

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION OF THE STATUS OF THESIS	
	SUPERVISOR'S DECLARATION	
	CERTIFICATION OF EXAMINATION	
	TITLE PAGE	i
	DECLARATION OF ORIGINALITY AND EXCLUSIVENESS	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	PREFACE	v
	ABSTRACT	vi
	ABSTRAK	vii
	TABLE OF CONTENTS	viii
	LIST OF TABLES	xii
	LIST OF SCHEMES	xiii
	LIST OF FIGURES	xiv
	LIST OF ABBREVIATIONS	xvi
	LIST OF APPENDICES	xviii
1	INTRODUCTION	
1.1	General Introduction	1
1.2	Objectives	2
1.3	Scope of Study	3
2	LITERATURE REVIEWS	
2.1	The Zingiberaceae Family	4

2.2	Botany and Distribution of <i>Kaempferia</i>	5
2.3	The Usages of <i>Kaempferia</i> Species	6
2.4	Phytochemicals of <i>Kaempferia</i> Species	7
2.4.1	Chalcones	7
2.4.2	Flavones	10
2.4.3	Flavanones	11
2.4.4	Pimarane Diterpenes	12
2.4.5	Cyclohexane Oxides	14
2.4.6	Cinnamates	16
2.4.7	Phenolics	17
2.4.8	Esters	18
2.4.9	Monoterpene	19
2.5	Bioactivity Studies on <i>Kaempferia</i> Species	19
2.6	Essential Oil Studies on <i>Kaempferia</i> Species	21
2.7	Biogenetic Pathway on Cyclohexane Oxides	22

3 CHEMICAL COMPOSITIONS OF KAEMPFERIA ROTUNDA OILS

3.1	The Essential Oils of <i>Kaempferia rotunda</i>	27
3.1.1	Rhizome Oil of Malaysian <i>Kaempferia rotunda</i>	27
3.1.2	Rhizome Oil of Indonesian <i>Kaempferia rotunda</i>	31
3.1.3	Comparison of the Compositions of Malaysian and Indonesian <i>Kaempferia rotunda</i>	33

4 PHYTOCHEMICAL AND BIOACTIVITY STUDIES OF KAEMPFERIA ROTUNDA

4.1	Phytochemical Study of Malaysian <i>Kaempferia rotunda</i>	36
4.1.1	Crotepoxide (54)	37
4.1.2	2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	38
4.1.3	4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	39
4.1.4	1,6-Desoxypipoxide (69)	41
4.1.5	2-(Benzoyloxymethyl)phenyl (3-O-acetyl)- β -glucopyranoside (131)	42

4.1.6	5-Hydroxy-7,4'-dimethoxyflavanone (Naringenin 4',7-dimethyl ether) (133)	51
4.1.7	12-Acetoxy-8 α ,13-dihydroxylab-14-en-7-one (Curcumrinol C) (134)	52
4.1.8	4-Benzoyloxymethyl-3-oxabicyclo[4.1.0]heptane-1,5,6,7-tetrol (3-Debenzoylrotepoxide A)(132)	56
4.2	Phytochemical Studies of Indonesian <i>Kaempferia rotunda</i>	64
4.2.1	Crotepoxide (54)	65
4.2.2	Benzyl Benzoate (82)	65
4.2.3	<i>trans</i> -Docosyl ferulate (137)	66
4.2.4	3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)-cyclohexa-4,6-diene (136)	69
4.2.5	6-Acetylzeylenol (68)	78
4.2.6	Benzoic Acid (138)	80
4.3	The Distribution of Compounds in Malaysian and Indonesian <i>Kaempferia rotunda</i>	81
4.4	Bioactivity Studies on <i>Kaempferia rotunda</i>	82
4.4.1	Antibacterial Activity	83
4.4.2	Antioxidant Activity	85

5 EXPERIMENTAL

5.1	General Experimental Procedures	87
5.2	Chemicals	88
5.3	Plant Materials	88
5.4	Essential Oil Extraction and Analysis	89
5.5	Extraction and Isolation of Malaysian <i>Kaempferia rotunda</i>	90
5.5.1	Crotepoxide (54)	90
5.5.2	2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	91
5.5.3	4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	92
5.5.4	1,6-Desoxypipoxide (69)	92
5.5.5	2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	93

5.5.6	Naringenin 4',7-dimethyl ether (133)	94
5.5.7	Curcumrinol C (134)	94
5.5.8	3-Debenzoylrotepoxyde A (132)	95
5.6	Extraction and Isolation of Indonesian <i>Kaempferia rotunda</i>	96
5.6.1	Crotepoxyde (54)	96
5.6.2	Benzyl benzoate (82)	96
5.6.3	<i>trans</i> -Docosyl ferulate (137)	97
5.6.4	3-Acetoxy-2-benzoyloxy-1-(benzyloxymethyl)-cyclohexa-4,6-diene (136)	97
5.6.5	6-Acetylzeylenol (68)	98
5.6.6	Benzoic Acid (138)	98
5.7	Bioactivity Studies	99
5.7.1	Chemicals	99
5.7.2	Microorganisms	99
5.7.3	Antimicrobial Assay	99
5.7.3.1	Microorganisms and Culture Media	99
5.7.3.2	Disc Diffusion Method	100
5.7.4	Antioxidant Screening (Free Radical Scavenging Activity (DPPH))	101
6	CONCLUSION AND RECOMMENDATION	103
	REFERENCES	105
	Appendices	117

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Biological Properties of Several <i>Kaempferia</i> Species	19
2.2	Biological Properties of Compounds Isolated from <i>Kaempferia</i> Species	20
3.1	Constituents of Malaysian of <i>Kaempferia rotunda</i> Rhizome Oil	29
3.2	Constituents of Indonesian <i>Kaempferia rotunda</i> Rhizome Oil	32
3.3	Comparison of the Rhizomes Oils of Malaysian and Indonesian <i>Kaempferia rotunda</i>	33
4.1	^1H , ^{13}C NMR and COSY Data of Compound (69)	42
4.2	^1H and ^{13}C NMR Data of Compound (131) and 2-(Benzoyl- oxymethyl)phenyl (3,6-di- <i>O</i> -acetyl)- β -glucopyranoside (135)	44
4.3	^1H , ^{13}C NMR, COSY and HMBC Data of Compound (134)	55
4.4	^1H , ^{13}C NMR, COSY and HMBC Data of Compound (132)	64
4.5	^1H , ^{13}C NMR, COSY and HMBC Data of Compound (137)	68
4.6	^1H , ^{13}C NMR, COSY and HMBC Data of Compound (136)	70
4.7	^1H , ^{13}C NMR, COSY and HMBC Data of Compound (68)	79
4.8	Compounds Isolated from Malaysian and Indonesian <i>Kaempferia rotunda</i>	82
5.1	The Inhibition Zones of Tested Samples	101
5.2	Percentage Inhibitions of Tested Samples	102

LIST OF SCHEMES

SCHEME NO.	TITLE	PAGE
4.1	The Elimination of Water Molecule from Compound (134) and the Formation of Acylium Ion	53
4.2	The Suggested Mass Fragmentation Pattern of Compound (137)	68

LIST OF FIGURES

FIGURES NO.	TITLE	PAGE
2.1	Biogenetic Pathway for the Formation of Crotepoxide (54)	23
2.2	Biogenetic Pathway for the Formation of Senepoxide (95) and Pipoxide (96)	24
2.3	Biogenetic Pathway for the Formation of (-)-Zeylenol (75), Senepoxide (95), Pipoxide (63), Seneol (106) and Zeylena (107)	25
2.4	Biogenetic Pathway for the Formation of (+)-Zeylenol (74) and (-)-Zeylenol (75)	26
4.1	IR Spectrum of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	45
4.2	^1H NMR Spectrum of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	46
4.3	CIMS Spectrum of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	47
4.4	^{13}C NMR Spectrum of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	48
4.5	^{13}C NMR and DEPT Spectra of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	49
4.6	HMBC Spectrum of 2-(Benzoyloxymethyl)phenyl (3- <i>O</i> -acetyl)- β -glucopyranoside (131)	50
4.7	IR Spectrum of 3-Debenzoylrotepoxide A (132)	57
4.8	^1H NMR Spectrum of 3-Debenzoylrotepoxide A (132)	58
4.9	^1H - ^1H COSY Spectrum of 3-Debenzoylrotepoxide A (132)	59
4.10	^{13}C NMR Spectrum of 3-Debenzoylrotepoxide A (132)	60
4.11	^{13}C NMR and DEPT Spectra of 3-Debenzoylrotepoxide A (132)	61

4.12	CIMS Spectrum of 3-Debenzoylrotepoxide A (132)	63
4.13	¹ H NMR Spectrum of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	72
4.14	¹ H- ¹ H COSY Spectrum of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	73
4.15	IR Spectrum of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	74
4.16	¹³ C NMR Spectrum of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	75
4.17	¹³ C NMR and DEPT Spectra of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	76
4.18	EIMS Spectrum of 3-Acetoxy-2-benzoyloxy-1-(benzoyloxymethyl)cyclohexa-4,6-diene (136)	77
4.19	Graft of Percentage Scavenging Capacity of DPPH by Vitamin C, 5-Deoxyquercetin, Crude Extracts and Essential Oils from <i>Kaempferia rotunda</i> Measured by UV Spectrometric Assay	86
5.1	<i>Kaempferia rotunda</i> cultivated in Kempas, Johor	90
5.2	<i>Kaempferia rotunda</i> imported from Indonesia	90
5.3	The Arrangement of the Sample Discs and Control Discs in Petri Dish	101

LIST OF ABBREVIATIONS

br	broad
CC	Column Chromatography
COSY	Correlation Spectroscopy
¹³ C	Carbon-13
CDCl ₃	Deuterated chloroform
CD ₃ COCD ₃	Deuterated acetone
CHCl ₃	Chloroform
CIMS	Chemical Ionization Mass Spectrometry
DPPH	2,2-Diphenyl-1-picrylhydrazyl
d	doublet
dd	doublet of doublet
ddd	doublet of doublet of doublet
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarization Transfer
D ₂ O	Deuterium oxide
EtOAc	Ethyl acetate
EIMS	Electron Impact Mass Spectrometry
Et ₂ O	Diethyl ether
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
¹ H	Proton
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Coherence
Hz	Hertz
IR	Infrared
IC ₅₀	Inhibition Concentration at 50%
<i>J</i>	coupling constant

KBr	Potassium bromide
KI	Kovats Index
lit.	Literature
<i>LWT</i>	Lebensm.-Wiss. u.-Technol / Food Science and Technology
MIC	Minimum Inhibition Concentration
MS	Mass Spectrometry
mM	millimolar
<i>m/z</i>	mass to charge ion
MeOH	Methanol
m.p.	melting point
MgSO ₄	Magnesium sulphate
MHz	Megahertz
m	multiplet
NMR	Nuclear Magnetic Resonance
nm	nanometer
NaOH	Sodium hydroxide
NaCl	Sodium chloride
Ph	Phenyl
PE	Petroleum ether
ppm	parts per million
q	quartet
R _f	retention factor
RP-VLC	Reversed Phase Vacuum Liquid Chromatography
SD	Standard Deviation
s	singlet
t	triplet
tr	trace
TLC	Thin Layer Chromatography
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
μM	micromolar
δ	chemical shift
c	concentration

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
1	GC Chromatogram of Malaysian <i>Kaempferia rotunda</i> (Rhizome) Oil	117
2	GC Chromatogram of Indonesian <i>Kaempferia rotunda</i> (Rhizome) Oil	118
3	CIMS Spectrum of Crotepoxide (54)	119
4	^1H NMR Spectrum of Crotepoxide (54)	120
5	^1H - ^1H COSY Spectrum of Crotepoxide (54)	121
6	HMBC Spectrum of Crotepoxide (54)	122
7	IR Spectrum of Crotepoxide (54)	123
8	^{13}C NMR Spectrum of Crotepoxide (54)	124
9	^{13}C NMR and DEPT Spectra of Crotepoxide (54)	125
10	HMQC Spectrum of Crotepoxide (54)	126
11	UV Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	127
12	IR Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	128
13	^1H NMR Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	129
14	^{13}C NMR Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	130
15	^{13}C NMR and DEPT Spectra of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	131
16	EIMS Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	132
17	HMBC Spectrum of 2'-Hydroxy-4,4',6'-trimethoxychalcone (1)	133
18	CIMS Spectrum of 4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	134
19	IR Spectrum of 4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	135

20	¹ H NMR Spectrum of 4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	136
21	¹³ C NMR Spectrum of 4-Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	137
22	HMBC Spectrum of Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	138
23	HMQC Spectrum of Benzoyloxymethyl-3,8-dioxatricyclo-[5.1.0.0 ^{2,4}]octane-5,6-diol 5-acetate (57)	139
24	EIMS Spectrum of 1,6-Desoxypipoxide (69)	140
25	¹³ C NMR Spectrum of 1,6-Desoxypipoxide (69)	141
26	¹³ C NMR and DEPT Spectra of 1,6-Desoxypipoxide (69)	142
27	IR Spectrum of 1,6-Desoxypipoxide (69)	143
28	¹ H NMR Spectrum of 1,6-Desoxypipoxide (69)	144
29	¹ H- ¹ H COSY Spectrum of 1,6-Desoxypipoxide (69)	145
30	IR Spectrum of Naringenin 4',7-dimethyl ether (133)	146
31	¹ H NMR Spectrum of Naringenin 4',7-dimethyl ether (133)	147
32	¹ H- ¹ H COSY Spectrum of Naringenin 4',7-dimethyl ether (133)	148
33	¹³ C NMR Spectrum of Naringenin 4',7-dimethyl ether (133)	149
34	¹³ C NMR and DEPT Spectra of Naringenin 4',7-dimethyl ether (133)	150
35	EIMS Spectrum of Naringenin 4',7-dimethyl ether (133)	151
36	IR Spectrum of Curcumrinol C (134)	152
37	¹³ C NMR Spectrum of Curcumrinol C (134)	153
38	¹³ C NMR and DEPT Spectra of Curcumrinol C (134)	154
39	EIMS Spectrum of Curcumrinol C (134)	155
40	¹ H NMR Spectrum of Curcumrinol C (134)	156
41	¹ H NMR Spectrum of Curcumrinol C (134) (Expansion)	157
42	¹ H- ¹ H COSY Spectrum of Curcumrinol C (134)	158
43	¹ H- ¹ H COSY Spectrum of Curcumrinol C (134) (Expansion)	159
44	HMBC Spectrum of Curcumrinol C (134)	160
45	¹ H NMR Spectrum of Benzyl Benzoate (82)	161
46	IR Spectrum of Benzyl Benzoate (82)	162
47	¹³ C NMR Spectrum of Benzyl Benzoate (82)	163
48	¹³ C NMR and DEPT Spectra of Benzyl Benzoate (82)	164

49	EIMS Spectrum of Benzyl Benzoate (82)	165
50	^{13}C NMR Spectrum of <i>trans</i> -Docosyl ferulate (137)	166
51	^{13}C NMR and DEPT Spectra of <i>trans</i> -Docosyl ferulate (137)	167
52	IR Spectrum of <i>trans</i> -Docosyl ferulate (137)	168
53	^1H NMR Spectrum of <i>trans</i> -Docosyl ferulate (137)	169
54	^1H - ^1H COSY Spectrum of <i>trans</i> -Docosyl ferulate (137)	170
55	HMBC Spectrum of <i>trans</i> -Docosyl ferulate (137)	171
56	EIMS Spectrum of <i>trans</i> -Docosyl ferulate (137)	172
57	IR Spectrum of 6-Acetylzeylenol (68)	173
58	^1H NMR Spectrum of 6-Acetylzeylenol (68)	174
59	CIMS Spectrum of 6-Acetylzeylenol (68)	175
60	^{13}C NMR Spectrum of 6-Acetylzeylenol (68)	176
61	^{13}C NMR and DEPT Spectra of 6-Acetylzeylenol (68)	177
62	^1H - ^1H COSY Spectrum of 6-Acetylzeylenol (68)	178
63	HMBC Spectrum of 6-Acetylzeylenol (68)	179
64	IR Spectrum of Benzoic Acid (138)	180
65	^1H NMR Spectrum of Benzoic Acid (138)	181
66	^{13}C NMR Spectrum of Benzoic Acid (138)	182