#### CONTENTS

	Page
บทคัดย่อ	(3)
Abstract	(6)
Acknowledgement	(9)
Contents	(11)
List of tables	(14)
List of illustrations	(16)
Abbreviations and symbols	(19)
Chapter	
1. Introduction	1
1.1 General Introduction	1
1.2 Review of Literatures	3
1.2.1 Plants of the family Zingiberaceae	3
1.2.2 Chemical constituents of the investigated species	6
1.2.2.1 Alpinia galanga	6
1.2.2.2 Boesenbergia pandurata	10
1.2.2.3 Curcuma longa	13
1.2.2.4 Kaempferia galanga	18
1.2.2.5 Zingiber officinale	19
1.2.3 Biological activities of the investigated species	24
1.2.3.1 Alpinia galanga	24
1.2.3.2 Boesenbergia pandurata	26
1.2.3.3 Curcuma longa	27
1.2.3.4 Kaempferia galanga	32
1.2.3.5 Zingiber officinale	34
1.3 Objectives	51

### **CONTENTS (continued)**

				Page
2.	Exp	oerime	ntal	52
	2.1	Chem	icals and Instruments	52
	2.2	Plant	Materials	53
	2.3	Extra	ction and Isolation	53
		2.3.1	Volatile oils	53
		2.3.2	Water extracts	54
		2.3.3	Methanol extracts	54
		2.3.4	Isolation of chemical constituents from Alpinia galanga	54
		2.3.5	Isolation of chemical constituents from Curcuma longa	55
		2.3.6	Isolation of chemical constituents from Zingiber officinale	56
	2.4	Physi	cal and Spectral Properties of the Isolated Compounds	57
	2.5	Assay	for Free Radical Scavenging Activity	59
	2.6	Assay	of for Cytotoxic Activity	60
		2.6.1	Human tumour cell lines	61
		2.6.2	Testing procedure	61
3.	Resi	ults ar	d Discussion	63
	3.1	Scree	ning of Biological Activity of Crude Extracts and Volatile Oils	63
		3.1.1	Free radical scavenging activity	65
		3.1.2	Cytotoxic activity	67
	3.2	Analy	sis of Chemical Composition of the Volatile Oils and Structure	72
		Deter	mination of the Isolated Compounds	
		3.2.1	Characterization of component of the volatile oils	72
		3.2.2	Structure elucidation of the isolated compounds	81
			3.2.2.1 AGM1	81
			3.2.2.2 CLM01	84
			3.2.2.3 CLM02	87
			3.2.2.4 CLM03	90

## **CONTENTS (continued)**

	Page
3.2.2.5 CLM06	93
3.2.2.6 ZOM0	95
3.2.2.7 ZOM1	98
3.2.2.8 ZOM3	101
3.3 Activity of the Isolated Compounds	104
4. Conclusions	109
Bibliography	111
Appendix	137
Vitae	188

#### LIST OF TABLES

Table		Page
1	Chemical constituents found in A. galanga	6
2	Chemical constituents found in B. pandurata	10
3	Chemical constituents found in C. longa	13
4	Chemical constituents found in K. galanga	18
5	Chemical constituents found in Z. officinale	19
6	Biological activities of A. galanga	24
7	Biological activities of B. pandurata	26
8	Biological activities of C. longa	27
9	Biological activities of K. galanga	32
10	Biological activities of Z. officinale	34
11	Yields and characteristics of the extracts and volatile oils from	64
	the fresh rhizomes of A. galanga, B. pandurata, C. longa,	
	K. galanga and Z. officinale	
12	Percent inhibition of the extracts and volatile oils of the fresh	66
	rhizomes of A. galanga, B. pandurata, C. longa, K. galanga	
	and Z. officinale tested by DPPH radical scavenging assay	
	(screening) at the final concentration of 100 $\mu$ g/ml	
13	EC <sub>50</sub> values of the methanol extracts of A. galanga (AGM),	67
	C. longa (CLM) and Z. officinale (ZOM) by DPPH radical	
14	Percent survival of LS174T tested with the extracts and	68
	volatile oils of the fresh rhizomes of <i>A. galanga</i> ,	
	B. pandurata, C. longa, K. galanga and Z. officinale by	
	SRB assay (screening) at final concentration of 100 $\mu$ g/ml	
15	Percent survival of MCF7 tested with the extracts and	69
	volatile oils of the fresh rhizomes of A. galanga, B. pandurata,	
	C. longa, K. galanga and Z. officinale by SRB assay	
	(screening) at final concentration of 100 $\mu$ g/ml	

## LIST OF TABLES (continued)

Table		Page
16	IC <sub>50</sub> values of the active methanol extracts and volatile oils	70
	tested against LS174T and MCF7 (mean <u>+</u> SEM) by	
	Sulphorhodamine B (SRB) assay	
17	Chemical constituents of the volatile oil from A. galanga	73
	(GC/MS analysis)	
18	Chemical constituents of the volatile oil from <i>B. pandurata</i>	74
	(GC/MS analysis)	
19	Chemical constituents of the volatile oil from C. longa	75
	(GC/MS analysis)	
20	Chemical constituents of the volatile oil from K. galanga	76
	(GC/MS analysis)	
21	Chemical constituents of the volatile oil from Z. officinale	77
	(GC/MS analysis)	
22	NMR spectral data (500 MHz for $^{1}$ H and 125 MHz for $^{13}$ C) of AGM1	83
23	NMR spectral data (500 MHz for <sup>1</sup> H and 125 MHz for <sup>13</sup> C) of CLM01	86
24	NMR spectral data (500 MHz for ${}^{1}$ H and 125 MHz for ${}^{13}$ C) of CLM02	89
25	NMR spectral data (500 MHz for <sup>1</sup> H and 125 MHz for <sup>13</sup> C) of CLM03	92
26	NMR spectral data (500 MHz for <sup>1</sup> H and 125 MHz for <sup>13</sup> C) of CLM06	94
27	NMR spectral data (500 MHz for $^{1}$ H and 125 MHz for $^{13}$ C) of ZOM0	97
28	NMR spectral data (500 MHz for ${}^{1}$ H and 125 MHz for ${}^{13}$ C) of ZOM1	100
29	NMR spectral data (500 MHz for ${}^{1}$ H and 125 MHz for ${}^{13}$ C) of ZOM3	103
30	$EC_{50}$ values against DPPH radical and $IC_{50}$ values against LS174T	108
	and MCF7 cells of the isolated compounds from A. galanga,	
	C. longa and Z. officinale	

### LIST OF ILLUSTRATIONS

Figure		Page
1	Structures of some chemical constituents found in A. galanga	37
2	Structures of some chemical constituents found in B. pandurata	39
3	Structures of some chemical constituents found in C. longa	40
4	Structures of some chemical constituents found in K. galanga	43
5	Structures of some chemical constituents found in Z. officinale	44
6	Structures of some major compounds detected in the volatile oils	80
	of A. galanga, B. pandurata, C. longa, K. galanga and Z. officinale	
7	Structure of the isolated compounds from the rhizome in	107
	A. galanga, C. longa and Z. officinale.	
8	GC chromatogram of volatile oil from A. galanga (water distillation)	137
9	GC chromatogram of volatile oil from <i>B. pandurata</i> (water distillation)	138
10	GC chromatogram of volatile oil from C. longa (water distillation)	139
11	GC chromatogram of volatile oil from K. galanga (water distillation)	140
12	GC chromatogram of volatile oil from Z. officinale (water distillation)	141
13	Ultraviolet spectrum of AGM1 (p-coumaryl-9-methyl ether)	142
14	IR spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	143
15	<sup>1</sup> H-NMR spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	144
16	<sup>13</sup> C-NMR spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	145
17	<sup>1</sup> H- <sup>1</sup> H COSY spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	146
18	HMQC spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	147
19	NOE spectrum of AGM1 ( <i>p</i> -coumaryl-9-methyl ether)	148
20	Mass spectrum (EI) of AGM1 (p-coumaryl-9-methyl ether) (GC/MS)	149
21	Mass spectrum (FAB) of AGM1 (p-coumaryl-9-methyl ether)	150
22	<sup>1</sup> H-NMR spectrum of CLM01 (ar-turmerone)	151
23	<sup>13</sup> C-NMR spectrum of CLM01 (ar-turmerone)	152

# LIST OF ILLUSTRATIONS (continued)

Figure		Page
24	<sup>1</sup> H- <sup>1</sup> H COSY spectrum of CLM01 (ar-turmerone)	153
25	HMQC spectrum of CLM01 (ar-turmerone)	154
26	HMBC spectrum of CLM01 (ar-turmerone)	155
27	Mass spectrum (EI) of CLM01 (ar-turmerone) (GC/MS)	156
28	<sup>1</sup> H-NMR spectrum of CLM02 (curcumin)	157
29	<sup>13</sup> C-NMR spectrum of CLM02 (curcumin)	158
30	HMQC spectrum of CLM02 (curcumin)	159
31	HMBC spectrum of CLM02 (curcumin)	160
32	Mass spectrum (FAB) of CLM02 (curcumin)	161
33	<sup>1</sup> H-NMR spectrum of CLM03 (demethoxycurcumin)	162
34	<sup>13</sup> C-NMR spectrum of CLM03 (demethoxycurcumin)	163
35	HMQC spectrum of CLM03 (demethoxycurcumin)	164
36	HMBC spectrum of CLM03 (demethoxycurcumin)	165
37	Mass spectrum (FAB) of CLM03 (demethoxycurcumin)	166
38	<sup>1</sup> H-NMR spectrum of CLM06 (bisdemethoxycurcumin)	167
39	<sup>13</sup> C-NMR spectrum of CLM06 (bisdemethoxycurcumin)	168
40	Mass spectrum (FAB) of CLM06 (bisdemethoxycurcumin)	169
41	<sup>1</sup> H-NMR spectrum of ZOM0 (6-shogaol)	170
42	<sup>13</sup> C-NMR spectrum of ZOM0 (6-shogaol)	171
43	<sup>1</sup> H- <sup>1</sup> H COSY spectrum of ZOM0 (6-shogaol)	172
44	HMQC spectrum of ZOM0 (6-shogaol)	173
45	HMBC spectrum of ZOM0 (6-shogaol)	174
46	Mass spectrum (FAB) of ZOM0 (6-shogaol)	175
47	<sup>1</sup> H-NMR spectrum of ZOM1 (6-dehydrogingerdione)	176
48	<sup>13</sup> C-NMR spectrum of ZOM1 (6-dehydrogingerdione)	177

# LIST OF ILLUSTRATIONS (continued)

Figure		Page
49	<sup>1</sup> H- <sup>1</sup> H COSY spectrum of ZOM1 (6-dehydrogingerdione)	178
50	HMQC spectrum of ZOM1 (6-dehydrogingerdione)	179
51	HMBC spectrum of ZOM1 (6-dehydrogingerdione)	180
52	Mass spectrum (FAB) of ZOM1 (6-dehydrogingerdione)	181
53	<sup>1</sup> H-NMR spectrum of ZOM3 (6-gingerol)	182
54	<sup>13</sup> C-NMR spectrum of ZOM3 (6-gingerol)	183
55	<sup>1</sup> H- <sup>1</sup> H COSY spectrum of ZOM3 (6-gingerol)	184
56	HMQC spectrum of ZOM3 (6-gingerol)	185
57	HMBC spectrum of ZOM3 (6-gingerol)	186
58	Mass spectrum (FAB) of ZOM3 (6-gingerol)	187

### ABBREVIATIONS AND SYMBOLS

amu	=	atomic mass unit
BHT	=	butylated hydroxytoluene
br.	=	broad (for NMR spectra)
br.d	=	broad doublet (for NMR spectra)
br.dq	=	broad doublet of quartets (for NMR spectra)
br.m	=	broad multiplet (for NMR spectra)
br.s	=	broad singlet (for NMR spectra)
br.t	=	broad triplet (for NMR spectra)
c	=	concentration (for optical rotations)
°С	=	degree Celsius
CDCl <sub>3</sub>	=	deuterochloroform
<sup>13</sup> C NMR	=	carbon-13 nuclear magnetic resonance
cm	=	centimetre
COSY	=	correlated spectroscopy
		( <sup>1</sup> H- <sup>1</sup> H COSY: <sup>1</sup> H- <sup>1</sup> H coupling)
d	=	doublet (for NMR spectra)
D.B.E.	=	double bond equivalence (degree of unsaturation)
dd	=	doublet of doublets (for NMR spectra)
dt	=	doublet of triplets (for NMR spectra)
DMSO	=	dimethyl sulphoxide
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
EC <sub>50</sub>	=	concentration causing 50 % effective activity
EDTA	=	ethylenediamine tetraacetic acid
EtOH	=	ethanol
FAB-MS	=	fast-atom bombardment mass spectroscopy
g	=	gram
GC/MS	=	gas chromatography/mass spectrometry
HMBC	=	heteronuclear multiple-bond correlation

## ABBREVIATIONS AND SYMBOLS (continued)

HMQC	=	heteronuclear multiple-quantum correlation
<sup>1</sup> H NMR	=	proton nuclear magnetic resonance
hr	=	hour
HR-FABMS	=	high resolution fast-atom bombardment mass
		spectrometry
Hz	=	hertz
IC <sub>50</sub>	=	concentration causing 50 % inhibitory effect
IR	=	infrared
IU	=	international unit
J	=	nuclear spin-spin coupling constant (in Hz)
KBr	=	potassium bromide
kg	=	kilogram
М	=	molar (concentration)
m	=	metre
m	=	multiplet (for NMR spectra)
MeOH	=	methanol
mg	=	milligram
MHz	=	megahertz
μg	=	microgram
μl	=	microlitre
min	=	minute
ml	=	millilitre
mm	=	millimetre
mM	=	millimolar
mol	=	mole
MS	=	mass spectroscopy
MW	=	molecular weight
m/z	=	mass to charge ratio

## ABBREVIATIONS AND SYMBOLS (continued)

nM	=	nanomolar
nm	=	nanometre
NMR	=	nuclear magnetic resonance
2D NMR	=	two dimentional nuclear magnetic resonance
NOE	=	nuclear Overhauser effect (change of signal
		intensities during decoupling experiments)
OD	=	optical density
PBS	=	phosphate buffered saline
ppm	=	parts per million
q	=	quartet (for NMR spectra)
S	=	singlet (for NMR spectra)
SD	=	standard deviation
sec	=	second
SEM	=	standard error of the mean
SRB	=	sulphorhodamine B
t	=	triplet (for NMR spectra)
TCA	=	trichloroacetic acid
TLC	=	thin-layer chromatography
TMS	=	tetramethylsilane
UV	=	ultraviolet
w/w	=	weight/weight
δ	=	chemical shift (in ppm, for NMR spectra)
3	=	molar absorptivity (for UV spectra)
λ	=	wavelength (for UV spectra)
ν	=	wavenumber (for IR spectra)