IN VITRO ANTIBACTERIAL ACTIVITY OF *Quercus infectoria* GALL EXTRACTS AGAINST MULTIDRUG RESISTANT BACTERIA

by

MASRAH BINTI MALIK

Dissertation submitted in partial fulfillment of the requirements for the

Degree of Bachelor of Health Sciences (Biomedicine)

2013

CERTIFICATE

This is to certify that the dissertation entitled "*In vitro* antibacterial activity of *Quercus infectoria* gall extracts against multidrug resistant bacteria" is the bona fide record of research work done by Miss Masrah Binti Malik during the period from July 2012 till June 2013 under my supervision.

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious and the Most Merciful

Alhamdulillah, all praises to Allah for His blessings as He gave me strength, courage and health to conduct this research and complete my dissertation well. Special appreciation goes to my supervisor who let me experience the microbiology research works beyond the textbooks, Dr. Wan Nor Amilah. A million thanks for all of her guidance, constant encouragement and moral support, patience and critical appraisal at every stage of this project. Her invaluable help of constructive comments and recommendations throughout my research and dissertation works have contributed to the success of this project.

I would also like to express my deepest gratitude and acknowledgement to Dr. See Too Wei Chun, the Coordinator of final year student research project for always being understanding and tolerance to us in completing our project.

My acknowledgement also goes to the laboratory staffs of Department of Microbiology and Parasitology, School of Medical Sciences especially Pn. Rosliza, Pn. Roziawati, Pn. Nur Fasihah and Pn. Amanina, laboratory staffs of School of Health Sciences especially Pn. Wan Razlin and Cik Kurunisa and all the postgraduate students especially Lukman, Zaharaini and Nurzila for sharing their immense knowledge and co-operations that they gave me during my research works.

Sincere thanks to all my friends especially Fatimah, Amira and Atiqah for their kindness and moral support during my research works and throughout completing this dissertation. Thanks for the friendship and memories.

Last but not least, my deepest gratitude goes to my beloved parents, Mr. Malik Bin Ali and Mrs. Hjh. Hajirah Bt. Kadir and also to my siblings for their endless love, prayers and encouragement. To those who indirectly contributed in my project, your kindness means a lot to me. Thank you very much.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AST – Antibiotic susceptibility testing

CA-MRSA – Community acquired - methicillin resistant *Staphylococcus aureus*

CDC – Centre for Disease Control and Prevention

CLSI – Clinical and Laboratory Standards Institute

DMSO – Dimethyl sulfoxide

ESBL - Extended spectrum beta lactamase

HA-MRSA - Hospital acquired - methicillin resistant Staphylococcus aureus

MBC – Minimum bactericidal concentration

MDR – Multidrug resistant

MHA – Mueller Hinton agar

MHB – Mueller Hinton broth

MIC – Minimum inhibitory concentration

MRCoNS - Methicillin resistant coagulase negative *Staphylococcus*

MRSA - Methicillin resistant *Staphylococcus aureus*

ABSTRAK

Kajian mengenai aktiviti antimikrob daripada tumbuh-tumbuhan telah lama dijalankan untuk mengenalpasti potensi ubat yang selamat digunakan pada masa hadapan bagi mengurangkan kesan rintangan mikroorganisma yang tidak diingini. Kajian ini dijalankan untuk menilai aktiviti antibakteria ekstrak biji manjakani (*Q. infectoria*) terhadap isolat klinikal bakteria rintangan pelbagai dadah (MDR) melalui kaedah penentuan kepekatan perencatan minimum (MIC) menggunakan teknik pencairan mikro bersiri berganda pada kepekatan antara 5.00 mg/ml - 0.01 mg/ml dan kepekatan bakterisidal minimum (MBC). Tiga kepekatan yang berbeza (1, 2 dan 5 mg/disk) daripada ekstrak akuas dan etanol biji manjakani telah digunakan untuk perbandingan kepekatan disk optimum semasa ujian saringan dengan lima isolat klinikal bakteria MDR iaitu MRSA, MRCoNS, MDR *Acinetobacter* sp., ESBL *E. coli* dan ESBL *K. pneumoniae*. Kami mendapati bahawa, diameter perencatan zon 5 mg/disk adalah lebih besar daripada diameter zon perencatan 1 mg/disk ekstrak akuas terhadap MRSA, MRCoNS dan MDR Acinetobacter sp. Begitu juga, terdapat perbezaan yang signifikan dari diameter zon diperhatikan antara 2 mg/disc dan 5 mg/disc ekstrak akuas terhadap ketiga-tiga jenis isolat. Untuk ekstrak etanol, diameter zon perencatan adalah lebih besar diperhatikan dengan 5 mg/disk terhadap MRSA dan MDR Acinetobacter sp. berbanding dengan 1 mg/disk. MRCoNS adalah bakteria yang paling menunjukkan kesan perencatan tertinggi diikuti oleh MRSA. Kedua-dua ekstrak menunjukkan kesan perencatan yang lemah terhadap MDR *Acinetobacter* sp. manakala tiada zon perencatan direkodkan untuk isolat ESBLs. Kesemua tiga kepekatan ekstrak menunjukkan saiz zon perencatan yang signifikan terhadap MRSA dan pada 5 mg/disc; terdapat perbezaan yang ketara dalam saiz zon antara kedua-dua ekstrak juga diperhatikan dalam MRCoNS ($p < 0.05$). Nilai MIC dan MBC daripada ekstrak adalah dari 0.08 mg/ml – 2.50 mg/ml.

Nilai untuk MIC ekstrak akuas dan etanol terhadap MRSA masing-masing adalah 0.08 mg/ml dan 0.16 mg/ml manakala nilai MIC untuk kedua-dua ekstrak terhadap MRCoNS adalah sama (0.08 mg/ml). Nilai MBC ekstrak akuas terhadap MRSA dan MRCoNS adalah lebih tinggi daripada nilai MIC manakala nilai MBC ekstrak etanol terhadap MRSA dan MRCoNS adalah sama dengan nilai MIC. Nilai MBC kedua-dua ekstrak terhadap semua bakteria Gram negatif adalah sama dengan nilai MIC iaitu MDR *Acinetobacter* sp. (0.63 mg/ml), ESBL *E. coli* (2.50 mg/ml) dan ESBL *K. pneumoniae* (1.25 mg/ml). Biji manjakani adalah sumber yang berpotensi baik sebagai ejen antimikrob kerana keberkesanan dalam aktiviti antibakteria terhadap bakteria rintangan pelbagai dadah.

ABSTRACT

Antimicrobial activities of plants have long been evaluated for their potential safe remedies in the future to minimize the unwanted resistance effects of microorganisms. The study was conducted to evaluate the antibacterial activity of *Quercus infectoria* gall extracts against multidrug resistance (MDR) bacterial clinical isolates by determination of minimum inhibitory concentration (MIC) using the twofold serial microdilution technique at concentration ranging from 5.00 mg/ml to 0.01 mg/ml and minimum bactericidal concentration (MBC) values. Three different concentrations (1, 2 and 5 mg/disc) of aqueous and ethanol extracts of *Q. infectoria* galls were used for comparison of optimum disc concentration during screening test with five MDR bacterial clinical isolates namely MRSA, MRCoNS, MDR *Acinetobacter* sp., ESBL *E. coli* and ESBL *K. pneumoniae.* We found that, the inhibition zones diameter of 5 mg/disc were significantly larger than inhibition zones diameter of 1 mg/disc aqueous extract against MRSA, MRCoNS and MDR *Acinetobacter* sp. Similarly, the significant difference of inhibitory zone diameter was observed between 2 mg/disc and 5 mg/disc of aqueous extract against all three isolate strains. For ethanol extract, larger inhibition zones diameter was observed with 5 mg/disc diffusion plate of MRSA and MDR *Acinetobacter* sp. as compared to 1 mg/disc diffusion plate. Among all tested bacteria, MRCoNS was the most susceptible followed by MRSA during screening. Both extracts showed weak inhibitory effects against MDR *Acinetobacter* sp. while there was no inhibition zone observed for ESBLs isolates. All of the three concentrations of extracts showed inhibition zone size which was significantly different against MRSA and at 5 mg/disc; there was a significant difference in the zone sizes between both extracts was also observed in MRCoNS ($p < 0.05$). The MIC and MBC values of the extracts ranged from 0.08 mg/ml to 2.5 mg/ml. The MIC values for aqueous and ethanol extracts

against MRSA were 0.08 mg/ml and 0.16 mg/ml respectively whereas the MIC value for both extracts against MRCoNS were the same (0.08 mg/ml). The MBC values of aqueous extracts against MRSA and MRCoNS were above their MIC values whereas the MBC values of ethanol extracts were the same with the MIC values against MRSA and MRCoNS. MBC values of both extracts against all Gram negative bacteria tested were the same with their MIC values; MDR *Acinetobacter* sp. (0.63 mg/ml), ESBL *E. coli* (2.50 mg/ml) and ESBL *K. pneumoniae* (1.25 mg/ml). The *Q. infectoria* gall extracts may be considered as a potentially good source of antimicrobial agents due to the effectiveness in their *in vitro* antibacterial activity against MDR bacteria.