

CONTENTS

	Page
Contents	(11)
List of Tables	(14)
List of Illustrations	(16)
List of Abbreviations and Symbols	(20)
Chapter	
1. Introduction	1
1.1 General Introduction	1
1.2 Introduction of cytotoxicity	2
1.3 Introduction of antioxidant	3
1.4 Introduction of apoptosis	4
1.5 Introduction to this study	4
1.6 Review of Literatures	5
1.6.1 <i>Bridelia ovata</i> Decne.	5
1.6.2 <i>Curcuma zedoaria</i> (Berg.) Roscoe	7
1.6.3 <i>Derris scandens</i> (Roxb.) Benth	10
1.6.4 <i>Dioscorea membranacea</i> Pirre	12
1.6.5 <i>Drynaria quercifolia</i> (Linn.)	13
1.6.6 <i>Erythrophleum teysmannii</i> Craib	15
1.6.7 <i>Moringa oleifera</i> Lamk.	16
1.6.8 <i>Nardostachys jatamansi</i> DC.	17
1.6.9 <i>Rhinacanthus nasutus</i> (L.) Kurz	18
1.6.10 <i>Sapindus rarak</i> DC.	19
1.6.11 <i>Smilax corbularia</i> Kunth	21
1.6.12 <i>Strychnos nux-vomica</i> Linn	22
1.7 Chemical constituents of the investigated species	24
1.8 Biological activities of the investigated species	94
1.9 Objective	116
2. Research Methodology	117
2.1 Instruments	117

CONTENTS (Continued)

	Page
2.2 Plant Materials	117
2.3 Preparation of plant extracts	118
2.3.1 Water extracts	118
2.3.2 Ethanol extracts	118
2.4 In vitro assay for cytotoxic activity	124
2.4.1 Human cell lines	124
2.4.2 Testing procedure	125
2.5 Assay for antioxidant activity	126
2.5.1 DPPH radical scavenging assay	126
2.5.2 Preparation, testing procedure and data processing	127
2.6 Bioassay-guided fractionation	128
2.7 Isolation of chemical constituents from <i>Curcuma zedoaria</i>	129
2.8 Isolation of chemical constituents from <i>Dioscorea membranacea</i>	129
2.9 Compounds from <i>Dioscorea membranacea</i>	130
2.10 Assay for apoptosis activity	130
2.10.1 Assay principle	130
2.10.2 Preparation of cells for the apoptosis assay	131
2.10.3 DeadEnd™ Colorimetric TUNEL System for apoptosis detection	132
3. Results and Discussion	134
3.1 Screening of biological activity of crude extracts	134
3.1.1 Cytotoxic activity	136
3.1.2 Free radical scavenging activity	144
3.2 Cytotoxic activity of bioassay-guided fractionation	146
3.3 Analysis of chemical composition and structure determination of the isolated compounds	148
3.3.1 Characterization of component of the oil	148
3.3.2 Structure elucidation of the isolated compounds	151
3.3.2.1 CZS1	151

CONTENTS (Continued)

	Page
3.3.2.2 CZS2	158
3.3.2.3 DMS1	165
3.3.2.4 DMS2	172
3.3.2.5 DMS3	179
3.4 Discussion on phytochemical investigation	186
3.5 Activities of the isolated compounds	188
3.5.1 Cytotoxic activity	190
3.5.2 Apoptosis assay	194
4. Conclusions	199
Bibliography	202
Vitae	221

LIST OF TABLES

Table		Page
1-1	Chemical constituents found in <i>Bridelia ovata</i> Dence	24
1-2	Chemical constituents found in <i>Curcuma zedoaria</i> (Berg.) Roscoe	25
1-3	Chemical constituents found in <i>Derris scandens</i> (Roxb.) Benth	32
1-4	Chemical constituents found in <i>Dioscorea membranacea</i> Pierre	38
1-5	Chemical constituents found in <i>Drynaria quercifolia</i> Linn	39
1-6	Chemical constituents found in <i>Moringa oleifera</i> Lamk	40
1-7	Chemical constituents found in <i>Nardostachys jatamansi</i> DC	43
1-8	Chemical constituents found in <i>Rhinacanthus nasutus</i> (L.) Kurz	45
1-9	Chemical constituents found in <i>Sapindus rarak</i> DC	49
1-10	Chemical constituents found in <i>Smilax corbularia</i> Kunth	50
1-11	Chemical constituents found in <i>Strychnos nux-vomica</i> L.	51
1-12	Biological activities of the investigated species	94
2-1	Plants and part of plants used in cancer preparation by folk doctor	119
3-1	%Yields of the ethanolic and water extracts from the investigated splices	135
3-2	% survival of cancerous cells (\pm SEM) (lung adenocarcinoma cell line = COR-L23 and prostate cancer cell line = PC3) treated with extract concentration 50 μ g/ml exposure time 72h	137
3-3	Cytotoxicity activity (IC_{50} μ g/ml \pm SEM) of plant extracts against two types of cancer cell (COR-L23, PC3) and one type of normal cells (10FS) at exposure time 72 hours	138
3-4	EC_{50} (μ g/ml) of plant extracts on DPPH assay	144
3-5	IC_{50} (μ g/ml) \pm SEM of the fraction from <i>Curcuma zedoaria</i> and <i>Dioscorea membranacea</i> separated by vacuum liquid chromatography against two cancer cell lines at exposure time 72 hours	147
3-6	Chemical constituent of the volatile oil from <i>Curcuma zedoaria</i> (GC/MS analysis)	149

LIST OF TABLES (Continued)

Table		Page
3-7	NMR spectral data (500 MHz for ^1H and 125 MHz for ^{13}C) of CZS1 and curcumin in CDCl_3	153
3-8	NMR spectral data (500 MHz for ^1H and 125 MHz for ^{13}C) of CZS2 and β -sitosterol 3- O - β -D-glucopyranoside in $\text{CDCl}_3+\text{CD}_3\text{OD}$	160
3-9	NMR spectral data (500 MHz for ^1H and 125 MHz for ^{13}C) of DMS1 and β -sitosterol 3- O - β -D-glucopyranoside in $\text{CDCl}_3+\text{CD}_3\text{OD}$	167
3-10	NMR spectral data (500 MHz for ^1H and 125 MHz for ^{13}C) of DMS2 and dioscorealide A in CDCl_3	174
3-11	NMR spectral data (500 MHz for ^1H and 125 MHz for ^{13}C) of DMS3 and dioscoreanone	181
3-12	IC_{50} value of compounds tested against lung and prostate cancer cell lines (COR-L23 and PC3), and normal cell line (fibroblast, 10FS)	192
3-13	Effects of compounds on the induction of apoptosis in lung cancer cell lines (COR-L23) and prostate cancer cell lines (PC3) at exposure time 48 hours	194

LIST OF ILLUSTRATIONS

Figure		Page
1-1	<i>Bridelia ovata</i> Dence	7
1-2	Bulb and finger of <i>Curcuma zedoaria</i> (Berg.) Roscoe the orange rhizome of <i>Curcuma longa</i> Linn (left side) compared with the yellow rhizome of <i>Curcuma zedoaria</i> (Berg.) Roscoe (right side)	9
1-3	The upper stem and flower of <i>Curcuma zedoaria</i> (Berg.) Roscoe	10
1-4	<i>Derris scandens</i> (Roxb.) Benth	11
1-5	<i>Dioscorea membranacea</i> Pirre	13
1-6	<i>Drynaria quercifolia</i> (Linn)	15
1-7	<i>Erythrophleum teysmannii</i> Craib	16
1-8	<i>Moringa oleifera</i> Lamk.	17
1-9	<i>Nardostachys jatamansi</i> DC.	18
1-10	<i>Rhinacanthus nasutus</i> (L.) Kurz	19
1-11	<i>Sapindus rarak</i> DC.	20
1-12	<i>Smilax corbularia</i> Kunth	22
1-13	<i>Strychnos nux-vomica</i> Linn.	23
1-14	Structures of some chemical constituents found in <i>B. ovata</i>	54
1-15	Structures of some chemical constituents found in <i>C. zedoaria</i>	55
1-16	Structures of some chemical constituents found in <i>D. scandens</i>	64
1-17	Structures of some chemical constituents found in <i>D. membranacea</i>	75
1-18	Structures of some chemical constituents found in <i>D. quercifolia</i>	77
1-19	Structures of some chemical constituents found in <i>M. oleifera</i>	78
1-20	Structures of some chemical constituents found in <i>N. jatamansi</i>	82
1-21	Structures of some chemical constituents found in <i>R. nasutus</i>	84
1-22	Structures of some chemical constituents found in <i>S. rarak</i>	89
1-23	Structures of some chemical constituents found in <i>S. nux-vomica</i>	91
2-1	Leaves of <i>Bridelia ovata</i> Dence	120
2-2	Rhizomes of <i>Curcuma zedoaria</i> (Berg.) Roscoe	120

LIST OF ILLUSTRATIONS (Continued)

Figure	Page
2-3 Stems of <i>Derris scandens</i> (Roxb.) Benth	120
2-4 Rhizomes of <i>Dioscorea membranacea</i> Pirre	121
2-5 Rhizomes of <i>Drynaria quercifolia</i> (Linn.)	121
2-6 Stems of <i>Erythrophleum teysmannii</i> Craib	121
2-7 Barks of <i>Moringa oleifera</i> Lamk.	122
2-8 Flowers of <i>Nardostachys jatamansi</i> DC.	122
2-9 Roots of <i>Rhinacanthus nasutus</i> (L.) Kurz	122
2-10 Fruit of <i>Sapindus rarak</i> DC.	123
2-11 Rhizomes of <i>Smilax corbularia</i> Kunth	123
2-12 Seeds of <i>Strychnos nux-vomica</i> Linn	123
2-13 Diagram of the DeadEnd™ Colorimetric TUNEL System end-labels the fragmented DNA	131
3-1 IC ₅₀ (µg/ml) of active crude extracts on cell lines exposure time 72h and using student t-test from Prism to compare significant difference between normal cell (10FS) and each cancer cell (COR-L23 and PC3)	140
3-2 Histogram comparing IC ₅₀ of each fractions against two cancer cell lines at exposure time 72 h.	147
3-3 Structures of compounds detected in the volatile oil of <i>C. zedoaria</i>	150
3-4 GC-MS spectrum of CZV in hexane	150
3-5 TLC of CZS1 in 2 hexane : 8 CHCl ₃ , 9 CHCl ₃ : 1 MeOH and 9 CHCl ₃ : 3 MeOH : 0.5 H ₂ O [1=CZS1, 2=Curcumin(Sigma)]	152
3-6 CZS1 (Curcumin)	152
3-7 IR spectrum of CZS1 in CHCl ₃	154
3-8 ¹ H NMR spectrum of CZS1 in CDCl ₃	155
3-9 ¹³ C NMR spectrum of CZS1 in CDCl ₃	156
3-10 EIMS spectrum of CZS1	157

LIST OF ILLUSTRATIONS (Continued)

Figure	Page
3-11 TLC of CZS2 in 9 CHCl ₃ : 1 MeOH and 9 CHCl ₃ : 3 MeOH : 0.3 H ₂ O (1=CZS2, 2=β-Sitosterol 3-O-β-D-glucopyranoside)	159
3-12 CZS2 (β-Sitosterol 3-O-β-D-glucopyranoside)	159
3-13 IR spectrum of CZS2 in CHCl ₃	161
3-14 ¹ H NMR spectrum of CZS2 in CDCl ₃ +CD ₃ OD	162
3-15 ¹³ C NMR spectrum of CZS2 in CDCl ₃ +CD ₃ OD	163
3-16 EIMS spectrum of CZS2	164
3-17 TLC of DMS1 in 9 CHCl ₃ : 1 MeOH and 9 CHCl ₃ : 3 MeOH : 0.3 H ₂ O (1=DMS1, 2=β-Sitosterol 3-O-β-D-glucopyranoside)	166
3-18 DMS1 (β-Sitosterol 3-O-β-D-glucopyranoside)	166
3-19 IR spectrum of DMS1 in CHCl ₃	168
3-20 ¹ H NMR spectrum of DMS1 in CDCl ₃ +CD ₃ OD	169
3-21 ¹³ C NMR spectrum of DMS1 in CDCl ₃ +CD ₃ OD	170
3-22 EIMS spectrum of DMS1	171
3-23 TLC of DMS2 in 9 CHCl ₃ : 1 MeOH and 9 CHCl ₃ : 3 MeOH : 0.3 H ₂ O (1=DMS2, 2=Dioscorealide A)	173
3-24 DMS2 (Dioscorealide A)	173
3-25 IR spectrum of DMS2 in CHCl ₃	175
3-26 ¹ H NMR spectrum of DMS2 in CDCl ₃	176
3-27 ¹³ C NMR spectrum of DMS2 in CDCl ₃	177
3-28 EIMS spectrum of DMS2	178
3-29 TLC of DMS3 in 2 hexane : 8 CHCl ₃ , 9 CHCl ₃ : 1 MeOH and 9 CHCl ₃ : 3 MeOH : 0.3 H ₂ O (1=DMS3, 2=dioscoreanone)	180
3-30 DMS3 (Dioscoreanone)	180

LIST OF ILLUSTRATIONS (Continued)

Figure		Page
3-31	IR spectrum of DMS3 in CHCl ₃	182
3-32	¹ H NMR spectrum of DMS3 in CDCl ₃	183
3-33	¹³ C NMR spectrum of DMS3 in CDCl ₃	184
3-34	EIMS spectrum of DMS3	185
3-35	The chemical structure of four compounds isolated from the ethanolic extracts of <i>Curcuma zedoaria</i> and <i>Dioscorea membranacea</i>	187
3-36	Structure of the isolated compounds from rhizomes of <i>Curcuma zedoaria</i> and <i>Dioscorea membranacea</i>	188
3-37	IC ₅₀ values (μ M) and SEM of cytotoxic compounds isolated from the ethanolic extracts of <i>C. zedoaria</i> and <i>D. membranacea</i> against lung and prostate cancer cell lines (COR-L23 and PC3) and normal cell line (10FS) at exposure time 72h	189
3-38	Compounds-induced apoptosis in COR-L23 cell after 48 h incubation	195
3-39	Compounds-induced apoptosis in PC3 cell after 48 h incubation	195
3-40	Apoptotic cells were demonstrated in PC3 and COR-L23 cells by colorimetric TUNEL assay after treatment with curcumin and diosgenin 3- O - α -L-rhamnopyranosyl(1→2)- β -D-glucopyranoside (DRG) for 48 hours and staining	196

LIST OF ABBREVIATIONS AND SYMBOLS

amu	=	atomic mass unit
BHT	=	butylated hydroxytoluene
br.s	=	broad singlet (for NMR spectra)
°C	=	degree Celsius
CC	=	column chromatography
CDCl ₃	=	deuterochloroform
CD ₃ OH	=	deuteromethanol
CHCl ₃	=	chloroform
¹³ C NMR	=	carbon-13 nuclear magnetic resonance
cm	=	centimeter
d	=	doublet (for NMR spectra)
dd	=	doublet of doublet (for NMR spectra)
DMSO	=	dimethyl sulphoxide
DNA	=	deoxyribonucleic acid
DPPH	=	1,1-diphenyl-2-picrylhydrazyl
EC ₅₀	=	concentration causing 50% effective activity
EDTA	=	ethylenediamine tetraacetic acid
EI-MS	=	electron impact mass spectroscopy
EtOH	=	ethanol
g	=	gram
FAB-MS	=	fast-atom bombardment mass spectrometry
Ft	=	foot (a measuring unit)
FTNMR	=	fourier transform nuclear magnetic resonance
GC/MS	=	gas chromatography/mass spectrometry
¹ H-NMR	=	proton nuclear magnetic resonance
hr	=	hour
Hz	=	hertz
IC ₅₀	=	concentration causing 50% inhibitory effect
In	=	inch

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

IR	=	infrared
<i>J</i>	=	nuclear spin-spin coupling constant (in Hz)
M	=	molar (concentration)
M+	=	molecular ion
m	=	meter
mg	=	milligram
MHz	=	megahertz
min	=	minute
ml	=	milliliter
mm	=	millimeter
mM	=	millimolar
mol	=	mole
MS	=	mass spectroscopy
MW	=	molecular weight
<i>m/z</i>	=	mass to charge ratio
μg	=	microgram
μl	=	microliter
nM	=	nanomolar
nm	=	nanometer
NMR	=	nuclear magnetic resonance
OD	=	optical density
PBS	=	phosphate buffer saline
ppm	=	part per million
s	=	singlet (for NMR spectra)
sec	=	second
SEM	=	standard error of the mean
SRB	=	sulphorhodamine B
TCA	=	trichloroacetic acid
TLC	=	thin-layer chromatography

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

TMS	=	tetramethylsilane
ROS	=	reactive oxygen species
UV	=	ultraviolet
VLC	=	vacuum liquid chromatography
w/w	=	weight/weight
δ	=	chemical shift (in ppm, for NMR spectra)
λ	=	wavelength (for UV spectra)
ν	=	wavenumber (for IR spectra)

There are several types of cancer which are the cause of three of the top ten causes of death. Among them, lung cancer is the most common cancer in the world (Ferlay et al., 2003) and it is the second cause of death in That cancer patient (National statistical office, 2003). Another type of cancer is prostate cancer, a serious problem of public health; it is the fifth leading cause of death among men in the world (Ferlay et al., 2003) including a major cause of death among men in European countries (Brenner et al., 1997). Prostate cancer occurs after lung cancer in male cancers worldwide and there were nearly 145 000 cases and 36 000 deaths in the European Union in 1998 (Brenner and Gabley, 2001). Prostate cancer incidence rates are strongly affected by diagnostic practices and therefore difficult to interpret, but mortality rates show that prostate cancer is about 10 times more common in North America and Europe than in Asia (Ferlay et al., 2003). Prostate cancer is estimated to account for about 1.6 million new cases worldwide in 2007 (World Health Organization Mortality Database, 2008). In Thailand, there were 0.4 100 000 deaths in 1999 and an increase to 0.6 100 000 in 2003 (National Statistical Office, 2003).