

**CHARACTERIZATION OF ANTIBACTERIAL  
COMPOUNDS FROM *Ganoderma boninense***



**SYAHRIEL BIN ABDULLAH**

**UMS**  
UNIVERSITI MALAYSIA SABAH

**FACULTY OF SCIENCE AND NATURAL  
RESOURCES  
UNIVERSITI MALAYSIA SABAH  
2018**

**CHARACTERIZATION OF ANTIBACTERIAL  
COMPOUNDS FROM *Ganoderma boninense***

**SYAHRIEL BIN ABDULLAH**



**UMS**  
UNIVERSITI MALAYSIA SABAH

**THESIS SUBMITTED IN FULFILLMENT  
FOR PHILOSOPHY OF DOCTORATE**

**FACULTY OF SCIENCE AND NATURAL  
RESOURCES  
UNIVERSITI MALAYSIA SABAH  
2018**

### UNIVERSITI MALAYSIA SABAH

#### BORANG PENGESAHAN TESIS

JUDUL : \_\_\_\_\_

\_\_\_\_\_

IJAZAH : \_\_\_\_\_

\_\_\_\_\_

SAYA : \_\_\_\_\_ SESI PENGAJIAN : \_\_\_\_\_

(HURUF BESAR)

Mengaku membenarkan tesis \*(LPSM/Sarjana/Doktor Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

SULIT (Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)

TERHAD (Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

TIDAK TERHAD

Disahkan oleh:

\_\_\_\_\_  
(TANDATANGAN PENULIS)

\_\_\_\_\_  
(TANDATANGAN PUSTAKAWAN)

Alamat Tetap: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_  
(NAMA PENYELIA)

TARIKH: \_\_\_\_\_

TARIKH: \_\_\_\_\_

**Catatan:**

\*Potong yang tidak berkenaan.

\*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.

\*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

## APPENDIX A

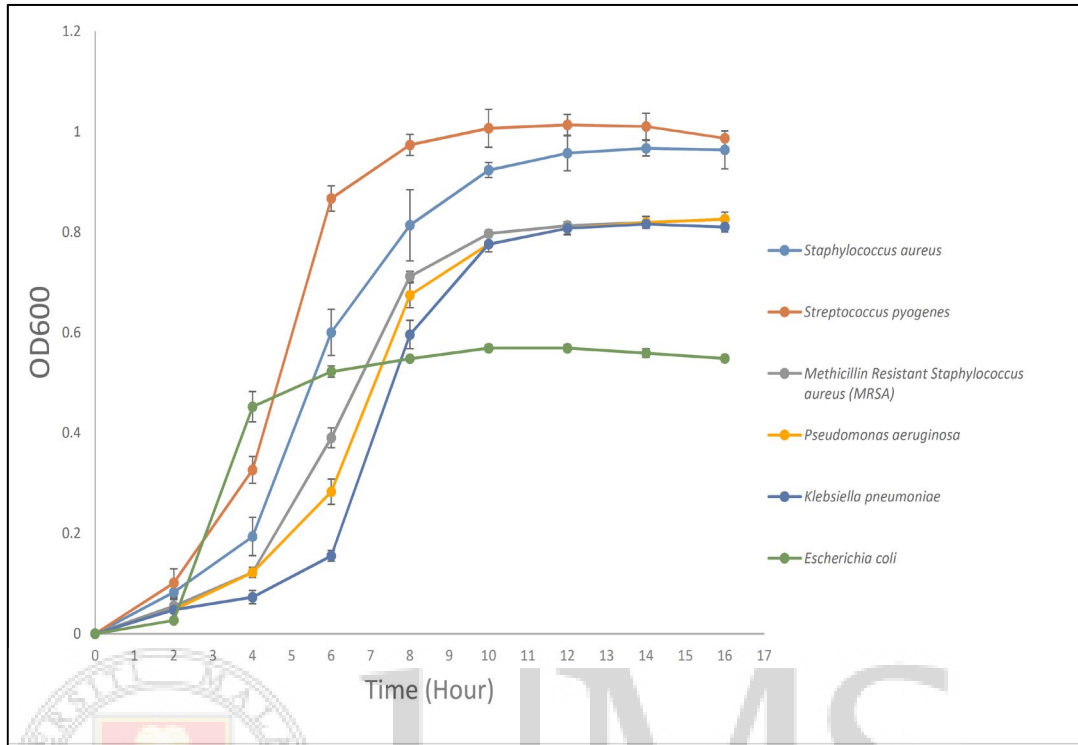


Figure A: Growth log phase determination for the six bacterial samples. Optical density (OD) reading at 600nm wavelength was recorded for every two hours until the reading reach stagnant value. Nutrient broth without bacterial suspension served as negative control and blank. OD<sub>600</sub> reading was plotted against time to construct bacterial growth curve. OD reading on the steepest curve were taken as the bacterial growth-log phase point.

## APPENDIX B

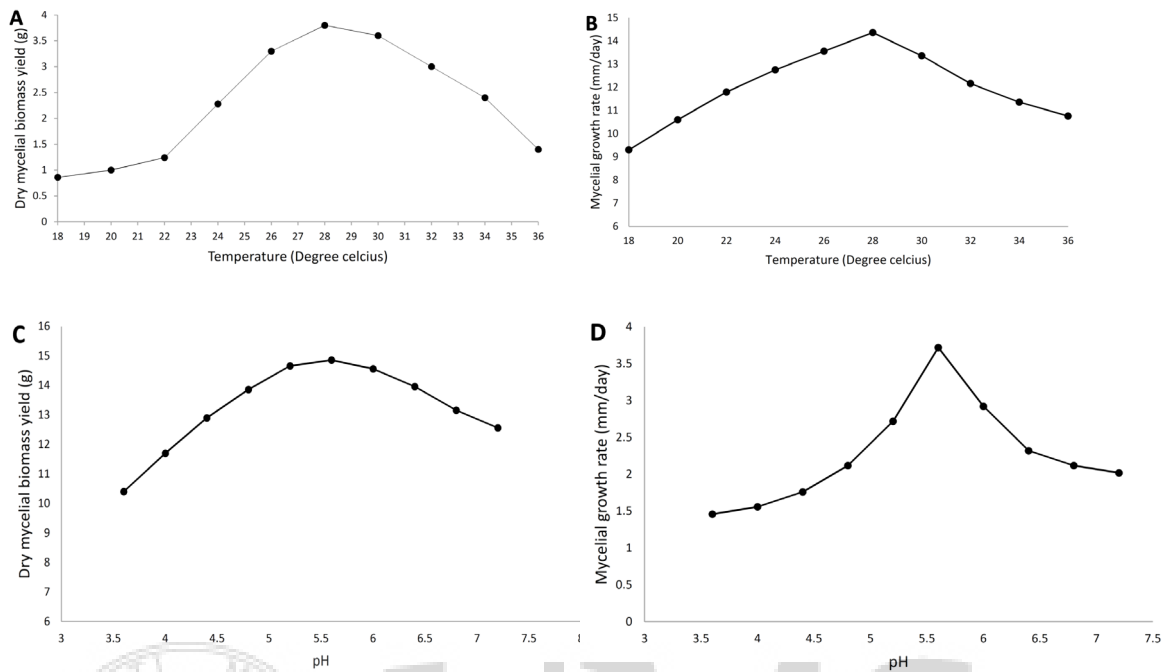
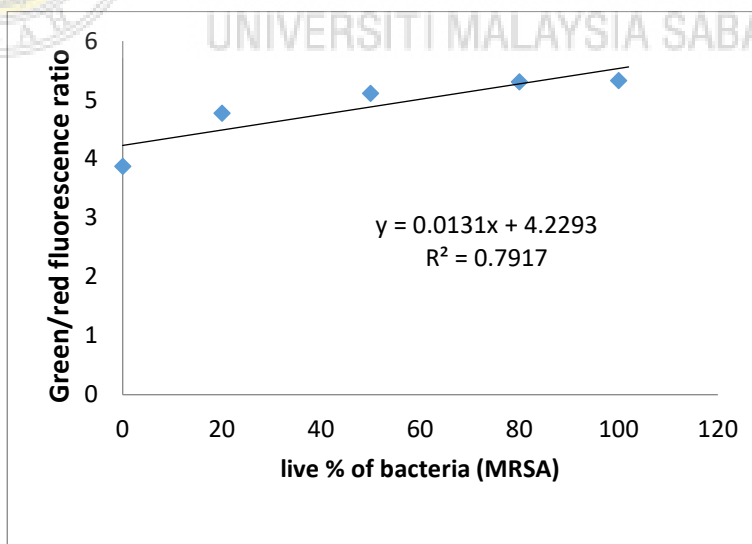
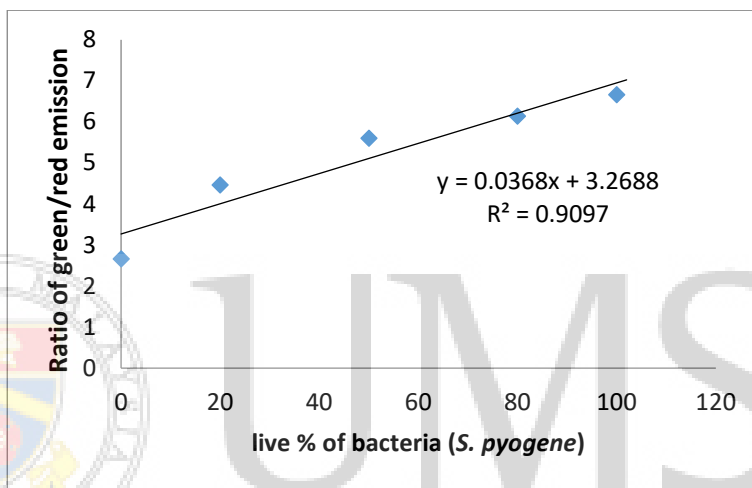
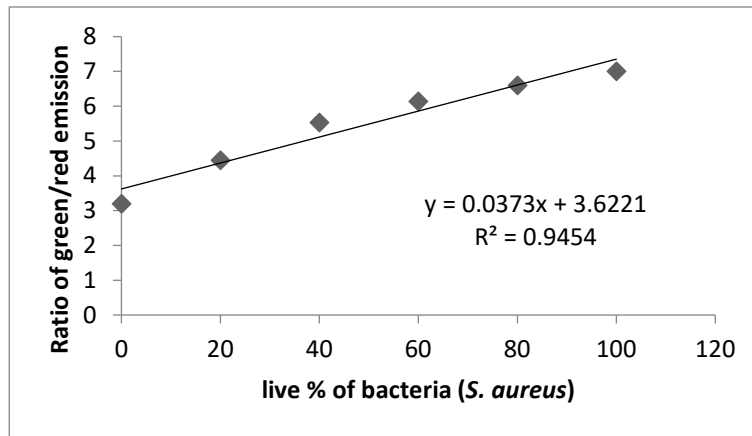
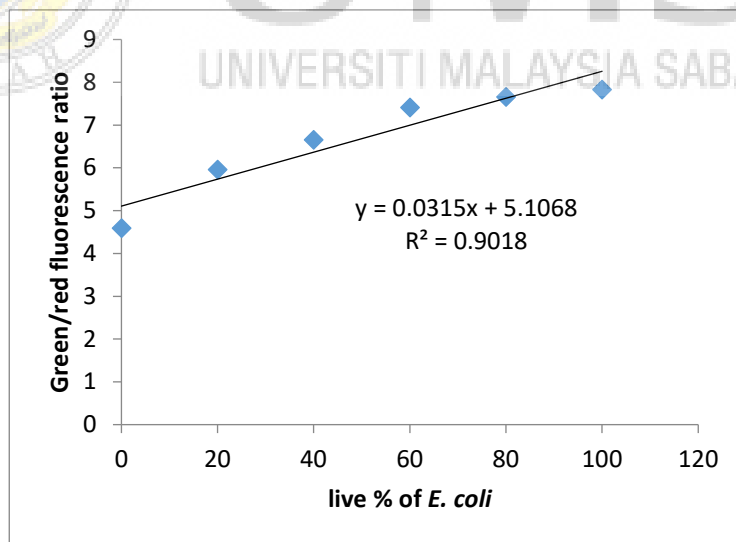
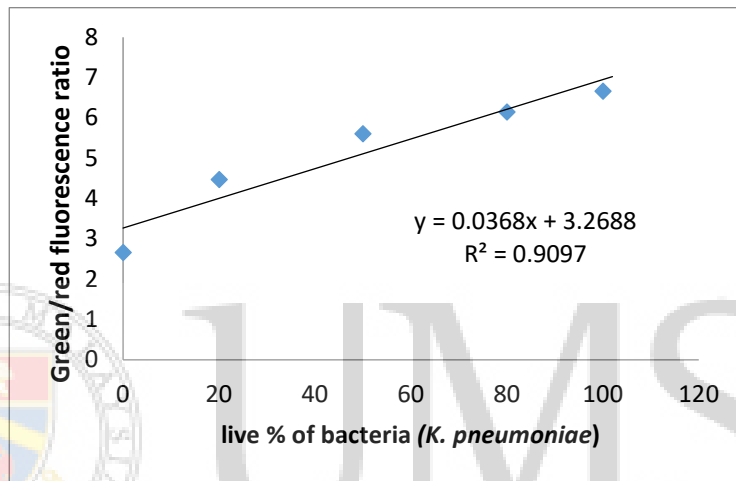
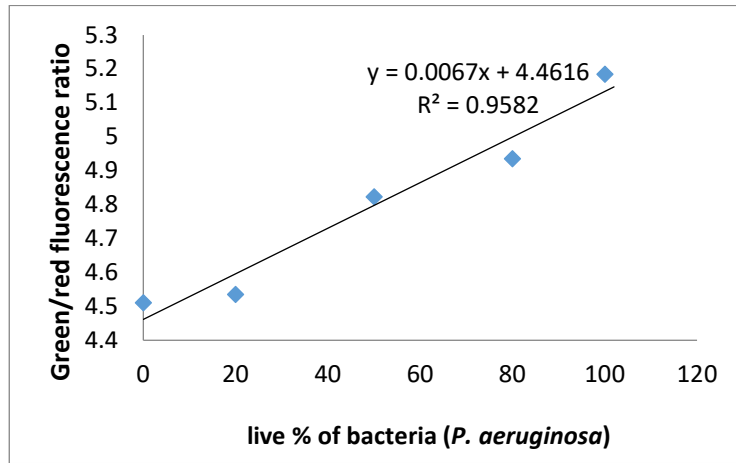


Figure B: Single factor experiment to identified effective range of incubation temperatures ( $^{\circ}\text{C}$ ) and acidity (pH). A) Effect of different temperatures on *G. boninense* dry mycelial biomass yield (g). B) Effect of different temperatures on *G. boninense* growth rate (mm/day). C) Effect of different pH on *G. boninense* dry mycelial biomass yield (g). D) Effect of different pH on *G. boninense* growth rate (mm/day).

### APPENDIX C





## APPENDIX D

Table D: Relative Luminescence activity of bacterial pathogens treated with Ergosterol and Ganoboninketal based on relative luminescence unit (RLU) obtained from luminometric bioassay.

<b>Bacterial Samples (treatment)</b>	<b>RLU</b>
<b>Ergosterol</b>	
<i>S. aureus</i> ATCC 25923	115872.96
<i>S. pyogenes</i> ATCC 19615	136578.552
Methicillin Resistant <i>S. aureus</i> (MRSA) NCTC 11939	130488.672
<i>P. aeruginosa</i> ATCC 9027	784541.784
<i>K. pneumoniae</i> ATCC 1705	1018393.176
<i>E. coli</i> ATCC 35218	380173.752
IPA (positive control)	106129.152
10% DMSO (negative control)	121733212.5
<b>Ganoboninketal</b>	
<i>S. aureus</i> ATCC 25923	118308.912
<i>S. pyogenes</i> ATCC 19615	141450.456
Methicillin Resistant <i>S. aureus</i> (MRSA) NCTC 11939	142668.432
<i>P. aeruginosa</i> ATCC 9027	801593.448
<i>K. pneumoniae</i> ATCC 1705	1106087.448
<i>E. coli</i> ATCC 35218	459342.192
IPA (positive control)	131706.648
10% DMSO (negative control)	121842830.4



## APPENDIX E

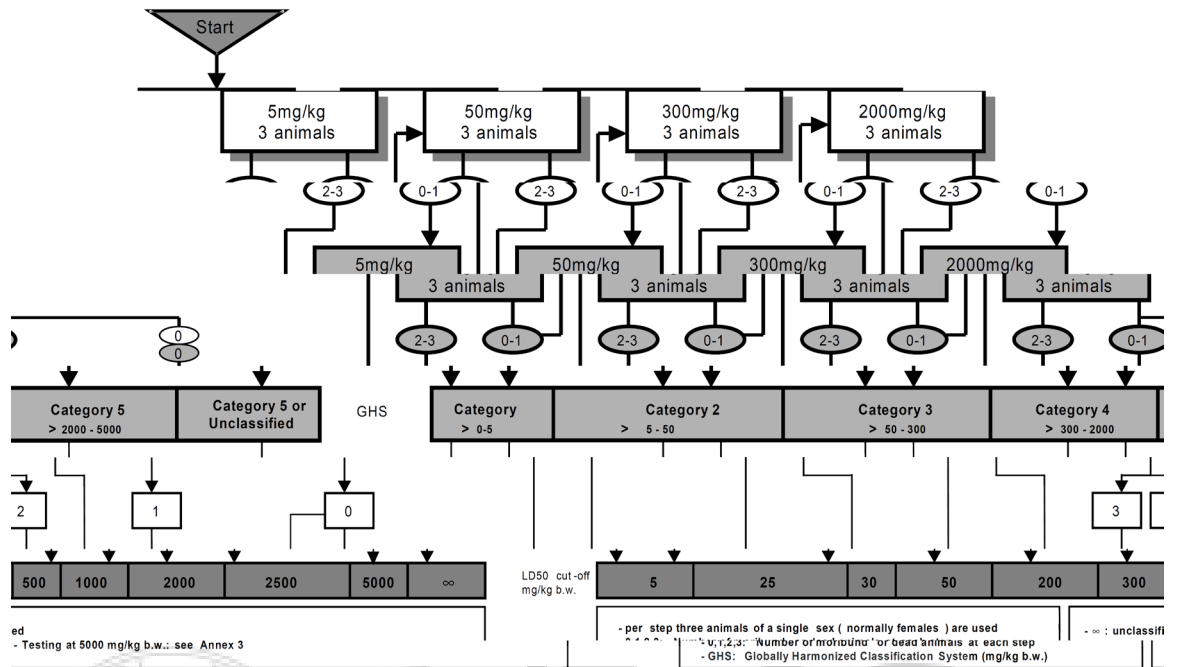
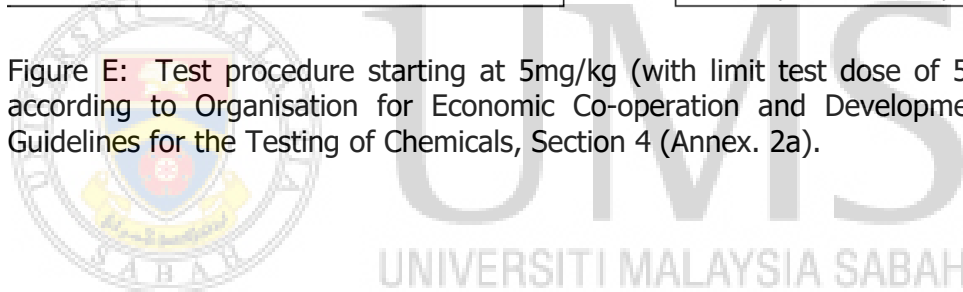


Figure E: Test procedure starting at 5mg/kg (with limit test dose of 5000mg/kg) according to Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Section 4 (Annex. 2a).



## APPENDIX F

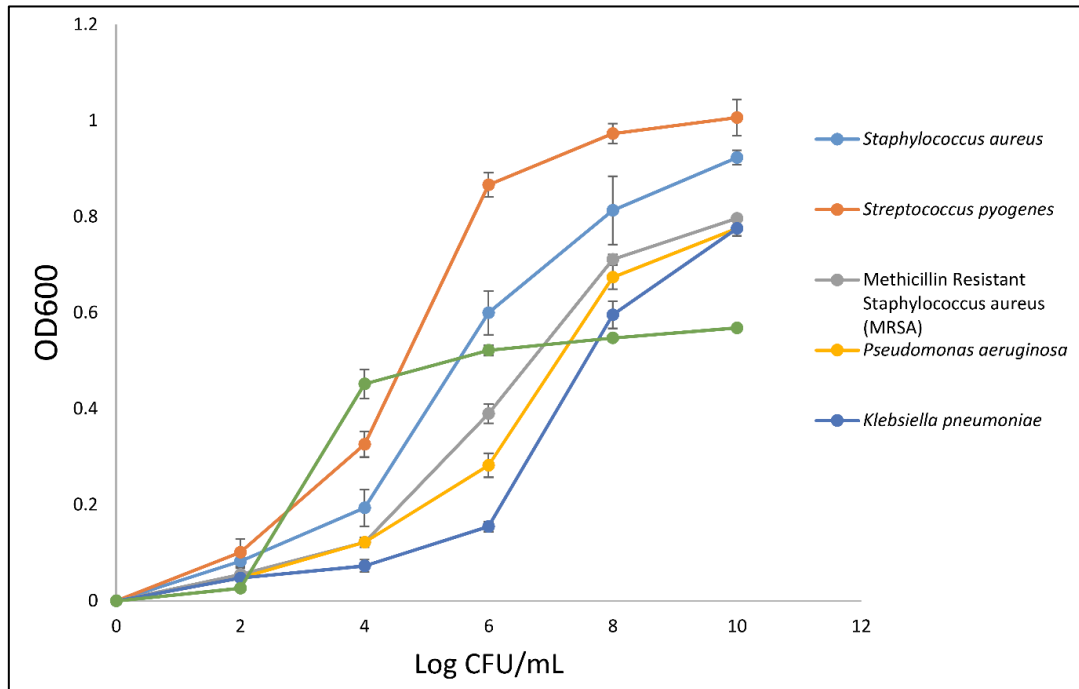
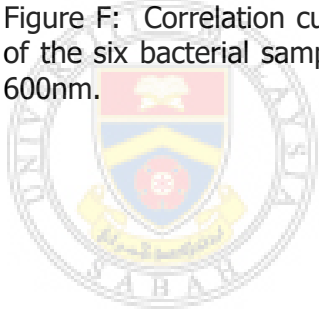


Figure F: Correlation curves between Colony Forming Unit (CFU) per mL (CFU/mL) of the six bacterial samples (Log transformed) and the absorbance (OD) reading at 600nm.



UMS  
UNIVERSITI MALAYSIA SABAH

## APPENDIX G

### List of Publications

#### Journal

1. Abdullah, S., Ling, Y.S., Daim, S.J., Alexander, A. and Chong, K.P., 2018. Ganoderma boninense isolated from Sabah, Malaysia exhibits potent antibacterial activity against clinically important bacterial pathogens. *Bangladesh Journal of Pharmacology*. **13**(1): 10-12.
2. Syahriel Abdullah, Arnnyitte Alexander, Chong Khim Phin. 2016. Early Detection and Management of *Ganoderma* Basal Stem Rot Disease: A Special Report from Sabah. *Transactions on Science and Technology*. **3**(3): 517-523.

#### Proceeding

1. Syahriel Abdullah, Ling Yee Soon, Chong Khim Phin. 2017. Optimization of *Ganoderma boninense* Growth using Response Surface Methodology (RSM). International Conference on Science and Natural resources (ICSNR) Second Edition. 5-6 April 2017. Grand Borneo Hotel, Kota Kinabalu, Malaysia
2. Syahriel Abdullah, Arnnyitte Alexander, Chong Khim Phin. 2016. Analysis of Metabolites from *Ganoderma boninense* Chloroform Extract using Gas Chromatography-Mass Spectrometry (GC-MS). International Conference on Plant Protection in The Tropics (ICPPT). 3-5 August 2016. Hilton Hotel Kuching Sarawak.



UMS  
UNIVERSITI MALAYSIA SABAH

## TABLE OF CONTENTS

	<b>Page</b>
<b>TITLE</b>	i
<b>DECLARATION</b>	ii
<b>CERTIFICATION</b>	iii
<b>ACKNOWLEDGEMENTS</b>	iv
<b>ABSTRACT</b>	v
<i>ABSTRAK</i>	vii
<b>LIST OF CONTENTS</b>	ix
<b>LIST OF TABLES</b>	xiii
<b>LIST OF FIGURES</b>	xvii
<b>LIST OF SYMBOLS AND ABBREVIATIONS</b>	xxi
<b>LIST OF APPENDICES</b>	xxiii
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Research Background	1
1.2 Problem Statement	4
1.3 Objectives	5
<b>CHAPTER 2: LITERATURE REVIEW</b>	6
2.1 Nosocomial Infection and the Emergence of Drug Resistant Bacterial Pathogens	6
2.1.1 Epidemiology of Bacterial Infections in Malaysia	6
2.1.2 <i>Staphylococcus aureus</i> and Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA)	7
2.1.3 <i>Streptococcus pyogenes</i> , a Group A <i>Streptococcus</i> (GAS)	8
2.1.4 <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , and Nosocomial Infection	9
2.2 Urgent Need for New Antibiotic	10
2.3 Fungi as Sources for New Therapeutic Drugs	11
2.3.1 Antibiotic Isolated from Fungi	12
2.4 <i>Ganoderma boninense</i>	12
2.4.1 <i>Ganoderma boninense</i> as a Plant Pathogen	14
2.4.2 Potential of <i>Ganoderma</i> species in Medicine	15
2.5 Review Method in Natural Product Discovery	16
2.5.1 Dereplication and Dereplication's Strategies	17
2.5.2 Hyphenated Instrumentation: Classical versus Hyphenated (on-line) Approaches	19
2.6 Toxicological Evaluation on Lead Compound	20
2.7 <i>In silico</i> and Qualitative Structural-Activity Relationship (QSAR) in Drug-Mode of Action Prediction	23
	25

**CHAPTER 4: PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EVALUATION OF *Ganoderma boninense* EXTRACTS**

3.1	Introduction	25
3.2	Methodology	26
3.2.1	<i>Ganoderma boninense</i> Collection from Fruiting Body	27
3.2.2	Molecular Identification of <i>Ganoderma boninense</i>	27
3.2.3	Preparation of <i>Ganoderma boninense</i> Culture on Solid and in Liquid Media	27
3.2.4	Bacterial Cultures	28
3.2.5	Log Phase Determination of Bacterial Cultures	29
3.2.6	Preparation of <i>Ganoderma boninense</i> for Solvent Extraction	30
3.2.7	<i>Ganoderma boninense</i> Crude Extracts Preparation	30
3.2.8	Phytochemical Analysis of GBFB, GBPDA, and GBPDB	31
3.2.9	Comparative Antibacterial Evaluation on GBFB, GBPDA, and GBPDB Extracts	33
3.2.10	Statistical analysis	34
3.3	Results and Discussion	34
3.3.1	Molecular Identification of Fruiting Body	34
3.3.2	Extraction yield of <i>Ganoderma boninense</i> from different sources	38
3.3.3	Preliminary Phytochemical Screening	40
3.3.4	Preliminary Antibacterial Evaluation of <i>Ganoderma boninense</i> Various Extract	42
3.3.5	Antibiotic Selection for Positive Control	44
3.3.6	Effect of Different Media on Antibiotic and GBPDA Chloroform Extract Inhibition	45
3.4	Conclusion	47

**CHAPTER 4: ISOLATION AND IDENTIFICATION OF POTENTIAL ANTIBACTERIAL COMPOUNDS FROM *Ganoderma boninense***

4.1	Introduction and Objectives	49
4.2	Methodology	50
4.2.1	Fungal and Bacterial Cultures	50
4.2.2	Liquid-liquid Extraction (LLE) of <i>Ganoderma boninense</i>	50
4.2.3	Antibacterial Assay for LLE extracts of <i>Ganoderma boninense</i>	51
4.2.4	Preparative High Performance Thin Layer Chromatography (PHPTLC) Profiling of Chloroform-Methanol (CM) fraction of <i>Ganoderma boninense</i> Extract	52
4.2.5	PHPTLC Antibacterial Bioautography Evaluation	53
4.2.6	Gas Chromatography – Mass Spectrometry (GC-MS)	53

	Analysis of the Potential Antibacterial Compounds	
4.2.7	Semi-Preparative High Performance Liquid Chromatography (SPHPLC) Analysis on Isolated Compounds from PHPTLC	54
4.2.8	Fourier Transform Infra-Red (FTIR) Spectroscopy Analysis on Lanostane and Ergosterol	55
4.2.9	Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis of Newly Potential Antibacterial Compound Isolated from <i>Ganoderma boninense</i>	56
4.2.10	Antibacterial Evaluation of Identified Compounds against the Bacterial Samples	56
4.3	Results and Discussions	56
4.3.1	Extraction Yield using LLE and Their Fractions' Antibacterial Properties	56
4.3.2	PHPTLC Profiles and Antibacterial bioautography of CM Fraction from <i>Ganoderma boninense</i> Extract	58
4.3.3	GC-MS Identification of CMRF04	61
4.3.4	SPHPLC Fractionation and Spiking Analysis on CMRF04	66
4.3.5	FTIR Spectroscopy Evaluation on Compound A, B and C	68
4.3.6	NMR Spectroscopy Evaluation for Structural Elucidation and Identification of Compound C	71
4.3.7	Antibacterial Properties of Lanostane, Ergosterol, and Ganoboninketal	78
4.4	Conclusion	80
<b>CHAPTER 5: MINIMUM INHIBITORY CONCENTRATION EVALUATION AND ANTIBACTERIAL MECHANISM OF ACTION OF ERGOSTEROL AND GANOBONINKETAL</b>		<b>81</b>
5.1	Introduction	81
5.2	Methodology	83
5.2.1	Bacterial Cultures Preparation	83
5.2.2	Ergosterol and Ganoboninketal Preparation	83
5.2.3	Minimum Inhibitory Concentration Study for Antibacterial Potential	84
5.2.4	Assessing on the Leakage of 260nm Absorbing Material	84
5.2.5	Disruption of Cell Wall and Membrane via Live/Dead BacLight Assay	85
5.2.6	Scanning Electron Microscope (SEM) Observation	86
5.2.7	Assessment on ATP activity via Bactiter™ Glo assay	87
5.2.8	Antibacterial Target Proteins, Ergosterol, and Ganoboninketal Structure Preparation	88
5.2.9	Antibacterial Target Proteins' Active Site Determination	89

5.2.10	Molecular Docking Protocol	90
5.2.11	Statistical Analysis	91
5.3	Results and Discussions	91
5.3.1	Minimum Inhibitory Concentration (MIC) Evaluation	91
5.3.2	Leakage of 260nm Absorbing Material	94
5.3.3	Disruption of Cell wall and Cell Membrane via Live/Dead Baclight Assay	95
5.3.4	Scanning Electron Microscope (SEM) Observation	96
5.3.5	Assessment on Bacterial ATP Activity via Bactiter™ Glo Assay	97
5.3.6	Receptor Active Site Determination using RaptorX Binding	100
5.3.7	Validation on Antibacterial Mechanism of Action using Molecular Docking	104
5.4	Conclusion	110
<b>CHAPTER 6: TOXICITY EVALUATIONS OF ERGOSTEROL AND GANOBONINKETAL</b>		111
6.1	Introduction	111
6.2	Methodology	112
6.2.1	Ergosterol and Ganoboninketal Preparation for Toxicity Study	112
6.2.2	<i>In silico</i> Toxicity Analysis	113
6.2.3	Test Animal for Toxicity Study	114
6.2.4	Median Dose Lethality (LD <sub>50</sub> ) Study	115
6.2.5	Acute Toxicity Study	115
6.2.6	Sub-acute Toxicity Study	116
6.2.7	Haematological and Biological Analysis	116
6.2.8	Histopathological Analysis	117
6.2.9	Statistical Analysis	117
6.3	Results and Discussion	118
6.3.1	<i>In silico</i> Toxicity Evaluation of Ergosterol and Ganoboninketal	118
6.3.2	<i>In vivo</i> Median Lethal Dose (LD <sub>50</sub> ) Study	119
6.3.3	Evaluation on <i>In vivo</i> Acute Toxicity	120
6.3.4	Evaluation on <i>In vivo</i> Sub-acute Toxicity	127
6.4	Conclusion	137
<b>CHAPTER 7: SUMMARY</b>		138
<b>REFERENCES</b>		141
<b>APPENDIX</b>		171

## LIST OF FIGURES

		Page
Figure 2.1	Fruiting bodies of <i>G. boninense</i> on infected stem of oil palm seeding	15
Figure 3.1	A) <i>G. boninense</i> cultured on Potato Dextrose Agar (PDA) at $28\pm 2^{\circ}\text{C}$ for 7 days old; B) <i>G. boninense</i> culture in Potato Dextrose Broth (PDB) with continuous shake at 120rpm, $28\pm 2^{\circ}\text{C}$ for 7 days.	28
Figure 3.2	Sample of fruiting body collected from infected oil palm tree in Sabah (Bar=1cm).	35
Figure 3.3	Polymerase Chain Reaction (PCR) amplification of DNA isolated from the fruiting body using ITS1 and ITS4 primers to amplify region on 5.8s ribosomal DNA. M=1kb ladder (Vivantis); Lane 1-3= Isolated DNA from the fruiting body in triplicates.	36
Figure 3.4	Evolutionary relationship of 14 taxa among the fruiting body DNA samples (labelled as unknown, highlighted in yellow). The evolutionary history was inferred using the Fast-Minimum Evolution method with maximum sequence difference at 0.75. The tree is drawn using slanted cladogram to infer the phylogenetic. The evolutionary distances were self-computed by BLAST pairwise alignment for distance tree result and are in the units of the number of base substitutions per site.	37
Figure 3.5	A) The effect of different media on Chloramphenicol inhibition against all the bacterial samples, measured in mm. B) The effect of different media on GBPDA chloroform extract against all the bacterial samples, measured in mm. Data denoted with same letter were not significantly different ( $p>0.05$ ). All data shown represent triplicates. Error bar represent standard deviation (std) from the triplicates. TSA=Tryptic Soy Agar; MHA= Muller-Hinton Agar; NA= Nutrient Agar; LBA= Luria-Bertani Agar. SA= <i>S. aureus</i> ; SP= <i>S. pyogenes</i> ; MRSA= Methicillin Resistant <i>S. aureus</i> ; PA= <i>P. aeruginosa</i> ; KP= <i>K. pneumoniae</i> ; EC= <i>E. coli</i> .	46
Figure 4.1	Antibacterial activity of both water-methanol (WM) and chloroform-methanol (CM) fractions of <i>G. boninense</i> extract. CM fraction shows greater antibacterial activity after 24h of incubation by producing inhibition zone to all the tested bacterial in contrast to hydro-methanol fraction, which only inhibiting <i>S. aureus</i> ATCC 25923 and <i>E. coli</i> ATCC 35218. Data denoted with same letter were not significantly different.	59



SA= *S. aureus*; SP= *S. pyogenes*; MRSA= Methicillin Resistant *S. aureus*; PA= *P. aeruginosa*; KP= *K. pneumoniae*; EC= *E. coli*. All data shown represent triplicates. All data shown represent triplicates. Error bar represent standard deviation (std) from the triplicates.

- Figure 4.2 PHPTLC separation of 1) Water-methanol (WM) and 2) Chloroform-methanol (CM) fractions visualized under A) UV254 and B) UV366, and pHPTLC antibacterial bioautography against C) MRSA (NCTC11939) and D) *S. aureus* (ATCC 25923). Fairly weak inhibition band was observed on band at Rf=0.4 against both MRSA and *S. aureus*. No visible bands were observed under visible (white) light. No inhibition was observed on other bacterial samples for pHPTLC antibacterial bioautography (not shown). 60
- Figure 4.3 GC chromatogram of fraction separated by HPTLC (Rt= 0.4) from Chloro-methanol fraction of *G. boninense* extract. Three (3) peaks were identified as compounds A (Rt<sub>min</sub> = 28.78), B (Rt<sub>min</sub> = 29.17), and C (Rt<sub>min</sub> = 29.89). 62
- Figure 4.4 Mass spectrum of compound A (Rt<sub>min</sub>= 28.78), identified as 4,4,14 $\alpha$ -Trimethylcholestane or Lanostane (m/z= 414.75) with 94% identity was identified from HPTLC (Rt= 0.4) of Chloro-methanol fraction of *G. boninense* extract. 63
- Figure 4.5 Mass spectrum of compound B (Rt<sub>min</sub>= 29.17), identified as Ergosta-5,7,22-trien-3 $\beta$ -ol or Ergosterol (m/z= 396.65) with 99% identity was identified from HPTLC (Rt= 0.4) of Chloro-methanol fraction of *G. boninense* extract. 64
- Figure 4.6 Mass spectrum of compound C (Rt<sub>min</sub>= 29.89) with molecular mass of 498.66, from HPTLC (Rt= 0.4) of Chloro-methanol fraction of *G. boninense* extract. Molecular Formula Identification based on Mass Hunter Agilent Algorithm predicted the molecular formula for Compound C is C<sub>30</sub>H<sub>42</sub>O<sub>6</sub> with 98.9% accuracy. However, no definite identity is available for compound C from the entire inquired mass spectrometry library. 65
- Figure 4.7 SPHPLC analysis of CMRF04 from *G. boninense* extract. The three compounds (A, B, and C) were recovered from PHPTLC after fractionation at their respective Rt (A= 39.21; B 38.55; C= 39.97). Compounds A and B were spiked with their respective standard (A= Lanostane, Sigma Aldrich; B=Ergosterol, Sigma Aldrich) to validate the identity of peaks A and B respectively. 66
- Figure 4.8 FTIR spectroscopy analysis of compound A (black spectrum) along with standard Lanostane (red spectrum). The analysis 68

shows high similarities of FTIR spectrum profile between compound A and the standard in addition to validate the identity of compound A as Lanostane.

- Figure 4.9 FTIR spectroscopy analysis of compound B (black spectrum) along with standard Ergosterol (red spectrum). The analysis shows high similarities of FTIR spectrum profile between compound B and the standard in addition to validate the identity of compound A as Ergosterol. 69
- Figure 4.10 FTIR spectroscopy analysis of compound C. 70
- Figure 4.11 A) HMBC spectrum of Compound C in pyridine. B) The NOESY excitation spectrums of Compound C in pyridine (500MHz) and C) the planar structure of Compound C deduced from NOESY spectrums in correlation with FTIR and HMBC data. 74
- Figure 4.12 A) COSY, HMBC and NOESY correlations of Compound C. The molecular models Compound C in minimal energy were generated by Conflex calculations *in silico* using MMFF94's force field. B) Experimental CD spectra of Compound C in Methanol and the calculated ECD spectra of 2a and 2b. Structures 2a and 2b with their respective configurations represent possible stereoisomers for Compound C. 75
- Figure 4.13 Hypothetical biogenetic pathway of Compound C, suggested that it generated through squalene and sterol metabolism, which explained the presence of Compound C along with Lanostane and Ergosterol. 77
- Figure 4.14 Antibacterial activity of Lanostane, Ergosterol, and Ganoboninketal against SA=*S. aureus*, ATCC 25923; SP= *S. pyogenes*, ATCC 19615; MRSA= MRSA, NCTC11939; PA= *P. aeruginosa*, ATCC 9027; KP= *K. pneumoniae*, ATCC 1705; and EC= *E. coli*, ATCC 35218. Inhibition data denoted with different letters are significantly differ at  $p < 0.05$ . 79
- Figure 5.1 Positive ligands used in MD study to validate the antibacterial mechanism of action of Ergosterol and Ganoboninketal against the antibacterial target proteins. a) PNM; b)DCS; c) ATP; d) ILA; e) NOV; f) LFX; g) XHP; h) TOP. 89
- Figure 5.2 Determination of MIC value of Ergosterol against the bacterial samples. The MIC value for *S. aureus* ATCC25923, *S. pyogenes* ATCC19615, and *E. coli* ATCC 35218 is 30ug/mL. Meanwhile, the MIC value for MRSA NCTC11939, *P. aeruginosa* ATCC9027, and *K. pneumoniae* ATCC1705 is 50ug/mL. 93

- Figure 5.3 Determination of MIC value of Ganoboninketal against the bacterial samples. The MIC value for *S. aureus* ATCC25923, *S. pyogenes* ATCC19615, and *E. coli* ATCC 35218 is 30ug/mL. Meanwhile, the MIC value for MRSA NCTC11939, *P. aeruginosa* ATCC9027, and *K. pneumoniae* ATCC1705 is 50ug/mL. 94
- Figure 5.4 SEM observations on A) *E. coli* treated with Ergosterol, B) *E. coli* treated with Ganoboninketal, C) untreated *E. coli* as control, and D) *E. coli* treated with isopropyl alcohol as positive control. Treatment by Ergosterol and Ganoboninketal caused reduction in cell size and corrugating the cell surface structure. Multiple blisters (indicated by yellow arrow) and protrusion of numerous small bubbles (indicated by red arrow) were also observed, indicating the symptoms of cell lysis. No damage was observed on *E.coli* treated with 10% DMSO (negative control). 97
- Figure 5.5 Molecular docking pose prediction of Ergosterol (green) and Ganoboninketal (yellow) superimposed with the positive control ligands (red) against (A) PBP1a, (B) Alr, (C) Ddl, (D) IARS (E) DNAG, and (F) TopoIV. All the receptor proteins are presented in cartoons (coloured based on secondary structure) while all ligands are presented in stick. Yellow dashed line indicate hydrogen-bond interaction Ergosterol, Ganoboninketal and positive ligands with amino acids residues in the receptors' binding site. 109
- Figure 6.1 Histopathological observation on control and Ganoboninketal treated rats in sub-acute toxicity study. (A) Normal control group; (B) Male liver treated with 1000mg/kg of Ganoboninketal. Red arrow shows sinusoidal dilatation followed by mild micronodular cirrhosis. C) Female liver treated with 1000mg/kg of Ganoboninketal shows more severe micronodular cirrhosis followed by obvious hepatocellular necrosis (indicated by green arrow) and lymphocytic infiltration (indicated by blue arrow). H & E observed at 100x magnification. 136

## LIST OF SYMBOL AND ABBREVIATIONS

-	-	minus
%	-	Percentage
/	-	divide by
+	-	plus
=	-	equals to
±	-	plus-minus
≥	-	more or equal to
µg	-	microgram
µl	-	microlitre
µM	-	micromolar
2D-NMR	-	Two Dimensional Nuclear Magnetic Resonance
ANOVA	-	Analysis of Variance
APT	-	Attached-Proton-Test
AR	-	Antibiotic resistant
ATCC	-	American Types of Culture Collection
BLAST	-	Basic Local Alignment Search Tool
CCD	-	Central Composite Design
CLSI	-	Clinical and Laboratory Standards Institute
CM	-	Chloroform-methanol
CMRF04	-	HPTLC separation of CM at Rt 0.4
COSY	-	Homonuclear Correlation Spectroscopy
DMSO	-	Dimethyl sulfoxide
ECD	-	Electronic Circular Dichroism

EDTA	-	Ethylene Diamine Tetra Acetic Acid
Eq.	-	Equation
FTIR	-	Fourier Transform Infra Red
g	-	gram
GAS	-	Group A <i>Streptococcus</i>
GBFB	-	<i>G. boninense</i> fruiting body
GBPDA	-	<i>G. boninense</i> growth on PDA
GC-MS,	-	Gas Chromatography – Mass Spectrometry
GHS	-	Global Harmonized System
HAI	-	Hospital-Acquired/Associated Infection
HMBC	-	Heteronuclear Multiple-Bond Correlation
Hz	-	Hertz
IMR	-	Institute of Medical Research
IUPAC	-	International Union of Pure and Applied Chemistry
kg	-	kilogram
L	-	litre
LD <sub>50</sub>	-	Median Lethal Dose
LLE	-	Liquid-liquid extraction
m	-	meter
M.F	-	Molecular Formula
m/z	-	Mass to charge ratio
MEA	-	Malt Extract Agar
mg	-	milligram
MIC	-	Minimum Inhibitory Concentration
ml	-	millilitre

mm	-	millimeter
MMFF94	-	Merck Molecular Force Field
mmhg	-	Millimeter of mercury
MRSA	-	Methicillin Resistant <i>Staphylococcus aureus</i>
NARS	-	National Antibiotic Resistance Surveillance
NCTC	-	National Collection of Type Cultures
NOESY	-	Nuclear Overhauser Effect Spectroscopy
°C	-	Degree Celcius
OECD	-	Organisation for Economic Co-operation and Development
PCR	-	Polymerase Chain Reaction
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
PHPTLC	-	Preparative High Performance Thin Layer Chromatography
pM	-	picomolar
ProTox	-	Prediction of Rodent Oral Toxicity
QSAR	-	Quantitative structure–activity relationships
R <sup>2</sup>	-	Coeffecient of correlation
RBA	-	Rose Bengal Agar
rpm	-	Round Per Minute
RSM	-	Response Surface Methodology
SAB	-	<i>Staphylococcus aureus</i> bacteremia
SPHPLC	-	Semi Preparative High Performance Liquid Chromatography
WHO	-	World Health Organization
WM	-	Water-methanol

$\beta$	-	Beta
$\delta_C$	-	$^1\text{H}$ NMR
$\delta_H$	-	$^{13}\text{C}$ Attached-Proton-Test NMR



UMS  
UNIVERSITI MALAYSIA SABAH

## LIST OF TABLES

		<b>Page</b>
Table 2.1	Summary of antibiotics discovered from fungal species.	13
Table 3.1	List of bacterial samples used throughout the work.	29
Table 3.2	Log phase point for each bacterial samples acquired from bacterial growth curve.	30
Table 3.3	BLAST similarity analysis shows top five results of the isolated fruiting body's DNA. It is confirmed the fruiting body sample collected from infected oil palm tree is <i>Ganoderma boninense</i> with 100% identity.	36
Table 3.4	Extraction yield of <i>G. boninense</i> from different sources (fruiting body, broth and mycelia) with different solvents.	39
Table 3.5	Biochemical test on <i>G. boninense</i> various extracts from different sources (fruiting body, broth and mycelia) to preliminary screen for the presence of bioactive compounds.	42
Table 4.1	Gradient solvent system used in PHPTLC separation on CM and WM fraction.	52
Table 4.2	NMR spectroscopic data for both $^1\text{H}$ NMR ( $\delta_C$ ) and $^{13}\text{C}$ Attached-Proton-Test (APT) NMR ( $\delta_H$ ) spectra of Compound C in Pyridine- $d_5$ .	73
Table 4.3	Final structure of Compound C based on NMR spectroscopy with COSY, HMBC, NOESY, and ECD Configuration analysis and their name according to IUPAC standard nomenclature.	76
Table 5.1	Relative percentage (%) of membrane leakage on bacteria based on 260nm absorbing materials.	95
Table 5.2	Effect of Ergosterol and Ganoboninketal (measured in %) at 3x of MIC on bacterial cell wall and cell membrane integrity using Live/Dead BacLight assay.	96
Table 5.3	Relative ATP Luminescence percentage (%) of bacterial samples after treated with Ergosterol and Ganoboninketal at 3x of MIC through luminometric bioassay.	99
Table 5.4	Receptors' binding site prediction using RaptorX binding	101