



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi

PHYTOCHEMICAL ANALYSIS OF *Murraya koenigii* (RUTACEAE)
AND *IN VITRO* CYTOTOXIC ACTIVITY OF ITS ISOLATED COMPOUNDS
AND THE SYNTHETIC ANALOGUES OF GIRINIMBINE AND MAHANIMBINE

TAN SIOW PING



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2015



05-4506832



pustaka.upsi.edu.my



Perpustakaan Tuanku Bainun
Kampus Sultan Abdul Jalil Shah



PustakaTBainun



ptbupsi



ABSTRACT

The objectives of the study are to determine the phytochemical constituents from the bark and leaves of *Murraya koenigii*, to develop synthetic analogues from girinimbine and mahanimbine via Lewis acid-mediated reactions, and to evaluate cytotoxic activities. The isolation and purification of the chemical compounds were done by using various chromatography methodologies. These compounds were then structurally identified by various spectroscopic techniques such as NMR, IR, UV and mass spectrometry. *In vitro* cytotoxic activities were evaluated against human promyelocytic leukemia (HL-60), cervical cancer cell lines (HeLa) and the normal mouse embryonic fibroblasts (NIH/3T3) cell lines via MTT assay. As the findings, phytochemical analysis on bark and leaves of *M. koenigii* afforded a total of 36 chemical compounds included six new carbazoles, viz., murrastanine-A, murrastanine-A, -B and -C, murrayatanine-A and bismahanimboline. Besides, two girinimbine analogues, viz., murranimbine and epoxygirinimbine; and three mahanimbine analogues, viz., bicyclomahanimbine, cyclomahanimbine and murrayazoline, were successfully derived. Five carbazoles and two non-carbazoles have shown very strong to moderate *in vitro* cytotoxic activities ($CD_{50} < 20 \mu\text{g/mL}$) against both HL-60 and HeLa cell lines, viz., murrayafoline-A, mahanine, murrayamine-J, murrastanine-C, murrayatanine-A, β -sitosterol and 2-hydroxy-4-methoxy-3,6-dimethylbenzoic acid. In conclusion, the isolated compounds from *M. koenigii* were found to possess potential *in vitro* cytotoxic activities against selected cancer cell lines. In fact, most of these results were first time reported. These findings have shown that *M. koenigii* is an important source for therapeutic discovery and may lead to the development of potential drugs.





ANALISIS FITOKIMIA *Murraya koenigii* (RUTACEAE) DAN AKTIVITI SITOTOKSIK *IN VITRO* BAGI SEBATIAN KIMIA YANG DIPENCILKAN DAN ANALOG SINTETIK GIRINIMBINA DAN MAHANIMBINA

ABSTRAK

Kajian ini bertujuan menentukan kandungan fitokimia daripada kulit kayu dan daun *Murraya koenigii*, membina analog sintetik daripada girinimbina dan mahanimbina menggunakan asid Lewis sebagai pengantara, dan menguji aktiviti sitotoksik. Pemencilan dan penulenan sebatian kimia dijalankan dengan menggunakan pelbagai kaedah kromatografi. Struktur sebatian kemudiannya dikenalpasti melalui teknik spektroskopi iaitu NMR, IR, UV dan spektrometri jisim. Aktiviti sitotoksik *in vitro* diuji ke atas sel promielositik leukemia manusia (HL-60), sel kanser servik (HeLa) dan sel normal tikus fibroblas embrio (NIH/3T3) dengan rawatan MTT. Dapatan kajian menunjukkan sebanyak 36 sebatian kimia termasuk enam karbazola baharu, iaitu murrastanina-A, murrastinina-A, -B dan -C, murrayatanina-A dan bismahanimbolina telah diperolehi. Selain itu, dua analog girinimbina, iaitu murranimbina dan epoksiginimbina dan tiga analog mahanimbina, iaitu bisiklomahanimbina, siklomahanimbina dan murrayazolinina, telah berjaya disintesis. Lima karbazola dan dua sebatian bukan karbazola menunjukkan aktiviti sitotoksik yang sangat aktif ke sederhana aktif ($CD_{50} < 20 \mu\text{g/mL}$) terhadap kedua-dua sel HL-60 dan sel HeLa, iaitu murrayafolina-A, mahanina, murrayamina-J, murrastinina-C, murrayatanina-A, β -sitosterol dan asid 2-hidroksi-4-metoksi-3,6-dimetilbenzoik. Kesimpulannya, sebatian yang dipencilkan daripada *M. koenigii* didapati mempunyai potensi aktiviti sitotoksik *in vitro* ke atas sel kanser yang dipilih. Malah, sebahagian besar daripada keputusan ini dilaporkan pertama kali. Hasil kajian menunjukkan *M. koenigii* merupakan sumber penting bagi penemuan terapeutik dan membawa kepada pembangunan ubat-ubatan yang berpotensi.



**CONTENTS**

	PAGE
DECLARATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
ABSTRAK	v
CONTENTS	vi
LIST OF TABLES	xi
LIST OF FIGURES	xv
LIST OF SCHEMES	xxv
ABBREVIATIONS	xxvii

**CHAPTER 1 INTRODUCTION**

1.1	General Introduction	1
1.2	Objectives of Study	5
1.3	Significance of Study	6

CHAPTER 2 LITERATURE REVIEW

2.1	The Plant - <i>Murraya koenigii</i> (Linn.) Spreng	8
2.1.1	Morphological Characteristics	9
2.1.2	Ethnobotanical Uses	13
2.2	Chemical Aspects	15
2.2.1	Carbazole Alkaloids	15
2.2.1.1	Occurrence and Structure Diversity	16
2.2.1.2	Biosynthesis	20



2.2.2	Terpenes	27
2.2.2.1	Occurrence and Structure Diversity	27
2.2.2.2	Biosynthesis	33
2.3	Phytochemistry and Pharmacological Aspects of <i>M. koenigii</i>	39
2.3.1	Phytochemistry	39
2.3.2	Pharmacological Properties	59

CHAPTER 3 MATERIALS AND METHODS

3.1	Phytochemical Analysis on <i>Murraya koenigii</i>	68
3.1.1	General Methods	68
3.1.2	Chemical Reagents	71
3.1.3	Plant Materials	72
3.1.4	Extraction Methods	72
3.1.5	Isolation and Purification	76
3.1.5.1	Chemical Constituents from Bark of <i>M. koenigii</i>	77
3.1.5.2	Chemical Constituents from Leaves of <i>M. koenigii</i>	83
3.1.6	Physical and Spectral Data of Isolated Chemical Constituents from <i>M. koenigii</i>	88
3.2	Development of Synthetic Analogues of Girinimbine and Mahanimbine	118
3.2.1	General Introduction	118
3.2.2	Oxidative Coupling/Cyclization Reaction Using Anhydrous Ferric (III) Chloride	121
3.2.3	Oxidative Coupling/Cyclization Reaction Using Boron Trifluoride Diethyletherate	124
3.2.4	Physical and Spectral Data of the Synthetic Analogues of	127

Girinimbine and Mahanimbine

3.3	Biological Activity	132
3.3.1	General Introduction	132
3.3.2	Materials and Chemical Reagents	133
3.3.3	Culture of Cells	134
3.3.4	Cytotoxic Assay	136

CHAPTER 4 RESULTS AND DISCUSSION

4.1	General Introduction	138
4.2	Chemical Constituents Isolated from <i>M. koenigii</i>	141
4.2.1	Tricyclic Carbazole Alkaloids	145
4.2.1.1	Murrayanine (Mk1), Murrayafoline-A (Mk2), Koenoline (Mk3) and 3-Formylcarbazole (Mk4)	145
4.2.1.2	2-Hydroxy-3-methylcarbazole (Mk5)	157
4.2.1.3	Mahanimbilol (Mk6), Euchrestine-B (Mk7) and Mukoenine-B (Mk8)	160
4.2.1.4	Murrastanine-A (Mk9)	169
4.2.2	Carbazolequinone Alkaloid	178
4.2.2.1	Murrayaquinone-A (Mk10) and Murrayaquinone-B (Mk11)	178
4.2.3	Pyrano[3,2-a]carbazole Alkaloids	185
4.2.3.1	Girinimbine (Mk12), Koenimbine (Mk13) and Murrayacine (Mk14)	185

4.2.3.2	Mahanimbine (Mk15), Mahanine (Mk16), Isomahanine (Mk17), Murrayamine-B (Mk18) and Murrayamine-J (Mk19)	195
4.2.3.3	Murrastinine-A (Mk20)	208
4.2.3.4	Murrastinine-B (Mk21)	219
4.2.3.5	Murrastinine-C (Mk22)	228
4.2.4	Cyclic Monoterpenoid Pyrano[3,2-a]carbazole Alkaloids	238
4.2.4.1	Murrayazolinol (Mk23) and Murrayakoeninol (Mk24)	238
4.2.4.2	Bicyclomahanimbine (Mk25)	245
4.2.4.3	Murrayatanine-A (Mk26)	249
4.2.5	Biscarbazole Alkaloids	260
4.2.5.1	Bismurrayafoline-E (Mk27)	260
4.2.5.2	Bispyrayafoline (Mk28)	264
4.2.5.3	Bisisomahanine (Mk29)	268
4.2.5.4	Bismahanimboline (Mk30)	272
4.2.6	Others	283
4.2.6.1	β -Sitosterol (Mk31)	283
4.2.6.2	Stigmast-4-ene-3-one (Mk32)	287
4.2.6.3	Squalene (Mk33)	291
4.2.6.4	α -Tocopherol (Mk34)	294
4.2.6.5	2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (Mk35)	298
4.2.6.6	Selin-11-en-4 α -ol (Mk36)	301
4.3	Synthesis of Girinimbine Analogues	304

4.3.1	Spectroscopic Analysis of Murranimbine (G1) and	305
-------	--	-----

Epoxygirinimbine (G2)

4.3.2	Mechanistic Studies	323
4.4	Synthesis of Mahanimbine Analogues	326
4.4.1	Spectroscopic Analysis of Bicyclomahanimbine (M1), Cyclomahanimbine (M2) and Murrayazolinine (M3)	327
4.4.2	Mechanistic Studies	346
4.5	Biological Activity	348
4.5.1	Cytotoxic Assay on the Isolated Chemical Constituents of <i>M. koenigii</i>	349
4.5.2	Cytotoxic Assay on the Synthetic Analogues of Girinimbine and Mahanimbine	357
4.5.3	Structure-activity Relationship (SAR) Analysis on Carbazole	359

Alkaloids

CHAPTER 5	CONCLUSIONS AND RECOMMANDATIONS	365
5.1	Introduction	365
5.2	Conclusions	365
5.3	Recommendations for Future Research	370

REFERENCES	372
-------------------	-----

APPENDICES	394
-------------------	-----

LIST OF TABLES

TABLE	PAGE
2.1 The Classification of Terpenes	32
2.2 Carbazole Alkaloids Isolated from <i>M. koenigii</i>	41
2.3 Pharmacological Properties of Carbazole Alkaloids Isolated from <i>M. koenigii</i>	65
3.1 Yields of Plant Materials and Crude Extracts	73
3.2 Synthesis of Girinimbine Analogues with Anhydrous FeCl ₃	122
3.3 Synthesis of Mahanimbine Analogues with Anhydrous FeCl ₃	123
3.4 Synthesis of Girinimbine Analogues with BF ₃ -O(C ₂ H ₅) ₂	125
3.5 Synthesis of Mahanimbine Analogues with BF ₃ -O(C ₂ H ₅) ₂	126
4.2 The Chemical Constituents Isolated from <i>M. koenigii</i>	142
4.2.1.1a ¹ H NMR [500 MHz, δ _H (J Hz)] of Murrayanine (Mk1), Murrayafoline-A (Mk2), Koenoline (Mk3) and 3-Formylcarbazole (Mk4)	151
4.2.1.1b ¹³ C NMR [125 MHz, δ _C] of Murrayanine (Mk1), Murrayafoline-A (Mk2), Koenoline (Mk3) and 3-Formylcarbazole (Mk4)	152
4.2.1.2 ¹ H NMR [500 MHz, δ _H (J Hz)] and ¹³ C NMR [125 MHz, δ _C] of 2-Hydroxy-3-methylcarbazole (Mk5)	158
4.2.1.3a ¹ H NMR [500 MHz, δ _H (J Hz)] of Mahanimbilol (Mk6), Euchrestine-B (Mk7) and Mukoenine-B (Mk8)	164
4.2.1.3b ¹³ C NMR [125 MHz, δ _C] of Mahanimbilol (Mk6), Euchrestine-B (Mk7) and Mukoenine-B (Mk8)	165
4.2.1.4 1D (¹ H, ¹³ C and DEPT) and 2D (COSY, HMQC and HMBC) NMR	172

Spectral Data of Murrastanine-A (**Mk9**)

4.2.2.1a ^1H NMR [500 MHz, δ_{H} (*J* Hz)] of Murrayaquinone-A (**Mk10**) and 181
Murrayaquinone-B (**Mk11**)

4.2.2.1b ^{13}C NMR [125 MHz, δ_{C}] of Murrayaquinone-A (**Mk10**) and 182
Murrayaquinone-B (**Mk11**)

4.2.3.1a ^1H NMR [500 MHz, δ_{H} (*J* Hz)] of Girinimbine (**Mk12**), 190
Koenimbine (**Mk13**) and Murrayacine (**Mk14**)

4.2.3.1b ^{13}C NMR [125 MHz, δ_{C}] of Girinimbine (**Mk12**), Koenimbine 191
(**Mk13**) and Murrayacine (**Mk14**)

4.2.3.2a ^1H NMR [500 MHz, δ_{H} (*J* Hz)] of Mahanimbine (**Mk15**), 201
Mahanine (**Mk16**), Isomahanine (**Mk17**), Murrayamine-B (**Mk18**)
and Murrayamine-J (**Mk19**)

4.2.3.2b ^{13}C NMR [125 MHz, δ_{C}] of Mahanimbine (**Mk15**), Mahanine 202
(**Mk16**), Isomahanine (**Mk17**), Murrayamine-B (**Mk18**) and
Murrayamine-J (**Mk19**)

4.2.3.3 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 212
Spectral Data of Murrastanine-A (**Mk20**)

4.2.3.4 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 222
Spectral Data of Murrastanine-B (**Mk21**)

4.2.3.5 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 232
Spectral Data of Murrastanine-C (**Mk22**)

4.2.4.1a ^1H NMR [500 MHz, δ_{H} (*J* Hz)] of Murrayazolinol (**Mk23**) and 241
Murrayakoeninol (**Mk24**)

4.2.4.1b ^{13}C NMR [125 MHz, δ_{H} (*J* Hz)] of Murrayazolinol (**Mk23**) and 242

Murrayakoeninol (**Mk24**)

4.2.4.2 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 247

Bicyclomahanimbine (**Mk25**)

4.2.4.3 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 253

Spectral Data of Murrayatanine-A (**Mk26**)

4.2.5.1 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 262

Bismurrayafoline-E (**Mk27**)

4.2.5.2 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 266

Bispyrayafoline (**Mk28**)

4.2.5.3 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 270

Bisisomahanine (**Mk29**)

4.2.5.4 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 276

Spectral Data of Bismahanimboline (**Mk30**)

4.2.6.1 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of β - 285

Sitosterol (**Mk31**)

4.2.6.2 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 289

Stigmast-4-ene-3-one (**Mk32**)

4.2.6.3 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 292

Squalene (**Mk33**)

4.2.6.4 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of α - 296

Tocopherol (**Mk34**)

4.2.6.5 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 2- 299

Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (**Mk35**)

4.2.6.6 ^1H NMR [500 MHz, δ_{H} (J Hz)] and ^{13}C NMR [125 MHz, δ_{C}] of 302



Selin-11-en-4 α -ol (**Mk36**)

- 4.3.1 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 309

Spectral Data of **G1**

- 4.3.2 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 316

Spectral Data of **G2**

- 4.4.1 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 331

Spectral Data of **M1**

- 4.4.2 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 336

Spectral Data of **M2**

- 4.4.3 1D (^1H , ^{13}C and DEPT) and 2D (COSY, HMQC and HMBC) NMR 341

Spectral Data of **M3**

- 4.5.1 CD_{50} Values ($\mu\text{g/mL}$) for Cytotoxic Activity of Carbazole 350



Alkaloids Isolated from *M. koenigii*

- 4.5.2 CD_{50} Values ($\mu\text{g/mL}$) for Cytotoxic Activity of the Synthetic 357

Analogues of Girinimbine and Mahanimbine



LIST OF FIGURES

FIGURE	PAGE
2.1 Bark of <i>M. koenigii</i>	11
2.2 Roots of <i>M. koenigii</i>	11
2.3 Leaves and Flowers of <i>M. koenigii</i>	12
2.4 Fruits of <i>M. koenigii</i>	12
3.1 NIH/3T3 Cell Lines	135
3.2 HL-60 Cell Lines	135
3.3 HeLa Cell Lines	135
4.2.1.1a ¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayanine (Mk1)	153
4.2.1.1b ¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayanine (Mk1)	153
4.2.1.1c ¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayafoline-A (Mk2)	154
4.2.1.1d ¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayafoline-A (Mk2)	154
4.2.1.1e ¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Koenoline (Mk3)	155
4.2.1.1f ¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Koenoline (Mk3)	155
4.2.1.1g ¹ H NMR Spectrum (CD ₃ OD, 500 MHz) of 3-Formylcarbazole (Mk4)	156
4.2.1.1h ¹³ C NMR Spectrum (CD ₃ OD, 125 MHz) of 3-Formylcarbazole (Mk4)	156
4.2.1.2a ¹ H NMR Spectrum (CD ₃ OD, 500 MHz) of 2-Hydroxy-3-methylcarbazole (Mk5)	159
4.2.1.2b ¹³ C NMR Spectrum (CD ₃ OD, 125 MHz) of 2-Hydroxy-3-methylcarbazole (Mk5)	159
4.2.1.3a ¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Mahanimbilol (Mk6)	166

 05-4506832			 pustaka.upsi.edu.my	 Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah	 PustakaTBainun	 ptbupsi
4.2.1.3b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Mahanimbilol (Mk6)	166				
4.2.1.3c	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Euchrestine-B (Mk7)	167				
4.2.1.3d	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Euchrestine-B (Mk7)	167				
4.2.1.3e	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Mukoenine-B (Mk8)	168				
4.2.1.3f	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Mukoenine-B (Mk8)	168				
4.2.1.4a	¹ H NMR Spectrum (CD ₃ OD, 500 MHz) of Murrastanine-A (Mk9)	173				
4.2.1.4b	NOE NMR Spectrum (CD ₃ OD, 500 MHz) of Murrastanine-A (Mk9)	173				
4.2.1.4c	¹³ C NMR Spectrum (CD ₃ OD, 125 MHz) of Murrastanine-A (Mk9)	174				
4.2.1.4d	DEPT-135 Spectrum (CD ₃ OD, 125 MHz) of Murrastanine-A (Mk9)	174				
4.2.1.4e	HMQC Spectrum (CD ₃ OD, 500 MHz) of Murrastanine-A (Mk9)	175				
4.2.1.4f	¹ H- ¹ H COSY Spectrum (CD ₃ OD, 500 MHz) of Murrastanine-A (Mk9)	175				
4.2.1.4g	HMBC Spectrum (CD ₃ OD, 500 MHz) of Murrastanine-A (Mk9)	176				
4.2.1.4h	Selected ¹ H- ¹ H COSY and HMBCs of Mk9	176				
4.2.1.4i	IR Spectrum of Murrastanine-A (Mk9)	177				
4.2.1.4j	Mass Spectrum of Murrastanine-A (Mk9)	177				
4.2.2.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayaquinone-A (Mk10)	183				
4.2.2.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayaquinone-A (Mk10)	183				
4.2.2.1c	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayaquinone-B (Mk11)	184				

 05-4506832  pustaka.upsi.edu.my  Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah  PustakaTBainun  ptbupsi			
4.2.2.1d	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayaquinone-B	184	
	(Mk11)		
4.2.3.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Girinimbine	192	
	(Mk12)		
4.2.3.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Girinimbine	192	
	(Mk12)		
4.2.3.1c	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Koenimbine	193	
	(Mk13)		
4.2.3.1d	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Koenimbine	193	
	(Mk13)		
4.2.3.1e	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayacine	194	
	(Mk14)		
4.2.3.1f	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayacine	194	
	(Mk14)		
4.2.3.2a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Mahanimbine	203	
	(Mk15)		
4.2.3.2b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Mahanimbine	203	
	(Mk15)		
4.2.3.2c	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Mahanine	204	
	(Mk16)		
4.2.3.2d	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Mahanine	204	
	(Mk16)		
4.2.3.2e	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Isomahanine	205	
	(Mk17)		
4.2.3.2f	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Isomahanine	205	
	(Mk17)		
4.2.3.2g	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayamine-B	206	
	(Mk18)		
4.2.3.2h	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayamine-B	206	
	(Mk18)		
4.2.3.2i	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayamine-J	207	
	(Mk19)		
4.2.3.2j	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayamine-J	207	
	(Mk19)		
4.2.3.3a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A	213	
	(Mk20)		
4.2.3.3b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-A	213	
	(Mk20)		
4.2.3.3c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-A	214	
	(Mk20)		
4.2.3.3d	HMQC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A	214	
	(Mk20)		
4.2.3.3e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A	215	
	(Mk20)		

05-4506832			pustaka.upsi.edu.my	Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah	PustakaTBainun	ptbupsi
4.2.3.3f	HMBC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A (Mk20)	215				
4.2.3.3g	TOCSY Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A (Mk20)	216				
4.2.3.3h	NOESY Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-A (Mk20)	216				
4.2.3.3i	Selected ¹ H- ¹ H COSY and HMBCs of Mk20	217				
4.2.3.3j	Selected NOESY Correlations and Relative Stereochemistry for Mk20	217				
4.2.3.3k	IR Spectrum of Murrastinine-A (Mk20)	218				
4.2.3.3l	Mass Spectrum of Murrastinine-A (Mk20)	218				
4.2.3.4a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-B (Mk21)	223				
4.2.3.4b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-B (Mk21)	223				
4.2.3.4c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-B (Mk21)	224				
4.2.3.4d	HMQC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-B (Mk21)	224				
4.2.3.4e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-B (Mk21)	225				
4.2.3.4f	HMBC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-B (Mk21)	225				
4.2.3.4g	Selected ¹ H- ¹ H COSY and HMBCs of Mk21	226				
4.2.3.4h	IR Spectrum of Murrastinine-B (Mk21)	226				
4.2.3.4i	Mass Spectrum of Murrastinine-B (Mk21)	227				
4.2.3.5a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-C (Mk22)	233				
4.2.3.5b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-C (Mk22)	233				
4.2.3.5c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of Murrastinine-C (Mk22)	234				
4.2.3.5d	HMQC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-C (Mk22)	234				
4.2.3.5e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-C (Mk22)	235				

 05-4506832			 pustaka.upsi.edu.my	 Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah	 PustakaTBainun	 ptbupsi
4.2.3.5f	HMBC Spectrum (CDCl ₃ , 500 MHz) of Murrastinine-B (Mk22)	235				
4.2.3.5g	Selected ¹ H- ¹ H COSY and HMBCs of Mk22	236				
4.2.3.5h	IR Spectrum of Murrastinine-C (Mk22)	236				
4.2.3.5i	Mass Spectrum of Murrastinine-C (Mk22)	237				
4.2.4.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayazolinol (Mk23)	243				
4.2.4.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayazolinol (Mk23)	243				
4.2.4.1c	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayakoeninol (Mk24)	244				
4.2.4.1d	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayakoeninol (Mk24)	244				
4.2.4.2a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Bicyclomahanimbine (Mk25)	248				
4.2.4.2b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Bicyclomahanimbine (Mk25)	248				
4.2.4.3a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	254				
4.2.4.3b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Murrayatanine-A (Mk26)	254				
4.2.4.3c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of Murrayatanine-A (Mk26)	255				
4.2.4.3d	HMQC Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	255				
4.2.4.3e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	256				
4.2.4.3f	HMBC Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	256				
4.2.4.3g	NOESY Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	257				
4.2.4.3h	Expanded NOESY Spectrum (CDCl ₃ , 500 MHz) of Murrayatanine-A (Mk26)	257				

**A (Mk26)**4.2.4.3i Selected ^1H - ^1H COSY and HMBCs of **Mk26** 2584.2.4.3j Selected NOESY Correlations and Relative Stereochemistry for **Mk26** 2584.2.4.3k IR Spectrum of Murrayatanine-A (**Mk26**) 2594.2.4.3l Mass Spectrum of Murrayatanine-A (**Mk26**) 2594.2.5.1a ^1H NMR Spectrum (CDCl_3 , 500 MHz) of Bismurrayafoline-E 263**(Mk27)**4.2.5.1b ^{13}C NMR Spectrum (CDCl_3 , 125 MHz) of Bismurrayafoline-E 263**(Mk27)**4.2.5.2a ^1H NMR Spectrum (CDCl_3 , 500 MHz) of Bispyrayafoline (**Mk28**) 2674.2.5.2b ^{13}C NMR Spectrum (CDCl_3 , 125 MHz) of Bispyrayafoline (**Mk28**) 2674.2.5.3a ^1H NMR Spectrum (CDCl_3 , 500 MHz) of Bisisomahanine (**Mk29**) 2714.2.5.3b ^{13}C NMR Spectrum (CDCl_3 , 125 MHz) of Bisisomahanine (**Mk29**) 2714.2.5.4a ^1H NMR Spectrum (CDCl_3 , 500 MHz) of Bismahanimboline 277**(Mk30)**4.2.5.4b ^{13}C NMR Spectrum (CDCl_3 , 125 MHz) of Bismahanimboline 277**(Mk30)**4.2.5.4c DEPT-135 Spectrum (CDCl_3 , 125 MHz) of Bismahanimboline 278**(Mk30)**4.2.5.4d HMQC Spectrum (CDCl_3 , 500 MHz) of Bismahanimboline (**Mk30**) 2784.2.5.4e ^1H - ^1H COSY Spectrum (CDCl_3 , 500 MHz) of Bismahanimboline 279**(Mk30)**4.2.5.4f HMBC Spectrum (CDCl_3 , 500 MHz) of Bismahanimboline (**Mk30**) 279

 05-4506832  pustaka.upsi.edu.my  Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah  PustakaTBainun  ptbupsi		
4.2.5.4g	NOESY Spectrum (CDCl ₃ , 500 MHz) of Bismahanimboline (Mk30)	280
4.2.5.4h	Selected ¹ H- ¹ H COSY and HMBCs of Mk30	280
4.2.5.4i	Selected NOESY Correlations and Relative Stereochemistry for Mk30	281
4.2.5.4j	IR Spectrum of Bismahanimboline (Mk30)	281
4.2.5.4k	Mass Spectrum of Bismahanimboline (Mk30)	282
4.2.6.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of β-Sitosterol (Mk31)	286
4.2.6.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of β-Sitosterol (Mk31)	286
4.2.6.2a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Stigmast-4-ene-3-one (Mk32)	290
4.2.6.2b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Stigmast-4-ene-3-one (Mk32)	290
4.2.6.3a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Squalene (Mk33)	293
4.2.6.3b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Squalene (Mk33)	293
4.2.6.4a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of α-Tocopherol (Mk34)	297
4.2.6.4b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of α-Tocopherol (Mk34)	297
4.2.6.5a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of 2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (Mk35)	300
4.2.6.5b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of 2-Hydroxy-4-methoxy-3,6-dimethylbenzoic acid (Mk35)	300
4.2.6.6a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of Selin-11-en-4α-ol (Mk35)	303
4.2.6.6b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of Selin-11-en-4α-ol (Mk35)	303

(Mk34)

4.3.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of G1	310
4.3.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of G1	310
4.3.1c	Expanded ¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of G1	311
4.3.1d	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of G1	311
4.3.1e	HMQC Spectrum (CDCl ₃ , 500 MHz) of G1	312
4.3.1f	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of G1	312
4.3.1g	HMBC Spectrum (CDCl ₃ , 500 MHz) of G1	313
4.3.1h	Expanded HMBC Spectrum (CDCl ₃ , 500 MHz) of G1	313
4.3.1i	NOESY Spectrum (CDCl ₃ , 500 MHz) of G1	314
4.3.1j	Selected ¹ H- ¹ H COSY and HMBCs of G1	314
4.3.1k	Selected NOESY Correlations and Relative Stereochemistry for G1	315
4.3.2a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of G2	317
4.3.2b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of G2	317
4.3.2c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of G2	318
4.3.2d	HMQC Spectrum (CDCl ₃ , 500 MHz) of G2	318
4.3.2e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of G2	319
4.3.2f	HMBC Spectrum (CDCl ₃ , 500 MHz) of G2	319
4.3.2g	NOESY Spectrum (CDCl ₃ , 500 MHz) of G2	320
4.3.2h	Selected ¹ H- ¹ H COSY and HMBCs of G2	320
4.3.2i	Selected NOESY Correlations and Relative Stereochemistry for G2	321
4.3.2j	IR Spectrum of Epoxygirinimbine (G2)	321
4.3.2k	Mass Spectrum of Epoxygirinimbine (G2)	322
4.4.1a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of M1	332

 05-4506832			 pustaka.upsi.edu.my	 Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah	 PustakaTBainun	 ptbupsi
4.4.1b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of M1					332
4.4.1c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of M1					333
4.4.1d	HMQC Spectrum (CDCl ₃ , 500 MHz) of M1					333
4.4.1e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of M1					334
4.4.1f	HMBC Spectrum (CDCl ₃ , 500 MHz) of M1					334
4.4.1g	Selected ¹ H- ¹ H COSY and HMBCs of M1					335
4.4.2a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of M2					337
4.4.2b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of M2					337
4.4.2c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of M2					338
4.4.2d	HMQC Spectrum (CDCl ₃ , 500 MHz) of M2					338
4.4.2e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of M2					339
4.4.2f	HMBC Spectrum (CDCl ₃ , 500 MHz) of M2					339
4.4.2g	Selected ¹ H- ¹ H COSY and HMBCs of M2					340
4.4.3a	¹ H NMR Spectrum (CDCl ₃ , 500 MHz) of M3					342
4.4.3b	¹³ C NMR Spectrum (CDCl ₃ , 125 MHz) of M3					342
4.4.3c	DEPT-135 Spectrum (CDCl ₃ , 125 MHz) of M3					343
4.4.3d	HMQC Spectrum (CDCl ₃ , 500 MHz) of M3					343
4.4.3e	¹ H- ¹ H COSY Spectrum (CDCl ₃ , 500 MHz) of M3					344
4.4.3f	HMBC Spectrum (CDCl ₃ , 500 MHz) of M3					344
4.4.3g	Selected ¹ H- ¹ H COSY and HMBCs of M3					345
4.5.1	Cytotoxic Effects (CD ₅₀) of Isolated Carbazole Alkaloids against HL-60 and HeLa along with NIH/3T3 Cell Lines via MTT Assay					352
4.5.2	Cytotoxic Effects (CD ₅₀) of Synthetic Analogues of Girinimbine and Mahanimbine against HL-60 and HeLa along with NIH/3T3					358

Cell Lines via MTT Assay

4.5.3 Structure of Tricyclic Carbazole Alkaloids and Their 360
Corresponding CD₅₀ Values against HL-60 and HeLa Cell Lines

4.5.4 Structure of Carbazolequinone Alkaloids and Their Corresponding 360
CD₅₀ Values against HL-60 and HeLa Cell Lines

4.5.5 Structure of Pyrano[3,2-a]carbazole Alkaloids and Their 361
Corresponding CD₅₀ Values against HL-60 and HeLa Cell Lines

4.5.6 Structure of Cyclic Monoterpenoid Pyrano[3,2-a]carbazole 363
Alkaloids and Their Corresponding CD₅₀ Values against HL-60 and
HeLa Cell Lines

4.5.7 Structure of Biscarbazole Alkaloids and Their Corresponding CD₅₀ 363
Values against HL-60 and HeLa Cell Lines

4.5.8 Important Structural Features of Carbazole Alkaloids for Cytotoxic 364
Activities against HL-60 and HeLa Cell Lines

LIST OF SCHEMES

SCHEME	PAGE
2.1 Shikimate Pathway	21
2.2 Biogenetic Pathway to Carbazole Through <i>N</i> -phenylated Anthranilic Acid	23
2.3 Narasimhan's Approach Biogenetic Pathway	24
2.4 Biogenetic Pathway to Carbazole Alkaloid Derivatives	25
2.5 Terpene Family	33
2.6 The Biogenesis Pathway from Aceto-CoA to the Formation of Geranyl Pyrophosphate (GPP)	35
2.7 Biosynthesis of Terpene Family	36
2.8 Biosynthesis of Triterpenes	38
3.1 Extraction Process of <i>Murraya koenigii</i> (Bark)	74
3.2 Extraction Process of <i>Murraya koenigii</i> (Leaves)	75
3.3 Isolation of Chemical Constituents of Hexane Crude Extract (Bark)	80
3.4 Isolation of Chemical Constituents of Dichloromethane Crude Extract (Bark)	81
3.5 Isolation of Chemical Constituents of Methanol Crude Extract (Bark)	82
3.6 Isolation of Chemical Constituents of Hexane Crude Extract (Leaves)	86
3.7 Isolation of Chemical Constituents of Dichloromethane Crude Extract (Leaves)	87
3.8 Synthesis of Girinimbine Analogues with Anhydrous FeCl ₃	122
3.9 Synthesis of Mahanimbine Analogues with Anhydrous FeCl ₃	123



3.10	Synthesis of Girinimbine Analogues with $\text{BF}_3\text{-O}(\text{C}_2\text{H}_5)_2$	124
3.11	Synthesis of Mahanimbine Analogues with $\text{BF}_3\text{-O}(\text{C}_2\text{H}_5)_2$	125
4.2.1.4	Proposed Biogenetic Pathway to Mk9	171
4.2.3.3	Proposed Biogenetic Pathway to Mk20	211
4.2.3.4	Proposed Biogenetic Pathway to Mk21	221
4.2.3.5	Proposed Biogenetic Pathway to Mk22	231
4.2.4.3	Proposed Biogenetic Pathway to Mk26	252
4.2.5.4	Proposed Biogenetic Pathway to Mk30	275
4.3.1	A Possible Mechanistic Pathway to G1	324
4.3.2	A Possible Mechanistic Pathway to G2	325
4.4.1	A Possible Mechanistic Pathway to M1, M2 and M3 . a) Anhydrous FeCl_3 , 25 °C; b) $\text{BF}_3\text{-O}(\text{C}_2\text{H}_5)_2$, 25 °C	347

