CHARACTERIZATION OF ANTIBACTERIAL COMPOUNDS FROM *Ganoderma boninense*

SYAHRIEL BIN ABDULLAH

FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH
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2018

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APPENDIX A

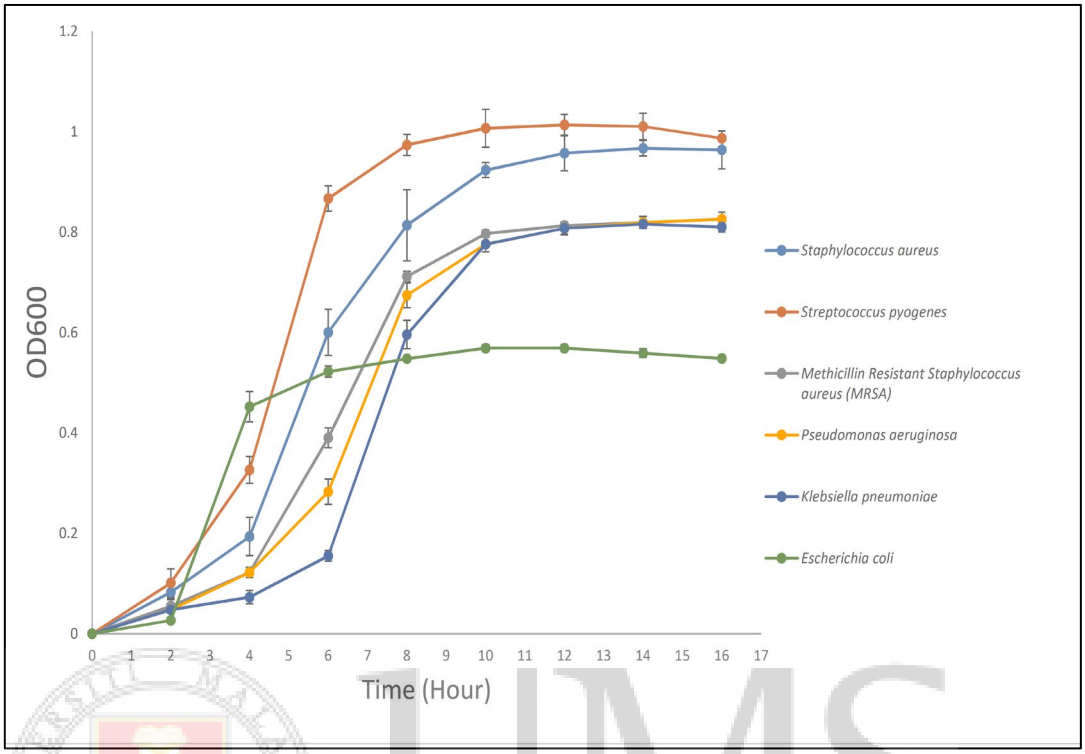


Figure A: Growth log phase determination for the six bacterial sampes. Optical density (OD) reading at 600nm wavelength was recorded for every two hours until the reading reach stagnant value. Nutrient broth without bacterial suspension served as negative control and blank. OD_{600} reading was plotted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

APPENDIX B

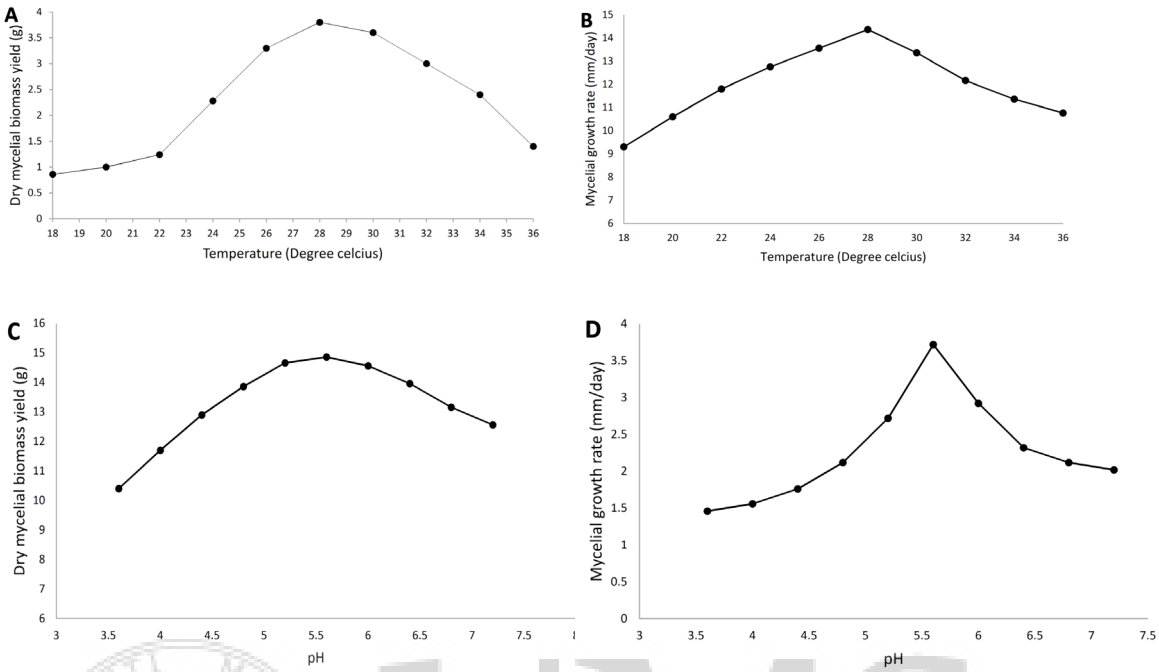
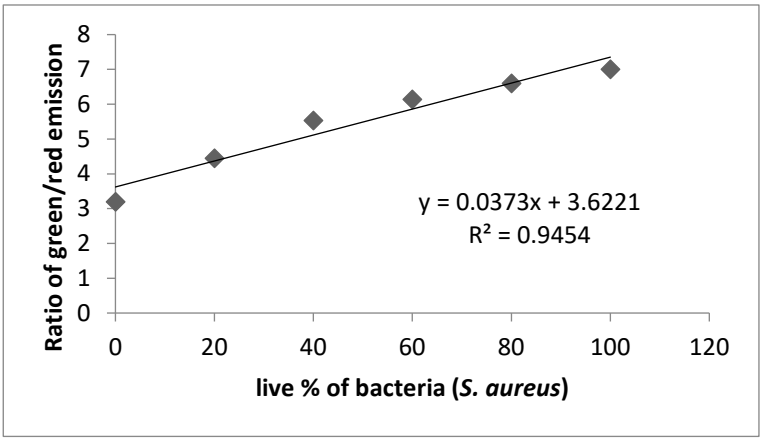
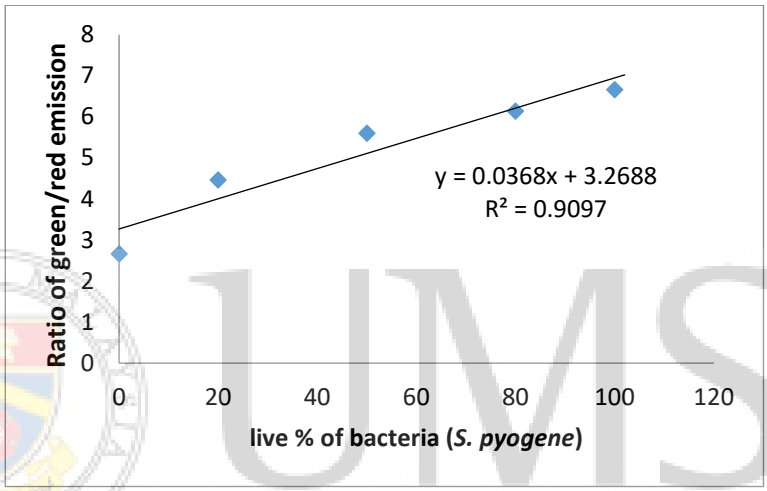
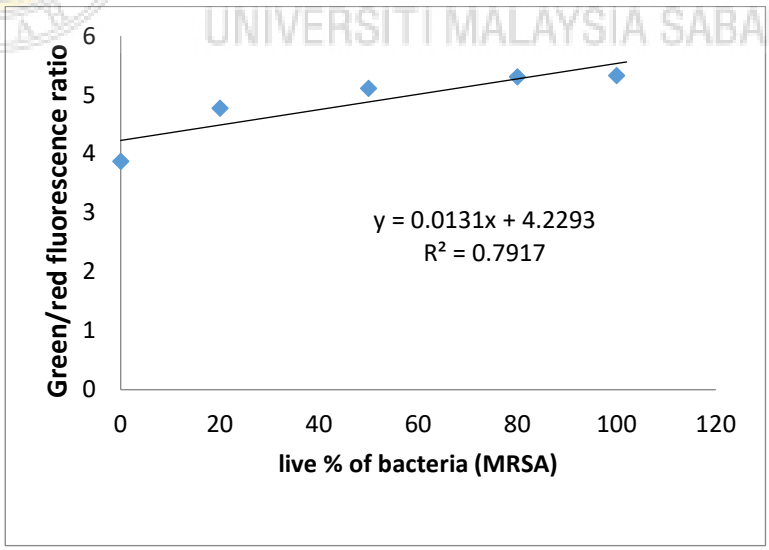


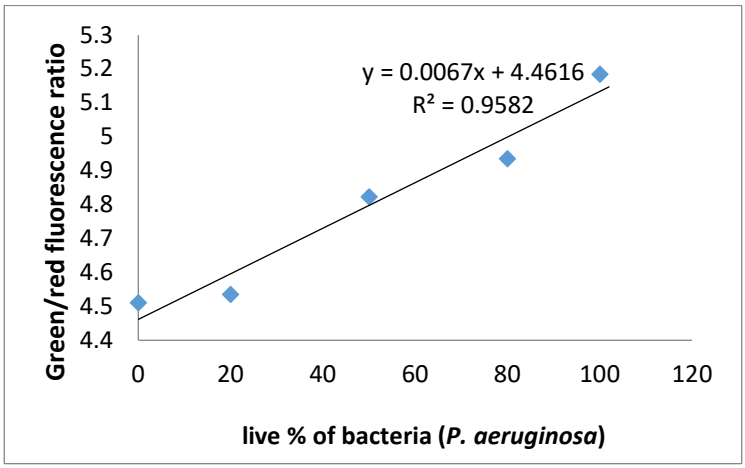
Figure B: Single factor experiment to identified effective range of incubation temperatures (^{O}C) and acidity (pH). A) Effect of different temperatures on *G. boninense* dry mycelial biomass yield (g). B) Effect of different temperatures on *G. boninense* growth rate (mm/day). C) Effect of different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

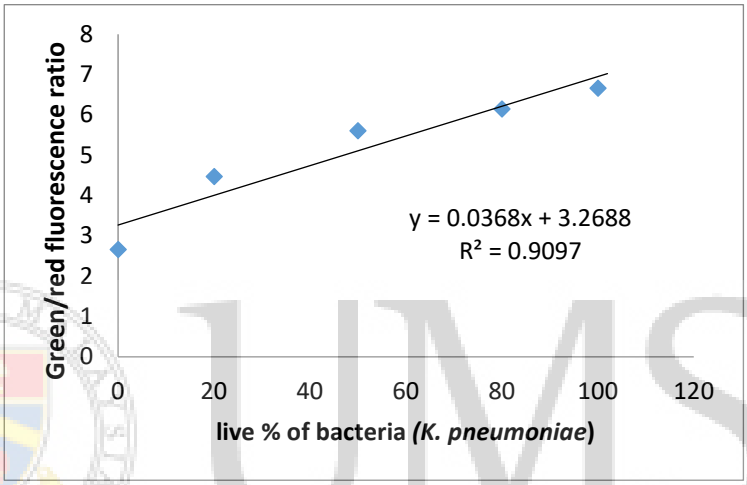
APPENDIX C

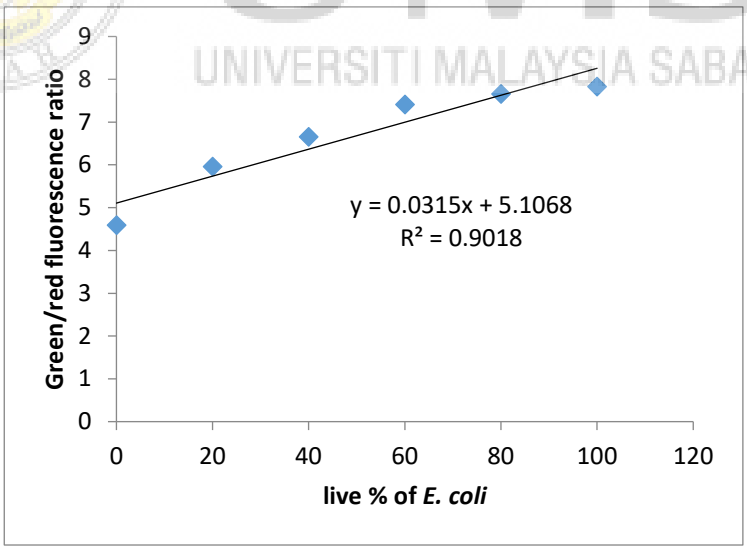












APPENDIX D

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

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

APPENDIX E

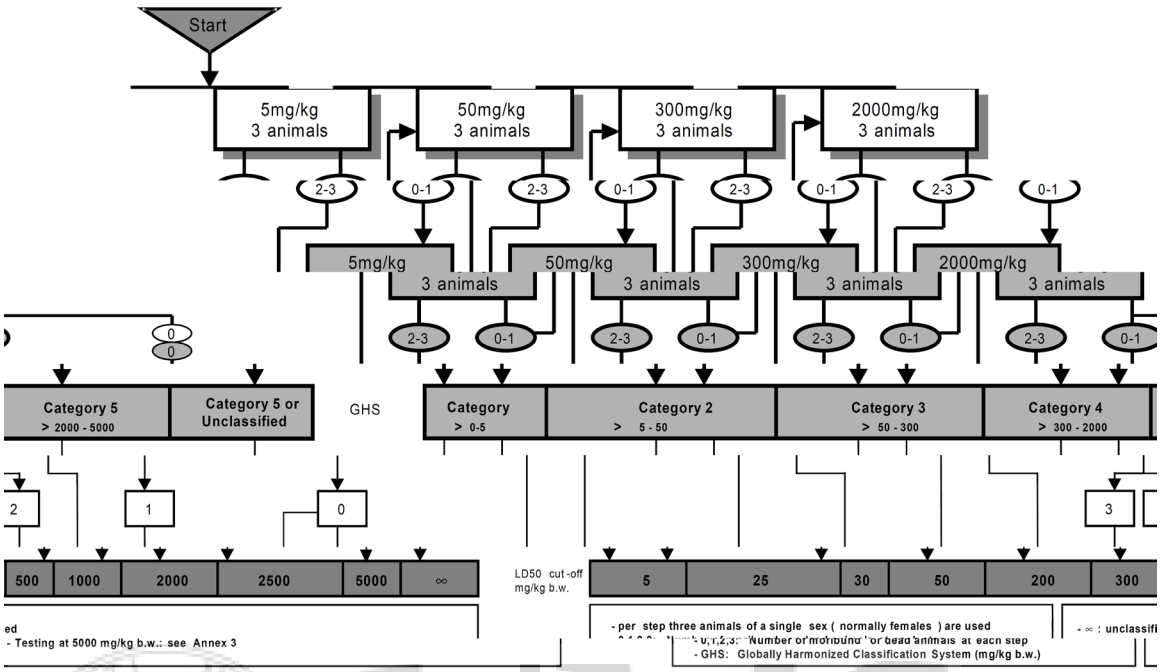


Figure E: Test procedure starting at 5mg/kg (with limit test dose of 5000mg/kg) according to Organisation for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, Section 4 (Annex. 2a).

APPENDIX F

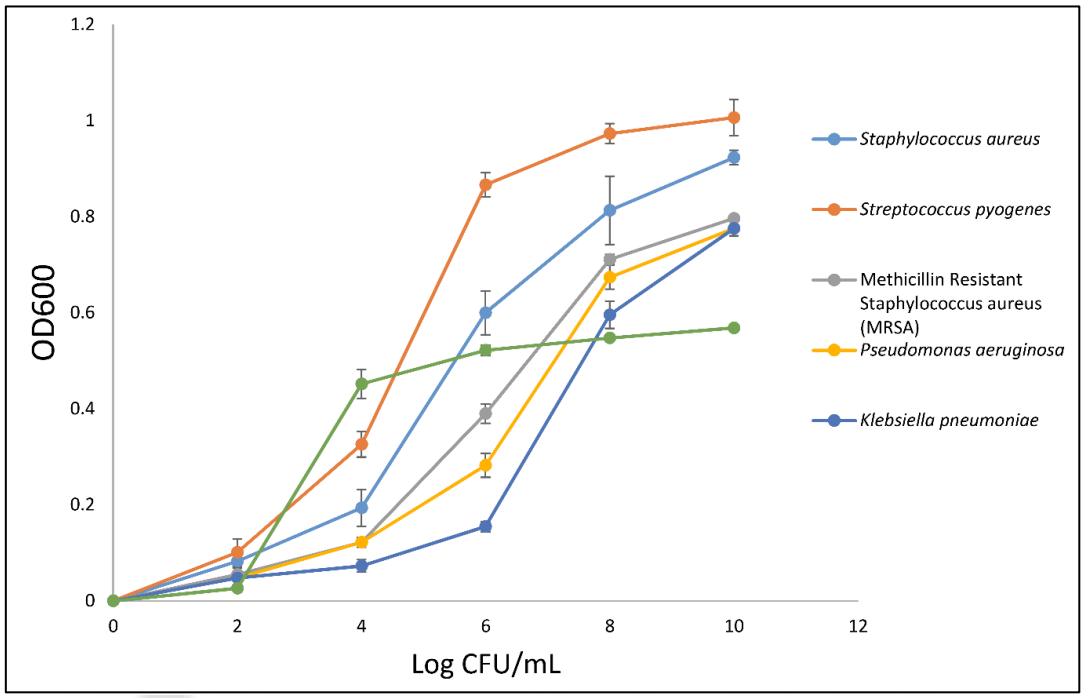


Figure F: Correlation curves between Colony Forming Unit (CFU) per mL (CFU/mL) of the six bacterial samples (Log transformed) and the absorbance (OD) reading at 600nm.

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.

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LIST OF FIGURES

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	-	Tw Dimensional Nuclear Magnetic Resonance
ANOVA	-	Analysis of Variant
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 *Streptococcus*
GBFB	-	*G. boninense* fruiting body
GBPDA	-	*G. boninense* 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_{50}	-	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 *Staphylococcus aureus*
NARS	-	National Antibiotic Resistance Surveillance
NCTC	-	National Collection of Type Cultures
NOESY	-	Nuclear Overhauser Effect Spectroscopy
^{O}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^2	-	Coeffecient of correlation
RBA	-	Rose Bengal Agar
rpm	-	Round Per Minute
RSM	-	Response Surface Methodology
SAB	-	*Staphylococcus aureus* bacteremia
SPHPLC	-	Semi Preparative High Performance Liquid Chromatography
WHO	-	World Health Organization
WM	-	Water-methanol

β	-	Beta
δ_C	-	^{1}H NMR
δ_H	-	^{13}C Attached-Proton-Test NMR

LIST OF TABLES