INHIBITION OF BIOFILM DEVELOPMENT BY USING *Solanum melongena* FRUIT EXTRACTS AGAINST SOME CLINICALLY IMPORTANT PATHOGENS

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THIS DISSERTATION IS SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
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ACKNOWLEDGEMENT

'I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me' said Isaac Newton. This world is grand and there lies an ocean of undiscovered findings that are waiting for eager and curious minds. During these 10 weeks of laboratory work, I found myself as a small fish in this deep ocean full of undetermined truth. I faced a lot unexpected challenges and the intense learning has enriched my life experiences, not only in terms of scientific knowledge but most importantly my mental virtues. Splendid encouragement and motivation from the surrounding people have given me strength to accomplish this project. First and foremost, I would like to sincerely thank my beloved supervisor, Dr. Geetha Subramaniam for her great guidance and valuable suggestions throughout these weeks. Her vision, passion and wisdom have been inspiring me not to be just a better researcher but also a better person with high dignity. Furthermore, I would like to show my sincere gratitude to all the lecturers especially Ms. Lalita Ambigai Sivasamugham, Dr. Yuka Hara and Dr. Ong Ghim Hock who have supported me by contributing their precious time and professionalism. Not forgetting my senior, Himashi Imanda Gurudeniya and my friend, Chia Zheng Yang who have held out their hands in friendship to provide me countless help and motivation during this project. Last but not least, I would like to express my greatest affection and sincere appreciation to my family who has always been there for me in the good and bad moments.

ABSTRACT

Antibiotic resistance has been a global issue because generally-prescribed antibiotics are no longer effective against the resistant bacteria. One of the factors that have contributed to antibiotic resistance is the formation of biofilms. Common nosocomial pathogens such as *Acinetobacter baumannii, Pseudomonas aeruginosa*, *Enterococci faecalis* and *Staphylococcus aureus* are capable of forming biofilms on dry hospital surfaces, indwelling medical devices and other areas of healthcare institutions. This causes difficulties in treatment of bacterial infections and further increases the rate of mortality of patients. Thus, there is an immediate need to discover alternative biotherapeutic agents with significant antibacterial and anti-biofilm properties. This study determined the potential role of *Solanum melongena* in helping to overcome antibiotic resistance and biofilm development in four clinically important pathogens namely *A. baumannii, P. aeruginosa, E. faecalis* and *S. aureus*. Fruit extracts of *S. melongena* were prepared by using three different organic solvents including acetone, ethanol and methanol in order to compare their effectiveness in inhibiting the growth of the bacterial strains mentioned above. Phytochemical screening was also done to qualitatively determine the phenolic compounds that were contained in the three different plant extracts. Both acetone and ethanolic extracts contained flavonoids, alkaloids and tannins while only flavonoids and tannins were found in methanolic extract. Agar well diffusion assay was carried out to determine antibacterial activity of *S. melongena* fruit extracts against the four bacterial strains. *P. aeruginosa* was the only bacteria tested to be susceptible towards methanolic and ethanolic extracts whereas other bacterial isolates were resistant towards all three extracts. Subsequently, both methanolic and ethanolic fruit extracts were used in microtiter plate biofilm assay to evaluate their anti-biofilm properties on *P. aeruginosa*. However, biofilm size observed was not reduced by the fruit extracts. Further investigation has to be carried out to determine the effect of *S. melongena* in inhibiting biofilm development by testing it on a wider range of bacteria.

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LIST OF ABBREVIATIONS

%	percent
°C	degree Celsius
µL	microlitre
µm	micrometer
A_{620}	absorbance reading at 620 nm
ANOVA	analysis of variance
CFU/ mL	colony-forming units per millilitre
EPS	extracellular polymeric substances
FeCl	ferric chloride
g	gram
H_2O_2	hydrogen peroxide
H_2S	hydrogen sulphide
H_2SO_4	sulphuric acid
HCl	hydrochloric acid
LB	lysogeny broth
MBL	metallo-beta-lactamases
mg	milligram
mL	millilitre
mm	millimeter
NaOH	sodium hydroxide
nm	nanometer
PBPs	penicillin-binding proteins
PBS	phosphate-buffered saline
pH	power of hydrogen
QS	Quorum sensing
rpm	revolutions per minute
UK	United Kingdom
USA	United States of America
v/v	volume per volume

CHAPTER 1

INTRODUCTION

Health care institutions provide patients with medical and surgical treatment for certain diseases with the help of medical specialists and sophisticated equipment (World Health Organization, 2017). However, pathogens such as *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Acinetobacter baumannii* which have been identified as common nosocomial pathogens have caused more severe infections and prolonged hospitalization among patients (Khan, Ahmad & Mehboob, 2015). Such clinically important pathogens are also capable of forming biofilms that further lead to chronic infections as a result of regulating the expression of virulence genes through quorum sensing (Waters, Lu, Rabinowitz, & Bassler, 2008). As a consequence, patients require prolonged therapy and higher dosage of antibiotics to prevent the initial health issue from becoming a potentially fatal illness. There is a high possibility for the symptoms to recur even after repeated treatment since biofilm-causing bacteria have shown high resistance to disinfectants and antibiotics as well as enhanced tolerance to the phagocytic action of immune systems (Hoiby, Bjarnsholt, Givskov, Molin & Ciofu, 2010). These resistance factors can be due to extracellular polymers and specific enzymes in the bacterial biofilms which not only prevent the penetration of antimicrobial agents, but also cause the inactivation of these drugs (Chadha, 2014). The resistance can be acquired through horizontal gene transfer or mutation in previously susceptible bacteria (Tenover, 2006).

The issue of drug resistance in bacterial biofilms has raised concerns globally due to the increased risk of mortality in patients with prolonged hospitalization and in-dwelling devices. Hence, there is an immediate need to discover alternatives with significant antibacterial and antibiofilm properties. Herbaceous plants are applied widely in medical field and are being used as starting material to formulate allopathic medicine (Palombo, 2011). Several compounds including flavonoids and tannins have been extracted from the plants and proved to have properties such as antibacterial, antioxidant, anti-inflammatory and others (Akter et al., 2016).

Solanum melongena, commonly known as eggplant, is ordinarily cultivated and consumed by every farm household and many families throughout the world (Knapp, Vorontsova & Prohens, 2013). Several studies have been done on this economically important crop and have shown that every part of this plant has significant antimicrobial, antifungal, antitumor and other properties due to the presence of compounds such as solanine, siloxane and linoleic acid (Al-Janabi & Al-Rubeey, 2010; Gao, Wang & Ji, 2006; Sitap, Tilawale, Nadaf & Ghosh, 2015; George Daye Mandy, Anthony & Kingsley, 2014). However, there is a lack of knowledge in its role of inhibiting bacterial biofilm development.

Based on these perspectives, this study was undertaken to determine the antibacterial activity against some clinically important bacteria, namely *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Entrococcus faecalis* and *Staphylococcus aureus* using fruit extracts of *Solanum melongena*. In addition, the efficacy of the fruit extracts of *S. melongena* in inhibiting the development of biofilms by these pathogens was studied. This study was also carried out to compare the effectiveness of *S. melongena* fruit extracts prepared using three different organic solvents which were acetone, ethanol and methanol in both the antimicrobial and antibiofilm assays.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacterial Strains

2.1.1 Gram-negative Bacteria

2.1.1.1 *Acinetobacter baumannii*

A.baumannii is a non-motile, Gram-negative bacterium that grows under aerobic condition (Kurcik-Trajkovska, 2009). It colonizes several parts of human body including the hand, forehead, throat and other parts (Almasaudi, 2016). However, it is also an opportunistic pathogen that mostly affects patients who have a weakened immune system and require prolonged medical and surgical treatment (Montefour et al., 2008). It is isolated in high concentrations from the mucous membrane of the oropharynx and respiratory tract as well as from the skin of affected individuals in intensive care units (Howard, O' Donoghue, Feeney & Sleator, 2012). Hospital-associated infections caused by these bacteria can be transmitted through nursing staff and hospital equipment. These infections include urinary tract infections, endocarditis, meningitis, bacteremia, pneumonia as well as skin and soft tissue infections (McConnell, Actis & Pachón, 2012). Before 1970s, these infections could be cured using antibiotics such as sulphonamides and beta-lactams (Gonzalez-Villoria & Valverde-Garduno, 2016). However, *A. baumannii* has currently become a more resilient pathogen due to its ability to acquire genes conferring antibiotic resistance (Peleg, Seifert & Paterson, 2008).

2.1.1.2 *Pseudomonas aeruginosa*

P. aeruginosa is a Gram-negative bacterium that can be ubiquitously isolated from river or lake waters (Mena & Gerba, 2009). Quorum sensing (QS), which is a system responsible for the regulation of several virulence traits, acts as a key factor in the pathogenicity of *P. aeruginosa* (Williams & Cámara, 2009). This opportunistic pathogen causes community- and healthcare-associated infections such as cystic fibrosis, pneumonia and neutropenia in immunocompromised patients (Chatzinikolaou et al., 2000). Infections caused by *P. aeruginosa* generally disseminate to other patients through contact with medical equipment and nursing staff (Yayan, Ghebremedhin & Rasche, 2015). *P. aeruginosa* is capable of forming biofilms and this has become a growing global concern (Tran et al., 2014). The biofilms which develop during the course of an infection could result in increased morbidity and mortality in patients who are seriously ill. In addition, the bacteria in these biofilms are resistant to various antibiotics including carbapenems, penicillin G, ceftazidime and quinolones (Tam et al., 2010). Furthermore, characteristics including production of periplasmic beta-lactamases, efflux system and restricted outer-membrane permeability also contribute to the antibiotic resistance which intrinsically a feature in these bacteria (Hancock & Speert, 2000).

2.1.2 Gram-positive Bacteria

2.1.2.1 *Enterococcus faecalis*

E. faecalis is non-motile, Gram-positive bacterium that is commonly found in the human intestine (Gajan et al., 2013). Among all of the species of enterococci, it is the third most common pathogen that is isolated from healthcare institutions and has become a serious public health issue (Hidron et al., 2008; Brooks, Carroll, Butel, Morse & Mietzner, 2013). *E. faecalis* usually infects wounds, blood and urinary tract of patients and can be transmitted from one patient to another through hospital personnel or contaminated medical equipment (Brooks et al., 2013). In addition, the advent of antibiotic treatment has led to the development of antibiotic resistance in *E. faecalis* since the 1960s (Miller, Munita, & Arias, 2014). The mechanism of horizontal gene transfer was first discovered in 1970s and showed that bacteria could acquire the antibiotic-resistant gene both intra-specifically as well as inter-specifically (Clewell & Franke, 1974). Strains of *E. faecalis* can be resistant to antibiotics including cephalosporins, monobactams and vancomycin (Brooks et al., 2013). They can also be resistant to the synergistic effect of aminoglycosides and some other antibiotics due to the expression of aminoglycoside-modifying enzymes (Brooks et al.,