



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
KAEMPFERIA ANGUSTIFOLIA, K. ROTUNDA, SPERMACOCE
ARTICULARIS AND S. EXILIS**

NEOH BEE KEAT.

FS 2006 12



**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
KAEMPFERIA ANGUSTIFOLIA, *K. ROTUNDA*, *SPERMACOCE ARTICULARIS*
AND *S. EXILIS***

By

NEOH BEE KEAT

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

January 2006



Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
KAEMPFERIA ANGUSTIFOLIA, *K. ROTUNDA*, *SPERMACOCE
ARTICULARIS* AND *S. EXILIS***

By

NEOH BEE KEAT

January 2006

Chairman: Associate Professor Mohd Aspollah Hj. Sukari, PhD

Faculty: Science

Kaempferia angustifolia, *K. rotunda* (Zingiberaceae), *Spermacoce articularis* and *S. exilis* (Rubiaceae) were chosen since they have long been used traditionally in medicinal treatment. In this research, isolation and separation work had been carried out using various solvents and chromatographic methods. The structure of these compounds were determined by using spectroscopic methods such as infrared (IR), nuclear magnetic resonance (^1H , ^{13}C and 2D- NMR), mass spectrometry (MS) and by comparison with the data reported previously. The crude extracts and some of the isolated compounds were subjected to the biological activity test: antioxidant, anti-microbial tests, cytotoxic screening and larvicidal activity.



Extracts from *Kaempferia angustifolia* have yielded a new compound angustifolienol (58), together with seven other compounds; crotepoxide (33), β -sitosterol (44), boesenboxide (34), 2'-hydroxy-4, 4', 6'-trimethoxychalcone (36), 6-methylzeylenol (59), and sucrose (60), and zeylenol (35). Fractionation of extracts from *K. rotunda* has yielded five pure compounds; crotepoxide (33), tetracosanoic acid (61), benzoic acid (62), stigmasterol (45) and β -sitosterol (44). Both tetracosanoic acid (61) and benzoic acid (62) are new to species. Extracts from *S. articularis* gave two pure compounds, identified as ursolic acid (63) and stigmasterol (45). While the extracts from *S. exilis* gave four compounds, benzo[g]isoquinoline-5,10-dione (64), stigmasterol (45), hexadecanoic acid (65) and ursolic acid (63). Phytochemical studies on later species had never been reported before.

Acetylations of new compound, angustifolienol (58) gave two derivatives, triacetoxiangustifolienol (66) and diacetoxiangustifolienol (67). Both are new compounds, and have never been reported previously either as natural product or synthesized compounds.

The major constituents of the essential oil of *K. angustifolia* was DL- camphor (20.57 %), while the major constituent of *K. rotunda* was benzyl benzoate (49.84 %). The major constituent of the essential oil of *S. articularis* was phytol (10.31 %), while the major constituent of *S. exilis* was oleic acid (20.9 %).



Cytotoxic screening showed that all the crude extracts of *K. angustifolia* and *K. rotunda* were not cytotoxic against HL-60 cell line. However, pure compounds of *K. angustifolia* showed strong activity. All crude extracts of *S. articularis* and *S. exilis* (except hexane extract of *S. exilis*) and ursolic acid (63) were strongly cytotoxic towards HL-60 and MCF-7 cell lines. All extracts and most of the pure compounds were subjected to four strains of microbes (MRSA, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*) in the antimicrobial test. Pet. ether extracts from *Kaempferia* species, all extracts from *S. exilis* and boesenboxide (34) were active towards some microbes. In antioxidant assay, *K. rotunda* was more active than *Kaempferia angustifolia* while both *Spermacoce* species were moderately active. Besides that, some of the compounds from *Kaempferia angustifolia* were subjected to the antioxidant assay and showed stronger antioxidant activity than α -tocopherol. In addition, all plants also showed moderate to low larvicidal activity.



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

**CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF
KAEMPFERIA ANGUSTIFOLIA, *K. ROTUNDA*, *SPERMACOCE
ARTICULARIS* AND *S. EXILIS***

Oleh

NEOH BEE KEAT

Januari 2006

Pengerusi: Profesor Madya Mohd Aspollah Hj. Sukari, PhD

Fakulti: Sains

Kaempferia angustifolia, *K. rotunda* (Zingiberaceae), *Spermacoce articularis* dan *S. exilis* (Rubiaceae) telah dipilih sebagai bahan kajian kerana telah lama digunakan sebagai ubatan tradisional. Dalam kajian ini, kerja pemencilan dijalankan menggunakan pelbagai pelarut dan kaedah kromatografi. Struktur sebatian dikenalpasti dengan kaedah spektroskopi seperti inframerah (IR), resonans magnet nukleus (^1H , ^{13}C and 2D NMR) dan spektroskopi jisim (MS) dan perbandingan dengan data yang telah dilaporkan. Ujian biocerakinan seperti ujian antioksidan, ujian antimikrob, ujian sitotoksik dan ujian aktiviti larva telah dilakukan ke atas kesemua ekstrak dan sebahagian sebatian tulen.

Ekstrak dari *K. angustifolia* menghasilkan satu sebatian baru angustifolienol (58), bersama tujuh sebatian lain; kroteposida (33), β - sitosterol (44), boesenboksida (34), 2'-hidroksi-4, 4', 6'-trimetoksikalkon (36), 6-metoksizeylenol (59), sukrosa (60) dan zeylenol (35). Pemencilan ekstrak- ekstrak dari *K. rotunda* menghasilkan lima sebatian tulen, krotepoksida (33), asid tetrakosanoik (61), asid benzoik (62), stigmasterol (45) dan β - sitosterol (44). Pemencilan kedua- dua sebatian asid tetrakosanoik (61) dan asid benzoik (62) belum pernah dilaporkan sebelum ini. Ekstrak dari *S. articularis* menghasilkan dua sebatian tulen iaitu asid ursolik (63) dan stigmasterol (45), sementara ekstrak *S. exilis* menghasilkan empat sebatian tulen iaitu benzo[g]isokuinolin-5,10-dion (64), stigmasterol (45), asid heksadekanoik (65) and asid ursolik (63). Kerja pemencilan ke atas *S. exilis* tidak pernah dilaporkan sebelum ini.

Proses pengasetatan ke atas sebatian baru angustifolienol (58) menghasilkan dua sebatian, triasetoksiangustifolienol (66) dan diasetoksiangustifolienol (67). Kedua- dua sebatian disyorkan sebagai sebatian baru dan tidak pernah dilaporkan sama ada sebagai hasil semulajadi atau sebatian hasil sintesis.

Sebatian utama dari minyak pati *K. angustifolia* adalah DL- kamfor dengan hasil 20.57 %, sementara sebatian utama *K. rotunda* adalah benzil benzoat dengan hasil 49.84 %. Sebatian utama dari minyak pati *S. articularis* adalah fitol dengan hasil 10.31 %, sementara sebatian utama *S. exilis* adalah asid oleik dengan hasil 20.93 %.

Ujian sitotoksik menunjukkan kesemua ekstrak dari *K. angustifolia* dan *K. rotunda* tidak aktif terhadap sel HL-60. Walau bagaimanapun, sebatian tulen dari *K. angustifolia* adalah aktif. Semua ekstrak *S. articularis* dan *S. exilis* (kecuali ekstrak heksana *S. exilis*) dan asid ursolik (63) sangat aktif terhadap sel HL-60 dan MCF-7. Semua ekstrak dan kebanyakan sebatian tulen disubjek kepada empat spesies mikrob (MRSA, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis*) dalam ujian anti- mikrob. Ekstrak petroleum eter dari kedua-dua spesis *Kaempferia* kesemua ekstrak dari spesis *Spermacoce* dan boesenboksida (34) adalah aktif terhadap sebilangan mikrob dalam aktiviti antimikrob. Dalam ujian antioksidasi, *K. rotunda* adalah lebih aktif berbanding *K. angustifolia* sementara kedua-dua spesis *Spermacoce* adalah sederhana aktif. Sebahagian daripada sebatian tulen dari *Kaempferia angustifolia* telah dijalani ujian antioksidasi dan menunjukkan aktiviti yang lebih kuat daripada aktiviti α -tokoferol. Keempat –empat tumbuhan menunjukkan keaktifan yang rendah ke sederhana dalam ujian aktiviti larva.

ACKNOWLEDGEMENT

I would like to express my appreciation to my supervisor, Assoc. Prof. Dr. Mohd Aspollah B. Hj. Sukari for his intellectual advice and for supplying me with materials required for this research. My sincere thanks and deepest appreciation is also extended to Assoc. Prof. Dr. Gwendoline Ee Cheng Lian for all her invaluable advises and guidance throughout the project. I'm also indebted to Prof. Dr. Mohd. Nordin B. Lajis and Prof. Dr. Abdul Manaf B. Ali for their invaluable suggestions. Financial support from PASCA and IRPA program are also greatly appreciated.

I wish to express my sincere gratitude to Dr. Nor Azah Mohamad Ali and En. Abu Said Ahmad from FRIM for their help on carried out the identification of essential oils. I am also indebted to my laboratory mates, especially Dr. Sugeng Riyanto, Puan Nur Yuhasliza Rashid, Wan Noor Fazliyatul Wahida Abd. Jalil, Suhaila Saad and Siti Zuraida Yaakob. They have made many useful suggestions and encouragement to me throughout my research. My special thanks also go to all the staff of Chemistry Department, especially Puan Rusnaini Bt. Amirudin, En. Zainal Kassim, En. Ahmad Zainuddin, En. Zainal Zahari B. Zakaria and En. Abas B. Abd. Rahman for all their help and co-operation during this research.

Lastly, I am grateful to my parents and family members, Chai Seng, Yvonne and beloved friends, whose help, encouragement and support were invaluable which I will never forget.




I certify that an Examination Committee met on 24th January 2006 to conduct the final examination of Neoh Bee Keat on her Doctor of Philosophy Thesis entitled “Chemical Constituents and Biological Activity of *Kaempferia angustifolia*, *K. rotunda*, *Spermacoce articularis* and *S. exilis*” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Ahmad Faujan, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mawardi Rahmani, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal examiner)

Taufiq- Yap Yun Hin, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Internal examiner)

Zuriati Zakaria, PhD
Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External examiner)



HASANAH MOHD. GHAZALI, PhD
Professor/ Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 MAR 2006

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

Mohd Aspollah Hj. Sukari, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohd Nordin Hj. Lajis, PhD

Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Gwendoline Ee Cheng Lian, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Abdul Manaf Ali, PhD

Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)



AINI IDERIS, PhD
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 13 APR 2006



DECLARATION

I hereby declare that the thesis is based on my own original work except for quotations and citations which have been duly acknowledgement. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.



NEOH BEE KEAT

Date: 02/03/06

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENT	viii
APPROVAL	ix
DECLARATION	x
LIST OF TABLES	xv
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxvii

CHAPTER

1. INTRODUCTION	1
1.1 General Introduction	1
1.2 Natural Products Research in Malaysia	7
1.2 Zingiberaceae	10
1.3.1 Uses and Commercial Importance	12
1.3.2 <i>Kaempferia</i>	13
1.3.3 <i>Kaempferia angustifolia</i>	14
1.3.4 <i>Kaempferia rotunda</i>	15
1.4 Rubiaceae	16
1.4.1 Uses and Commercial Importance	18
1.4.2 <i>Spermacoce</i>	20
1.4.3 <i>Spermacoce articularis</i>	21
1.4.4 <i>Spermacoce exilis</i>	22
1.5 Essential Oils and Their Production	23
1.6 Bioassays	25
1.6.1 Cancer and Cytotoxic Drugs	25
1.6.2 Antimicrobial Agents	27
1.6.3 Antioxidant and α -Tocopherol	30
1.6.4 Dengue and Mosquitoes Repellant	32
1.7 Choice of Plants	34
1.8 Overall Objectives	35
2. LITERATURE REVIEW	36
2.1 Plant Chemistry of Zingiberaceae	36
2.1.1 Literature review of <i>Kaempferia</i> species	37
2.1.2 Literature review of <i>Kaempferia angustifolia</i>	43
2.1.3 Literature review of <i>Kaempferia rotunda</i>	45
2.2 Plant Chemistry of Rubiaceae	48



2.2.1 Literature Review of <i>Spermacoce</i> Species	49
3. MATERIAL AND METHODS	51
3.1 Instruments	51
3.2 Chromatographic Methods	53
3.3 Experimental Methods	54
3.3.1 Plant Materials	54
3.3.2 Extraction and Fractionation of Extracts	54
3.3.3 Isolation of Chemical Constituents of <i>Kaempferia angustifolia</i>	56
3.3.4 Isolation of Chemical Constituents of <i>Kaempferia rotunda</i>	67
3.3.5 Isolation of Chemical Constituents of <i>Spermacoce articularis</i>	73
3.3.6 Isolation of Chemical Constituents of <i>Spermacoce exilis</i>	77
3.4 Acetylation of Angustifolienol (72)	81
3.4.1 Preparation of Triacetoxiangustifolienol (80)	81
3.4.2 Preparation of Diacetoxiangustifolienol (81)	82
3.5 Essential Oils From <i>Kaempferia angustifolia</i> , <i>Kaempferia rotunda</i> , <i>Spermacoce articularis</i> and <i>Spermacoce exilis</i>	84
3.5.1 Isolation of Essential Oil	84
3.5.2 Chemical Analysis	85
3.5.3 Kovats Retention Indices	85
3.6 Bioassays Procedures	88
3.6.1 Cytotoxic Screening	88
3.6.2 Antimicrobial Test	89
3.6.3 Antioxidant Assay	90
3.6.4 Larvicidal Activity	91
4. RESULTS AND DISCUSSIONS	92
4.1 Isolated and Structural Elucidation of Compounds From <i>Kaempferia angustifolia</i>	92
4.1.1 Characterization of Crotepoxide (33)	95
4.1.2 Characterization of Boesenboxide (34)	108
4.1.3 Characterization of 2'- Hydroxy- 4;4', 6'-trimethoxychalcone (36)	120
4.1.4 Characterization of β -Sitosterol (44)	131
4.1.5 Characterization of Angustifolienol (58)	136
4.1.6 Characterization of 6- Methylzeylenol (59)	152
4.1.7 Characterization of Sucrose (60)	166
4.1.8 Characterization of Zeylenol (35)	176
4.1.9 Characterization of Compound KA10	187
4.2 Isolated and Structural Elucidation of Compounds From <i>Kaempferia rotunda</i>	187
4.2.1 Characterization of Tetracosanoic acid (61)	190
4.2.2 Characterization of β -Sitosterol (44)	193
4.2.3 Characterization of Stigmasterol (45)	194
4.2.4 Characterization of Benzoic Acid (62)	199
4.2.5 Characterization of KR2	202
4.2.6 Characterization of KR8	203



4.2.7 Characterization of Crotepoxide (33)	203
4.2.8 Characterization of KR9	204
4.2.9 Characterization of KR10	204
4.3 Isolated and Structural Elucidation of Compounds From <i>Spermacoce</i> <i>articularis</i>	205
4.3.1 Characterization of Ursolic Acid (63)	207
4.3.2 Characterization of Stigmasterol (45)	224
4.3.3 Characterization of Ursolic Acid (63) and SA4	224
4.3.4 Characterization of Ursolic Acid (63) and SA10	225
4.4 Isolated and Structural Elucidation of Compounds From from <i>Spermacoce exilis</i>	225
4.4.1 Characterization of Benzo[g]isoquinoline-5,10-dione (64)	228
4.4.2 Characterization of Hexadecanoic acid (65)	239
4.4.3 Characterization of Stigmasterol (45)	243
4.4.4 Characterization of Ursolic acid (63)	243
4.4.5 Characterization of SE4	243
4.5 Acetylation of Angustifolienol	244
4.5.1 Preparation of Triacetoxyangustifolienol (66)	245
4.5.2 Preparation of Diacetoxyangustifolienol (67)	258
4.6 Chemical Compositions of Essential Oil	271
4.6.1 Essential Oils of <i>Kaempferia angustifolia</i>	272
4.6.2 Essential Oils from <i>Kaempferia rotunda</i>	277
4.6.3 Essential Oils of <i>Spermacoce articularis</i>	283
4.6.4 Essential Oils of <i>Spermacoce exilis</i>	286
4.7 Cytotoxic Screening	289
4.7.1 Cytotoxic Screening Results of <i>Kaempferia</i> Species	289
4.7.2 Cytotoxic Screening Results of <i>Spermacoce</i> Species	291
4.8 Antimicrobial Test	293
4.8.1 Antimicrobial Test Results of <i>Kaempferia</i> Species	293
4.8.2 Antimicrobial Test Results of <i>Spermacoce</i> Species	295
4.9 Antioxidant Assay	296
4.9.1 Antioxidant Assay Results of <i>Kaempferia</i> Species	296
4.9.2 Antioxidant Assay Results of <i>Spermacoce</i> Species	300
4.10 Larvicidal Activity	303
4.10.1 Larvicidal Activity of <i>Kaempferia</i> Species	303
4.10.2 Larvicidal Activity of <i>Spermacoce</i> Species	304
 5. CONCLUSIONS	 306
 BIBLIOGRAPHY	 310
APPENDICES	316
BIODATA OF THE AUTHOR	339



LIST OF TABLES

Table		Page
1.1	Modern Medication in Market Produced from Traditional Herbs	5
1.2	Medicinal Plants and Associated Ailments	6
1.3	Genera of Zingiberaceae in Malesia (1998)	11
1.4	Classification of Rubiaceae According to Subfamily	18
2.1	Antibacterial Activity of <i>Kaempferia galanga</i> Rhizomes and Root Essential Oils	43
2.2	LC ₅₀ and EC ₅₀ Values of Insecticidal Constituents Isolated from Rhizomes of <i>Kaempferia rotunda</i> against Neonate Larvae of <i>S. littoralis</i> .	47
4.1	¹³ C NMR (125 MHz, CDCl ₃) and ¹ H NMR (500MHz, CDCl ₃) Assignments for Crotepoxide (33)	98
4.2	Coupling Constants of COSY (¹ H- ¹ H Correlation) Assignments for Crotepoxide (33)	99
4.3	¹³ C NMR (125 MHz, CDCl ₃) and ¹ H NMR (500MHz, CDCl ₃) Assignments for Boesenboxide (34)	111
4.4	Coupling Constants of COSY (¹ H- ¹ H Correlation) Assignments for Boesenboxide (34)	112
4.5	¹³ C NMR (125 MHz, Acetone) and ¹ H NMR (500MHz, Acetone) Assignments for 2'-Hydroxy-4,4',6'-trimethoxychalcone (36)	123
4.6	¹³ C NMR (125 MHz, CDCl ₃) and ¹ H NMR (500MHz, CDCl ₃) Assignments for β- Sitosterol (44)	133
4.7	¹³ C NMR (125 MHz, Acetone) and ¹ H NMR (500MHz, Acetone) Assignments for Angustifolienol (58)	140
4.8	Coupling Constants of COSY and NOESY (¹ H- ¹ H Correlation) Assignments for Angustifolienol (58)	140
4.9	¹³ C NMR (125 MHz, CDCl ₃) and ¹ H NMR (500MHz, CDCl ₃) Assignments for 6-Methylzeylenol (59)	155



4.10	Coupling Constants of COSY and NOESY (^1H - ^1H Correlation) Assignments for 6-Methylzeylenol (59)	156
4.11	^{13}C NMR (125 MHz, DMSO) and ^1H NMR (500MHz, DMSO) Assignments for Sucrose (60)	167
4.12	Coupling Constants of COSY (^1H - ^1H Correlation) Assignments for Sucrose (60)	168
4.13	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (500MHz, CDCl_3) Assignments for zeylenol (35)	178
4.14	Coupling Constants of COSY (^1H - ^1H Correlation) Assignments for zeylenol (35)	179
4.15	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (400MHz, CDCl_3) Assignments for Tetracosanoic acid (61)	191
4.16	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (400MHz, CDCl_3) Assignments for Stigmasterol (45)	196
4.17	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (500MHz, CDCl_3) Assignments for Benzoic Acid (62)	200
4.18	^{13}C NMR (125 MHz, DMSO) and ^1H NMR (400MHz, DMSO) Assignments for Ursolic acid (63)	210
4.19	Coupling Constants of COSY (^1H - ^1H Correlation) Assignments for Ursolic acid (63)	211
4.20	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (500MHz, CDCl_3) Assignments for benzo[g]isoquinoline-5,10-dione (64)	230
4.21	Coupling Constants of COSY (^1H - ^1H Correlation) Assignments for benzo[g]isoquinoline-5,10-dione (64)	231
4.22	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (400MHz, CDCl_3) Assignments for Hexadecanoic acid (65)	240
4.23	^{13}C NMR (125 MHz, CDCl_3) and ^1H NMR (400MHz, CDCl_3) Assignments for Triacetoxyangustifolienol (66)	248
4.24	Coupling Constants of COSY (^1H - ^1H Correlation) Assignments for Triacetoxyangustifolienol (66)	249



4.25	¹³ C NMR (125 MHz, CDCl ₃) and ¹ H NMR (400MHz, CDCl ₃) Assignments for Diacetoxyangustifolienol (67)	260
4.26	Coupling Constants of COSY (¹ H- ¹ H Correlation) Assignments for Diacetoxyangustifolienol (67)	261
4.27	Weight and Percentage of Essential Oils Produced From Zingiberaceae and Rubiaceae Species	271
4.28	Composition of Essential Oil from <i>Kaempferia angustifolia</i>	274
4.29	Composition of Essential Oil from <i>Kaempferia rotunda</i>	279
4.30	Composition of Essential Oil from <i>Spermacoce articularis</i>	285
4.31	Composition of Essential Oil from <i>Spermacoce exilis</i>	288
4.32	Cytotoxic Activity of Crude Extracts of <i>Kaempferia</i> species Against HL-60 Cell Lines (T- Promyelocytic Leukemia)	290
4.33	Cytotoxic Activity of Pure Compounds of <i>Kaempferia angustifolia</i> Against HL-60 Cell Lines (T- Lymphoblastic Leukemia)	290
4.34	Cytotoxic Activity of Crude Extracts of <i>Spermacoce</i> Species Against HL-60 Cell Lines (T- Promyelocytic Leukemia) and MCF-7 (Human Breast Cancer)	291
4.35	Diameter Inhibition Zone of Various Target Microorganism Test Against Crude Extracts (<i>Kaempferia</i> Species)	293
4.36	Diameter Inhibition Zone of Various Target Microorganism Test Against Pure Compounds (<i>Kaempferia</i> Species)	294
4.37	Diameter Inhibition Zone of Various Target Microorganism Test Against Crude Extracts (<i>Spermacoce</i> Species)	295
4.38	Antioxidant Assay of Crude Extracts of <i>Kaempferia species</i>	297
4.39	Antioxidant Assay of Pure Compounds of <i>Kaempferia angustifolia</i>	297
4.40	Antioxidant Assay of Crude Extracts of <i>Spermacoce species</i>	301
4.41	Lethal Concentration 50 % (LC ₅₀) and Lethal Concentration 90 % (LC ₉₀) of <i>Kaempferia</i> Species Crude Extracts on <i>Aedes aegypti</i> Activity	304
4.42	Lethal Concentration 50 % (LC ₅₀) and Lethal Concentration 90 % (LC ₉₀) of <i>Spermacoce</i> Species Crude Extracts on <i>Aedes aegypti</i> Activity	305



LIST OF FIGURES

Figure		Page
1.1	Main Pathway Leading to Secondary Metabolites	3
1.2	Several Pathways of Secondary Metabolites Derive from Precursors in the Shikimate Pathway	4
1.3	Plant of <i>Kaempferia angustifolia</i>	15
1.4	Plant of <i>Kaempferia rotunda</i>	16
1.5	Plant of <i>Spermacoce articularis</i>	22
1.6	Plant of <i>Spermacoce exilis</i>	23
2.1	3- caren-5-one (1)	37
2.2	Diterpenes from <i>Kaempferia Species</i>	38
2.3	Diterpenes from <i>Kaempferia pulchra</i>	39
2.4	Some Terpenoids Products from <i>Kaempferia</i> and <i>Curcuma Species</i>	40
2.5	Chemical Constituents Obtained from <i>Kaempferia angustifolia</i>	44
2.6	Cyclohexane Compounds from <i>Kaempferia rotunda</i>	45
2.7	Chemical Constituents Obtained from <i>Kaempferia rotunda</i>	46
2.8	Benzyl Benzoate (46)	47
2.9	Some Alkaloids from <i>Spermacoce verticillata</i>	49
2.10	Some Compounds Isolated from <i>Spermacoce articularis</i>	50
3.1	Flow Chart of Plant Extraction and Isolation Chemical Constituents of <i>Kaempferia angustifolia</i> and <i>K. rotunda</i>	57
3.2	Flow Chart of Plant Extraction and Isolation of <i>Spermacoce articularis</i> and <i>S. exilis</i>	74
3.3	Flow Chart of Essential Oil Extraction	87



4.1	Isolation of Compounds from <i>Kaempferia angustifolia</i>	93
4.2	Selected COSY Correlation of Crotepoxide (33)	96
4.3	Selected HMBC Correlation of Crotepoxide (33)	97
4.4	Mass Fragmentation Patterns of Crotepoxide (33)	100
4.5	EI-MS Spectrum of Crotepoxide (33)	101
4.6	Infrared Spectrum of Crotepoxide (33)	102
4.7	¹ H NMR Spectrum of Crotepoxide (33) (500MHz, CDCl ₃)	103
4.8	COSY Spectrum of Crotepoxide (33) (¹ H- ¹ H Coupling)	104
4.9	¹³ C NMR Spectrum of Crotepoxide (33) (125 MHz, CDCl ₃)	105
4.10	HMQC Spectrum of Crotepoxide (33) (<i>J</i> ₁ Coupling)	106
4.11	HMBC Spectrum of Crotepoxide (33) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling)	107
4.12	Selected COSY Correlation of Boesenboxide (34)	109
4.13	Selected HMBC Correlation of Boesenboxide (34)	110
4.14	FAB-MS Spectrum of Boesenboxide (34)	113
4.15	Infrared Spectrum of Boesenboxide (34)	114
4.16	¹ H NMR Spectrum of Boesenboxide (34) (500MHz, CDCl ₃)	115
4.17	COSY Spectrum of Boesenboxide (34) (¹ H- ¹ H Coupling)	116
4.18	¹³ C NMR Spectrum of Boesenboxide (34) (125MHz, CDCl ₃)	117
4.19	HMQC Spectrum of Boesenboxide (34) (<i>J</i> ₁ Coupling)	118
4.20	HMBC Spectrum of Boesenboxide(34) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling)	119
4.21	Selected HMBC Correlation of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36)	122
4.22	Mass Fragmentation Patterns of 2'- Hydroxy- 4,4', 6'-trimethoxychalcone (36)	124



4.23	FAB-MS Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36)	125
4.24	Infrared Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36)	126
4.25	¹ H NMR Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36) (500MHz, Acetone)	127
4.26	¹³ C NMR Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36) (125MHz, Acetone)	128
4.27	HMQC Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36) (<i>J</i> ₁ Coupling)	129
4.28	HMBC Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (36) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling)	130
4.29	EI-MS Spectrum of β- Sitosterol (44)	134
4.30	Infrared Spectrum of β- Sitosterol (44)	134
4.31	¹ H NMR Spectrum of β- Sitosterol (44) (500MHz, CDCl ₃)	135
4.32	¹³ C NMR Spectrum of β- Sitosterol (44) (125MHz, CDCl ₃)	135
4.33	Selected COSY Correlation of Angustifolienol (58)	137
4.34	Selected HMBC Correlation of Angustifolienol (58)	138
4.35	Selected NOESY Correlation of Angustifolienol (58)	139
4.36	Mass Fragmentation Patterns of Angustifolienol (58)	141
4.37	FAB-MS Spectrum of Angustifolienol (58)	142
4.38	HRCI-MS Spectrum of Angustifolienol (58)	143
4.39	Infrared Spectrum of Angustifolienol (58)	144
4.40	¹ H NMR Spectrum of Angustifolienol (58) (500MHz, Acetone)	145
4.41	COSY Spectrum of Angustifolienol (58) (¹ H- ¹ H Coupling)	146
4.42	NOESY Spectrum of Angustifolienol (58) (¹ H- ¹ H Coupling)	147



4.43	¹³ C NMR Spectrum of Angustifolienol (58) (125 MHz, Acetone)	148
4.44	¹³ C NMR Spectrum of Angustifolienol (58) (125 MHz, Acetone) (Expanded)	149
4.45	HMQC Spectrum of Angustifolienol (58) (<i>J₁</i> Coupling)	150
4.46	HMBC Spectrum of Angustifolienol (58) (<i>J₂, J₃, J₄</i> Coupling)	151
4.47	Selected COSY Correlation of 6- Methylzeylenol (59)	153
4.48	Selected HMBC Correlation of 6- Methylzeylenol (59)	154
4.49	Selected NOESY Correlation of 6- Methylzeylenol (59)	154
4.50	Mass Fragmentation Patterns of 6-Methylzeylenol (59)	157
4.51	FAB-MS Spectrum of 6- Methylzeylenol (59)	158
4.52	Infrared Spectrum of 6- Methylzeylenol (59)	159
4.53	¹ H NMR Spectrum of 6- Methylzeylenol (59) (500MHz, CDCl ₃)	160
4.54	COSY Spectrum of 6- Methylzeylenol (59) (¹ H- ¹ H Coupling)	161
4.55	NOESY Spectrum of 6- Methylzeylenol (59) (¹ H- ¹ H Coupling)	162
4.56	¹³ C NMR Spectrum of 6- Methylzeylenol (59) (125 MHz, CDCl ₃)	163
4.57	HMQC Spectrum of 6- Methylzeylenol (59) (<i>J₁</i> Coupling)	164
4.58	HMBC Spectrum of 6- Methylzeylenol (59) (<i>J₂, J₃, J₄</i> Coupling)	165
4.59	Selected HMBC Correlation of Sucrose (60)	167
4.60	EI-MS Spectrum of Sucrose (60)	169
4.61	Infrared Spectrum of Sucrose (60)	170
4.62	¹ H NMR Spectrum of Sucrose (60) (400MHz, DMSO)	171
4.63	COSY Spectrum of Sucrose (60) (¹ H- ¹ H Coupling)	172
4.64	¹³ C NMR Spectrum of Sucrose (60) (125 MHz, Acetone)	173



4.65	HMQC Spectrum of Sucrose (60) (J_1 Coupling)	174
4.66	HMBC Spectrum of Sucrose (60) (J_2, J_3, J_4 Coupling)	175
4.67	Selected COSY Correlation of Zeyleenol (35)	177
4.68	Selected HMBC Correlation of Zeyleenol (35)	178
4.69	FAB-MS Spectrum of Zeyleenol (35)	180
4.70	Infrared Spectrum of Zeyleenol (35)	181
4.71	^1H NMR Spectrum of Zeyleenol (35) (500MHz, CDCl_3)	182
4.72	COSY Spectrum of Zeyleenol (35) (^1H - ^1H Coupling)	183
4.73	^{13}C NMR Spectrum of Zeyleenol (35) (125 MHz, CDCl_3)	184
4.74	HMQC Spectrum of Zeyleenol (35) (J_1 Coupling)	185
4.75	HMBC Spectrum of Zeyleenol (35) (J_2, J_3, J_4 Coupling)	186
4.76	Isolation of Compounds from <i>Kaempferia rotunda</i>	188
4.77	EI-MS Spectrum of Tetracosanoic Acid (61)	191
4.78	Infrared Spectrum of Tetracosanoic Acid (61)	192
4.89	^1H NMR Spectrum of Tetracosanoic Acid (61) (500MHz, CDCl_3)	192
4.80	^{13}C NMR Spectrum of Tetracosanoic Acid (61) (125MHz, CDCl_3)	193
4.81	EI-MS Spectrum of Stigmasterol (45)	197
4.82	Infrared Spectrum of Stigmasterol (45)	197
4.83	^1H NMR Spectrum of Stigmasterol (45) (500MHz, CDCl_3)	198
4.84	^{13}C NMR Spectrum of Stigmasterol (45) (125MHz, CDCl_3)	198
4.85	EI-MS Spectrum of Benzoic Acid (62)	200
4.86	Infrared Spectrum of Benzoic Acid (62)	201



4.87	¹ H NMR Spectrum of Benzoic Acid (62) (500MHz, CDCl ₃)	201
4.88	¹³ C NMR Spectrum of Benzoic Acid (62) (125MHz, CDCl ₃)	202
4.89	Isolation of Compounds from <i>Spermacoce articularis</i>	206
4.90	Selected COSY Correlation of Ursolic acid (63)	208
4.91	Selected HMBC Correlation of Ursolic acid (63)	209
4.92	Mass Fragmentation Patterns of Ursolic acid (63)	212
4.93	EI-MS Spectrum of Ursolic acid (63)	213
4.94	Infrared Spectrum of Ursolic acid (63)	214
4.95	¹ H NMR Spectrum of Ursolic acid (63) (400MHz, DMSO)	215
4.96	¹ H NMR Spectrum of Ursolic acid (63) (400MHz, DMSO) (Expanded)	216
4.97	COSY Spectrum of Ursolic acid (63) (¹ H- ¹ H Coupling)	217
4.98	¹³ C NMR Spectrum of Ursolic acid (63) (125 MHz, DMSO)	218
4.99	¹³ C NMR Spectrum of Ursolic acid (63) (125 MHz, DMSO) (Expanded)	219
4.100	HMQC Spectrum of Ursolic acid (63) (<i>J</i> ₁ Coupling)	220
4.101	HMQC Spectrum of Ursolic acid (63) (<i>J</i> ₁ Coupling) (expanded)	221
4.102	HMBC Spectrum of Ursolic acid (63) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling)	222
4.103	HMBC Spectrum of Ursolic acid (63) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling) (Expanded)	223
4.104	Isolation of Compounds from <i>Spermacoce exilis</i>	227
4.105	Selected HMBC Correlation of Benzo[g]isoquinoline-5,10-dione (64)	230
4.106	EI-MS Spectrum of Benzo[g]isoquinoline-5,10-dione (64)	232
4.107	Infrared Spectrum of Benzo[g]isoquinoline-5,10-dione (64)	233



4.108	¹ H NMR Spectrum of Benzo[g]isoquinoline-5,10-dione (64) (500MHz, CDCl ₃)	234
4.109	COSY Spectrum of Benzo[g]isoquinoline-5,10-dione (64) (¹ H- ¹ H Coupling)	235
4.110	¹³ C NMR Spectrum of Benzo[g]isoquinoline-5,10-dione (64) (125 MHz, CDCl ₃)	236
4.111	HMQC Spectrum of Benzo[g]isoquinoline-5,10-dione (64) (<i>J</i> ₁ Coupling)	237
4.112	HMBC Spectrum of Benzo[g]isoquinoline-5,10-dione (64) (<i>J</i> ₂ , <i>J</i> ₃ , <i>J</i> ₄ Coupling)	238
4.113	EI-MS Spectrum of Hexadecanoic acid (65)	241
4.114	Infrared Spectrum of Hexadecanoic acid (65)	241
4.115	¹ H NMR Spectrum of Hexadecanoic acid (65) (400MHz, CDCl ₃)	242
4.116	¹³ C NMR Spectrum of Hexadecanoic acid (65) (125 MHz, CDCl ₃)	242
4.117	Selected COSY Correlation of Triacetoxyangustifolienol (66)	246
4.118	Selected HMBC Correlation of Triacetoxyangustifolienol (66)	247
4.119	Mass Fragmentation Patterns of Triacetoxyangustifolienol (66)	249
4.120	EI-MS Spectrum of Triacetoxyangustifolienol (66)	250
4.121	HRCI-MS Spectrum of Triacetoxyangustifolienol (66)	251
4.122	Infrared Spectrum of Triacetoxyangustifolienol (66)	252
4.123	¹ H NMR Spectrum of Triacetoxyangustifolienol (66) (400MHz, CDCl ₃)	253
4.124	COSY Spectrum of Triacetoxyangustifolienol (66) (¹ H- ¹ H Coupling)	254
4.125	¹³ C NMR Spectrum of Triacetoxyangustifolienol (66) (125 MHz, CDCl ₃)	255

