ANDROGRAPHIS PANICULATA AS POTENTIAL SENSITIZING AGENT TOWARDS ANTIBIOTIC

CHUNG CHEE HOOI

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

JANUARY 2018

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CHUNG CHEE HOOI	*Dr. WONG KOK KEE*
I15009076	(SUPERVISOR)
9th / APR / 2018	

ACKNOWLEDGEMENT

I would like to take this opportunity to express my gratitude to my supervisor, Dr Wong Kok Kee for his continuous guidance, support, suggestion and motivation throughout the entire final year project. The guidance and advices given by him greatly assisted me in completing my final year project on time.

Besides that, I would also like to express my gratitude to Faculty of Health and Life Sciences (FHLS) along with laboratory technicians Ms Quah Hui Hsien and Mr Ng Peng Wah for their help throughout the entire project: Aside from that, I sincerely appreciate all the lecturers and my peers that offered me their help during the project.

Lastly, I would like to thank my parents for their unconditional support and those who indirectly supported me which contributed to the completion of my final year project.

ABSTRACT

Antibiotic resistance is the ability of microbes to resist antibiotic action despite of increasing concentration, and plant extracts have shown potential as the antibiotic sensitizing agent to reduce the dosage of antibiotic use. So, in this study, ethanolic extract of *Andrographis paniculata* was screened for antimicrobial property using modified Kirby Bauer disc diffusion assay. The results showed significant growth inhibition ($p<0.05$) of *Pseudomonas aeruginosa,* exhibited through the formation of zone of inhibition. The subsequent minimum inhibitory concentration (MIC) for the ethanolic extract of *A. paniculata* was determined to be 25 mg/mL, which was the minimum extract concentration that significantly inhibited ($p<0.05$) the growth of *P. aeruginosa*. This MIC of *A. paniculata* was used to proceed with anti-adhesion assay and the results reported no significant ($p>0.05$) inhibition on bacterial cells adhesion, thus suggesting no inhibition on the biofilm formation of *P. aeruginosa*. Although *A. paniculata* ethanolic extract did not exhibit anti biofilm formation as the mode of action to inhibit growth of *P. aeruginosa*, the overall results suggest that *A. paniculata* can still be a good candidate as a sensitizing agent towards antibiotics due to the strong antimicrobial activity demonstrated.

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LIST OF ABBREVIATIONS

CFU/mL	Colony-forming units per millilitre
EPS	Extracellular polymeric substances
mg	Milligram
mg/mL	Milligram per litre
MIC	Minimum Inhibitory Concentration
mm	Millimetre
mL	Millilitre
NaCl	Sodium chloride
nm	Nanometre
OD	Optical density
PBS	Phosphate buffer saline
rpm	Rounds per minute
µg	Microgram
µg/mL	Microgram per litre
µL	Microlitre
°C	Celsius

CHAPTER 1

INTRODUCTION

According to Centers for Disease Control and Prevention [CDC] (2017), at least 2 million people had been infected with antibiotic resistant bacteria each year in United States and approximately 23000 people died because of this infection. In year 2017, WHO has listed out some of the antibiotic resistant pathogens that pose a threat to humanity such as methicillin resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* that is carbapenem resistant, *Neisseria gonorrhoeae* that is cephalosporin and fluoroquinolone resistant and many more. (World Health Organization [WHO], 2017). Thus, due to the rise of antibiotic resistance crisis, natural products have now been use as an alternative for antibiotic.

Natural products from plant particularly received a lot of attention since there are numerous plants that exhibit antimicrobial activity and are widely available. One of the example is *Andrographis paniculata. A. paniculata* has been used in traditional Asian medicine due to its multiple medicinal properties such as hepatoprotective effect, antimicrobial effect, antiparasitic effect and many more (Jarukamjorn & Nemoto, 2008; Jayakumar, Hsieh, Lee & Shen, 2013). One study even showed that andrographolide which is one of the active compound present in *A. paniculata* exhibits anticancer property and might be applicable in future cancer treatment (Kumar, Sridevi, Kumar, Nanduri & Rajagopal, 2004).

Research findings have demonstrated that *A. paniculata* exhibits antimicrobial activity (Jarukamjorn & Nemoto, 2008; Jayakumar et al., 2013). It was reported that 100 µg/mL of ethanolic extract of *A. paniculata* was able to inhibit the growth of Gram positive bacteria such as *Staphylococcus aureus, Bacillus licheniformis* and also Gram negative bacteria such as *Escherichia coli, Vibrio cholera, Salmonella typhi*, comparable to 100 µg/mL of ciprofloxacin (Mishra, Mishra, Kumari, Murthy & Naik, 2009).

Antimicrobial activities can be attributed to different mode of action, for example, inhibition of cell wall synthesis, inhibition of protein synthesis, inhibition of nucleic acid synthesis, alteration of cell membranes and so on (Rollins & Joseph, 2000). In the case of antibiotic, the most common mechanism for antimicrobial action is inhibition of peptidoglycan synthesis that disrupt bacterial cell wall structure (Rollins & Joseph, 2000). However, plants might have different ways of preventing bacteria growth. One of the research had shown that *Pongamia pinnata*, a traditional herb, exhibits antimicrobial activity by preventing the formation of biofilm (Abraham et al., 2012). According to Cernakova and Kostalova (2002), *Mahonia aquifolium*, another traditional herb used for treating chronic skin conditions, contains a bioactive compound known as berberine that can prevent bacterial growth by intercalating with DNA at the same time inhibiting protein biosynthesis. Aside from traditional herbs, the common garlic, *Allium sativum* was shown to contain allicin, that interferes with RNA and lipid synthesis and, thus affecting the protein production and formation of phospholipid bilayer which are crucial for bacterial growth (Kumar et al., 2014). To date, no research had been carried out yet to determine the mode of antimicrobial action of *A. paniculata*.

Hence, the first aim in this study is to re-confirm the antimicrobial properties of ethanolic extract of *A. paniculata* by doing preliminary screening via disc diffusion and dilution method on *P. aeruginosa* and using results obtained from dilution method to determine minimum inhibitory concentration (MIC). The second aim is to determine whether *A. paniculata* exhibit anti-biofilm formation, as the mode of action in preventing bacterial growth, using anti-adhesion assay.

CHAPTER 2

LITERATURE REVIEW

2.1 ANTIBIOTIC RESISTANCE

What is antibiotic resistance? Antibiotic resistance is the microbe's ability to develop resistance towards the effect of antibiotic (CDC, 2017). According to WHO (2017), antibiotic resistance poses a great threat to the survival of mankind. The function of antibiotic is to protect us against bacterial infection and if antibiotic loses its effects against bacteria, it will greatly affect the survival rate of mankind as procedure such as organ transplantation will be dangerous to perform due to the possibility of contracting a severe bacterial infection (Theguardian, 2017). One of the report stated that approximate 700,000 of people died annually due to antibiotic resistant infections. If this phenomenon continues to progress, 10 million of people might die annually by the year of 2050 (The guardian, 2017). Hence, it is important to study the sources and causes of antibiotic resistance in order for us to take actions against antibiotic resistance.

Antibiotic resistance can come from two sources which are human and animal. In the case of human, if a person did not consume all the antibiotic prescribed or overuse antibiotic, this person might develop antibiotic resistance within the body and when this person travels from places to places, there might be a chance of spreading antibiotic resistance especially in hospital whereby resistant germs can directly spread to other patients (CDC, 2017). On the other hand, antibiotic is commonly used by farmers to improve the efficiency and rate of gain in animals such as cattle, swine and poultry (National Research Council, 1980). The increasing use of antibiotic in animal production accelerates the development of antibiotic resistance within the animal's gut. When these animals are consumed by human, this will in turn spread the antibiotic resistance to human as well (CDC, 2017). Study carried out on *Neisseria gonorrhoeae* (Figure 2.1) indicated that *N. gonorrhoeae* has developed new resistant towards fluoroquinolone and cefixime in the recent years, increasing the urgency of finding new strategies to combat antibiotic resistance (CDC, 2013).

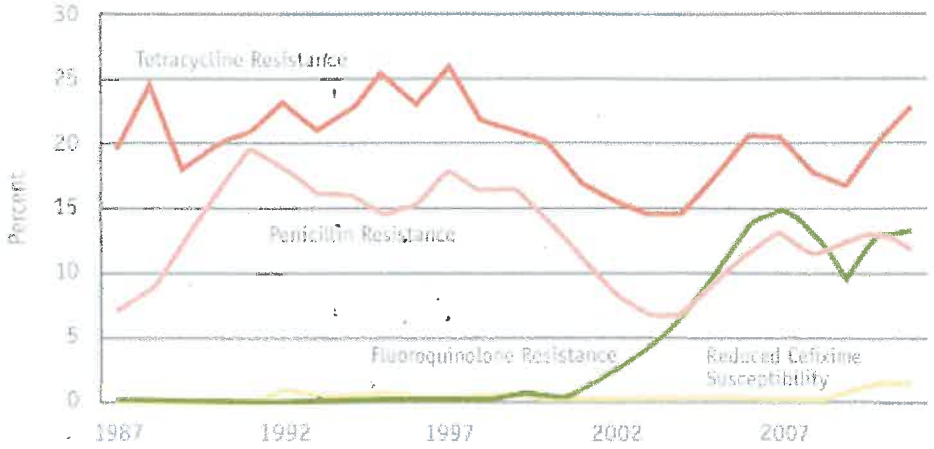

Figure 2.1 Development of the multiple antibiotic resistance by *Neisseria gonorrhoeae* Source: Adopted from CDC, 2013

The current strategies of minimizing antibiotic resistance are establishing a guideline of the proper use of antibiotics and also having a stringent law in the antibiotic use for animal production (Lee, Cho, Jeong & Lee, 2013). Education also can play a role in reducing antibiotic resistance. However, the strategies stated above requires a long duration of time in order to have an effect in reducing antibiotic resistance. Thus, the more effective way will be developing novel antibiotics (Lee et al., 2013). To develop novel antibiotics, we must first understand the mode of action of different antibiotic resistant bacteria which will be summarized in Table 2.1. Although development of novel antibiotics is a more effective way, it requires extensive research and development and also financing in order to develop one new drug that is able to combat antibiotic resistance. Hence, a better alternative is using plant extracts as a sensitizing agent towards antibiotic to reduce the dosage of antibiotic use. This will also be the reason why *A. paniculata* is chosen as the study subject for this research, as it is reported to exhibit antimicrobial property (Jayakumar et al., 2013).

Table 2.1 Mode of action of different bacteria in resisting the effects of different antibiotic.

Antibiotic	Resistant bacteria	Mode of action
Aminoglycosides	*Providencia, stuartii* *Serratia marcescens*	• Produces aminoglycosides acetyltransferase, a modifying enzyme. • To modify the amino/ hydroxyl group of aminoglycosides • Cause steric hindrance and thus decreasing avidity of antibiotic to bacteria
Penicillin	*Staphylococcus aureus*	• Produces β-lactamase • To destroy the amide bond in the β-lactam ring present of the penicillin • β-lactam ring is the core structure of penicillin, if the core structure is destroyed, antibiotic will lost its effect as well
Tetracycline	*Pseudomonas aeruginosa*	• activation of MexAB-OprM efflux pump • To pump out tetracycline • Tetracycline would not be able to inhibit bacterial protein synthesis
Vancomycin	*Enterococcus faecium*	• activation of *van* gene clusters • To modify the D-Ala in the peptidoglycan cell wall with D-lactate • Prevents vancomycin from binding and disrupting the cell wall
Quinolone	*Klebsiella pneumoniae*	• Activation of protein Qnr, a DNA homologue • To compete with DNA for the binding site of DNA gyrase. • Through this competition, reduces the formation of DNA gyrase-DNA, which in turn reduces the chance of quinolone being able to form gyrase cleaved DNA-quinolone complex which can kill the bacteria cell.

Source: Adapted from Munita & Arias, 2016