ANTIMICROBIAL ACTIVITIES AGAINST MULTIDRUG RESISTANT and FOODBORNE ORGANISMS and ACUTE TOXICITY STUDY of *Peperomia pellucida* (KETUMPANG AIR) AERIAL PART EXTRACTS

by

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LIST of SYMBOLS AND ABBREVIATIONS

AMR	Antimicrobial resistance
ANOVA	One-way analysis of variance
ARASC	Animal Research and Service Centre
AST	Antimicrobial Susceptibility Testing
BW	Body weight
CC 398	CLONE COMPLEX 398
CDC	Centre for Disease Control and Prevention
CMC	Carboxymethyl cellulose
CRE	Carbapanem-Resistant *Enterobactericeae*
DMSO	Dimethyl sulfoxide
EAEC	Enteroaggretive *E. coli*
EDTA	Ethylenediaminetetraacetic acid
EHEC	Enterohaemorrhagic *E. coli*
EPEC	Enteropathogenic *E. coli*
ESBL	Extended Spectrum Beta-Lactamase
ETEC	Enterotoxigenic *E. coli*
LD_{50}	The lethal dose for 50 percent of the animals tested
MDROs	Multi-drug resistant organisms
MR	Methyl red
MRSA	Methicillin-Resistant *Staphylococcus aureus*
OECD	Organisation for Economic Cooperation and Development
PPAP	*Peperomia Pellucida* aerial parts
SE	Staphylococcal enterotoxin
SIM	Sulfide, indole, motality
TSI	Triple sugar iron
VIM	Verona Integron-Mediated
VP	Voges praskauer

VRE	Vancomycin-Resistant *Enterococcus*
^{o}C	Degree celcius
g	Gram
kg	Kilogram
mg	Milligram
ml	Millilitre
mm	Millimetre
mg/ml	Milligram per milimitre
mg/kg	Milligram per kilogram
µg	Micro gram
µl	Micro litre
w/w	Weigh per weight
%	Percent

Aktiviti Antimikrob Terhadap Organisma Tahan Multi-Ubat dan Bakteria Bawaan Makanan serta Kajian Ketoksikan Akut Ekstrak Bahagian Atas (*Peperomia pellucida*) Ketumpang air

ABSTRAK

Peperomia pellucida telah digunakan dalam perubatan tradisional untuk merawat pelbagai penyakit. Walaubagaimanapun, maklumat saintifik yang tidak mencukupi mengenai potensi antibakteria dan kebimbangan tentang keselamatannya telah menyegerakan pelaksanaan kajian ini. Matlamat kerja semasa adalah untuk menilai aktiviti antibakteria dalam tabung uji oleh ekstrak metanol dan air bahagian atas *P. pellucida* (BAPP) terhadap organisma tahan multi-ubat (OTMU) (Kajian I) dan patogen bawaan makanan (Kajian II). Tambahan pula, hanya ekstrak terbaik diuji untuk ketoksikan akut dalam organisma hidup dalam Kajian III. OTMU yang diuji dalam Kajian I terdiri daripada Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococcus* (VRE), Extended Spectrum Beta Lactamase (ESBL) dan Carbapenem-Resistant *Enterobacteriaceae* (CRE). Manakala dalam Kajian II, strain patogen bawaan makanan yang diperiksa termasuk *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* dan *Salmonella typhimurium*. Kedua-dua kajian menggunakan kaedah ujian yang sama di mana potensi antibakteria ekstrak tumbuhan dinilai pada dua kepekatan (250mg/ml dan 500mg/ml) menggunakan teknik penyerapan disk Kirby-Bauer. Hanya bakteria yang memperlihatkan kesan selanjutnya ditentukan untuk Kepekatan Minimum Penghalang (KMP) dan Kepekatan Minimum Membunuh (KMM) menggunakan kaedah pencairan mikro. Penemuan yang diperoleh menunjukkan

bahawa pemeriksaan awal zon perencatan menggambarkan bahawa semua strain bakteria tahan terhadap ekstrak air *P. pellucida*. Sebaliknya, hanya ekstrak methanol *P. pellucida* didapati sebagai perencat yang kuat terhadap *Bacillus cereus*. Dari pemeriksaan awal ini, *Bacillus cereus* kemudiannya tertakluk kepada KMP dan pertumbuhan bakteria dihalang pada 3.91mg/ml ekstrak methanol *P. pellucida*. Selain itu, KMM ekstrak tumbuhan ini ialah 7.81mg/ml. Kajian III, sebaliknya, telah dijalankan untuk menyiasat kesan dos tunggal 5000mg/kg ekstrak metanol BAPP pada tikus Sprague Dawley betina dengan megguna pakai garis panduan OECD No 425. Hasil yang diperoleh dari Kajian III menunjukkan bahawa tidak ada kematian atau morbiditi dan tiada keabnormalan terhadap penampilan fizikal dan tingkah laku bagi semua tikus yang diperiksa. Begitu juga, pemeriksaan kasar menunjukkan keadaan normal untuk semua rongga terbuka, tulang tengkorak, toraks dan organ dalaman. Berat badan, pengambilan makanan dan berat organ juga kelihatan setanding. Mengambil kira semua data kumulatif, kajian ini menunjukkan bahawa ekstrak metanol BAPP akan menjadi sumber yang menjanjikan halangan terhadap pertumbuhan *B. cereus* dalam makanan tetapi ia hanya menunjukkan aktiviti antibakteria yang lemah terhadap OTMU dan patogen bawaan makanan yang selebihnya. Di dalam organisma hidup, dos maut median akut (LD_{50}) bagi ekstrak metanol BAPP dalam tikus betina dijangkakan lebih tinggi daripada 5000mg/kg.

Antimicrobial Activities Against Multidrug Resistant and Foodborne Organisms and Acute Toxicity Study of *Peperomia pellucida* (Ketumpang air) Aerial Part Extracts

ABSTRACT

Peperomia pellucida has been used in ethnomedicines for the treatment of various illnesses. However, the scarcity of scientific informations on its antibacterial potential and safety concern have enhanced the urgency of conducting this study. The aims of the current work were to evaluate the *in vitro* antibacterial activities of methanol and aqueous extracts of *P. pellucida* aerial part (PPAP) against multi-drug resistant organisms (MDROs) (Study I) and foodborne pathogens (Study II). Further, the best extract was tested for *in vivo* acute toxicity in Study III. The MDROs tested in Study I involved Methicillin-Resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant *Enterococcus* (VRE), Extended Spectrum Beta-Lactamase (ESBL) and Carbapenem-Resistant *Enterobacteriaceae* (CRE). Whilst in Study II, the foodborne pathogen strains examined including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhimurium*. Both studies applied similar methods of testing where the antibacterial potentiality of the plant extracts were evaluated at two concentrations (250mg/ml and 500mg/ml) using Kirby-Bauer's disk diffusion assay. Only bacteria that showed susceptibility were further determined for Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using microbroth dilution method. Findings obtained indicated that the preliminary screening of the inhibition zone depicted that all bacteria strains were resistant to *P. pellucida* aqueous extract. In

contrast, only *P. pellucida* methanol extract was found to be a potent inhibitor towards *Bacillus cereus*. From this initial screening, *Bacillus cereus* was subsequently subjected to MIC and the bacteria activities was inhibited at 3.91mg/ml of *P. pellucida* methanol extract. Additionally, the MBC of this plant extract was 7.81mg/ml. Study III, in contrast, was conducted to investigate the effects of a single dose of 5000mg/kg PPAP methanol extract on female Sprague Dawley rats by adopting the OECD guidelines No 425. Results acquired from Study III revealed that there were neither mortality nor morbidity and absent of abnormalities on the physical appearances and behaviour of all the rats examined. Likewise, the gross examination displayed normal condition of all orifices, cranium, thorax, abdomen cavities and visceral organs within. The body weight, food consumption and organ weight were also looked comparable. Taking all the cumulative data together, this study suggests that PPAP methanol extract would be a promising source to inhibit *B. cereus* growth in foods but it only displayed weak antibacterial activities against other tested MDROs and foodborne pathogens. *In vivo*, the approximate acute median lethal dose (LD_{50}) of PPAP methanol extract in female rats was estimated to be higher than 5000mg/kg.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

"Superbug" is a common term used to refer to any microorganisms such as bacteria, fungi, viruses and parasites that develop antimicrobial resistance. Antimicrobial resistance (AMR) occurs when these microorganisms change in a way that render medications used to cure the infections such as antibiotics, antifungals, antivirals, antimalarials and antihelmintics turn out to be ineffective. As a consequence, infections persist and increase the risk of worse clinical outcomes (WHO, 2016).

Despite current medical advances, AMR poses a dramatic elevation of threats over the past decade (O'Neill, 2014). Moreover, the same author has also revealed that there is an estimated 700 thousand deaths related to AMR at present. If this issue remains uncontrolled, the death toll is expected to rise up to 10 million deaths by the year 2050. AMR has caused the emergence of multi-drug resistant organisms (MDROs) which are labelled as such because of resistance to more than one antimicrobial agents (Martin-Loeches *et al*., 2015). This marks a vital issue of global concern due to the elevation of mortality rates and healthcare costs as resulted from the threats of resistant pathogens (Janahiraman *et al*., 2015). It is estimated that 25,000 patients died each year in European Union countries from the infections caused by MDROs and had caused 1.5 billion euro spent for treatment purposes (Leung *et al*., 2011).

In other parts of the world, the United States recorded 26,000 deaths whereas approximately 96,000 deaths were reported in the Southern Asia region (Khan *et al*., 2016). Malaysia had spent most of the budget allocated in the year 2006 and 2007 to dispense antibiotics for treatment (Lim & Teh, 2012). This was ranked as the eighth most utilized therapeutic groups based on the survey done by Malaysia National Medicine (Siti Fauziah *et al*., 2014). Moreover, this number continued to rise up as there was 16% increment in annually antimicrobial consumption from the year 2009 to 2010 in the country (Sing *et al*., 2016).

In the meantime, foodborne diseases caused by pathogenic bacteria also remain a global burden and continuous concern to the community worldwide. An estimation made by Centre for Disease Control and Prevention (CDC), approximately 76 million people suffers due to ingestion of contaminated foods and beverages while 325,000 being warded and 5000 had died each year in America alone despite their high standard of living (Scallan *et al.*, 2011). In developing countries, the cases are even greater although most break out are under-reported (Law *et al*., 2013).

Both antimicrobial resistance and foodborne illnesses are regarded as relatively recent medical challenge which require considerable attention from scientific communities. Therefore, this present study is one of the few efforts aimed to find a solution towards the aforementioned issues by screening the antimicrobial activity using a local herb. The plant of interest, chosen for this study is *Peperomia pellucida.* It is one of the species recorded under the second largest genus of *Peperomia* under the Piperaceae family. Other species of this genus that had been scientifically proven to

have various medicinal properties are *P. serpent* (Young *et al*., 2006), *P. villipetiola* (Malquichagua *et al*., 2005) and *P. blanda* (Velozo *et al*., 2009). *P. pellucida* is locally known as a shiny bush, slate pencil plant or rat's ear in English or 'ketumpang air' among Malaysian (Wei *et al.,* 2011). This plant is commonly ingested raw as a salad because of its crispiness like carrot stick and celery as well as able to produce a mustard-like odor when crushed (Majumder *et al.,* 2011). *P. pellucida* has also become a herb of choice among old folks due to its pivotal role in providing cure for various types of illnesses such as fever, headache, eczema, abdominal pain while the aerial parts can be utilized for wound dressing (Munhoz *et al*., 2000). Further, through Mutee *et al.,* (2010), their study had proven that the anti-inflammatory properties of petroleum ether extract of this plant could significantly reduce the carrageenan-induced hind paw edema in male Sprague Dawley rats. Moreover, *P. pellucida* has also revealed its potent anticancer properties by significantly inhibiting human breast adenocarcinoma (MCF-7) cell line (Wei *et al.,* 2011).

Apart from the above, the medicinal properties of *P. pellucida* have been documented in several studies in exhibiting significant antibacterial properties against wide range of bacteria such as *Edwardsiella tarda*, *Flavobacterium* sp., *Pseudomonas aeruginosa*, *Vibrio cholera*, *Klebsiella* sp., *Vibrio alginolyticus* (Wei *et al*., 2011), *Bacillus subtilis*, *Candida albicans* (Abere *et al*., 2012) and aquatic bacteria such as *Streptococcus agalactiae*, *Aeromonas hydrophila* and *Enterobacter cloa* (Raina and Hassan, 2016). Besides that, the aqueous extract of *P. pellucida* of up to 5000mg/kg was found not to influence the behavior and body weight of both male and female Swiss mice in the previous acute toxicity study (Arrigoni-blank *et al*., 2004).

Although there are numerous research concerning the vast pharmacological activities of *P. pellucida*, none of the studies has ever been performed specifically on antibacterial activities of this plant against MDROs and foodborne pathogens. Realising the importance of filling up these lacunae, our current study was carried out in order to further explore the usage of *P. pellucida* whether it has potential to cure bacterial infections. Additionally, the potential toxicity of this herb was also evaluated using rats as the surrogate.

The use of herbal medicines has tremendously increased over the past decades with not less than 80% of people over the globe relying on them. These products are generally safe and effective with less reported toxicity (Nasri and Shirzad, 2013). However, it is not surprised that many plants contain potentially poisonous compounds that are highly toxic to human and animals when either acutely or subchronically administered (Ernst, 2002; Atsamo *et al*., 2011; Nordin & Selamat, 2013). In this regard, *P. pellucida* is not an exception and should not be left ignored. This is because the methanol, hexane and ethyl acetate fractions of this plant were reported to be toxic in the *in vitro* experiment with its median lethal concentration (LC_{50}) was less than 1000mg/ml (Oloyede *et al*., 2011).

1.2 Scope of the study

Generally, the current study was performed to evaluate *P. pellucida* aerial parts (PPAP) for its *in vitro* antibacterial activities and *in vivo* acute toxicity study. This herb was chosen due to its wide availability and can be easily obtained throughout the region. Furthermore, this herb has demonstrated reliable pharmacological actions in exhibiting hypotensive effects (Nwokocha *et al*., 2012), antihyperglycaemic, anti-inflammatory and analgesic properties (Sheikh *et al*., 2013) as well as promotes fracture healing osteoblast (Ngueguim *et al*., 2013). In addition, PPAP has been broadly tested for its antibacterial properties against various clinical strains of bacteria and fungal organisms (Oloyede *et al*., 2011; Abere *et al*., 2012; Igwe *et al*., 2014; Idris *et al*., 2016).

Despite the fact that there are considerable amount of evidences available for this plant on its antibacterial activities, this present study is certainly different in various aspects. As there is no similar experiment reported at the moment, we decided to evaluate both aqueous and methanol extracts of PPAP for its antibacterial properties specifically against four multidrug-resistant organisms and four common foodborne pathogens. The MDROs tested consist of two gram positive; Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant Enterococcus (VRE) and two gram negative; Extended Spectrum Beta-Lactamase (ESBL) and Carbapanem-Resistant *Enterobactericeae* (CRE). Meanwhile, the four selected common foodborne strains comprising of two gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) and two gram negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) were further tested following the first experiment on MDROs.

These studies were designed to test both categories of bacteria because the effects of bioactive compounds within the herb might vary towards gram positive and gram negative due to dissimilarities in the cell wall compositions (Nazzaro *et al*., 2013). Additionally, both types of bacteria are correspondingly involved in the emergence of antimicrobial resistance as well as become a causal factor in foodborne illnesses (Magiorakos *et al*., 2011).

Two types of plant extraction; aqueous and methanol extracts were selected in this study. The aqueous extract of fresh PPAP was selected as a resemblance of regular dietary intake of this herb as a raw salad (ulam). In contrast, the methanol extract was chosen because of its ability in extracting a great amount of bioactive compounds mainly polyphenol as compared to water (Mohammedi & Atik, 2011). The purpose of selecting two types of extracts was to compare which extract might responsible for significant antibacterial activities.

Further, the experiment was proceeded to investigate the effects of a single dose administration of the best extract on female Sprague Dawley rats via acute toxicity study. This study was conducted according to the Organisation for Economic Cooperation and Development (OECD) Guideline No 425 (OECD, 2008). This part of experiment was designed to provide preliminary toxicity findings of PPAP *in vivo*.

Overall, the current study was separated into three main stages as listed below:

a) Study I: - *In vitro* antibacterial activities of the aqueous and methanol extracts of PPAP against four selected MDROs.

   These four MDROs were selected due to a high frequency of detection being reported generally in Asian countries for MRSA and VRE (Kang & Song, 2013) and specifically in Southeast Asia region for ESBL and CRE (Suwantarat & Carroll, 2016).

b) Study II: - *In vitro* antibacterial activities of the aqueous and methanol extracts of PPAP against four selected common foodborne pathogens.

   These four strains chosen were the common bacteria isolated in the foodborne illnesses globally including in Malaysia (Petrus *et al.*, 2011; Law *et al.*, 2013; Noor Ifatul *et al.*, 2016).

c) Study III: - *In vivo* acute toxicity study of the PPAP methanol extract in Sprague Dawley rats.

   This final stage of the experiment was conducted to observe the possible acute toxicity effects at the limit dose of 5000mg/kg body weight of the PPAP methanol extract on female SD rats.

In this regard, this set of study could pose as preliminary evidence in providing alternative treatment from a natural source in order to maintain the community health as

well as could serve as an initial platform to boost the level of public awareness towards the safety of natural herbs.

1.3 Research objectives

This study was conducted to achieve the following general and specific objectives.

1.3.1 General objectives

The general aim of this study was to evaluate the *in vitro* antibacterial activities and the *in vivo* acute toxicity study of *Peperomia pellucida* aerial parts (PPAP) extracts.

1.3.2 Specific objectives

The specific objectives of this study are listed as follows:

I. To evaluate the antibacterial activities of both aqueous and methanol extracts of PPAP against four MDROs by measuring the zone of inhibition using disc diffusion assay by means of Study I.

II. To assess the antibacterial activities of both aqueous and methanol extracts of PPAP against four common foodborne pathogens by measuring the zone of inhibition using disc diffusion assay by means of Study II.

III. To determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the tested bacteria (Study I and II) that

showed susceptibility towards both aqueous and methanol extracts of PPAP by microbroth dilution techniques.

IV. To evaluate the *in vivo* acute toxicity of PPAP methanol extract on female Sprague Dawley rats by means of Study III.

V. To determine the safe limit dose of PPAP methanol extract from Study III for a subsequent repeated dose toxicity study.

1.4 Importance of study

With the growing prevalence of resistance in bacteria, there is no doubt that the community worldwide would face post-antibiotic era. Apart from this, the foodborne illnesses caused by the infection of resistant bacteria via the food chain may worsen and yet threaten nationwide productivity. These phenomena would be a catastrophe to the health sector as the statistic of morbidity and mortality would climb up fast as there will be limited treatment available to cure both MDR and foodborne related diseases.

In this regard, the present study had taken the initiative to incentivize the screening process for a new antimicrobial candidate from a natural source. Eventhough the journey is still far ahead, this effort is hope to expand new knowledge particularly on the potential aspects of PPAP in antimicrobial related studies. Coupled to this, the limited scientific literature pertaining to the safety and toxicity related data on PPAP enhance the urgency of conducting the present *in vivo* acute toxicity experiment.

Evaluation of the possible effects of PPAP in a complete body system of a rat model is of high importance to provide broad justification of toxicity rather than being performed in *in vitro* alone. The findings achieved from this study could serve as an eye opener to the public regarding the broad usage of herbal medicine. As there are growing divergence of opinions in scientific literatures related to the safety and undesirable side effects of natural herb (Zhang *et al*., 2012; Nasri & Shirzad, 2013; Silvanathan & Low, 2015), the acute toxicity data from this present study could pose as a reference for a subsequent toxicity study as well as able to aid in stimulating community perception regarding the safety evaluation of natural herbs.

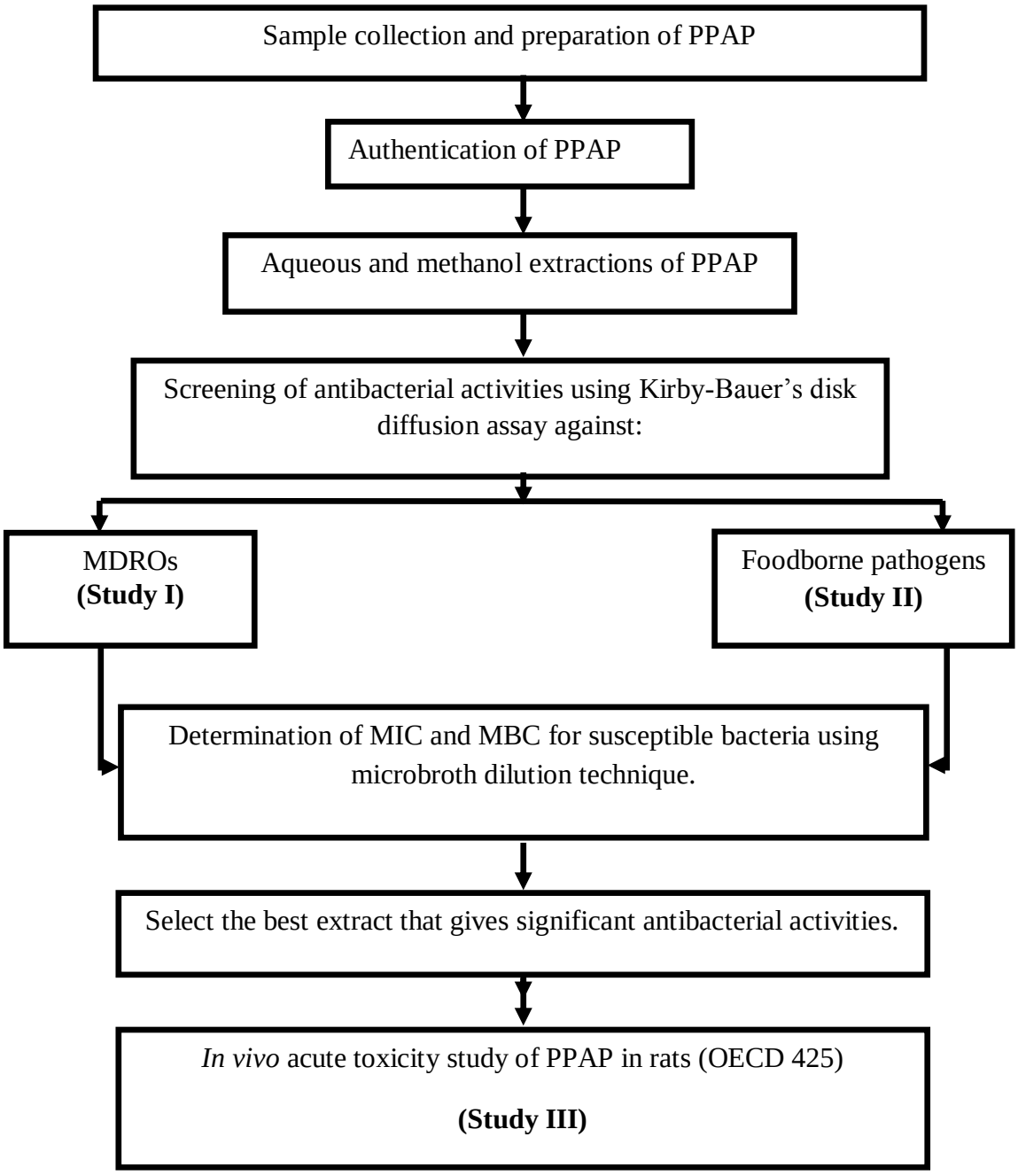

Figure 1.1 Experimental design of the overall study. The experiment consisted of evaluation of *in vitro* antibacterial activities and *in vivo* acute toxicity of PPAP extract.

CHAPTER 2

LITERATURE REVIEW

2.1 Antimicrobial Resistance (AMR)

During the 21st century, antimicrobial resistance has emerged on the forefront of global public health concern (Hwang and Gums, 2016). The authors also reported that this phenomena arise due to the natural evolutionary process of microorganisms against commonly prescribed antibiotics to ensure its own survival.

2.1.2 Mechanisms of antimicrobial resistance

Understanding the mechanisms involved has become a priority when combating the antimicrobial resistance threats that had breached the biosecurity of human health. Transformation, transduction and conjugation are the possible natural mechanisms of genetic mutations incorporates by the bacteria to produce resistance (Holmes *et al.*, 2016).

Transformation occurs when the bacteria take up free DNA from the environment and embodied it into their chromosome. An example of transformation is the acquisition of penicillin resistance which takes place through the recombination of foreign DNA from *Streptococcus mitis* to *Streptococcus pneumonia* (Hwang and Gums, 2016). As for transduction process, DNA materials being transferred from donor bacteria to the recipient bacteria via bacteriophages during infection. This genetic transfer has

resulted in the isolation of bacteriophages conferring genes encoded for β-lactamase and methicilin resistance, found in a sewage water plant and in human feces samples (Colomer-Lluch *et al*., 2011; Quirós *et al.*, 2014). Conjugation, on the other hand, involved the direct transmission of genetic material through the transfer of plasmid between neighboring bacteria. This process has been portrayed in the dissemination of genes encoding for ESBL and carbapenemases which resulted in less treatment option available (Walsh, 2010; Doumith *et al.*, 2012).

Another driven forces that contribute to the AMR are the utilization of antimicrobial in non-medical areas mainly veterinary medicine (Rantala *et al*., 2004), animal foods and fish production (Cabello, 2006). In addition, over utilisation and unnecessary prescriptions of antimicrobial drugs are the cofactors of the emergence of AMR (Levy *et al.*, 2004). Furthermore, the spread of AMR is assisted via human-human transmissions, such as improper sanitation practice among hospital workers (Chamchod and Ruan, 2012), through fecal-oral route (Zhang *et al.*, 2013) and also via sexual encounters (Lewis, 2013).

2.1.3 MDR strains tested

Therefore, our study accentuated to evaluate the antibacterial activities of these four MDR strains which are characterized as follows:

2.1.3.1 Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Prolong stay in the hospital, longer duration of exposure to contaminants of the healthcare workers and continuous contact with the individuals who have been colonised with MRSA are among the risk factors of the resistant type associated with this bacteria (Chamchod and Ruan, 2012). Aminoglycosides, quinolones, macrolides, tetracyclines and β-lactams are among the example of drugs that no longer effective to MRSA (Zuo *et al.*, 2016). Consequently, only fewer treatments are available to combat MRSA related infections on the multiple body sites, frequently on the skin, soft tissue, joints and bloodstream (Cadena *et al.*, 2016).

Moreover, recent evidence suggests the presence of clone strain of MRSA, that is clone complex 398 (CC 398) which has been resided in livestock animals primarily pigs, cattle and horses (Parisi *et al.*, 2016). In fact, this MRSA clone has been isolated from meats (Lim *et al.*, 2010), milk, dairy products and ice cream (Normanno *et al.*, 2007; Kamal *et al.*, 2013*)*. This situation leads to a potential risk of MRSA colonisation on human either direct contact with the infected animals or via food consumption (Köck *et al.*, 2013).

2.1.3.2 Vancomycin-Resistant *Enterococcus* (VRE)

Enterococcus is a gram positive, facultatively anaerobic bacteria that resides in the intestinal tracts of human and animal as the natural flora (Ranotkar *et al.*, 2014). Additionally, this particular organism is able to tolerate with stringent environmental conditions such as in 6.5% sodium chloride, wide range of pH and temperature from 10ºC to 45ºC (Arias and Murray, 2012). Further, these characteristics allow them to adapt well to any environmental surfaces and may elevate the prevalence of VRE through the horizontal gene transfer of resistance to another microbe (Tacconelli and Cataldo, 2008).

Other risk factors that contributed to VRE nosocomial infections are; individuals with compromised immune systems, patient in ICU, prolonged length of hospital stay, haemodialysis, undergo surgical procedures (indwelling of the urinary catheter) and frequent exposure to antimicrobial agent particularly the use of vancomycin (Monteserin and Larson, 2016).

2.1.3.3 Extended Spectrum Beta-Lactamase (ESBL)

ESBL can be defined as a plasmid-mediated enzyme that is capable to hydrolyze and inactivate the β-lactam ring. This process results in ineffectiveness of several antibiotics such as trimethoprim, sulfamethoxazole, aminoglycosides and quinolones (Gudiol *et al*., 2009). In addition, ESBL has also been reported to confer resistance to other antimicrobials such as penicillin and cephalosporin groups with an exception of cephamycin (Geser *et al*., 2012).

Improper food handling is one of the causal factor of a recent large outbreak caused by CTX-M-15-producing *E. coli* isolated within bean sprouts in Germany (Radosavljevic *et al*., 2016). Furthermore, this pathogen has secured its potential reservoir in the retail chickens, meats and poultry products (Hall *et al.*, 2011). This indicates that the mode of transmission of this pathogen are either through direct contacts to colonized animals, person-to-person transmission or through ingestion of the contaminated foods.

2.1.3.4 Carbapanem-Resistant *Enterobacteriaceae* (CRE)

Centre of Disease Control and Prevention (CDC) has nominated CRE as an urgent threat of antibiotic resistant. This is due to nearly half of patients who develop CRE bacteremia were reported dead (CDC, 2017). Carbapenem once has served as a crucial alternative antimicrobial class to treat resistant strain of ESBL (Gupta *et al.*, 2011). However, the comprehensive utilisation of this antibiotic has contributed to an increase of the CRE resistant strains globally (Borer *et al.*, 2012).

Metallo-β-lactamases such as Verona Integron-Mediated (VIM) types have been isolated in *Enterobacteriaceae* in Southern Europe (Nordmann *et al*., 2011) and *P. aeruginosa* in Asia (Tsutsui *et al*., 2011). Whereas, the New Delhi Metallo-β-lactamase which originate in India has already been disseminated in almost every continent in the world through the migration of travelers (Dortet *et al*., 2014). Oxacillinase-48 (Oxa-48) is another product of carbapenem mutation which has been identified in Mediterranean and European countries (Evans and Amyes, 2014).

2.2 Foodborne pathogens tested

The most common pathogens associated with foodborne illnesses in Malaysia are further portrayed as follows.

2.2.1 *Staphylococcus aureus*

Individual who suffers staphylococcal foodborne poisoning usually shows symptoms of abdominal cramps, nausea and vomiting with or without diarrhea. These symptoms may show up approximately 3-5 hours upon ingestion of *S. aureus* contaminated foods and approximately revolve within 48 hours (Kadariya *et al*., 2014). This intoxication process occurs mainly due to ingestion of heat-tolerance staphylococcal enterotoxin (SE) (Balaban and Rasooly., 2000). Additionally, SE also confers resistant to low pH of gastric stomach fluid and proteolytic enzymes (pepsin and trypsin), thus secure its activities within the gastrointestinal tract (Argudín *et al*., 2010).

Furthermore, food handlers who colonised with SE are regarded as the main source of food contamination either through direct contact or via respiratory secretion. As well established, *S. aureus* is a common human commensal particularly on the skin and mucosal membrane (Kluytmans and Wertheim, 2005). Meat, poultry product, eggs, milk, dairy products, salad and sandwich filling (salted hams) are among the list of foods that frequently served an optimum condition for this pathogen (Bennett *et al*., 2013). Additionally, several possible causal factors of staphylococcal food poisoning are including an improper food handling (prolong exposure at room temperature, inadequate heat and duration of cooking) as well as cross-contamination in the vicinity of food

preparation (insufficient cleaning of cooking utensils and equipment and do not practice proper hand washing) (Bennett *et al*., 2013).

2.2.2 *Bacillus cereus*

Diarrhoea and emesis are the two types of foodborne illnesses caused by a gram positive facultative anaerobic endospore producing bacterium, *B. cereus* (Arnesen *et al*., 2008). The diarrhoeal disease presented by abdominal pains with watery diarrhoea approximately 8-10 hours upon ingestion and normally resolves within 24 to 48 hours. These symptoms developed through the activities of three potential enterotoxins which are hemolysin, nonhemolytic enterotoxin and cytotoxin K (Zhou *et al.*, 2014). Whereas, the emetic disease which is cause by a toxin known as cereulide is characterised by nausea and vomiting approximately 30 minutes to 6 hours upon ingestion and resolve within 24 hours. This toxin demonstrates resistant to heat, acid, alkali and proteolytic enzymes which aid in its virulence (Beer and McKillip, 2014).

2.2.3 *Escherichia coli*

E. coli, a gram negative rod-shaped bacterium is a commensal organism that resides within gastrointestinal tract in human (Campos *et al*., 2004). There are five categories of diarrhoeagenic *E. Coli* which possess virulence as an intestinal pathogen; enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enteroaggretive *E. coli* (EAEC). Based on Croxen *et al* (2013) the mechanisms of virulence of each strains and symptoms associated are summarized in Table 2.1.

Table 2.1 Features of *E. coli* gastroenteritis strains.

Types of *E. coli* strains	Mechanisms of virulence	Clinical pictures
Enteropathogenic *E. coli* (EPEC)	Possess plasmid-encoded adhesions in the small intestine; causing loss of microvilli and thickening of the cell surface with the release of protein (invade phagocytosis of macrophage).	Major cause of diarrheal outbreaks in hospital nurseries and in bottle–fed infants with fever, vomiting, watery diarrhoea containing mucus.
Enterohaemorrhagic E. coli (EHEC)	Same as EPEC except the location took place in the large intestine and produce Shiga toxin instead of protein. Most cases due to single serological type, O157: H7.	Fever, abdominal cramps, bloody diarrhoea and some may develop haemolytic uremic syndrome.
Enterotoxigenic *E. coli* (ETEC)	Possess adhesions that allow them to colonize the intestinal epithelium, where they secrete either one or both toxins (heat-labile or heat-stable enterotoxin).	A common cause of traveler's diarrhoea with signs of nausea, vomiting, abdominal cramps, massive watery diarrhoea leading to dehydration.
Enteroinvasive *E. coli* (EIEC)	Invade the intestinal epithelium causing cell destruction of the large intestine. This invasive pathogen adaptable to low gastric pH, temperature and changes in osmolarity.	Fever, abdominal cramps and presence of pus and blood in feces.
Enteroaggregative *E. coli* (EAEC)	Adherence to the intestinal mucosa with further production of enterotoxin and cytotoxin causing mucosal inflammation.	Watery secretory diarrhoea often with mucus, low-grade fever and accompanied with vomiting, abdominal pain and occasionally bloody stool.

(Croxen *et al.*, 2013)

The commensal *E. coli* is a common intestinal microbiota of most mammals and birds. Therefore, the zoonotic transmission may occur via the faecal-oral route through ingestion of contaminated water or undercook poultry products, milk and vegetable fertilized with colonized poultry litters (Markland *et al.*, 2015). It was reported that an outbreak to spinach crops was known to be contaminated with this pathogen only within 10 miles proximity to a poultry farm (Park *et al.*, 2013).

2.2.4 *Salmonella typhimurium*

Salmonellosis refers to a foodborne infection caused by a zoonotic pathogen, *Salmonella* species (Pires *et al*., 2014). *S. typhimurium*, one of the most frequently isolated species has caused approximately 90 million cases of gastroenteritis worldwide (Majowicz *et al.*, 2010). This disease is characterised by the presence of nausea, vomiting, abdominal cramps and foul-smelling loose stool, approximately 6 to 48 hours upon ingestion of the contaminated foods (Rahman and Othman, 2017). Foods of animal origin such as meats, milks, poutry, eggs, dairy products as well fruits and vegetables harvested near the cattle farm are the primary source of human salmonellosis food poisoning (Awang Salleh *et al.*, 2003).

Furthermore, another vehicle of salmonellosis includes inadequate cooked or raw meat, unpasteurized milk, direct or indirect contact with animals colonized with *Salmonella* either during a visit to the farm or during slaughtering process at the abattoir (Kemal, 2014; Muluneh and Kibret, 2015). The virulence of *Salmonella* is related to specific O antigen presence within. This antigen has the ability to invade the host cell

and withstand both digestions by macrophage and destruction of the complement body system. Therefore, this enables the bacteria to travel pass through the stomach and intestine and elicit food poisoning (Kemal, 2014).

2.3 Antimicrobial activity of medicinal plants

Valuable antimicrobial responses of plant resources have been recorded via the co-evolved relationship with pathogens since time immemorial (Datta *et al.*, 2011). To date, research sector has deviated their attention to discover novel potential drugs from medicinal plants around the world. Related to our scope, Table 2.2 displays data on the potential antibacterial activities of several valuable plants available in Malaysia.

Table 2.2 Malays medicinal plants with scientific findings related to the potential in exhibiting antimicrobial properties.

Scientific name	Local names	Types of extract	Scientific antimicrobial findings	References
Aloe vera	"Lidah buaya"	Acetone, aqueous and ethanol	Acetone extracts show maximum antibacterial activities and antifungal activities against *Aspergillus flavus* and *Aspergiluus niger*.	(Arunkumar and Muthuselvam, 2009)
Andrographis paniculata Nees	"Hempedu bumi"	Methanol and acetone	Shows antibacterial activities in wide strains of bacteria (*M. smegmatis,S. aureus, S. thermophilus, B. subtilis, E. coli, K. pneumonia* and *P. aeruginosa*).	(Arifullah *et al.*, 2013)
Averrhoa bilimbi L.	"Belimbing buluh"	Fresh juice	Ability to reduce both populations of *L. monocytogenes* Scott A and *S. typhimurium* ATCC 14028 (common isolates on raw shrimps) immediately after washing with the fresh juice.	(Wan Norhana *et al.*, 2009)
Centella asiatica	"Pegaga"	Petroleum ether, ethanol, chloroform,n-hexane and aqueous	Only petroleum ether, ethanol and chloroform extract demonstrated higher antimicrobial activites against four human pathogenic microbes and two fungus strains.	(Dash *et al.*, 2011)
Cosmos caudatus Kunth.	"Ulam raja"	n-hexane, diethyl-ether and ethanol	Shows potential antibacterial activities against five microbial strains which comprised of two gram positive, two gram negative and one fungi.	(Rasdi *et al.*, 2010)
Curcuma longa	"Kunyit"	Methanol	*S. flexneri* and *C. albicans* depict the maximum antibacterial activities against methanol extract of this plant.	(Rao and Mittal, 2014)
Eugenia polyantha	"Daun salam"	Methanol	*E. polyantha* extract confers antibacterial activity against two spore-forming bacteria; *B. cereus* and *B. subtilis*.	(Lau *et al.*, 2014)
Polygonum minus	"Daun kesum"	Ethanol extract	Exhibit strong inhibitory effect for both *S. aureus* and *E. coli*.	(Imelda *et al.*, 2014)

2.4 *P. pellucida*

2.4.1 General description

Peperomia pellucida (L) Kunth is a herbaceous plant that belongs to a family of Piperaceae, a genus of greater than 1000 species of herbs distributed in America as well as in Asian countries (Abere and Okpalaonyagu, 2015). The geographical distributions of this plant are described in details in a study done by Majumder *et al*., (2011) which stated that this plant is usually found at elevation of sea level to about 400m in Fiji and 700m in Samoa, New Guinea especially along roadsides, shady places near houses, and rarely along the forest trails.

P. pellucida is commonly known by its vernacular names which varies according to countries such as 'ketumpang air' among Malaysian (Wei *et al*., 2011), 'pak-krasang' in Thailand (Phongtongpasuk and Poadang, 2014), 'cang-cua' and 'ulasimang bato' among Vietnamese and Philippines people respectively (Ooi *et al*., 2012).

2.4.2 Botanical characteristics

Shallow-fibrous roots of *P. pellucida* can grow up to 15 to 45cm and have a shiny waxy surface of heart-shaped green leaves on the upper surface and appeared whitish-green on the lower surface. The leaves were alternately arranged with 1.5 to 5cm long and 1 to 3.3cm width. Besides that, *P. pellucida* is characterised by the presence of pale green threadlike but angular trailing stem and able creep up to 6 to 9cm above the surface (Majumder, 2012). These botanical descriptions can be clearly presented in

Figure 2.1. Further, attached to the several fruiting spikes are the tiny dot-like seeds (Figure 2.2) which is able to produce a mustard-like odor when crushed (Majumder *et al*., 2011). In addition, this plant grows in clumps, thriving in loose and humid soils in the tropical climate (Plate 1.1)(Arrigoni-blank *et al.*, 2004; Akinnibosun *et al*., 2008).