Table of Contents

Abstract	ii
Abstrak	iii
Acknowledgements	iv
List of Figures	xi
List of Tables	xii
List of Abbreviations	xiii
List of Appendices	xvii
1.0 INTRODUCTION	1
1.1 General introduction	1
1.2 Objectives	3
2.0 LITERATURE REVIEW	4
2.1 Antioxidant	4
2.1.1 Oxidant and antioxidant	4
2.1.2 Types of antioxidants	8
2.1.2.1 Endogenous antioxidants	9
2.1.2.2 Exogenous dietary antioxidants	11
2.1.3 Detection of exogenous and endogenous antioxidants	12
2.2 Cancer	16
2.2.1 Formation of cancer	16
2.2.2 Cancer statistics	19
2.2.3 Current treatment for cancer	20
2.2.4 Detection of anticancer agents	22

2.3 Ficus spp.	24
2.3.1 <i>Ficus</i> taxonomy	24
2.3.2 Ficus deltoidea	25
2.4 Genomics and Proteomics	28
2.4.1 Genomics	28
2.4.1.1 Reverse transcription- polymerase chain reaction (RT-PCR)	28
2.4.2 Proteomics	29
2.4.2.1 Two-dimension polyacrylamide gel electeophoresis (2-D	29
PAGE)	
2.4.2.2 Matrix assisted laser desorption/ionization (MALDI)	30
3.0 METHODOLOGY	31
3.1 List of Equipments	31
3.2 List of chemicals and materials	33
3.3 Plant sample extraction	34
3.3.1 Crude sample extraction	34
3.3.2 Semi-purification of samples	34
3.3.3 Protein estimation	35
3.4 Preliminary antioxidative studies	36
3.4.1 Total phenolic content assay	36
3.4.1.1 Preparation of reagents	36
3.4.1.2 Assay protocol	36
3.4.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay	37
3.4.2.1 Preparation of reagents	37
3.4.2.2 Assay protocol	37

vi

3.4.3 Lipid peroxidation	38
3.4.3.1 Preparation of reagents	38
3.4.3.2 Assay protocol	39
3.5 Cell culture	40
3.5.1 Preparation of media, buffer and sample solution	40
3.5.2 Cell culture procedure	41
3.6 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)	43
assay	
3.6.1 Preparation of MTT solution	43
3.6.2 Assay protocol	43
3.7 Endogenous antioxidative enzymes	44
3.7.1 Catalase	45
3.7.1.1 Preparation of reagents	45
3.7.1.2 Assay protocol	45
3.7.2 Glutathione peroxidase	46
3.7.2.1 Preparation of reagents	46
3.7.2.2 Assay protocol	46
3.7.3 Superoxide dismutase	47
3.7.3.1 Preparation of reagents	47
3.7.3.2 Assay protocol	47
3.8 DNA fragmentation	48
3.8.1 Preparation of reagents	48
3.8.2 Assay protocol	49

3.9 Proteomics	50
3.9.1 Sample extraction	50
3.9.1.1 Preparation of lysis buffer	50
3.9.1.2 Extraction procedure	50
3.9.2 Two-dimensional (2D) gel	51
3.9.2.1 IEF	51
3.9.2.1.1 Preparation of buffers	51
3.9.2.1.2 IEF procedure	51
3.9.2.2 2-D PAGE	52
3.9.2.2.1 Preparation of buffers	52
3.9.2.2.2 Preparation of 11% electrophoresis gel	54
3.9.2.2.3 Gel electrophoresis procedure	54
3.9.2.3 Silver staining	55
3.9.2.3.1 Preparation of staining solution	55
3.9.2.3.2 Silver staining procedure	56
3.9.3 Image Master analysis	56
3.9.4 Matrix assisted laser desorption/ionization (MALDI)	56
3.9.4.1 Preparation of solutions	56
3.9.4.2 Sample preparation	58
3.9.4.3 Sample clean up procedure	58
3.9.4.4 Sample spotting and mass spectrometry analysis	59
3.10 Real-time quantitative reverse transcription-PCR (RT-PCR)	60
3.10.1 RNA extraction	60
3.10.2 Reverse transcription of RNA to cDNA	60

3.10.3 Gene expression analysis	60
4.0 RESULTS	61
4.1 Plant protein extraction via ammonium sulfate precipitation	61
4.2 Preliminary antioxidative studies	63
4.2.1 Total phenolic content assay	63
4.2.2 DPPH assay	65
4.2.3 Lipid peroxidation assay	67
4.3 MTT assay	69
4.4 Endogenous antioxidants	72
4.4.1 Catalase	72
4.4.2 Glutathione peroxidase	74
4.4.3 Superoxide dismutase	76
4.5 DNA fragmentation	78
4.6 Gel image analysis	81
4.7 MALDI	113
4.8 Real-time RT-PCR	132
5.0 DISCUSSION	136
5.1 Plant sample extraction	136
5.2 Preliminary antioxidative studies	137
5.3 MTT assay	138
5.4 Endogenous antioxidants	139
5.5 DNA fragmentation	140
5.6 Gel image analysis	141
5.7 Identification of protein by MALDI and validation by RT-PCR	141

6.0 CONCLUSION	146
References	148
Appendices	154

List of Figures

Figure 2.1	Generation of ROS in the mitochondria of living organism	7
Figure 2.2	Formation of MDA-TBA complex in lipid peroxidation assay	13
Figure 2.3	Cancer formation processes	18
Figure 2.4	Ficus deltoidea plant with fruits	26
Figure 2.5	Ficus deltoidea female and male leaves	26
Figure 4.1	Protein concentration of <i>Ficus deltoidea</i> samples following different percentage of ammonium sulfate precipitation	62
Figure 4.2	Total phenolic content in F. deltoidea samples	64
Figure 4.3	IC ₅₀ value of <i>Ficus deltoidea's</i> extracts in DPPH assay	66
Figure 4.4	IC_{50} values for <i>Ficus deltoidea's</i> samples and ascorbic acid in lipid peroxidation assay	68
Figure 4.5	IC_{50} values for SF semi-purified fractions towards three different cell lines	70
Figure 4.6	IC_{50} values of three different cell lines following treatment with doxorubicin	71
Figure 4.7	Catalase concentrations in untreated (control) and treated cells	73
Figure 4.8	GPx activities in untreated (control) and treated cells	75
Figure 4.9	Percentage inhibition of NBT in untreated (control) and treated cells	77
Figure 4.10	DNA fragmentations following treatment with doxorubicin	79
Figure 4.11	DNA fragmentations following treatment with semi-purified and crude sample in different cell lines	80
Figure 4.12	Deregulated proteins with at least two fold changes following	83
	different treatments at IC ₅₀ values for Hep G2 cells	
Figure 4.13	Deregulated proteins with at least two fold changes following	94
	different treatments at IC50 values for Ca Ski cells	
Figure 4.14	Deregulated proteins with at least two fold changes following	103
	different treatments at IC ₅₀ values for Chang Liver cells	

xi

List of Tables

Table 2.1	List of reactive oxygen species	5
Table 2.2	Types of endogenous and exogenous dietary antioxidants	8
Table 2.3	List of antiapoptotic and proapoptotic molecules	21
Table 3.1	Nomogram for ammonium sulfate saturation for 1L at 25 °C, 4.1M	35
Table 3.2	IEF steps	52
Table 3.3	11% gel recipe	54
Table 3.4	Gel electrophoresis steps	55
Table 4.1	Number of deregulated proteins following treatment at IC_{50} values	82
Table 4.2	Image of deregulated proteins following different treatments in Hep	84
	G2 cells	
Table 4.3	Image of deregulated proteins following different treatments in Ca	95
	Ski cells	
Table 4.4	Image of deregulated proteins following different treatments in	104
	Chang Liver cells	
Table 4.5	Identified proteins in Hep G2 cells	114
Table 4.6	Identified proteins in Ca Ski cells	121
Table 4.7	Identified proteins in Chang Liver cells	126
Table 4.8	RT-PCR (relative quantification) of selected proteins in Hep G2	133
	cells following treatments	
Table 4.9	RT-PCR (relative quantification) of selected proteins in Ca Ski cells	134
	following treatments	
Table 4.10	RT-PCR (relative quantification) of selected proteins in Chang Liver	135
	cells following treatments	

List of Abbreviation

%	Percentage
$\times g$	Times gravity
\mathfrak{C}	Degree celcius
µg/ml	Microgram per milliliter
μΙ	Microliter
μm	Micrometer
2-D PAGE	Two-dimensional polyacrylamide gel electrophoresis
BCA	Bicinchoninic acid
BF	Big type fruits
BHA	Butylhydroxyanisole
BHT	Butylhydroxytoluene
bp	Base pair
cDNA	Complementary DNA
CHCA	α-Cyano-4-hydroxycinnamic acid
Cu	Copper
DFF	DNA fragmentation factor
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1,1-diphenyl-2-picrylhydrazyl
FBS	Foetus bovine serum
g	Gram
GPx	Glutathione peroxidase
GR	Glutathione reductase

GSH	Reduced glutathione
GSSG	Oxidized glutathione
H ₂ O	Water
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IC ₅₀	Half maximal inhibitory concentration
IEF	Isoelectric focusing
IL-6	Interleukin-6
K_2SO_4	Potassium sulfate
KMnO ₄	Potassium permanganate
L	Liter
LC-MS	Liquid chromatography- mass spectrometry
LL	Large type leaves
Μ	Molar
m/z	Mass-to-charge ratio
MALDI	Matrix assisted laser desorption/ionization
MDA	Malondialdehyde
mg	Milligram
mg/ml	Milligram per milliliter
ML	Medium type leaves
ml	Milliliter
mM	Millimolar
Mn	Manganese

MnSO ₄	Manganese sulfate
M _r	Molecular weight/ size
mRNA	Messenger ribonucleic acid
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
mU	Milliunit
NADPH	Reduced β -nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
nm	Nanometer
O ₂	Oxygen
PBS	Phosphate buffer saline
per	For every
pI	Charge
PTM	Post-translationally modified
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RQ	Relative quantification
RT-PCR	Reverse transcriptase polymerase chain reaction
SDS	Sodium dodecyl sulphate
SELDI	Surface-enhanced laser desorption/ionization
SF	Small type fruits
SL	Small type leaves
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TEMED	Tetramethylethylenediamine

TNFα	Tumor necrosis factor-alpha
TOF	Time of flight
U	Unit
UV	Ultraviolet
Zn	Zinc

List of Appendices

Appendix A	Real-t	-time quantitative reverse transcription-PCR (RT-PCR)		
	A1	RNA extraction		
	A2	Reverse transcription of RNA to cDNA		
	A3	Gene expression analysis		
Appendix B	Graph of gallic acid standard			
Appendix C	Dose-	response curve of samples and ascorbic acid in DPPH assays		
	C1	Crude sample curve		
	C2	Fraction 30 curve		
	C3	Fraction 60 curve		
	C4	Fraction 90 curve		
	C5	Ascorbic acid curve		
Appendix D	Dose-	response curve of samples and ascorbic acid in lipid peroxidation		
Appendix D	Dose-	response curve of samples and ascorbic acid in lipid peroxidation		
Appendix D	Dose- assays D1	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve		
Appendix D	Dose- assays D1 D2	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve		
Appendix D	Dose- assays D1 D2 D3	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve		
Appendix D	Dose- assays D1 D2 D3 D4	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve Fraction 90 curve		
Appendix D	Dose- assays D1 D2 D3 D4 D5	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve Fraction 90 curve Ascorbic acid curve		
Appendix D	Dose- assays D1 D2 D3 D4 D5 Viable	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve Fraction 90 curve e cells curve upon different treatment		
Appendix D Appendix E	Dose- assays D1 D2 D3 D4 D5 Viable E1	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve Fraction 90 curve Ascorbic acid curve e cells curve upon different treatment Treatment of SF crude and fractions towards Ca Ski cells		
Appendix D Appendix E	Dose- assays D1 D2 D3 D4 D5 Viable E1 E2	response curve of samples and ascorbic acid in lipid peroxidation Crude sample curve Fraction 30 curve Fraction 60 curve Fraction 90 curve Ascorbic acid curve e cells curve upon different treatment Treatment of SF crude and fractions towards Ca Ski cells Treatment of SF crude and fractions towards Hep G2 cells		

	E4	Treatment of doxorubicin towards different cell lines		
Appendix F	Catalase standard curve			
Appendix G	DNA fragmentation results			
	G1	Ca Ski cells upon different treatments		
	G2	Hep G2 cells upon different treatments		
	G3	Chang Liver cells upon different treatments		
Appendix H	2D gels of treated cells upon SF fractions treatments			
	H1	Hep G2 cells treated with SF30		
	H2	Hep G2 cells treated with SF60		
	Н3	Hep G2 cells treated with SF90		
	H4	Hep G2 cells treated with doxorubicin		
	H5	Ca Ski cells treated with SF30		
	H6	Ca Ski cells treated with SF60		
	H7	Ca Ski cells treated with SF90		
	H8	Ca Ski cells treated with doxorubicin		
	H9	Chang Liver cells treated with SF30		
	H10	Chang Liver cells treated with SF60		
	H11	Chang Liver cells treated with SF90		
	H12	Chang Liver cells treated with doxorubicin		