TABLE OF CONTENTS

		page
ACKNOWI	LEDGEMENT	iii
ABSTRACT	rab	iv
LIST OF TA	ABLES	xi
LIST OF FI	IGURES	xii
LIST OF A	BBREVIATIONS	xv
CHAPTER	s	
532	GENERAL INTRODUCTION	1
	Introduction to polyphenols	1
	Health benefits of polyphenols	1
	Potential use of polyphenolic compounds in cancer prevention	2
	Potential use of polyphenolic compounds in cancer intervention	3
	Cellular drug resistance in cancer chemotherapy	3
	Polyphenols modulate the cellular energetic state	4
	Polyphenols as inhibitors of MDR transporters	5
15	OBJECTIVES	6
	THE ORGANIZATION OF THE THESIS DISSERTATION	7
	REFERENCES	8
II	RED WINE POLYPHENOLS PROMOTE NORMAL	14
	MYOCYTE GROWTH BUT EXHIBIT ANTICANCER AND	
	APOPTOSIS-INDUCING ACTIVITIES AGIANST CANCER	O
	MULTIDRUG-RESISTANT CELLS	
	INTRODUCTION	15
	MATERIALS AND METHODS	17

	Siamois wine making	17
	Isolation of red wine polyphenols	17
	Establishment of primary culture of human myocytes	18
	Cell lines, cell culture and cytotoxicity assay	19
	Induction of apoptosis	20
	Cytofluorometric staining of the cells	20
	RESULTS	20
	SRPE promoted myocyte growth	21
	Anticancer activity	24
	Apoptosis-inducing activity	26
	DISCUSSION	28
	Normal human myocytes	28
	Cancer cell lines	29
	ACKNOWLEDGMENT	29
	REFERENCES	29
III	SPONTANEOUS MITOCHONDRIAL POTENTIAL	32
	CHANGE DURING APOPTOTIC INDUCTION	
	BY QUERCETIN IN K562 AND K562/ADR CELLS	
	INTRODUCTION	33
	MATERIALS AND METHODS	34
	Drug preparation	34
	Radiolabeled compounds	34
	Cell culture and cytotoxicity assay	35
	Induction of apoptosis	35
	Cytofluorometric staining of the cells	35
	Measurement of mitochondrial membrane potential ($\Delta \Psi_m$)	35
	Determination of cellular uptake of 99mTc-tracers	36
	RESULTS	37

	Cytotoxicity of quercetin against K562 and K562/adr cells	37
	Quercetin induced apoptosis	38
	Modulation of mitochondrial membrane potential by quercetin	39
	DISCUSSION	42
	ACKNOWLEDGEMENTS	43
	REFERENCES	43
IV	QUERCETIN, EXTRACTS OF MAMOA WOOD	46
	ANDSIAMOIS® RED WINE INDUCE APOPTOSIS IN	
	HUMAN BREAST CANCER MDA-MB-435 CELLS	
	XENOGRAFTS IN VIVO	
	INTRODUCTION	47
	MATERIALS AND METHODS	49
	Siamois® red wine vinification	49
	Preparation of extracts of mamoa wood	49
	Cell culture and apoptotic induction assay	49
	Cytofluorometric staining of the cells	50
	Animal experiments	50
	Preparation of 99m Tc-hynic-rh-Annexin V	50
	Radionuclide imaging	51
	Tissue preparation and immuno-histochemical analysis	51
	Statistical analyses	52
	RESULTS Y Chiang Mai University	52
	DISCUSSION AND CONCLUSION	58
	ACKNOWLEDGEMENTS	59
	REFERENCES	59
V	GENERAL DISCUSSION AND CONCLUSION	62

APPENDICES

A	SPECTROFLUOROMETRIC DETERMINATTIOIN	64
	OF INTRACELLULAR LEVELS OF REACTIVE	
	OXIGEN SPECIES IN DRUG-SENSITIVE AND	
	DRUG-RESISTANT CANCER CELLS USING	
	2', 7'-DICHLOROFLUORESCEIN ACETATE ASSAY	
	INTRODUCTION	66
	MATERIALS AND METHODS	67
	Drugs and chemicals	67
	Cell culture and cytotoxicity assay	68
	Physicochemical properties of DCHF-DA	68
	Determination of the intracellular DCF concentration	69
	Theoretical approach	71
	RESULTS AND DISCUSSION	73
	ACKNOWLEDGEMENTS	77
	REFERENCES	77
В	P-GLYCOPROTEIN-MEDIATED EFFLUX AND	80
	LYSOSOMAL SEQUESTRATION OF DRUGS CONFER	
	ADVANTAGES OF K562 MDR SUBLINES TO SURVIVE	
	PROLONGED EXPOSURE TO CYTOTOXIC AGENTS	
	INTRODUCTION S I C S E I V E	81
	MATERIALS AND METHODS	82
	Drugs and chemicals	82
	Cell culture and cytotoxicity assay	83

	Selection of MDR cells	83
	Theoretical approach for intracellular $pH(pH_i)$	83
	and luminal pH (pH $_v$) of lysosome determination	
	Subcellular distribution of acridine orange	84
	Cellular uptake and the Pgp-mediated efflux of pirarubicin	85
	Fluorescence micrograph and flow cytofluorometric assay	86
	RESULTS	86
	Cytotoxic effects of acridine orange and THP	86
	Cellular distribution of AO	86
	Determination of the lysosomal concentration (C_v)	86
	and mean influx coefficient (k_+) of AO	
	P-glycoprotein-mediated efflux of pirarubicin and AO	90
	DISCUSSION	91
	ACKNOWLEDGEMENTS	93
	REFERENCES	93
CURRICUL	LUM VITAE	95

ลิ**ปสิทธิมหาวิทยาลัยเชียงใหม** Copyright[©] by Chiang Mai University All rights reserved

LIST OF TABLE

page

APPENDIX A

Table 1 Mean rate constant (k_e) of the DCHF-DA deacetylation catalyzed by 76 intracellular esterases (pseudo-first-order reaction).

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

LIST OF FIGURES

		page
CHAPTE	N 31 21 26 0	
Figure 1	Fluorescence micrograph of 3 rd passage myoblasts.	22
Figure 2	Effects of SRPE, MPE, Siamois® and doxorubicin on myocyte cell	23
	growth.	
Figure 3	Effects of SRPE and/or MPE on cancer cell growth.	24
Figure 4	Effects of MPE and Siamois® on MDA-MB-435 cell growth.	25
Figure 5	Representative biparametric histogram of an Annexin V-FITC	26
	versus PI of MDA-MB-435 cells.	
Figure 6	Apoptosis-inducing activities of MPE and Siamois® against MDA-	27
	MB-435 cells.	
Figure 7	Apoptosis-inducing activities of SRPE	28
	PIULIVERS	
CHAPTE	RIII	
Figure 1	Chemical structure of quercetin.	37
Figure 2	Monoparametric histogram of cell bound radiolabeled compound	37
	Annexin V-FITC.	
Figure 3	Variation of percentage of early apoptotic cells as a function of time.	38
Figure 4	Detection of early apoptotic cells using 99mTc-Annexin V.	39
Figure 5	Accumulation of ^{99m} Tc-MIBI in cancer cells.	40
Figure 6	Absolute value of mitochondrial membrane potential ($\Delta \Psi_m$)	41

Figure 7	Variation of absolute value of $\Delta\Psi_m$ and an early apoptotic cell.	41
СНАРТЕ	RIV	
Figure 1	Micrograph of tumor section and tumor growth curve.	53
Figure 2	Apoptosis-inducing activities of quercetin, extracts of mamoa wood	54
	and Siamois® red wine on MDA-MB-435 in cell culture system.	
Figure 3	Gamma scintigraphic images showing the enhanced accumulation of	55
	99mTc-annexin V in the MDA-MB-435 breast tumor xenografted in	
	athymic nude mice.	
Figure 4	Ex vivo results of 99mTc-Annexin V of tumor activity.	55
Figure 5	Ex vivo histological tumor tissue.	56
Figure 6	Ex vivo histological liver.	57
APPENDIX A		
Figure 1	Absorption spectra of DCHF-DA in aqueous solution.	69
Figure 2	Excitation and emission spectra of DCF in HEPES-Na ⁺ buffer.	70
Figure 3	Mechanism of action of 2', 7'-dichlorofluorescein diacetate (DCHF-	71
	DA).	
Figure 4	Kinetics of DHCF-DA oxidation.	74
Figure 5	Kinetics of DCHF-DA oxidation performed for different	75
	concentrations of K562 cells.	

APPENDIX B

Figure 1	Drug response pattern of MDR cell lines.	8
Figure 2	Fluorescence micrograph of human myoblastic cells showing	88
	lysosomes (orange or red fluorescence in circles) and cytoplasm	
	(green fluorescence).	
Figure 3	Quantitative measurement of lysosomal acridine orange	88
	concentration.	
Figure 4	A typical acridine orange uptake, initial rate of uptake and	89
	intralysosmal concentration of AO in K562, K562/adr and	
	K562/10000 cell lines.	
Figure 5	The Pgp-mediated efflux of pirarubicin and AO determined from	90
	K562/adr and K562/10000 cells.	
Figure 6	Determination of intracellular pH and intraluminal pH of lysosomes	93
	of K562, K562/adr and K562/10000 cell lines.	

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

LIST OF ABBREVIATIONS

adr or ADR

Adriamycin

AO

Acridine orange

antiCD95/CD95L/FasL

CD95 ligand/Fas ligand

ATP

Adenosine triphosphate

CD56-PE

CD56-Phycoerythrin

 $\Delta \Psi_m$

mitochondrial membrane potential

DAB

DNA-biotinylated-diaminobenzidine

DCHF-DA

2',7'-dichlorofluorescein diacetate

DCHF

2',7'-dichlorofluorescein

DCF

dichlorofluorescein

Dox

Doxorubicin

EDTA

Ethelenediaminetetra acetic acid

FITC

Fluoresceinisothiocyanate

GG918

Acridone carboxamide derivative

H&E

Hematoxyline and Eosin

ITLC

instant thin-layer chromatography

MDR

Multidrug resistance

MPE

Mamoa wood extract

MRP1

multidrug-associated Protein

MTT

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

bromide

m.f.p. Mammary fat pad

NCAM/CD56 myocyte-associated neural cell adhesion molecule

PI Propidium iodide

Pgp P-glycoprotein

PS phosphatidylserine

RF Resistance factor

ROI Region-of-interest

ROS_i Intracellular reactive oxygen species

Siamois® The mixture of SRPE and MPE

s.c. subcutaneous

SRPE Siamois® red wine polyphenol extract

TUNEL Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick

End Labeling

THP Theprarubicin/pirarubicin

TW Tumor weight

K562 human erythromyelogenous leukemic drug-sensitive cell

K562/adr human erythromyelogenous leukemic drug-resistant cell

GLC4 human small cell lung carcinoma drug-sensitive cell

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

MDA-MB-435 human breast carcinoma cell