TABLE OF CONTENTS

्रे भुश्चामण	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS AND SYMBOLS	xvii
CHAPTER 1 INTRODUCTION	1
1. Rationale	1
2. Objectives of the study	6
CHAPTER 2 LITERATURE REVIEWS	7
1. Evaluation of non-cytotoxic polyphenols as putative	
new drugs in cancer chemotherapy	7
2. Biochemistry and physiology of normal and cancer	}
เปลาธิแมหาวิทยาลยเชียง	10
Copyrigh ^{2.1 Energetic state} hiang Mai Univer	rsit ¹
2.2 Buffering system 2.3 Oxidative stress	12
3. Classification of polyphenols	17
4. Antioxidant activities of polyphenols	21

5.	Effect of polyphenols on cellular biochemistry and	
	Physiology	23
	5.1 Apoptosis-inducing activity against cancer cells	23
	5.2 Multidrug resistant cancer cells	28
	5.3 NFκB-regulated gene expression in cancer cells	35
CHAPTER 3 MA	ATERIALS AND METHODS	41
j.	Chemicals and Reagents	41
2.	Characterization of Siamois® polyphenols	42
	2.1 Preparation of Siamois® crude extracts	42
503	2.2 Preparative liquid column chromatography and	
	HPLC	44
10	2.3 Acid-Butanol hydrolysis	44
3.	Cells and Culture conditions	45
	3.1 Cancer cell lines	45
	3.2 Establishment of primary human myoblasts	
	cultures	46
	3.3 Detection of desmin and CD56 co-expression	47
3 a 3 m 4.	Fluorescence micrographs of living cells	48
	Cytotoxicity assay	48
Copyrigh	5.1 Cancer cells Chiang Mai University	48
AII	5.2 Normal myoblasts	49
6.	Determination of intracellular ATP, ADP and AMP	
	levels	49
7.	Determination of mitochondrial membrane potential	50
8.	Apoptosis assay	52

Ģ	9.	Reporter Gene Analysis	54
	10.	RNA isolation and real-time Q-PCR analysis	55
	11.	Western blot analysis	56
	12.	Electrophoretic Mobility Shift Assay (EMSA)	56
	13.	Measurement of caspase-3 activity	57
CHAPTER 4 R	RES	ULTS	58
	1.	Polyphenol composition of Siamois [®] , Siamois 1	
9		and Siamois 2	58
	2.	Visualization of potential intracellular targets of	
502		Siamois [®] , Siamois 1 and Siamois 2 crude extracts	
		in normal cells	64
	3.	Cytotoxicity of Siamois [®] , Siamois 1 and Siamois 2	
1 =		crude extracts against normal cells	66
	4.	Anticancer activity of Siamois [®] and Siamois 1 crude	
		extracts	67
:	5.	Effects of Siamois [®] , Siamois 1 and Siamois 2 crude	
		extracts on cellular ATP, ADP, and AMP levels in	
22	2	cancer cells	70
adan	6.	Apoptosis-inducing activity of Siamois® crude extracts	72
Copyrig	7.	Putative active polyphenols in Siamois®, Siamois 1	ty
AII	r	and Siamois 2 crude extracts	72
8	8.	Modulation of mitochondrial membrane potential	
		$(\Delta \Psi_m)$ and Apoptotic induction by quercetin	73
<u>(</u>	9.	Pure polyphenols present in Siamois® crude extract	
		dose dependently inhibited NFκB-driven reporter	

gene expression in murine L929sA cells	74
10. Pure polyphenols present in Siamois® crude extract	
inhibit similarly endogenous NFκB target gene	
expression in K562 and K562/Adr cells, irrespective	
of doxorubicin sensitivity	76
11. Pure polyphenols present in Siamois® crude extract	
all prevent IkB degradation but selectively interfere	
with p38, ERK MAPK, MEK1 and Akt kinase	
activation	81
12. K562 and K562/Adr cells reveal distinct nuclear	
regulation of NFκB, AP1, Nrf2 and Sirt1 proteins	84
13. NFκB, AP1 DNA-binding profiles in K562 and	
K562/Adr cells show qualitative and quantitative	
differences	85
14. Quercetin and eriodictyol both present in Siamois®	
crude extract strongly inhibit DNA binding of NFκB,	
AP1 and Nrf2	86
15. Pure polyphenols present in Siamois® crude extract	KJ I
reduce cell viability in both K562 and K562/Adr cells	89
16. Pure polyphenols present in Siamois® crude extract	ty
induce early apoptosis in both K562 and K562/Adr	d
cells but only late apoptosis in the former	90
17. Pure polyphenols present in Siamois® crude extract	
induce caspase-3 activation in K562 but not K562/Adr	

cells			93
CHAPTER 5 DISCUSSIONS A	AND CONCLUSIONS		95
REFERENCES			103
APPENDICES	091613160		130
APPENDIX A	Differential chemosensitisation of		
790	P-glycoprotein overexpressing K562/Adr		
	cells by Withaferin A and Siamois		
	polyphenols	92	131
APPENDIX B	The intracellular targets and apoptosis-		
502	inducing activity of Siamois® in drug-	502	
	sensitive and drug- resistant cancer cells	200	169
CURRICULUM VITAE		76	198

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

TAI UNIVERSIT

LIST OF TABLES

TABL	E 0 9/3/21/00	PAGE
	0000	
1	Chemical structures of phenolic acids	17
2	Chemical structure of flavonoids	19
3	Major ABC transporters associated with MDR, chemotherapy	
	substrates and MDR inhibitors common other systematic	
	substrates inhibitors	31
4	Primers sequences used in the real-time Q-PCR	55
5	IC50 value of Siamois [®] , Siamois 1 and Siamois 2 crude extracts,	
	quercetin, and doxorubicin in K562, K562/Adr, GLC4 and	
	GLC4/Adr cells	69
6	Resistance factor (RF) of Siamois [®] , Siamois 1 and Siamois 2	
	crude extracts, quercetin, and doxorubicin in K562, K562/Adr,	
	GLC4 and GLC4/Adr cells	70
7	IC50 values of pure polyphenols present in Siamois® crude	hIJ
op	extract in K562 and K562/Adr cells after 72 h incubation	90
	l rights reserve	e d

LIST OF FIGURES

FIC	GUI	RE 978181969 PA	.GE
	1	The scheme of conventional cancer therapy and therapy	
	/	targeted against cancer stem cells	10
	2	Carbonic buffer plays a role in providing additional mobile	
		buffer for facilitating intracellular proton flux	13
	3	Chemical structure of tannins	21
	4	Hallmarks of the apoptotic and necrotic cell death process	24
	5	Schematic representation of some major apoptotic signalling	
		pathways	25
	6	Cellular factors that cause drug resistance	30
	7	Structures of ABC transporters known to confer drug resistance	30
	8	Schematic diagram of NFκB/Rel protein structure	37
	9	NFκB signaling pathways	37
9	10	Cancer cell lines; K562, K562/adr, GLC4, and GLC4/adr	45
O	11	Typical kinetics of cellular rhodamine B uptake recorded from	tv
		Spectrofluorometer ts reserve	52
	12	Annexin V-FITC binding with phosphatidyl serine (PS) on cell	
		membrane	53
	13	Biparametric histogram of annexin V-FITC versus PI of MDA-	

	MB435 cells	54
14	HPLC chromatogram of mixture of standard polyphenols	
	solution, Siamois®, Siamois 1 and Siamois 2	59
15	Absorption spectra of the fractions obtained from Sephadex®	
	G-50 open column chromatography of Siamois [®] , Siamois 1	
	and Siamois 2	61
16	Acid butanol hydrolysis of Siamois® polyphenols	62
17	HPLC chromatogram of Siamois® crude extracts obtained from	
	solid/liquid extraction	63
18	HPLC chromatograms of pigments and flavonoids present in	
	Siamois® crude extract	64
19	Fluorescence micrograph of myoblasts	66
20	Effects of Siamois [®] , Siamois 1 and Siamois 2 and doxorubicin	
	on myoblast cell growth	67
21	Effects of Siamois® and Siamois 1 on K562 and K562/adr, and	
	GLC4 and GLC4/adr cell growth	68
22	Effects of Siamois 2 on cellular ATP, ADP and AMP levels in	
	cancer cells	71
23	24 h apoptosis-induced activities of Siamois® crude extract	HU
Cop	against K562, K562/Adr, GLC4 and GLC4/Adr cells	72
24	Spontaneous change of absolute value of $\Delta\Psi_m$ and apoptosis-	d
	induced activity in the presence of quercetin.	74
25	Pure polyphenols present in Siamois® crude extract dose-	
	dependently inhibited NFkB-dependent reporter gene expression	

	in murine L929sA cells	75
26	Pure polyphenols present in Siamois® crude extract inhibited	
	endogenous NF κ B-dependent transcription in K562 and K562/Adr	
	cells	77
27	Selective effects of pure polyphenols present in Siamois® crude	
	extract on the NFκB signaling pathway	82
28	K562 and K562/adr cells reveal different nuclear regulation of	
	NFκB, AP1, Nrf2 transcription factors and Sirt1 cofactors	85
29	K562 and K562/adr cells show qualitative and quantitative	
0	differences in NFκB and AP1/DNA-binding profiles	88
30	Apoptosis induced-activities of pure polyphenols present in	
	Siamois® crude extract in K562 and K562/Adr cells	92
31	Caspase3 activation by pure polyphenols present in Siamois®	
	crude extract in K562 and K562/Adr cells	94
	MAI UNIVERSI	

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved

LIST OF ABBREVIATIONS AND SYMBOLS

AO Acridine orange

Rho B Rhodamine B

ATP Adenosine triphosphate

ADP Adenosine diphosphate

AMP Adenosine monophosphate

CD56-PE CD56-Phycoerythrin

Dox Doxorubicin

EDTA Ethelenediaminetetra acetic acid

EMSA electrophoretic mobility shift assay

FITC Fluoresceinisothiocyanate

GLC4 human small cell lung carcinoma drug-sensitive cell

GLC4/Adr human small cell lung carcinoma drug-resistant cell

K562 human erythromyelogenous leukemic drug-sensitive cell

K562/Adr human erythromyelogenous leukemic drug-resistant cell

NFκB nuclear factor kappa B

IκB inhibitory subunit of NFκB

IKK IκB kinase

IL Interleukin

h hour

min minute

 $M \mod L^{-1}$

MDR Multidrug resistance

MRP1 multidrug-associated Protein

MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide

NCAM/CD56 myocyte-associated neural cell adhesion molecule

P-gp P-glycoprotein

PI Propidium iodide

PMA Phorbol-12-myristate-13-acetate

PS phosphatidylserine

RF Resistance factor

ROS_i Intracellular reactive oxygen species

THP Theprarubicin/pirarubicin

TNF tumour necrosis factor

 $\Delta \Psi_{\rm m}$ mitochondrial membrane potential

pH_i intracellular pH

pH_v lysosomal pH

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved