

DEDICATION

This thesis is dedicated to the people I love and respect for their
untiring support and encouragement

ACKNOWLEDGEMENT

I would like to take this opportunity to express my gratitude and appreciation to all the people whom have helped me throughout this project. I am especially grateful to my supervisors, Professor Zulqarnain Mohamed and Dr. Chang Li Yen for giving me this opportunity to carry out this research project under their supervision. I owe a huge debt of gratitude to both of them for their invaluable help, patience, continuing encouragement and advice. I would also like to offer my greatest thanks for their guidance and support of my research endeavours.

Also, the members of Dr. Chang's laboratory, Aziyah, Kim Kee, Shu Meng Hooi, Asyura, Jeffree, Han Wei, Connie Lam, Eva Tiong to whom I am forever thankful for their guidance and help. I would also like to thank the Department of Medical Microbiology especially Prof Sazaly Abu Bakar for the use of the facilities.

Finally, to my family and friends, especially to my beloved father; Shoib Abdullah and husband; Mohd Izwan Hussin, thank you for your love, support and encouragement.

TABLE OF CONTENT

	PAGE
ACKNOWLEDGMENTS	ii
TABLE OF CONTENTS	iii
LIST OF FIGURES	v
ABBREVIATIONS	vi
ABSTRACT	viii
1.0 INTRODUCTION	1
1.1 General	1
1.2 Plant proteomics	2
1.3 Leaves senescence	3
1.4 Secondary metabolites	4
1.5 Bioapplication	5
1.6 Biosynthetic pathway	6
1.7 Objective of study	7
2.0 MATERIALS AND METHODS	9
2.1 Plant sample	9
2.2 Sample preparation	9
2.2.1 Sample harvesting	9
2.2.2 Protein extraction from the kesum leaves	10
2.2.3 Determination of protein concentration	12
2.3 Electrophoresis	13
2.3.1 Preparation of SDS-PAGE gel	13
2.3.2 One-dimensional SDS-PAGE separation	13
2.3.3 Two-dimensional SDS-PAGE separation	14

2.3.3.1	First dimension protein separation	14
2.3.3.1 (a)	Rehydration	14
2.3.3.1 (b)	Isoelectric focusing (IEF)	14
2.3.3.2	Second dimension protein separation	15
2.3.3.2 (a)	Equilibration	15
2.3.3.2 (b)	Vertical SDS-PAGE separation	15
2.4	Staining of SDS-PAGE gels	16
2.4.1	Silver staining	16
2.4.2	Colloidal Coomassie Brilliant Blue staining	17
2.5	Computational analysis of the established protein profiles	17
2.6	Protein identification by MALDI TOF/TOF mass spectrometry	18
2.6.1	Peptide mass fingerprinting (PMF)	18
2.6.2	Mass spectrometry (MS) data analysis	19
2.7	Chemicals and reagents	19
3.0	RESULTS	20
3.1	Establishment of the protein profile of kesum leaves from different groups	20
3.2	Computational analysis of the differential expressed proteins in kesum leaves	22
3.3	Analyses of proteins using mass spectrometry (MALDI-TOF)	26
4.0	DISCUSSION	27
	REFERENCES	32

LIST OF FIGURES

		PAGE
Figure 1.1	Kesum or <i>Persicaria minus</i> at four weeks old.	1
Figure 2.1	Marking of nodes on kesum plants to facilitate the harvesting on kesum leaves at different age	11
Figure 3.1	One dimensional-polyacrylamide gel electrophoresis (1D-PAGE) separation of kesum leaves protein at four weeks, six weeks and eight weeks old on 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).	21
Figure 3.2	Coomassie blue stained, two-dimensional-polyacrylamide gel electrophoresis (2D-PAGE) of kesum leaves proteins on broad range pH 4-7, 18 cm gel	23
Figure 3.3	Composite gel image of the two-dimensional PAGE protein pattern profiles of kesum leaves using PDQUEST™ software (Bio-Rad, USA) for the quantitative analysis between four weeks old and six weeks old.	24
Figure 3.4	Composite gel image of the two-dimensional PAGE protein pattern profiles of kesum leaves using PDQUEST™ software (Bio-Rad, USA) for the quantitative analysis between six weeks old and eight weeks old.	25

ABBREVIATIONS

µg	microgram
µl	microliter
1-DE	one-dimensional electrophoresis
2-DE	two-dimensional electrophoresis
APS	ammonium persulphate
BCA	bichinchoninic acid
CHAPS	3-(3-cholamidopropyl)-dimethylammonio-propane-sulfonate
cm	centimetre
DTT	dithiothreitol
<i>et al.</i>	Et alia (and others)
IEF	isoelectric focusing
IPG	immobilize pH gradient
kDa	kilo Dalton
l	litre
M	molar
mg	milligram
ml	millilitre
mM	millimolar
nm	nanometre
PAGE	polyacrylamide gel electrophoresis
pI	isoelectric point
RT	room temperature
SDS	sodium dodecyl sulphate
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
TEMED	N,N,N',N'-tetramethylethylenediamine

V voltage
Vhr volt-hour

ABSTRACT

Proteomics has been applied in various fields such as in medicine and pharmaceutical. Although proteomics has been introduced for almost a decade, the application of proteomics to protein expression in plants can still be considered as new. This present study is aimed at proteins that are involved in leaves senescence. Proteins were extracted from the leaves of the kesum plants at different age. The extracted protein samples were then subjected to separation on one-dimensional electrophoresis (1-DE) and 2-DE. Separation was performed on broad range pH (pH 3-10) gels of 7 cm and narrow range pH (pH 4-7) gels of 7 cm and 18 cm. The gels were silver stained and results showed that the narrow range gels offered a better resolution of spots. The established 2-DE protein profiles were then analysed using PDQUEST™. A total of 223 protein spots were detected in the four weeks old kesum protein sample. In the six weeks old and eight weeks old sample, 200 and 100 protein spots were detected respectively. From quantitative analysis, at least nine protein spots were up-regulated in the six weeks old sample in comparison with the four weeks old sample and four protein spots were up-regulated in the eight weeks old sample in comparison with the six weeks old sample. On the other hand, eight proteins spots were down-regulated in the six weeks old sample in comparison with the four weeks old and another six protein spots were down-regulated in the eight weeks old sample in comparison with the six weeks old sample. These differentially expressed proteins may play a role in plant growth and maturation, or plant senescence. These proteins should be further identified and characterized as it will help to better understand the function and role of these proteins in leave senescence or its possible involvement in metabolic pathways of secondary metabolites.