

2.3.2	Biogenesis / biosynthesis of Zingiberaceae terpenes	34
2.4	Structural elucidation : General methods and theory	39
2.4.1	Ultra violet spectra of aromatic or benzenoid compounds	40
2.4.2	Mass spectroscopy of aromatic or benzenoid compounds	42
2.4.3	¹ HNMR spectroscopy of aromatic or benzenoid compounds	44
2.4.4	¹³ CNMR spectroscopy of aromatic or benzenoid compounds	46

CHAPTER 3

	Smooth muscle relaxants	49
3.1	The contractile process and the regulation of calcium in the smooth muscle	49
3.2	Mechanism of actions of vascular smooth muscle relaxants	56
3.2.1	α_1 -Adrenoceptor antagonists	56
3.2.2	Calcium antagonists	59
3.2.3	Angiotensin converting-enzyme inhibitors and angiotensin antagonists	60
3.2.4	Drugs that act by increasing cyclic nucleotides concentration	61
3.2.5	Potassium channel openers	65
3.2.6	Contractile protein modulators	66

CHAPTER 4

	Results and discussions	67
4.1	Bioassay-guided study of the CH ₂ Cl ₂ extract of <i>Kaempferia galanga</i> Linn.	67
4.1.1	Brine shrimp lethality bioassay on crude CH ₂ Cl ₂ extract	69
4.1.2	Screening of crude CH ₂ Cl ₂ extract for antihypertensive activity	70
4.1.3	Isolation of compound A	73
4.1.3.1	Vasorelaxant activity of compound A	77

4.1.4	Structural elucidation and determination of compounds isolated from the CH ₂ Cl ₂ extract of <i>Kaempferia galanga</i> Linn.	82
4.1.4.1	Compound A	82
4.1.4.2	Compound B	95
4.2	Chemical study of the pet. ether extract of <i>Kaempferia galanga</i> Linn.	108
4.2.1	Structural elucidation and determination of compounds isolated from the pet. ether extract of <i>Kaempferia galanga</i> Linn.	108
4.2.1.1	Volatile components of <i>Kaempferia galanga</i> Linn.	108
4.2.1.2	Compound C	111
4.3	Mechanisms of vasorelaxant action of compound A and comparison of its activity with other structurally similar compounds	123
4.3.1	Effects of compound A and related compounds on the contractions induced by high K ⁺ and PE in endothelium intact rat aorta	125
4.3.2	Time course of the inhibitory effects of compound A and related compounds on the contractions induced by high K ⁺ and PE in endothelium intact rat aorta	129
4.3.3	Effects of compound A and related compounds on the PE-induced transient contractions of the rat aorta	131
4.3.4	Effects of compound A and related compounds on the contractions induced by PE in endothelium intact and denuded preparation of the rat aorta	133
4.3.5	Effects of methylene blue and indomethacin on the relaxant actions of compound A and related compounds on the contractions induced by PE in the endothelium intact rat aorta	133

4.4	Cytotoxicity effects of compounds A and C on KB cells	136
CHAPTER 5		
	Conclusions	139
CHAPTER 6		
	Experimental	143
6.1	Plant materials	143
6.2	Phytochemical analysis	145
6.2.1	Instrumentation	145
6.2.1.1	Column chromatography	146
6.2.1.2	Preparative thin layer chromatography	147
6.2.1.3	Visualising reagents	147
6.2.1.4	Recrystallization of pure compounds	148
6.2.2	Extraction of plant material	148
6.2.2.1	Bioassay-guided fractionation of the crude CH ₂ Cl ₂ extract	149
6.2.2.2	Study of the crude pet. ether extract	149
6.3	Biological assay	153
6.3.1	Brine shrimp lethality bioassay	153
6.3.2	Antihypertensive activity on anaesthetized rats	155
6.3.3	Vasorelaxant activity on smooth muscles of rat aorta	155
6.3.3.1	Effects of compound A and related compounds on the contraction induced by high K ⁺ and PE in endothelium intact rat aorta	157

6.3.3.2	Time course of the inhibitory effects of compound A and related compounds on contractions induced by high K ⁺ and PE in endothelium intact rat aorta	159
6.3.3.3	Effects of compound A and related compounds on contractions induced by PE in endothelium intact and denuded preparation of the rat aorta	159
6.3.3.4	Effects of methylene blue and indomethacin on the relaxant actions of compound A and related compounds on the contractions induced by PE in the endothelium intact rat aorta	159
6.3.3.5	Effects of compound A and related compounds on the PE-induced transient contractions of the rat aorta	160
6.3.4	Cytotoxicity assay on KB cells	160
6.4	Physical and spectral data of isolated compounds	161
	REFERENCES	164

LIST OF FIGURES

Figure		Page
2.1	Ring current effects in benzene	44
3.1	Mechanism of contraction of vascular smooth muscle	52
3.2	Calcium movements in smooth muscle	54
4.1	Schematic procedure of isolation of the chemical constituents from <i>Kaempferia galanga</i> Linn.	68
4.2	Effect of the dichloromethane extract of <i>Kaempferia galanga</i> on the mean arterial pressure (MAP) of anaesthetized rats	72
4.3	Detection and isolation of the bioactive compound of <i>Kaempferia galanga</i> Linn.	74
4.4	Gas chromatograms of the active fractions 1, 2, 3 and 4	75
4.5	Effect of compound A on the contractions induced by high K ⁺ and PE in endothelium intact rat aorta	80
4.6	Time course of the inhibitory effect of compound A on the contractions induced by high K ⁺ and PE in endothelium intact rat aorta	81
4.7	Mass spectrum of compound A	83
4.8	IR spectrum of compound A	86
4.9	¹ HNMR spectrum of compound A	87
4.10	¹³ CNMR spectrum of compound A	89
4.11	DEPT spectrum of compound A	91
4.12	COSY spectrum of compound A	92
4.13	HETCOR spectrum of compound A	93
4.14	HMBC spectrum of compound A	94

4.15	IR spectrum of compound B	96
4.16	Mass spectrum of compound B	98
4.17	¹ HNMR spectrum of compound B	100
4.18	¹³ CNMR spectrum of compound B	102
4.19	DEPT spectrum of compound B	104
4.20	COSY spectrum of compound B	105
4.21	HETCOR spectrum of compound B	106
4.22	HMBC spectrum of compound B	107
4.23	Gas chromatogram of volatile components of <i>Kaempferia galanga</i> Linn.	109
4.24	Mass spectrum of compound C	112
4.25	IR spectrum of compound C	114
4.26	¹ HNMR spectrum of compound C	116
4.27	¹³ CNMR spectrum of compound C	117
4.28	DEPT spectrum of compound C	119
4.29	COSY spectrum of compound C	120
4.30	HETCOR spectrum of compound C	121
4.31	HMBC spectrum of compound C	122
4.32(a)	Effects of compound A and related compounds on the contractions induced by high K ⁺ in endothelium intact rat aorta	126
4.32(b)	Effects of compound A and related compounds on the contractions induced by PE in endothelium intact rat aorta	127
4.33	Time course of the inhibitory effects of compound A and related compounds on the contractions induced by high K ⁺ and PE in endothelium intact rat aorta	130

4.34	Effects of compound A and related compounds on the PE-induced transient contractions of the rat aorta	132
4.35	Effects of compound A and related compounds on the contractions induced by PE in endothelium intact and denuded preparations of the rat aorta	134
4.36	Effects of methylene blue and indomethacin on the relaxant actions of compound A and related compounds on the contractions induced by PE in the endothelium intact rat aorta	135
4.37	Toxicity effects of compounds A and C on KB cells	137
5.1	The proposed biogenetic relationship between the phenolics of <i>Kaempferia galanga</i> Linn.	140
6.1	The organ bath and instrumentation for recording muscle contraction	158

Diagram

4.1	Tonic and phasic contractions of the rat aorta	78
6.1	<i>Kaempferia galanga</i> Linn.	144

Plate

6.1	<i>Kaempferia galanga</i> Linn.	144
-----	---------------------------------	-----

LIST OF SCHEMES

Scheme	Page
1.1 Classification of Zingiberaceae	8
2.1 Biosynthesis of phenolic compounds via shikimic acid pathway	27
2.2 Reactions of chorismic acid	29
2.3 Outline on the formation of phenylalanine and tyrosine, and subsequent formation of phenylpropanoids from chorismic acid	30
2.4 Normal biosynthesis of <i>para</i> -hydroxybenzoic acid in plants	32
2.5 A proposed biosynthetic pathway for ubiquinones	33
2.6 Biosynthesis of caffeic acid	35
2.7 Biosynthesis of chloramphenicol	36
2.8 Biosynthesis of terpenes	38
4.1 The mass fragmentation patterns of compound A	84
4.2 The mass fragmentation patterns of compound B	99
4.3 The mass fragmentation patterns of compound C	113
6.1 Flowchart for the bioassay-guided isolation of the bioactive compound of <i>Kaempferia galanga</i> Linn.	151

LIST OF TABLES

Table	Page
1.1 Genera of Zingiberaceae in the Malaysian forest	9
1.2 Distribution of species in genera of the tribe Hedychieae	11
1.3 Different species of the genus <i>Kaempferia</i> and their distribution	12
1.4 List of some of the Zingiberaceous plants used in traditional treatments of several ailments	14
1.5 The ingredients of jamu used for body endurance and protection	16
2.1 The classification of the phenolic constituents in plants	18
4.1 Preliminary screening for biological activity of crude CH ₂ Cl ₂ extract using the brine shrimp lethality bioassay	71
4.2 Tables showing the chemical constituents of the active fractions 1, 2, 3 and 4, respectively, as elucidated from the GC-MS	76
4.3 Volatile components of <i>Kaempferia galanga</i> elucidated from GC-MS	110
4.4 IC ₅₀ values of compounds A-I for their vasorelaxant activities	128
5.1 Postulation on the structure-activity relationship of ethyl cinnamate and other structurally similar compounds against their vasorelaxant activities	142
6.1 Percentage yields of the crude pet. ether and CH ₂ Cl ₂ extracts of the rhizomes of <i>Kaempferia galanga</i> Linn.	150
6.2 Phenolics isolated from the rhizomes of <i>Kaempferia galanga</i> Linn.	152

ABBREVIATIONS

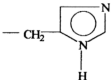
ATP	adenosine triphosphate
$(\text{Ca}^{2+})_i$	intracellular Ca^{2+}
$[\text{Ca}^{2+}]_i$	intracellular Ca^{2+} concentration
CaCl_2	calcium chloride
cAMP	cyclic adenosine monophosphate
CCl_4	carbon tetrachloride
CDCl_3	deuterated chloroform
cGMP	cyclic guanosine monophosphate
CH_2Cl_2	dichloromethane
CHCl_3	chloroform
cm	centimetre
cm^{-1}	per centimetre
COSY	H-H correlation spectroscopy
δ	chemical shift
DAHP	3-deoxy- <i>D</i> -arabino-heptulosonic acid-7-phosphate
DEPT	distortionless enhancement by polarisation transfer
DMSO	dimethylsulphoxide
$\Delta\nu$	difference in frequency
ϵ	molar absorptivity
ED_{50}	effective dose at 50% activity
EDRF	endothelium-derived relaxing factors
EDTA	ethylenediamine-tetraacetic acid
EGTA	ethyleneglycol- <i>bis</i> -(β -aminoethylether)tetraacetic acid
FT	fourier transform
g	gram
GC	gas chromatogram
HETCOR	heteronuclear chemical shift correlation
high $[\text{K}^+]$, high K^+	high K^+ concentration
HMBC	heteronuclear multiple bond connectivity
Hz	Hertz

IC ₅₀	concentration required to inhibit 50% of muscle contraction
IP ₃	inositol-1,4,5-triphosphate
IR	infra red
<i>J</i>	coupling constant (Hz)
KCl	potassium chloride
LC ₅₀	concentration required to kill 50% of shrimps
L-type	long lasting type
m	metre
M	molar
m/z	mass/charge
MAP	mean arterial pressure
max	maximum
MeOH	methanol
mg ml ⁻¹	milligram per millilitre
MgCl ₂	magnesium chloride
MHz	megaHertz
ml	millilitre
MLC	myosin light-chain
MLCK	myosin light-chain kinase
MLCP	myosin light-chain phosphatase
mm	millimetre
mM	millimolar
MS	mass spectrum
mV	millivolt
µg ml ⁻¹	microgram per millilitre
µl	microlitre
NaCl	sodium chloride
NaHCO ₃	sodium hydrogen carbonate
nm	nanometre
nM	nanomolar
NMR	nuclear magnetic resonance
NO	nitric oxide

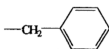
PE	phenylephrine
pet. ether	petroleum ether
ppm	parts per million
R _f	retention factor
ROC	receptor-operated non-selective Ca ²⁺ channel
R _t	retention time
SR	sarcoplasmic reticulum
tlc	thin layer chromatography
TMS	tetramethylsilane
2D	two dimensions
UV	ultra violet
v/v	volume per volume
var	variants
VDC	voltage-dependent Ca ²⁺ channel

Amino acids

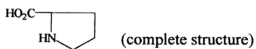


<u>abbreviation</u>	<u>name</u>	<u>structure of R</u>
Ala	alanine	-CH ₃
Arg	arginine	-CH ₂ CH ₂ CH ₂ NHC(=NH)NH ₂
Asp	aspartic acid	-CH ₂ CO ₂ H
Gly	glycine	-H
His	histidine	
Ile	isoleucine	-CH(CH ₃)CH ₂ CH ₃
Leu	leucine	-CH ₂ CH(CH ₃) ₂

Phe phenylalanine



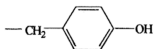
Pro proline



Ser serine



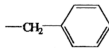
Tyr tyrosine



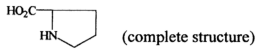
Val valine



Phe phenylalanine



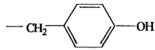
Pro proline



Ser serine



Tyr tyrosine



Val valine

