

TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	ii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	vi
LIST OF FIGURES	xi
LIST OF TABLES	xii
LIST OF PLATES	xiii
LIST OF SYMBOLS AND ABBRERATIONS	xiv
CHAPTER ONE	
GENERAL INTRODUCTION	1
CHAPTER TWO	
LITERATURE REVIEW	
2.1 Medicinal mushrooms	6
2.2 Pharmacological potential of medicinal mushrooms	7
2.3 Cultivation of medicinal mushrooms	8
2.4 <i>Ganoderma</i>	10
2.4.1 <i>Ganoderma australe</i>	11
2.5 Proteins from mushroom	12
2.5.1 Antimicrobial proteins	12

2.5.2 Other proteins with biological activities from mushroom	14
2.6 Isolation, purification and characterization of proteins	15
2.6.1 Extraction	16
2.6.2 Ammonium sulfate precipitation of proteins	16
2.6.3 Chromatographic techniques for protein purification	17
2.6.4 Sodium dodecyl sulfate - polyacrylamide gel electrophoresis	19
2.6.5 Protein sequencing and identification	20
2.7 Antimicrobial activity	22
2.7.1 Pathogenic bacteria	22
2.7.2 Pathogenic yeast	23
2.7.3 Pathogenic fungi	25
2.7.4 Importance of antimicrobial agent	27
2.7.5 Drug resistance of microbes	27
2.7.6 Antimicrobial compounds from medicinal mushroom	28
2.8 Anti Human Immunodeficiency Virus-1 Reverse Transcriptase activity	30
2.8.1 Human Immunodeficiency Virus (HIV)	31
2.8.2 Anti HIV compounds	32
2.8.3 Anti HIV-1 reverse transcriptase activity	32
2.9 Hemolytic activity	34

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1	Chemicals and Disposables	35
3.1.2	Instrumentations	38
3.1.3	Samples	39
3.1.4	Test Organisms	39

3.2 METHODS

3.2.1	Media preparation	40
3.2.2	Submerged cultivation for mycelia production	40
3.2.3	Preparation of extracts	41
3.2.4	Ammonium sulfate precipitation of proteins	41
3.2.5	Primary screening of antimicrobial activities	44
3.2.6	Purification of proteins	45
3.2.7	Protein quantification by absorbance assay at 280 nm	46
3.2.8	Tricine Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis	47
3.2.9	Staining of SDS PAGE gel	
3.2.9.1	Colloidal Coomassie blue staining (MALDI-TOF compatible)	48
3.2.9.2	Silver staining	48
3.2.9.3	Modified silver staining for mass spectrometry	49
3.2.10	Protein visualization	49
3.2.11	Identification of purified protein	50

3.2.12 Bioactive properties of purified protein	
3.2.12.1 Determination of minimal inhibitory concentration (MIC)	52
3.2.12.2 HIV-1 Reverse Transcriptase inhibition activity assay	52
3.2.12.3 Deoxyribonuclease activity	53
3.2.12.4 Hemolysis test	54
3.2.12.5 Antifungal activity	54
3.2.12.6 Ribonuclease activity	55
3.2.13 Statistical analysis	56

CHAPTER FOUR

RESULTS

4.1 Fungal strain	57
4.2 Antimicrobial activities of powdery materials (PM) from ammonium sulfate precipitation.	
4.2.1 Antibacterial activity in primary screening	58
4.2.2 Antifungal activity	61
4.3 Purification of protein with antimicrobial activity from <i>Ganoderma ausrale</i> strain KUM60813	61
4.4 Identification of purified protein	69
4.5 Bioactive properties of purified protein	70
4.5.1 Minimal inhibitory concentration (MIC) of protein against <i>Escherichia coli</i> O157:H7 and <i>Salmonella typhi</i>	70
4.5.2 Anti HIV-1 Reverse Transcriptase activity	73
4.5.3 Deoxyribonuclease activity	75

4.5.4 Hemolytic activity	75
4.5.5 Antifungal and ribonuclease activity	76
CHAPTER FIVE	
DISCUSSION	
5.1 Cultivation of <i>Ganoderma australe</i> mycelia in submerged culture	78
5.2 Preparation of extract for antimicrobial activity test	78
5.3 Isolation, purification and identification of protein	79
5.4 Antimicrobial activity of PM and protein fractions	81
5.5 Antimicrobial activity of purified protein	85
5.6 Other biological activities of purified protein	88
5.7 Comparison of antimicrobial protein from <i>Ganoderma australe</i> with other <i>Ganoderma</i> species	91
CHAPTER SIX	
CONCLUSIONS	
6.1 Recommendation for Further Studies	93
6.2 Conclusions	94
REFERENCES	95
APPENDIX A	112
APPENDIX B	114
APPENDIX C	115
APPENDIX D	119
APPENDIX E	130