COMBINATORIAL EFFECTS OF CRUDE PLANT EXTRACTS OF *Murraya koenigii* AND SELECTED ANTIBIOTICS ON METHICILLIN-RESISTANT *Staphylococcal* SPECIES.

HIMASHI IMANDA GURUDENIYA

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
BACHELOR OF BIOTECHNOLOGY (HONOURS)

FACULTY OF HEALTH AND LIFE SCIENCES
INTI INTERNATIONAL UNIVERSITY
PUTRA NILAI, MALAYSIA
MAY 2017

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HIMASHI GURUDENIYA	DR. S. GEETHA SUBRAMANIUM
I14006389	(SUPERVISOR)
24/07/2017	MS. LALITHA AMBIGAI SIVASAMUGHAM
	(CO-SUPERVISOR)

ABSTRACT

Antibiotic resistance is an alarming phenomenon worldwide, challenging the effectiveness of antibiotics which are used to treat mankind. One of the recommended ways to treat antibiotic resistance is combinatorial therapy or synergistic therapy which combines crude medicinal plant extracts and standard antibiotics. The pure cultures of nine different MRSA (methicicllin-resistant *Staphylococcus aureus*), seven different MRSE (Methicillin-resistant *Staphylococcus epidermidis*), two *Staphylococcus aureus,* and two *Staphylococcus epidermidis* isolates were obtained from INTI International University culture collection. The combinatorial effect of curry leaves (*Murraya koenigii*) extracts (aqueous, ethanolic, methanolic) and standard antibiotics ampicillin and gentamicin were examined against selected MRSA and MRSE strains. The susceptibility of curry leaf extracts alone, antibiotics alone and in combination against MRSA and MRSE were observed using the agar well diffusion method. Water, ethanol and methanol were used as negative controls in the respective plates and each experiment was done in triplicates. In this study, methanolic extracts exhibited better antimicrobial effect on MRSA and MRSE compared to aqueous and ethanolic extracts. One of the MRSA strains (N12) was resistant to gentamicin but was susceptible to the combination of gentamicin and *M. koenigii*. All *S. epidermidis* isolates were resistant to ampicillin and did not exhibit any susceptibility to the methanolic extracts, alone or in combination with antibiotics. In conclusion, there is a possible combinatorial effect exhibited by *M. koenigii* and gentamicin, in MRSA isolates resistant to gentamicin, but this has to be tested with a larger number of bacterial isolates, including MRSA.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AMC	Ampicillin
AQU	Aqueous *M. koenigii* extract
°C	degrees Celsius
cm	Centimetre
CN	Gentamicin
EtOH	Ethanolic *M. koenigii* extract
g	Grams
HCl	Hydrochloric acid
min	Minutes
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensative *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
MSSE	Methicillin-sensitive *Staphylococcus epidermidis*
M. koenigii	*Murraya koenigii*
mm	Millimetre
MtOH	Methanolic *M. koenigii* extract
NaOH	sodium hydroxide
rpm	Revolution per minutes
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
WHO	World Health Organization

(w/v)	Percent of weight of solution in the total volume of solution
VRSA	vancomycin-resistant *Staphylococcus aureus*
%	Percentage

CHAPTER 1

INTRODUCTION

The discovery of antibiotics in the 1950s (Bérdy, 2012) is unquestionably one of the most significant discoveries that has benefited mankind. As a result, some lethal infectious diseases have been controlled, leading to an increase in the average lifespan of man. However, these infectious microorganisms when faced with the challenge of survival against antibiotics, have developed the ability to overcome the effects of antibiotics through the process called natural selection leading to antibiotic resistance (Cantley, 2016). Furthermore, the effect of antibiotics decreases against these surviving bacteria which is the basis of antibiotic resistance. This is due to the changes in a microorganism's structure which could be the result of overuse or misuse of antibiotics. The infections caused by these "superbugs" (WHO, 2016) such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant *Staphylococcus epidermidis*, vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant *Enterococcus* (VRE) (CDC, 2013) are generally difficult to treat. MRSA is resistant to methicillin, oxacillin, penicillin (Malone, 2005) and many other antibiotics. Critical MRSA infections could lead to pneumonia, fever, impetigo, gangrene and many other serious infections (Federal Bureau of Prisons Clinical Practice Guidelines, 2012). Symptoms of skin infections caused by MRSA include bump filled with pus or other drainage sites and the infected area appears swollen, reddish, painful and warm to touch (Center for Disease Control, 2013).

S. epidermidis that are also name as methicillin-resistant *Staphylococcus epidermidis* (MRSE) have become an alarming medical problem because these bacterial infections crucial to eliminate from the colonized devices. MRSE also leads to bacteremia and nosocomial infections spread through clinical devices such as heart valves (R, Chovanová, M. Mikulášová & S.Vaverková, 2013). The authorities responsible for detecting and responding to antibiotic-resistance threats are limited even though the emergence of antibiotic-resistance is an alarming healthcare problem throughout the world (CDC, 2013). According to the U.S. Centers for Disease control and Prevention (2013) even for the critical pathogens such as carbapenem-resistant

Enterobacteriaceae (CRE) and *Neisseria gonorrhoeae,* authorities do not have clear information on the prevalence, domestic incidence, increased cost of healthcare services, morphology, and mortality. Globally, antibiotic resistance is an alarming threat to human and animal health. Traditional herbal medicine has been used for treatment and therapeutic purposes long before it was recorded in history. *Murraya koenigii* a member of the family of Rutaceae, (Rao et al., 2013) is used as an analgesic or painkiller, and the treat skin rashes, fever (febrifuge), diarrhea and dysentery. Previous studies have shown that curry leaves have several therapeutic effects including anti-diabetic, anti-diarrheal, and antioxidant properties. Therefore, one potential method to overcome antibiotic resistance is combinatorial therapy, which is a method to increase the antimicrobial spectrum, inhibit the occurrence of antibiotic resistance and decrease toxicity. The synergistic effects of commonly used antibiotics and crude plant extracts is fast becoming a key therapeutic agent against antibiotic resistance (Valli & Shankar, 2013). A study carried out by Faculty of Science, University of Nottingham Malaysia Campus , able to increase the efficacy of ampicillin by combining *Acalypha wilkesiana* extract and this combination able to suppress the production of PBP2a or completely inactivated it (Santiago, Pang, Lim, Loh, & Ting, 2014). Furthermore, according to Santiago, Pang, Lim, Loh, & Ting, (2015) study the synergism between *Duabanga grandiflora* with ampicillin also able to suppress MRSA growth via PBP2a inhibition. Drug combinations may exhibit synergistic or antagonistic effects and rational design of synergistic drug combinations remains a challenge despite active experimental and computational efforts. (Yin et al., 2014).

In this project, the combinatorial effect of dried curry leaf (*M. koenigi*) extracts prepared with different solvents (aqueous, ethanol, methanol) with selected antibiotics (gentamicin, ampicillin) was compared and tested against MRSA and MRSE isolates obtained from INTI International University culture collection. This was to determine if *M. koenigii* had potential antimicrobial effect to be used in combination with gentamicin and ampicillin in the treatment of infections caused by MRSA and MRSE. In addition, semi- qualitative analysis of phytochemicals present in different solvent preparations (aqueous, ethanol, methanol) of *M. koenigii* were also carried out to determine the best solvent for this type of study and for any future commercial application.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE

Antibiotics are biological substances that can treat infectious diseases caused by bacteria and fungi, however, they do not have any effect on viruses (CDC, 2015). Antibiotics include antibacterial substances produced by other microorganisms such as chloramphenicol and erythromycin (from *Streptomyces* sp.) and synthetically produced chemotherapeutic substances (Rao, 2005). Technically, the term "antibiotic" refers to naturally produced substances such as chemicals secreted by microorganisms while the term "antimicrobial" refers to a combination of product and man-made (synthetic) components. There are two types of antibiotics based on their activity: bactericidal and bacteriostatic. Bactericidal antibiotics include gentamicin, penicillin, and ceftriaxone, which can directly kill the bacteria. On the other hand, bacteriostatic antibiotics such as tetracycline and chloramphenicol, inhibit the multiplication of bacteria (CDC, 2013). Nevertheless, in practice, both types of antibiotics have the ability to stop the progression of a bacterial infection. Sir Alexander Fleming accidentally discovered that the chemical secreted by the mold, *Penicillium notatum,* could kill bacteria (*Staphylococcus aureus*). However, sulphonamides were the first successful antibiotics used against bacterial infection in mankind as early as the 1930's (Hardy & Diagnostics, 1980).

The main drawback of using antibiotics as a therapeutic agent is the development of antibiotic resistance, which is defined as the ability of bacteria to develop resistance against or reduce the effectiveness of chemicals, drugs or other substances designed to treat a bacterial infection (CDC, 2013). This occurs when there is antibiotic overuse or misuse, or inappropriate infection prevention. The U.S. Department of Health and Human Services have categorized antibiotic resistant bacteria to the world as "nightmare bacteria" hence it is one of the most pressing public health problems globally, today (CDC, 2013).