EXTRACTION AND ISOLATION OF ANTI-MRSA EXTRACELLULAR COMPOUNDS FROM *Pseudomonas aeruginosa* STRAIN MSO(Y)

SHU CHAI CHING

BACHELOR OF SCIENCE (HONS)
BIOTECHNOLOGY

FACULTY OF SCIENCE
UNIVERSITI TUNKU ABDUL RAHMAN
SEPTEMBER 2015

EXTRACTION AND ISOLATION OF ANTI-MRSA EXTRACELLUAR COMPOUNDS FROM *Pseudomonas aeruginosa* STRAIN MSO(Y)

By

SHU CHAI CHING

A project report submitted to the Department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman

In partial fulfillment of the requirements for the degree of

Bachelor of Science (Hons) Biotechnology

September 2015

ABSTRACT

EXTRACTION AND ISOLATION OF ANTI-MRSA EXTRACELLULAR COMPOUNDS FROM *Pseudomonas aeruginosa* STRAIN MSO(Y)

SHU CHAI CHING

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) is threatening the public health. Thus, there is a need to search for new antibiotics and treatments that can combat MRSA. This project was carried out to evaluate the anti-MRSA activity of the extracellular compounds produced by *Pseudomonas aeruginosa* MSO(Y). The spent culture supernatant of *P. aeruginosa* MSO(Y) was obtained after 72 hours of incubation at 37ºC and subjected to liquid-liquid extraction using dichloromethane. After verifying the presence of anti-MRSA activity using Kirby-Bauer test, the compounds of interest in dichloromethane phase (DP) were isolated using normal and reversed phase chromatography. All of the fractions were collected and tested on anti-MRSA activity using Kirby-Bauer test. Results obtained showed that the compounds with polarities ranging from 3.1-3.7 and 6.63-8.44 are positive for anti-MRSA activity. However, anti-MRSA compounds eluted with mobile phase of polarity 3.3 using normal phase chromatography showed the strongest anti-MRSA activity. This

fraction was then further tested on the viability of MRSA and their time response growth curves were plotted. The result obtained indicated that the higher the concentration of anti-MRSA compounds, the stronger the inhibition effect. It is found that the growth of MRSA was only inhibited for a certain period of time and the effect reduced with prolonged incubation indicating the bacteriostatic effect of the isolated compounds. The subsequent HPLC analysis showed the possible presence of four phenazine compounds which are phenazine-1-carboxylic acid, phenazine-1-carboxyamide, pyocyanin and 1-hydroxyphenazine were a comparison was made with the retention times of reference compounds. From this study, *P. aeruginosa* MSO(Y) is found to be able to produce various types of secondary metabolites which possess anti-MRSA activity. Further research is needed to purify the isolated anti-MRSA compounds followed by further evaluation on their anti-MRSA potential.

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere gratitude to my final year project supervisor, Assistant Professor Dr Kho Chiew Ling for her guidance and advice throughout the whole project. Her effort, support and patient enable to motivate me to accomplish my bench work and thesis in most presentable way. I would also like to extent my appreciation to Dr Tong Kim Suan for his invaluable knowledge and suggestion which help me a lot in bench work. Besides, I would also like to thank the master student, Lim Sin Yun for her knowledge and experience sharing.

Next, I would like to thank all the lab offices form both biological science and chemistry departments for their help and assistance in completing the bench work. In addition, I would also like to thank to all my lab mates for their sharing, supporting and encouraging throughout this project.

Last but not least, a million thank to my family members in giving me moral support and encouraging me during the completion of this project. Thank you.

DECLARATION

I hereby declare that the project report is based on my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

_____

SHU CHAI CHING

APPROVAL SHEET

This project entitled **"EXTRACTION AND ISOLATION OF ANTI-MRSA EXTRACELLULAR COMPOUNDS FROM *Pseudomonas aeruginosa* STRAIN MSO(Y)"** was prepared by SHU CHAI CHING and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) in Biotechnology at Universiti Tunku Abdul Rahman.

Approved by:

_____

(Assistant Professor Dr. Kho Chiew Ling) Date:……

Supervisor

Department of Biological Science

Faculty of Science

Universiti Tunku Abdul Rahman

FACULTY OF SCIENCE

UNIVERSITI TUNKU ABDUL RAHMAN

Date: _____

PERMISSION SHEET

It is hereby certified that **SHU CHAI CHING** (ID No: **13ADB00284**) has completed this final year project entitled **"EXTRACTION AND ISOLATION OF ANTI-MRSA EXTRACELLULAR COMPOUNDS FROM *Pseudomonas aeruginosa* STRAIN MSO(Y)"** under the supervision of Dr Kho Chiew Ling from the Department of Biological Science, Faculty of Science.

I hereby give permission to the University to upload the softcopy of my final year project in pdf format into the UTAR Institutional Repository, which may be made accessible to the UTAR community and public.

Yours truly,

_____

(SHU CHAI CHING)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS

1-OHPHZ	1-hydroxyphenazine
2,4-DAPG	2,4-diacetylphloroglucinol
API	Analytical profile index
ATCC	American type culture collection
CA-MRSA	Community-acquired methicillin resistant *Staphylococcus aerues*
CF	Cystic fibrosis
CFF	Cystic Fibrosis Foundation
DP	Dichloromethane phase
FTIR	Fourier transform infrared spectroscopy
HA-MRSA	Healthcare associated methicillin resistant *Staphylococcus aerues*
HPLC	High performance liquid chromatography
LB	Luria Bertani
LC-MS	Liquid chromatography-mass spectrum
$MgSO_4$	Magnesium sulphate
MH	Muller-Hinton

MRSA	Methicillin resistant *Staphylococcus aureus*
MS	Mass spectrum
MSSA	Methicillin-sensitive *Staphylococcus aerues*
NMR	Nuclear magnetic resonance
PBP2a	Penicillin binding protein 2a
PCA	Phenazine-1 carboxylic acid
PCN	Phenazine-1-carboxyamide
PVL	Panton-Valentine leukocidin
PYO	Pyocyanin
rRNA	Ribosomal ribonucleic acid
TLC	Thin layer chromatography
TMP-SMX	Trimethoprim-sulfamethoxazole
UV	Ultraviolet
v/v	volume per volume
VISA	Vancomycin insensitive *Staphylococcus aureus*