THE COMBINATORIAL EFFECT OF *Azadirachta indica* (NEEM) PLANT WITH AMIKACIN AND TETRACYCLINE AGAINST CLINICALLY IMPORTANT BACTERIA

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

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I hereby declare that the work in this dissertation is my own except for quotations and summaries which have been duly acknowledged, and completed under the supervision of Ms. Lalita Ambigai Sivasamugham.

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ABSTRACT

Emergence and rapid spread of antibiotic resistance is due to the overuse and incorrect prescription of antibiotics. Antibiotic resistance has brought negative impacts such as difficulty in treating common infections, increase in mortality rate and prolonged suffering. Current treatments to treat diseases caused by antibiotic-resistant bacteria have become increasingly limited. Therefore, alternative approaches such as the use of plant extracts and combined therapy have been studied to improve the effectiveness of treatment. Plant extracts such as *Azadirachta indica* (neem) has shown promising use as an antimicrobial agent because it produces various secondary metabolites such as alkaloids and flavonoids. In this study, the combinatorial effect of neem leaf extracts and antibiotics against clinically important pathogens was investigated. The checkerboard assay was carried out to determine the minimum inhibitory concentration (MIC) of the neem leaf extract and amikacin as well as tetracycline. However, no MIC could be determined due to the technical error of the microplate reader. Agar well and disc diffusion assay was performed to study the combinatorial effect of neem leaf extract and amikacin as well as tetracycline. An antagonistic effect was observed when *Bacillus subtilis* was exposed to the neem leaf extract and amikacin as a significant reduction ($p<0.05$) of zone of inhibition from the neem leaf extract and amikacin combination was observed compared to that of amikacin alone. The combined effect of neem leaf extract and amikacin showed no synergistic effect against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae* and *Pseudomonas aeruginosa* as the diameter of zone of inhibition obtained was the same as that of amikacin alone. No zone of inhibition was observed with *Serratia marcescens* when tested with neem leaf extract and amikacin combination. On the other hand, synergism between neem leaf extract and tetracycline against *Propionibacterium acnes, Bacillus subtilis* and *Streptococcus pneumoniae* were obtained which showed significant enlargement ($p<0.05$) of zone of inhibition as compared to that of tetracycline alone. The combinatorial effect of neem leaf extract and tetracycline showed indifference against *Streptococcus faecalis, Staphylococcus epidermidis, Enterococcus faecalis* and *Staphylococcus aureus* compared to that of neem leaf extract alone and tetracycline alone. No zone of inhibition was observed with *Pseudomonas aeruginosa* and *Serratia marcescens* when tested with neem leaf extract and tetracycline combination. In conclusion, neem leaf extract showed a better combinatorial effect with tetracycline compared to

amikacin due to the synergistic action between neem leaf extract and tetracycline. Antagonism between neem leaf extract and amikacin suggests that combining these agents will reduce the efficacy of amikacin.

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LIST OF ABBREVIATIONS

CO_2	carbon dioxide
CFU/mL	colony forming unit per millilitre
°C	degree Celsius
g	gram
h	hour
H_2O_2	hydrogen peroxide
H_2S	hydrogen sulfide
µL	microlitre
mL	millilitre
mm	millimeter
min	minute
%	percent
pH	power of hydrogen
KOH	potassium hydroxide
rpm	revolutions per minute
s	second
v/v	volume per volume

CHAPTER 1

INTRODUCTION

Antibiotic resistance is an emerging threat and a global concern to public health (WHO, 2016a). The development of antibiotic resistance is aggravated by the overuse and inappropriate use of antibiotics leading to the increase in mortality due to infections caused by antibiotic resistant bacteria (FAO, 2016). The emergence and spread of resistance mechanisms such as horizontal transfer of resistance through mcr1-gene has been discovered by Chan (2016). This horizontal transfer of resistance encourages the rapid transfer of antibiotic resistance genes among bacteria and thus, increases the difficulty in treating the diseases caused by antibiotic resistant bacteria (WHO, 2016a).

The increase of antibiotic resistance to a dangerously high level urges the need to discover and develop alternative antimicrobial agents from other potential sources such as plants. The use of plants for medicinal purposes has been recorded in ancient Egyptian and Chinese history as early as 3,000 B.C (University of Virginia, 2007). Studies show that estimated up to 80% of people worldwide use plants for primary health care (Ehrlich, 2015). Medicinal plants such as *Allium sativum* (garlic) and *Prosopis juliflora* (mesquite) contain phytoconstituents such as alkenyl phenols, alkaloids and flavonoids which possess antimicrobial properties (Emad, 2011). Plant extracts have additional benefits as they are low in toxicity. In addition, the cost of production as an alternative antimicrobial agent is also low (Teka et al., 2015).

Azadirachta indica from the family *Meliaceae*, commonly known as neem, is used as traditional medicine due to its antimicrobial and other medicinal properties. Studies have shown that different parts of neem plants exhibit antimicrobial activities (Raja Ratna Reddy et al., 2013). The synergistic effect of neem extracts with other medicinal plants and antibiotics against several clinically important bacteria have been carried out to further study the therapeutic properties and effects of neem extracts. Neem leaf extracts with other medicinal plants such as Aloe Vera and curry leaf extracts as well as antibiotics have shown synergistic effect against clinically important bacteria such as *Bacillus subtilis* (Rasha, Hatil, & Aisha, 2015). The synergistic effect helps to improve the efficacy of antimicrobial activity, reduce toxicity and reduce the development of antibiotic resistance in bacteria. Therefore, it is crucial to study the synergistic effect of neem extracts with antibiotics as it can enhance the antimicrobial activity of antibiotic for therapeutic purposes. Previous study done by Kushalini (2016) revealed that neem leaf extract inhibited the growth of several clinically-important bacteria such as *Streptococcus faecalis, Enterococcus faecalis, Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Bacillus subtilis, Serratia marcescens* and *Pseudomonas aeruginosa.*

Thus, this study was aimed to analyze the combinatorial effect of *Azadirachta indica* (neem) leaf extract with amikacin and tetracycline against nine strains of clinically important bacteria as stated by Kushalini using the checkerboard assay and the agar well and disc diffusion assay.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE

Antibiotics are antimicrobial drugs which are commonly used to prevent and treat bacterial infections in both animals and humans (CDC, 2015). The ability of bacteria to adapt and survive from the antimicrobial effect has led to the emergence of antibiotic resistance (APUA, 2014). Antibiotic resistance is one of the most vital public health concern worldwide as the rapid emergence and spread of antibiotic resistance has threatened the use of antibiotic to treat common infections (WHO, 2016a).

According to the Review on Antimicrobial Resistance, approximately 700,000 people die from infectious disease caused by antibiotic-resistant bacteria every year (O'Neill, 2016) and 200,000 people die from diseases caused by multidrug-resistant bacteria such as drug-resistant tuberculosis (XDR-TB) (WHO, 2017). About 60,000 deaths of newborns have been reported due to antibiotic-resistant neonatal infections in India in 2013 (Laxminarayan et al., 2013). It is estimated that death attributed to antibiotic resistance may increase up to 10 million people by the year of 2050 as shown in Figure 1 (O'Neill, 2016).