), fridelane (81), lupine (82), oleanane (83), ursane (84), hopane (85)	38
2.8	Backbone skeleton of steroid	39
2.9	A different skeleton of steroids; pregnane (86), cholestane (87), stigmastane (88), ergostane (89), cholane (90) and androstane (91)	39
2.10	The biosynthesis of sterols and triterpenes	41
3.1	Flow process of the colorimetric assay	98
3.2	The structure of PPL-CLP-TGME (PDB-ID: 1ETH) obtained from PDB	101
4.1	LC-MS spectrum of Compound ASBD1	107
4.2	IR spectrum of compound ASBD1	108
4.3	UV spectrum of compound ASBD1	108
4.4	¹ H-NMR spectrum of compound ASBD1	109

4.5	^{13}C -NMR spectrum of compound ASBD1	110
4.6	UV spectrum of compound ASBD2	113
4.7	IR spectrum of compound ASBD2	113
4.8	LC-MS spectrum of compound ASBD2	114
4.9	^1H -NMR spectrum of compound ASBD2	115
4.10	^{13}C -NMR spectrum of compound ASBD2	116
4.11	UV spectrum of compound ASBD3	118
4.12	IR spectrum of compound ASBD3	119
4.13	LC-MS spectrum of compound ASBD3	119
4.14	^1H -NMR spectrum of compound ASBD3	121
4.15	^{13}C -NMR spectrum of compound ASBD3	121
4.16	LC-MS spectrum of compound ASBD4	123
4.17	IR spectrum of compound ASBD4	124
4.18	UV spectrum of compound ASBD4	125
4.19	^1H -NMR spectrum of compound ASBD4	126
4.20	^{13}C -NMR spectrum of compound ASBD4	127
4.21	LC-MS spectrum of compound ASBD5	129
4.22	IR spectrum of compound ASBD5	130
4.23	^1H NMR spectrum of compound ASBD5	131
4.24	^{13}C -NMR spectrum of compound ASBD5	132
4.25	IR spectrum of compound ASBD6	135
4.26	LC-MS spectrum of compound ASBD6	135
4.27	^1H -NMR spectrum of Compound ASBD6	136
4.28	^{13}C -NMR spectrum of compound ASBD6	137

4.29	LC-MS spectrum of compound ASBD7	140
4.30	IR spectrum of compound ASBD7	140
4.31	¹ H-NMR spectrum of compound ASBD7	141
4.32	¹³ C-NMR spectrum of compound ASBD7	142
4.33	COSY spectrum of compound ASBD7	145
4.34	HMQC spectrum of compound ASBD7	145
4.35	HMBC spectrum of compound ASBD7	146
4.36	COSY and HMBC correlations of compound ASBD7	146
4.37	IR spectrum of compound ASBD8	148
4.38	UV spectrum of compound ASBD8	148
4.39	LC-MS spectrum of compound ASBD8	149
4.40	¹ H-NMR spectrum of compound ASBD8	150
4.41	¹³ C-NMR spectrum of compound ASBD8	150
4.42	IR spectrum of compound ASLD1	153
4.43	LC-MS spectrum of compound ASLD1	153
4.44	¹ H-NMR spectrum of compound ASLD1	155
4.45	¹³ C-NMR spectrum of compound ASLD1	155
4.46	IR spectrum of compound ASLD2	158
4.47	LC-MS spectrum of compound ASLD2	158
4.48	¹ H-NMR spectrum of compound ASLD2	159
4.49	¹³ C-NMR spectrum of compound ASLD2	160
4.50	IR spectrum of compound ASLD3	163
4.51	LC-MS spectrum of compound ASLD3	163
4.52	¹ H-NMR spectrum of compound ASLD3	164

4.53	^{13}C -NMR spectrum of compound ASLD3	165
4.54	IR spectrum of compound ASLD4	168
4.55	LC-MS spectrum of compound ASLD4	168
4.56	^1H -NMR spectrum of compound ASLD4	170
4.57	^{13}C -NMR spectrum of compound ASLD4	170
4.58	IR spectrum of compound ASLD5	173
4.59	LC-MS spectrum of compound ASLD5	173
4.60	^1H -NMR spectrum of compound ASLD5	174
4.61	^{13}C -NMR spectrum of compound ASLD5	175
4.62	UV spectrum of compound ASLD6	177
4.63	IR spectrum of compound ASLD6	177
4.64	LC-MS spectrum of compound ASLD6	178
4.65	^1H -NMR spectrum of compound ASLD6	179
4.66	IR spectrum of compound ASLD7	182
4.67	LC-MS spectrum of compound ASLD7	182
4.68	^1H -NMR spectrum of compound ASLD7	183
4.69	^{13}C -NMR spectrum of compound ASLD7	184
4.70	DEPT spectrum of compound ASLD7	185
4.71	COSY spectrum of compound ASLD7	187
4.72	TOCSY spectrum of compound ASLD7	188
4.73	HMQC spectrum of compound ASLD7	189
4.74	HMBC spectrum of compound ASLD7	190
4.75	Expanded HMBC spectrum of compound ASLD7	191
4.76	COSY, ^1H and ^{13}C correlations observed in HMBC spectrum of ASLD7	192

4.77	IR spectrum of compound ASLD8	195
4.78	LC-MS spectrum of compound ASLD8	195
4.79	¹ H-NMR spectrum of compound ASLD8	197
4.80	¹³ C-NMR spectrum of compound ASLD8	197
4.81	COSY spectrum of compound ASLD8	199
4.82	HMQC spectrum of compound ASLD8	200
4.83	HMBC spectrum of compound ASLD8	201
4.84	COSY, ¹ H and ¹³ C correlations observed in HMBC spectrum of ASLD7	202
4.85	IR spectrum of compound ASLD9	205
4.86	LC-MS spectrum of compound ASLD9	205
4.87	¹ H-NMR spectrum of compound ASLD9	206
4.88	¹³ C-NMR spectrum of compound ASLD9	208
4.89	DEPT spectrum of compound ASLD9	208
4.90	COSY spectrum of compound ASLD9	211
4.91	HMQC spectrum of compound ASLD9	212
4.92	HMBC spectrum of compound ASLD9	213
4.93	COSY, ¹ H and ¹³ C correlations observed in HMBC spectrum of ASLD9	214
4.94	The percentage of lipase activity (U/mL) of six crude extracts from <i>A. subintegra</i> and PPL (positive control). Results stated as mean ± SD (n=3), *p<0.05	222
4.95	Percentage (%) lipase activity of isolated compounds and PPL (positive control). Results stated as mean ± SD (n=3), *p<0.05	224
4.96	Ligand molecules docked onto the PPL receptor. Ligands are shown in the stick model meanwhile active site of PPL shown in yellow colour.	229

4.97	The percentage of inhibition of DPPH radicals by different concentrations of extracts in comparison to ascorbic acid standard. Data expressed as mean \pm SD, n=3.	235
4.98	The percentage of inhibition of DPPH radicals by different concentrations of the isolated compound in comparison to ascorbic acid standard. Data expressed as mean \pm SD, n=3.	237
5.1	Summary phytochemical isolated from bark and leaf of <i>A. subintegra</i>	243

LIST OF SCHEMES

Scheme No.		Page
3.1	Extraction procedure from the bark of <i>Aquilaria subintegra</i>	73
3.2	Extraction procedure from the leaf of <i>Aquilaria subintegra</i>	74
3.3	Flow chart for the purification process of <i>A. subintegra</i> bark extracts	77
3.4	Flow chart for the purification process of <i>A. subintegra</i> leaf extracts	84

LIST OF ABBREVIATIONS

α	-	Alpha
AA	-	Ascorbic acid
Abs	-	Absorbance
ASBH	-	<i>n</i> -hexane crude of bark <i>A. subintegra</i>
ASBD	-	Dichloromethane crude of bark <i>A. subintegra</i>
ASBM	-	Methanol crude of bark <i>A. subintegra</i>
ASLH	-	<i>n</i> -hexane crude of leaf <i>A. subintegra</i>
ASLD	-	Dichloromethane crude of leaf <i>A. subintegra</i>
ASLM	-	Methanol crude of leaf <i>A. subintegra</i>
β	-	Beta
br	-	broad
δ	-	Chemical Shift
CC	-	Column Chromatography
^{13}C -NMR	-	Carbon NMR
CDCl_3	-	Chloroform-D
CD_3OD	-	Methanol-D
CH_2Cl_2	-	Dichloromethane
COSY	-	Correlation spectroscopy
1D	-	1-dimensional
2D	-	2-dimensional
<i>d</i>	-	Doublet
<i>dd</i>	-	Doublet of doublet
DEPT	-	Distortionless Enhancement by Polarization Transfer
DMSO	-	Dimethyl Sulfoxide
DL	-	Dried Leaves
DPPH	-	2,2-diphenyl-1-picrylhy-drazyl
EIMS	-	Electron Ionization Mass Spectrometry

ESI-MS	-	Electron Spray Ionization Mass Spectrometry
EtOAc	-	Ethyl acetate
<i>n</i> -Hex	-	Hexane
HCl	-	Hydrochloric acid
H ₂ SO ₄	-	Sulfuric acid
Hz	-	Hertz
¹ H-NMR	-	Proton NMR
HMBC	-	Heteronuclear Multiple Bond Correlation
HMQC	-	Heteronuclear Multiple Quantum Coherence
IR	-	Infrared
<i>J</i>	-	Coupling constant
<i>M</i>	-	Multiplet
MeOH	-	Methanol
<i>m/z</i>	-	Mass to charge ion
MS	-	Mass Spectrometry
mg	-	miligram
mL	-	mililiter
min	-	minute
NMR	-	Nuclear Magnetic Resonance
nm	-	nanometer
PPL	-	Porcine Pancreatic Lipase
<i>s</i>	-	Singlet
sec	-	second
SD	-	Standard deviation
SiO ₂	-	Silica gel
<i>t</i>	-	triplet
TLC	-	Thin Layer Chromatography
UV	-	Ultra Violet
μL	-	microliter
μmole	-	micromole
PHE	-	Phenylalanine
ILE	-	Isoleucine

ASP	-	Aspartate
TYR	-	Tyrosine
HIS	-	Histidine
SER	-	Serine
LEU	-	Leucine
ALA	-	Alanine
PRO	-	Proline
ILE	-	Isoleucine
PHE	-	Phenylalanine
TRP	-	Tryptophan
ARG	-	Arginine
VAL	-	Valine
GLU	-	Glutamate
THR	-	Threonine

LIST OF APPENDICES

- A Standard Curve of Free Fatty Acid
- B Anti-lipase activity of crude extracts and isolated compounds of *A. subintegra*
- C 2-D illustration of docking study using Ligplot+
- D Standard Curve of Antioxidant Activity
- E Dose-response Plot Based on Non-linear Regression Analysis
- F Publication(s) to be derived from this work
- G Conference Attendance

CHAPTER 1

INTRODUCTION

1.1 General Introduction

Natural products (NPs) give a massive impact on science because of the discovery of plentiful medicinal drugs precursor (Crane & Gademann, 2016). NPs universally refer to substances which are isolated from living organism, and it has usually formed in secondary metabolites. NPs have always been a keystone in finding new lead molecules for drug candidates (Orhan, 2016). They are representing a vast family of diverse chemical constituents with their valuable biological activities, particularly in human, veterinary medicine, and agriculture, as well as significant agents in pharmaceuticals, herbicides, and insecticides (Katz & Baltz, 2016). NPs have brought significant effects of economic due to their role in pharmaceuticals, and fragrances (Anulika, Ignatius, Raymond, Osasere, & Abiola, 2016).

Historically, since 60,000 years ago, NPs such as plants, animals, microorganisms and marine organisms have been used as a medicinal treatment. However it faced a massive challenge in the early stage to the humans because of the consumption risks such as vomiting, diarrhoea, coma or other toxic reactions and perhaps led to the death; still, early humans knowledgeable about edible materials and natural medicines (Yuan, Ma, Ye, & Piao, 2016).

The NPs such as bacterial, fungal, and the plant has served as front line therapeutics to treat diseases. For example, antibiotics, chemotherapeutics, immunosuppressants, cholesterol-lowering agents and anaesthetics. These molecules derive from large groups of chemicals including polyketides, nonribosomal peptides, saccharides, alkaloids, terpenoids, and comprise a staggering diversity of chemical scaffolds (Medema & Fischbach, 2015; Tang, Zou, Watanabe, Walsh, & Tang, 2017).

Today, natural medicines have been used principally in medical treatment, especially in developing countries (Greenwell & Rahman, 2015). Herbal plants, either one part (flowers, leaves, branches or roots) or entire parts of it might use in the treatment of acute and chronic diseases. Also, in the development of dietary products due to continuous advantages, showed minimal side effect contrasting to synthetic medicine which contributes a severe impact for the long term uses (Farzaneh & Carvalho, 2015). Therefore, in the 21st century, medicinal herbs gained attention as an alternative way to replace the usage of synthetic drugs.

The statistical data from the World Health Organization (WHO), showed about 80% of people in the world depend on traditional herbal medicine for their health care needs. Traditional Chinese Medicine, Indian Ayurvedic Medicine, and Unani Medicine of Arab cultures were globally used as references for herbal medicine (Rehman, Choe, & Yoo, 2016).

Most researchers attract Malaysian herbs due to the massive areas of rainforest and tropical weather. In Malaysia, approximated 15,000 known plant species, in which 2,000 species have medicinal values, and the rest species are not exploited and cultivated (Saad et al., 2015). Some examples of medicinal plants reported in Malaysia shown in Table 1.1 (Madaleno, 2015).

Medicinal plants reported in Malaysia

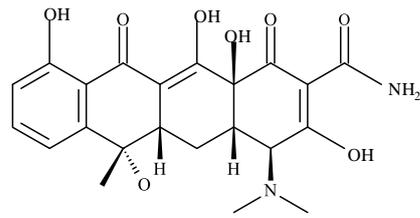
Plant Species	Part	Medicinal action
<i>Strobilanthes crispus</i> (L.) Bremek (Pecah Kaca)	Leaf	Anticancer, antioxidant, antidiabetic
<i>Cananga odorata</i> (Lam.) Hook. F. & Thomson (Ylang-ylang)	Flower	As a relaxing body oil
<i>Cuminum cyminum</i> L. (Cumin)	Seed	Herbal supplement for fatigue
<i>Cocos nucifera</i> L. (Chamomile)	Fruit	As a healing oil
<i>Plectranthus barbatus</i> Andrews (Long Pepper)	Whole plant	Healing body oils

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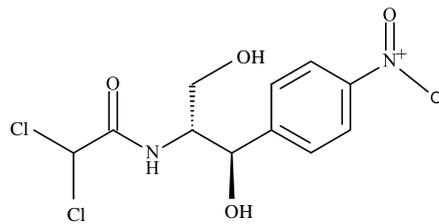
Table 1.1 (continued)

Plant Species	Part	Medicinal action
<i>Pinellia nti-in</i> (Thunb.) Ten. Ex Breitenb. (Musli)	Rhizome	Chinese remedy for cough and cold
<i>Mentha arvensis</i> L. (Jamun)	Leaf	Chinese remedy for pimples, skin rashes and bruises
<i>Cinnamomum camphora</i> (L.) J. Presl (PolygalaRhubarb)	Bark, leaf	As nti-inflammatory cream for itching and burns

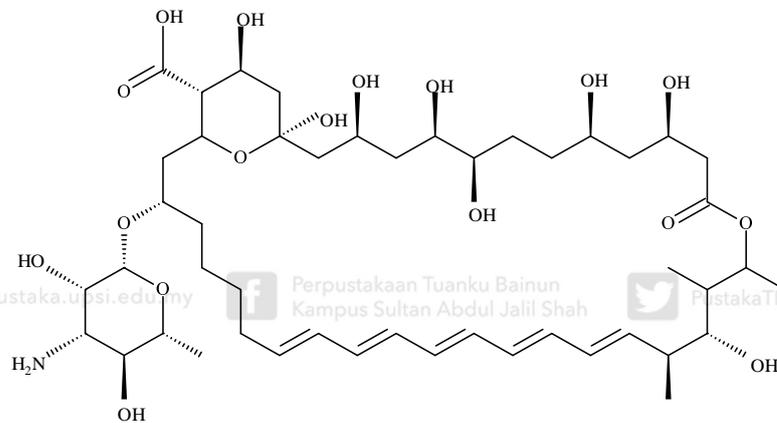
The US Food and Drug Administration approved 1453 new NPs in the year 2013, and prominently used in medicinal treatment. For example, tetracycline (1) and chloramphenicol (2) as antibacterial, amphotericin B (3) (antifungal and antiparasitic), mitomycin C (4) and daunorubicin (5) as antitumor agents (Katz & Baltz, 2016). Thus, the significance of NPs are undoubtedly enormous and contribute massive benefits. Therefore, the scientist worldwide has a vital responsibility to identify medicinal plants which valuable to commercialize as herbal medicines.



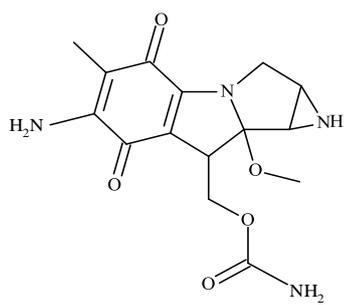
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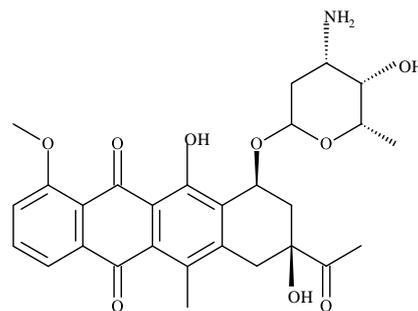
(2)



(3)



(4)



(5)

1.2 Problem Statement

Aquilaria sp. is a fragrant tree which widely distributed in Asia including, Malaysia, Thailand, and Indonesia. This species was valuable due to the production of scented and aromatic wood which known as gaharu or agarwood. Besides that, agarwood tea was produced from *Aquilaria* leaves which mainly manufactured in China, Malaysia, and Indonesia. Yet, there is no official report commenced date of agarwood tea became as a commercial product, however, in 2007, there was first reported (Wu et al., 2007) toward toxicological safety of agarwood tea in the local market Hainan, China (Adam, Lee, & Mohamed, 2017).

However, there is a deficiency information specific population of *A. subintegra*.

A. subintegra has found in Thailand, Narathiwat province, with an altitude range between 300 -500 m asl (Lee & Mohamed, 2016).

Furthermore, there is a limited number of phytochemical and pharmacological studies of *A. subintegra*. To the best of our knowledge, there is a tiny reference for isolation of *A. subintegra* in the phytochemical study. Besides, an inadequate study on the pharmacological study of the isolated compound from *A. subintegra* triggered us to reveal the chemical constituents and expected to obtained new bioactive potential compounds from this plant.

1.3 Significance of Study

This study revealed the chemical constituents obtained for the first time and examined their biological activities. Current results would be able to enhance knowledge, especially on species of study, the diversity of its chemical compounds derived from this plant, and its potential as a precursor to modern medicine.

1.4 Objectives of Study

The main objectives of the current study are:

- i-  To extract, isolate, and purify chemical constituents from the crude extracts of *A. subintegra* bark and leaf using chromatographic techniques.
- ii- To identify and elucidate the chemical structures of the isolated compounds using several spectroscopic analyses such as nuclear magnetic resonances (NMR), ultraviolet-visible spectroscopy (UV), Fourier transform infrared spectroscopy (FTIR) and mass spectrometry (MS).
- iii- To investigate biological properties including antioxidant, anti-lipase, and anticancer toward crude extracts and isolated compounds. Antioxidant activity was determined using DPPH free radical scavenging assay. The anti-lipase activity was measured by colorimetric assay; meanwhile,

anticancer activity was examined using Cell Counting Kit-8 (CCK-8)/
WST-8 toward breast cancer cell, MCF-7.

- iv- To determine the interaction of isolated compounds with receptor using
computational study.