

TABLE OF CONTENT

| | |
|--|-------------|
| THESIS | i |
| RATIFICATION PAGE | ii |
| CERTIFICATE OF AUTHENTICATION | iii |
| ACKNOWLEDGEMENT | iv |
| DEDICATION | v |
| TABLE OF CONTENT | vi |
| LIST OF FIGURES | viii |
| LIST OF TABLES | ix |
| LIST OF APPENDICES | x |
| ABSTRACT | xi |
| INTISARI | xii |
| CHAPTER I | 1 |
| INTRODUCTION | 1 |
| I.1 Background | 1 |
| I.2 Research Purposes | 2 |
| I.3 Research Benefits | 2 |
| CHAPTER II | 3 |
| LITERATURES REVIEW AND HYPOTHESIS | 3 |
| II.1 Literatures Review | 3 |
| II.1.1 Endophytic fungi | 3 |
| II.1.2 <i>Fusarium</i> sp. | 3 |
| II.1.3 Isolation of secondary metabolites from <i>Fusarium</i> sp. | 5 |
| II.1.4 Structure elucidation of metabolites with NMR spectroscopy | 7 |
| II.1.5 Antioxidant | 8 |
| II.2 Hypothesis and Experimental Design | 9 |
| II.2.1 Rationale I | 9 |
| II.2.2 Hypothesis I | 10 |
| II.2.3 Rationale II | 10 |
| II.2.4 Hypothesis II | 10 |
| II.2.5 Experimental design | 10 |
| CHAPTER III | 12 |
| RESEARCH METHOD | 12 |
| III.1 Materials | 12 |
| III.1.1 Plant material | 12 |
| III.1.2 Culture media | 12 |
| III.1.3 Chromatography and organic solvents | 12 |
| III.2 Equipments and Instruments | 13 |
| III.3 Procedures | 13 |
| III.3.1 Culture media and solution preparation | 13 |
| III.3.2 Isolation of endophytic fungi | 15 |
| III.3.3 Small fermentation and extraction | 15 |
| III.3.4 The screening of endophytic fungi | 16 |

| | |
|---|-----------|
| III.3.5 Scale up fermentation and extraction | 17 |
| III.3.6 Partition and isolation of compounds using chromatography | 17 |
| III.3.7 Structure elucidation | 19 |
| III.3.8 Antioxidant activity | 19 |
| CHAPTER IV | 21 |
| RESULTS AND DISCUSSIONS | 21 |
| IV.1 Isolation of Endophytic Fungi | 21 |
| IV.2 Screening of Endophytic Fungi | 22 |
| IV.3 Isolation of Selected Secondary Metabolites | 23 |
| IV.4. Structural Elucidation of Selected Compounds | 25 |
| IV.4.1 Compound 1 | 25 |
| IV.4.2 Compound 2 | 29 |
| IV.4.3 Compound 3 | 33 |
| IV.4.4 Compound 4 | 37 |
| IV.5. Antioxidant test | 45 |
| CHAPTER V | 48 |
| CONCLUSIONS | 48 |
| V.1 Conclusions | 48 |
| V.2 Suggestions | 48 |
| REFERENCES | 49 |
| APPENDICES | 54 |

LIST OF FIGURES

| | | |
|--------------|---|----|
| Figure II.1 | Structure some of the active metabolites from <i>Fusarium</i> sp. Bostrycoidin (1), Karaquinone A (2), Karaquinone B (3), Beauvericin (4) | 5 |
| Figure II.2 | Structure of Bassiatin | 8 |
| Figure II.3 | X-ray crystallography of Bassiatin | 8 |
| Figure II.4 | Scheme scavenging reaction between DPPH and an antioxidant, (R:H)=antioxidant radical scavenger, R=antioxidant radical | 9 |
| Figure IV.1 | <i>Fusarium napiforme</i> GCN-3 | 21 |
| Figure IV.2 | TLC profile of 13 fractions from ethyl acetate fraction | 21 |
| Figure IV.3 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 1 | 23 |
| Figure IV.4 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 1 | 24 |
| Figure IV.5 | Structure of compound 1, ¹ H- ¹ H COSY (black line) and HMBC correlation (red arrow) | 24 |
| Figure IV.6 | The structure of Bassiatin | 26 |
| Figure IV.7 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 2 | 27 |
| Figure IV.8 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 2 | 28 |
| Figure IV.9 | Structure of 2, ¹ H- ¹ H COSY (black line) and HMBC correlation (red arrow) | 28 |
| Figure IV.10 | Structure of 3 <i>R</i> ,6 <i>R</i> -Beauvericin | 30 |
| Figure IV.11 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 3 | 31 |
| Figure IV.12 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 3 | 32 |
| Figure IV.13 | Structure of 3, HMBC correlations (red arrow) | 32 |
| Figure IV.14 | Structure of 8- <i>O</i> -methyl-fusarubin | 34 |
| Figure IV.15 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 4 | 35 |
| Figure IV.16 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 4 | 36 |
| Figure IV.17 | DEPT spectrum of compound 4 | 37 |
| Figure IV.18 | HMQC spectrum of compound 4 | 38 |
| Figure IV.19 | ¹ H- ¹ H COSY spectrum of compound 4 | 38 |
| Figure IV.20 | The HMBC spectrum of 4 | 39 |
| Figure IV.21 | Structure of compound 4, ¹ H- ¹ H COSY (black line) and HMBC correlation (red arrow) | 39 |
| Figure IV.22 | Two unit (a) and (b) of compound 4 | 40 |
| Figure IV.23 | Struture of compound 4 | 40 |
| Figure IV.24 | Curve of DPPH scavenging activity (%) vs concentration of compound 4 | 42 |
| Figure IV.25 | Curve of DPPH scavenging activity (%) vs concentration of ascorbic acid | 43 |

LIST OF TABLES

| | | |
|------------|--|----|
| Table IV.1 | NMR data comparison of Bassiatin with compound 1 in CDCl ₃ | 25 |
| Table IV.2 | NMR data comparison of compound 2 with (3 <i>R</i> ,6 <i>R</i>) isomer of Bassiatin in CDCl ₃ | 29 |
| Table IV.3 | NMR data comparison between compound 3 and 8- <i>O</i> -methyl-fusarubin in CDCl ₃ | 33 |
| Table IV.4 | The comparison of NMR data between compound 4 and 8- <i>O</i> -methyl-fusarubin (3) in CDCl ₃ | 41 |
| Table IV.5 | The result of measurement DPPH scavenging activity (%) of compound 4 | 42 |
| Table IV.6 | The result of DPPH scavenging activity (%) measurement of ascorbic acid | 43 |

LIST OF APPENDICES

| | | |
|-------------|--|----|
| Appendix 1 | Scheme of fungal isolation | 51 |
| Appendix 2 | Total fungi collections | 52 |
| Appendix 3 | Scheme of screening endophytic fungi | 52 |
| Appendix 4 | Result of chemical profiling by TLC | 53 |
| Appendix 5 | Result of antimicrobial assay | 55 |
| Appendix 6 | List selected of fungi | 56 |
| Appendix 7 | Scheme of second fermentation | 57 |
| Appendix 8 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 1 | 58 |
| Appendix 9 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 1 | 58 |
| Appendix 10 | DEPT spectrum of compound 1 in CDCl ₃ | 59 |
| Appendix 11 | ¹ H- ¹ H COSY spectrum of compound 1 in CDCl ₃ | 59 |
| Appendix 12 | HMQC spectrum of compound 1 in CDCl ₃ | 60 |
| Appendix 13 | HMBC spectrum of compound 1 in CDCl ₃ | 60 |
| Appendix 14 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 2 | 61 |
| Appendix 15 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 2 | 61 |
| Appendix 16 | DEPT spectrum of compound 2 in CDCl ₃ | 62 |
| Appendix 17 | ¹ H- ¹ H COSY spectrum of compound 2 in CDCl ₃ | 62 |
| Appendix 18 | HMQC spectrum of compound 2 in CDCl ₃ | 63 |
| Appendix 19 | HMBC spectrum of compound 2 in CDCl ₃ | 63 |
| Appendix 20 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 3 | 64 |
| Appendix 21 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 3 | 64 |
| Appendix 22 | DEPT spectrum of compound 3 in CDCl ₃ | 65 |
| Appendix 23 | ¹ H- ¹ H COSY spectrum of compound 3 in CDCl ₃ | 65 |
| Appendix 24 | HMQC spectrum of compound 3 in CDCl ₃ | 66 |
| Appendix 25 | HMBC spectrum of compound 3 in CDCl ₃ | 66 |
| Appendix 26 | ¹ H NMR (600 MHz, CDCl ₃) spectrum of compound 4 | 67 |
| Appendix 27 | ¹³ C NMR (600 MHz, CDCl ₃) spectrum of compound 4 | 67 |
| Appendix 28 | DEPT spectrum of compound 4 in CDCl ₃ | 68 |
| Appendix 29 | ¹ H- ¹ H COSY spectrum of compound 4 in CDCl ₃ | 68 |
| Appendix 30 | HMQC spectrum of compound 4 in CDCl ₃ | 69 |
| Appendix 31 | HMBC spectrum of compound 4 in CDCl ₃ | 69 |
| Appendix 32 | IR spectrum of compound 4 | 70 |
| Appendix 33 | Specific rotation data of compound | 70 |
| Appendix 34 | Picture of appearance compound | 70 |
| Appendix 35 | HRESITOFMS spectrum of compound 4 | 71 |
| Appendix 36 | UV (MeOH) spectrum of compound 4 | 71 |
| Appendix 37 | Fungal identification of GCN-3 | 72 |
| Appendix 38 | Calculation concentration of compound 4 for antioxidant assay | 73 |
| Appendix 39 | Calculation concentration of DPPH 0.2 mM | 74 |
| Appendix 40 | Calculation concentration of ascorbic acid for antioxidant assay | 75 |
| Appendix 41 | Calculation of IC ₅₀ | 77 |
| Appendix 42 | HPLC chromatogram of compound 1 | 78 |
| Appendix 43 | HPLC chromatogram of compound 2 | 78 |
| Appendix 44 | HPLC chromatogram of compound 3 | 79 |
| Appendix 45 | HPLC chromatogram of compound 4 | 79 |