UNIVERSITI TEKNOLOGI MARA

THE EFFECTS OF *Chromolaena Odorata* CHLOROFORM EXTRACT ON *Pseudomonas aeruginosa* Biofilm

MOHAMAD SHAMIL ANWAR BIN IBRAHIM

Thesis submitted in fulfillment
of the requirements for degree of
Master of Science

Faculty of Applied Sciences

December 2016

ABSTRACT

Pseudomomas aeruginosa infection is especially prevalent among patients with burn wound, cystic fibrosis, acute leukemia, organ transplants and intravenous-drug addiction. The most serious infections include malignant external otitis, endhophthalmitis and more. At the same time, there are several reports on antibacterial properties of *Chromolaena odorata* plant extract. However, it remains uncertain whether *C. odorata* could control *P. aeruginosa* infections especially the biofilm growth. It is still unknown how the *C. odorata* plant extract potentially modulate the proteome level in *P. aeruginosa* biofilm. This study was carried out to determine the antibiofilm activity of *C.odorata* chloroform extracts (COCE) against *P.seudomonas aeruginosa* under aerobic and anaerobic conditions. There are several reports on antibacterial properties of COCE. Phytochemical screening using gas chromatography mass spectrometry (GCMS) revealed one of the major compound in COCE was Germacrene D. Based on micro broth dilution assay performed, oxygen level did not show any effect towards the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of COCE against *P. aeruginosa.* However, antibacterial susceptibility test showed that the antibacterial activity of COCE against *P. aeruginosa* under anaerobic condition higher than aerobic conditions. Colony forming unit (CFU) counting of biofilm cells demonstrated that COCE had greater antibiofilm activity against *P. aeruginosa* under aerobic condition. Subsequent, treatment *C. odorata* chloroform extract resulted in changes in the biochemical composition of *P. aeruginosa* biofilm fraction under both experimental conditions, as indicated by variation in infrared (IR) spectra in the region between 1700 cm^{-1} and 900 cm^{-1}. The cytoplasmic proteins expressed by *P. aeruginosa* biofilm upon treatment with COCE extract were profiled. The treatment of COCE resulted in changes in the profile of cytoplasmic proteins of *P. aeruginosa* biofilm under aerobic and anaerobic condition. The treatment of COCE resulted in changes in the profile of cytoplasmic proteins of *P. aeruginosa* biofilm under aerobic and anaerobic condition. By using MALDI-TOF-TOF for protein profiling, about 24 proteins was identified. For example phosphogluconate, fumarate hydratase class II 1, elongation factor Tu 1 and more.

ACKNOWLEDGEMENT

Assalammualaikum w.b.t

Alhamdulillah and praise to Allah, the Almighty God for His mercy has given me strength, patience and blessings to complete this study and research. Here, I would like to express my sincere to every single person for their effort and support that contributed to help me in completing all the process of the research and development activities.

First and foremost, I would like to express my sincere thanks to my supervisors; Dr Umi Marshida Abdul Hamid and Mr. Fakharulzaman Raja Yahya for their patience, supervision, encouragements and thoughtful guidance towards the completion of this thesis.

I am also indebted to the Ministry of High Education, Ministry of Education Malaysia, Universiti Teknologi MARA (UiTM) and Faculty of Applied Sciences for funding my MSc study and providing the instrument facilities. I take this opportunity to express my special appreciation to all laboratory assistants and staff from the Department of Biology, Research Department of IPSIS and Faculty of Applied Sciences Universiti Teknologi MARA for assistance in handling instrument and also supplying relevant literature and information.

Last but not least, heartfelt appreciation is given to my beloved family especially to my father Mr. Haji Ibrahim bin Abdul Rahman and family for their support, endless love, prayers and most precious care. I also place on record, a very warm thank you to my friends who directly or indirectly have lent their helping hand in this venture. I also wish to express my special appreciation and gratitude to my “postgraduate team” for sincere and valuable guidance and encouragement extended to me.
Thank you.

Mohamad Shamil Anwar Bin Ibrahim

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

1.1 RESEARCH BACKGROUND

Pseudomonads are broadly resistant to antibiotics and are opportunistic pathogens of plants and animals. One of the bacteria in *Pseudomonads* group is *Pseudomonas aeruginosa*. It is a monoflagellated gamma proteobacterium which can be found in soil, water, and the normal human microflora. It is also the leading cause of mortality in cystic fibrosis (CF) (Pradeep *et al.*, 2000).They are also capable of growing on various hydrocarbons, including tar, oil, or jet fuel.

A biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface. It may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings (Allison, 2000; Lynch *et al.*, 2003). The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism which by contrast, are single-cells that float or swim in a liquid medium. The adherent biofilm cells are frequently embedded within a self-produced matrix of extracellular polymeric substances (EPS). The EPS anchor the cells to their substrate and protect the bacteria from host defenses such as macrophages and antibodies and impart antibiotic resistance (Shih and Huang, 2002).One of several EPS secreted by *P. aeruginosa* is a repeating polymer of mannuronic and glucuronic acid that referred to as alginate. In lungs, the polysaccharide alginate is the major part of the *P. aeruginosa* biofilm matrix (Niels *et al.*, 2010).

The number of emerging multi-drug resistant microbial strains is continuously increasing gradually and has become one of the most serious threats to successful treatment of infectious diseases (Kalyani *et al.*, 2011). This increase is mainly attributed to indiscriminate use of broad-spectrum antibiotics (Suffredini *et al.*, 2004). The use of synthetic drugs is not only expensive but often found with adulterations and side effects. Therefore, new formulations of antimicrobial agents derived from natural plant products is required to address this current issue (Dabur *et al.*, 2007, Maji *et al.*, 2010). For example, by using herbal plant that already has the essential oils that contain various bioactive compounds that can give infections instead of drug. Plus, by using natural