Antibacterial Activity of *Leucas Zeylanica* Leaves Extract in Elimination of Bacteria

By

NUR HANIS ALIAA BINTI ZULKARNAIN

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DECLARATION

I hereby declare that this thesis entitled "Antibacterial Activity of *Leucas Zeylanica* Leaves Extract in Elimination of Bacteria" is the result of my own research and except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree

Signature :

Name : NUR HANIS ALIAA BINTI ZULKARNAIN

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ABSTRACT

Leucas zeylanica, herbal medicinal plants belong to the Lamiacea family, genus Leucas are common being used widely in traditional medicinal to treat various diseases and bacterial infection. The antibacterial activity of most of the medicinal plant produced is commonly due to the constituents of active compounds. The aim of the study is to determine the antibacterial activity of *L.zeylanica* leaves extract in inhibition of gram positive and gram negative bacteria, *E. coli* and *S. Aureus*. The extraction of plant leaves is produced by soxhlet extraction and rotary evaporator. The solvent being used in this study is methanol. Different concentration of plant extract was used against two different bacteria strain. Antibacterial susceptibilities of *L. zeylanica* leaves extract were performed by using Disc-Diffusion method. The antibacterial activity was being assessed with diameter zone of inhibition on agar plate. In this study, the result then compared with standard antibiotic, Gentamycin as positive control and methanol solvent as negative control. The result obtained shows that 600 µg/µL concentration give maximum inhibition zone for both bacterial strain with range of (12.1±0.5mm) against *E.coli* and (16.3±0.9) against *S.aureus*. Active compound contain in medicinal plant that shows therapeutic potency in inhibit infection potentially believe able to further use in pharmaceutical field within future research.

ABSTRAK

Leucas zeylanica adalah salah satu tumbuhan ubatan herba milik keluarga Lamiacea, genus Leucas biasa digunakan secara meluas dalam perubatan tradisional untuk merawat pelbagai penyakit dan jangkitan bakteria. Aktiviti antibakteria yang dihasilkan kebanyakan adalah disebabkan oleh adanya sebatian aktif. Tujuan kajian ini adalah untuk menentukan aktiviti antibakteria daun *L.zeylanica* ekstrak dalam penghambatan bakteria gram positif dan gram, *E. coli* dan *S. Aureus*. Pengekstrakan daun tumbuhan dihasilkan oleh pengekstrakan soxhlet dan penyejat berputar. Pelarut yang digunakan dalam kajian ini adalah metanol. Kepekatan ekstrak tumbuhan yang berbeza digunakan terhadap dua jenis bakteria yang berlainan. Kerentanan antibakteria daun ekstrak *L. zeylanica* dilakukan menggunakan kaedah Disc-Diffusion. Aktiviti antibakteria dinilai dengan zon diameter penghambatan pada plat agar. Dalam kajian ini, hasilnya dibandingkan dengan antibiotik standard, Gentamycin sebagai kawalan positif dan pelarut metanol sebagai kawalan negatif. Keputusan yang diperoleh menunjukkan bahawa kepekatan 600 μg / μL memberikan zon penghambatan maksimum bagi kedua-dua strain bakteria dengan bacaan (12.1 ± 0.5mm) terhadap *E. coli* dan (16.3 ± 0.9) terhadap *S.aureus*. Sebatian aktif yang terdapat dalam tumbuhan ekstrak menunjukkan potensi terapeutik dalam menghalang jangkitan berpotensi untuk terus berkembang dalam bidang farmaseutikal dalam penyelidikan masa depan.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Varience
BCE	Before Century
B. subtilis	*Bacillus subtilis*
B. cereus	*Bacillus cereus*
CPS	Coagulase Positive Staphylococci
CNS	Coagulase Negative Staphylococci
EHEC	Enteroheamorraghic *E. coli*
E.coli	*Escherichia coli*
GRAS	Generally Recognized as Safe
HUS	Haemolytic Anaemia Syndrome
L. zeylanica	*Leucas zeylanica*
L. aspera	*Leucas aspera*
MRSA	Methicilin Resistant *S.aureus*
NAM	Nutrient Agar Medium
P. aeruginosa	*Pseudomonas aeruginosa*
P. Vulgaris	*Proteus Vulgaris*
S. typhi	*Salmonella typhi*
S. aureus	*Staphylococcus aureus*
STEC	Shiga Toxin-Producing *E. coli*
SEs	Staphylococcal Enterotoxins
TSS	Toxic Shock Syndrome
VRSA	Vancomycin Resistant *S.aureus*
V. cholerae	*Vibrio cholerae*
WHO	World Health Organization

LIST OF SYMBOL

◦C	Degree celcius
pH	Potential Hydrogen
aW	Water Quality
CFU / g	Colony-forming units per gram
g	Gram
µg	Microgram
µL	Microlitre
µm	Micrometer
ml	Millimeter
mm	Millimeter
cm	Centimetre
SD	Standard deviation
min	Minute
%	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Leucas zeylanica (L.zeylanica) that belongs to the *Lamiaceae* family is an upright, hispid, terestial, annual herbs. In India, well-known Ceylon slitwort is recognized by local name as Kusha (Khanam & Hassan, 2005). The genus Leucas a member of *Lamiacae* are used mainly wide in traditional medicine to treat various diseases such as cough, cold, diarrhea and inflammation of skin disorders. In Asia and Africa, Leucas genus plants have been widely used in their tribes (Chouhan & Singh, 2011). This plant species grows with maximum height of 30 cm particular at sandy soils area. It is also normally being applied as a cure for insects and poisonous bite, healing wounds jaundice and as well as for stomach ache relief especially due to roundworms for children (Napagoda *et al.*, 2016). The plant morphology consists of its leaves showed in Figure 1.1.

Figure 1.1 : *L. zeylanica* (ketumbit) leaves.

Nowdays, due to unintelligible exploitation, the destruction of natural disasters, cutting and overgrazing many important medical plants are rapidly exhausting and lead to the possible extinction of some of these species (Ishtiaq *et al.*, 2006). Most tribal people still rely on local herbs for treatment of different diseases using the knowledge of herbal treatments they obtain from their folk ancestors. But this ethno-medical knowledge as well as medicinal plants declines at an alarming rate due to modern medical facilities and other socio-economic factors.

Detailed research with the use of medicinal plant extract for antimicrobial theraphy and various diseases was been conducted as it show to be a potential effective medicines since ancient times in Chinese, Ayurvedic, Arab and Unani medicines (Marasini *et al.*, 2015). 80% human population of the world estimated by World Health Organization (WHO) relies on the traditional system of medicine that used plant-based, for their primary health-care needs. Active constituents contribute to effectiveness of antimicrobial, anti-inflammatory, antioxidant, analgesic, antidiarrhoeal, even insecticidal activities due to different composition content and various constituents of complex chemical substances in medicinal plants that causes the plants to have the different potential therapheutic properties (Trivedi *et al.*, 2006).

Herbs medicinal plants have been identified in growing interest as natural foods additives because herb plant are Generally Recognized As Safe (GRAS) and excellent alternative for additional ingredients. The major activities of extracts and herbs from medicinal plants are antimicrobial, anti-inflammatory, bactericidal, antiviral, antifungal and preservative for foods. The use of natural preservatives to reduce infection and food poisoning is promising method since many herbs plant show antioxidant and antimicrobial properties. Increased demand by consumers for good health without consume of artificial antibiotic, possible free of chemical additives, open opportunities to these bioactive secondary metabolites from medicinal plants as alternative performance enhancers (Nieto, 2018).

Antibacterial is a category of antimicrobial activities that used to inhibit the growth of bacterial and to treat antibacterial infections that cause some diseases to human. Antibiotics are a broader scope of antimicrobial component which able to inhibit on fungi, bacteria, microorganism, viruses and other more compounds. Antibiotics can be divided into the effects that able to kill bacteria, bactericidal or effects that inhibit bacteriostatic activity. However both have the effect of disabling bacteria from spreading in the body system.

1.1 Problem statement

Antibiotics is a medicine that inhibits the growth or eliminate microorganisms, types of medicines that treat patients from serious bacterial infections, but mostly often misused the intake of antibiotics. Excessive usage of artificial antibiotics in daily can cause a negative impact on the human body immune system where antibiotics invokes

the body prone to illness and easily susceptible to infections due to lowering the body's immune system. Generally antibiotics also have side effects such as allergies, addiction and to severe ones such as kidney, bowel and liver damage and convulsions. The use of antibiotics may be beneficial, but frequent use may lead to another issue that is appearance of bacteria resistant strains.

In contrast with plant-derived medicines that naturally do not harm the body system. Herbal plant able to act as antibiotics have no major harmful side effects, do not block beneficial active compound, less toxicity, no addiction or minor chemical allergic phenomena and such more. Traditional medicines availability much wider as it been used by the lower middle class especially in promoting health. In addition, plants as natural antibiotic, medicinal plant often been used in spices as culinary additive that enhances the effectiveness of medical treatment, intended to reduce food infection while maintain its flavour and. In this regard, herbs in development of food products as ingredients been widely used, especially those related to health care such as preventing, treating diseases, maintaining or improving health. In the preparation or cooking, herbs are commonly used as additives ingredients to supplement flavour or aroma. In this context, added herbs as additional ingredients have antibacterial constituents able to eliminated bacteria in food products.

Infection problems arising from the consume of bivalve mollusc species is a public health concern in mostly countries. Introduction of herbs medicinal extract as additional ingredients as a food paste, could help alleviate in this food infection, provide alternative sources of eliminating bacteria while at the same time maintaining the aroma flavour of the food product.

Bivalve molluscan shellfish like etok, clams and oysters are commonly eaten raw or partially cooked. Fresh raw mollusc species highly risk contamination as they generally expose in open field of water with potential exposure to enteric pathogens from soil, irrigation and contaminated water, sewage and other sources. This filter feeding species due to their living habitat can contain bacteria or viruses that can cause illness as if we consume it as food. Eating raw shellfish without sufficient heat, using low heat temperatures such as traditional cooking methods that are still used by community for instance smoked technique could not eliminate pathogen in food and the presence of bacteria could lead to food infection.

Statistic by World Health Organization (WHO) in 2015 estimates foodborne illnesses caused by 31 agents including bacteria, viruses, parasites, toxins and chemicals, resulting in 600 million cases or almost one in 10 people in the world falling sick every year last year consuming marine contaminated food. This crisis issues of food security when patient experience severe diarrhea symptoms from the habit of consuming raw or less cooked foods.

Thus, as a natural alternative, herbal extract as an additional ingredients could be applied in eliminating bacterial infection in etok tissue. Herbal product that derived from plant extract are widely used in traditional medicine plus the extracts of these plants were found to be antimicrobial in nature and have the potential to be used in clinical field.

Therefore, this research will be conducted to identify the natural alternative product in the form of plants to determine the antibacteria activity *L. zeylanica* plants leaves extract in eliminate bacteria such as *Escherichia coli* (*E. coli)* and *Staphylococcus aureus* (*S. aureus)* that can be applied as additional ingredients paste in treat and help controlling food borne-disease outbreaks especially infection involved bivalve mollucs species that serve as human food.

1.3 Objectives

The objective of this research is:

i) To determine the antibacterial activity of leaves extract of *L.zeylanica* in elimination of bacteria.

1.4 Scope of Study

Scope of research include the knowledge of antibacterial actvity of *L.zeylanica* . Different concentration of plant extract of *L.zeylanica* were used in eliminated different kinds of bacteria *(E.coli, S.aureus .)* The bacteria that used in this study are from stock culture bacteria. Soxhlet extractor and rotary evaporator were used in this study to get the concentrated extracts and use of disc diffusion method.

1.5 Significant of Study

Overall, the reason for conducting this research is to determine antibacterial activity of *L. zeylanica* extract against different bacteria. Filter-feeder species or bivalve mollusc always serve as a food consumed by the human-kind. This food production through the process of traditional cooking method for instance smoked at low temperatures that does not give guarantee pathogenic bacteria in this filter-feeder species was eliminate. Thus, the antibacterial properties in *L. zeylanica* has strong inhibition against bacteria, able to act as antibacterial to this filter-feeder whereas reduce food infection and guarenteeing food safety from contamination of pathogenic bacteria especially those that present in raw fresh products. *L. zeylanica* also known as herbal medicine used in treating influenza, inflammation, treats anorexia, flatulence and to treat malaria. Therefore, it is necessary to continuing this research to gain more data and research study about medicinal plant extract that can act as antibacterial and can potentially be effective in the treatment of these problematic bacterial infections.

CHAPTER 2

LITERATURE REVIEW

2.1 *Leucas zeylanica sp.*

L.zeylanica is one of the herbal medicinal plants belong to the Lamiacea family, genus Leucas are common being used widely in traditional medicine to treat various diseases and bacterial infection. Plants of the genus Leucas is a shrubs, herbs plants with woody roots and stems. *L. zeylanica* can be identified by the shape of the leaves with an oval-shaped lobe with tapered tip, petiolated or sometimes without stalks interfering. Further taxonomy classification of *L. zeylanica* been explained in Table 2.1. The growth of the axillary or terminal is unidentified. Bracteoles almost upright. The calyx shape of the genus, ususally tubular, several times the petals grow into fruit. Calyx consists of five straight joints from one upper, two sides and two lower and 5 - 20 secondary lobes (Chouhan & Singh, 2011). *L zeylanica* known as herbal medicine used in treating influenza, inflammation, and especially distributed in southern China (Zhang *et al.*, 2016).

Table 2.1: Taxonomy classification of *L. zeylanica sp*

KINGDOM	**PLANTAE**
PHYLUM	Tracheophyta
CLASS	Magnoliopsida
ORDER	Lamiales
FAMILY	Lamiaceae
GENUS	Leucas
SPECIES	*Leucas zeylanica*

(Parte, 2018)

Chouhan and Singh (2011) analyzed several active compound that have been isolated from Leucas species include lignans, flavonoids, coumarins, steroids, terpenes, fatty acids and aliphatic long chain compounds. From the extract of the plant and their phytoconstituents have been reported contain capabilities of anti-inflammatory, analgesic, antidiarrhoeal, antimicrobial, antioxidant and insecticidal activity that cure many disease. According to traditional customs medicine, plants from the genus Leucas show various medicinal activities (Babu *et al.*, 2016). Ceylon slitwort' or called as *Leucas zeylanica* are being used in the treatment of inflammation and urination within the scope of traditional medicine, antirheumatic, wild-crafted, coughs, colds, abdominal pains and thrombolytic activity (Babu *et al.*, 2016).

2.2 Herbal Medicinal Plant

Over thousands of years, medicinal plants not only been used for food preservation to inhibit the growth of microorganisms, moreover medicinal plants been aware in treat health disorder and prevent diseases such as epidemics (Singh, 2015). Nowdays, medicinal plants are taking crucial role in pharmaceutical medicine industry. It is also important for the discovery and identification of new therapeutic compounds, using extraction method, method that separate and identified of different phytochemicals from herbs, and screening plant extracts for novel leads (Gupta *et al.*, 2012).

The local people preferred medicinal plants to cured various ailments. Plant parts such as fruit, stem, seed, leaves and bark been used as traditional medicine. For ancient, plants have been played a key role in health care of vast majority of worldwide population. About 2 million of practioners traditional of health use medicinal plants in cured various illness. The extraction of alkaloids with alcohol ruptured the intrinsic therapheutic properties of herbal drugs. Indigineous communities of Uttarakhand have been grown up in their particular environment mereka, suitable life and culture, common habits of life. Ayurvedic drug are known naturally suited better and much safer than western medicines with chemical content. In Ayurveda, plants are major element used in preparing medicines. However, shortage of supply of this plany ingredients affected the presence used of these traditional age-old medicine in pharmaceutical system (Kala, 2010).

Plants been familiar used as it naturally effective and low cost sources of drugs. The synthesizes diverse active compound in plant are precious in treatment and control of various diseases. These compound are principally secondary metabolites. Active compound that occur either combine with inactive substances inhibit greatly to the life processes of pathogenic microbes. Medicinal plants also clarified as renewable sources of pharmacologically active substances (Alabi *et al.*, 2012).

Medicinal plants mostly contain remedial properties in different parts of plants that are proved to act as antibacterial, antifungal, antimicrobial and analgesic. Developing countries in Asia including India, Bangladesh, China, Burma dan Sri Lanka raised the important applied traditional medicine in their country. Medicinal plants have been used for long centries ago due to medicinal plants contain components of therapeutic value that are good for human diseases treatment. In India, they have been practices used on medicinal plants as traditional medicine, Medicinal plants in pharmacological avtivites. Native plant such as *Cassia alata* Linn to Ghana and Brazil now been distributed widely all over Africa and Americas (Nayak *et al.*, 2015).

21,000 of plant species are state to be useful in the preparation of this plant-based medicine. Various constituents of different composition that have complex substances contain in medicinal plants causes the medicinal to have the therapheutic properties. This constituents are found as secondary plant metabolites in one or more parts of these plants (Trivedi *et al.*, 2006).

Along current development, widespread of faith towards natural medicine that more secure and more natural beneficial than synthetic drug that provides adverse side effect and costly creates revival revolution, open interest research on plant-derived drugs. This evolution could lead to a discovery new drug or advance with the use of indigenous herbal medicines and potentail to developed it as pharmaceutical product. The study of traditional plants and their medicinal value is is getting more concern over the last few decades, this is due to ability in potent pharmacological activities, low toxicity and economic viability, able to opening up opportunities for medicinal plants for further research (Chew *et al.*, 2012).

2.3 Herbal Extract as Additional Ingredient In Food

Through generations of humans have been commonly used herbs and spices as ingredients in food and to cure illness. Researches estimated that medicinal properties contain in these herbs and spices that alleviate to this symptoms or disease. Frequently used herbs and spices such as garlic, black cumin, cloves, cinnamon, thyme, bay leaves, and rosemary, are believe able to possess antimicrobial properties that, in some cases, can be used therapeutically (Lai & Roy, 2004). Tapsell *et al.* (2006) stated that culinary herbs usually used leaf of plant, in contrast with another parts of plants are

suitable to prepared as spices, after been dried or in form of powder. Herbs and spices besides being used in culinary use, it also offer in provide health benefits, as well as acting as preservatives.

The demand for medicinal herb products recently has begun to rise and gain oppurtunity. The potential for use of medicinal herbs as natural antimicrobial additives for foods, 15 medicinal herbs were examined for antimicrobial activity. Fresh extracts and oil extracts of all medicinal herbs in this study were tested against *Bacillus cereus (B.cereus) , Salmonella typhi (S. typhi)* and *S.aureus by* using Agar Diffusion Method. The researches indicated the possibility of using medicinal herbs from Thai food ingredients as natural antimicrobial agents been approved (Chaisawadi *et al.,* 2005).

Herbs are often regarded as friendly safe to eat as it is natural plant. However, several studies found that some traditional medicinal plant and herbs can cause side effects on the kidneys, cardiovascular system, spinal cord, liver or lung for long-term intake at high doses. To ensure the safety of herbal products, the risk level of a chemical component in it needs to be analyzed and a safe measure for intake needs to be identified. This is especially important because herbal products are usually linked to health foods and this indirectly encourage long-term use or excessive consumption.

Toxicity is one of the methods of determining the level of safety of chemical components in food. It is performed to see any reaction that occurs in the body when exposed to a chemical for a period of time. It can be scientifically carried out using laboratory animals (in vivo) or bacteria or culture cells (in vitro). The development of

medicinal and aromatic herbal food products industry is a potential industry to be developed in this country. Hence, in order to ensure competitiveness with other countries, the development of herbal-based food products must always ensure quality assurance, efficacy and safety requirements (Pin, 2013).

2.4 Bacteria Caused Disease

2.4.1 *Escherichia coli* (*E. coli*)

E. coli is a type bacterium that is usually found in the human guts and warm-blooded animals (WHO, 2018). *E. coli*, Gram-negative bacterium, shape straight rod, size of 1-3 µ x 0.4-0.7 µ, arranged either singly or in pairs. Motility of peritrichous flagellae, eventhough on some strains might are non-motile. Do not formed spores but capsules in form and have fimbriae that usually found in some strains (Sangwan, 2016).

Enteroheamorraghic E.coli (EHEC) one of the six *E.coli* groups recognized as etiological agents of diarrhea. It produces cytotox which is referred to as verocytotoxin or toxin such as Shiga that responsible for heamorrhagic colitis. Back in 1982, this organism identified as first causes of the diseases and it infection been reported with increasing frequency. It is now a public health concern due to it is easily isolated from the rest of the human and animal polluting environment (Smith *et al.*, 2003).

Shiga Toxin-Producing *E. coli* (STEC) is one of the toxin produce *E. coli* which commanly become cause to severe foodborne diseases. Human consume contaminated food for instance, raw or undercooked products, such as meat contaminated vegetables and sprouts being the medium for theses toxin to transmitted. Shiga-toxins similarly to the toxins that produced by *Shigella dysenteriae.* Temperature to be range that STEC able to spread active from 7 °C to 50 °C, with an optimum temperature of 37 °C. Some type of STEC able to grow well in several acidic conditions, pH 0f 4.4 and down, and with 0.95 minimum water activity (*aW*). STEC able to eliminate with extreme condition of heat temperature up to70 °C or higher.

Thus, through the cooking process of food need to ensure maximum heat temperature exceeded to avoid transmitted of STEC. STEC serotype that commonly reported with human health is *E. coli O157:H7*. However, other serotypes of these toxins also classified as pathogenic and frequently involved in infection cases and outbreaks (WHO, 2018).

2.4.2 *E.coli* Infection

E.coli infection carried by etiological agent that carries features gram-negative, do not formed spores, facultatively anaerobic rods of family *Enterobacteriaceae* (Angulo *et al.*, 2006). To prove the contamination and persistence of *E. coli* recently past few years, Ishii *et al.* (2006) stated that the *E.coli* natural populations in the soil able to reach up to 105 CFU / g in northern temperate areas during the hottest months.

According to Sin Bin *et al.* (2014) stated that *E.coli* act as a surrogate marker, where become the hygiene indicator organisms. Hygienic quality of food can be observed as used bacteria as indicator. Its presence in food generally indicates direct or indirect faecal contamination. General lack of hygiene in handling and improper management indicated substantial number of *E.coli*. *Enterobacteriaceae* is a major group of biochemically and genetically related bacteria that been used to assess the general hygiene status of a food product. Their presence in food indicates inadequate heat required for cooking process or post-processing contamination.

Rising death in developing country cause of illness due to food infection that caused poisoning to human population is become serious concern. (Doughari *et al.*, 2007). Gram negative pathogen like *E.coli and Pseudomonas aeruginosa (P. aeruginosa)* commonly reported to be associated with bacterial contamination. Whereas, Gram positive pathogen was recognized to become the causal agents of food borne diseases, for instance *S. aureus* and *B.cereus.* Natural alternatives by considered plant extract as another sources to become antimicrobial agents, approved as nutritionally safe and natural degradable (Ashraf A Mostafa *et al.*, 2017).

Human pathogenic caused due to bacteria such as *E. coli* have been aware and recognized. It characterization, habitat, toxicity have been discover include the infection symptom to human such as diarrhoea, meningitis or involve severe conditions leads to fatality. During past century, numerous food infection outbreaks and incidents that have been attributable with *E. coli* were been recorded. Outbreak reported associated with bacterial infection caused by *E. coli* group to identified the

primary cause of transmitted infection, which is commonly due to inadequate sanitation, poor hygienic concern and low standard of food preparation (Bell & Kyriakides, 2010).

Haemolytic uraemic syndrome (HUS) is another diseases due to infection of pathogenic bacteria. It is recorded that 10% of patients end up with a fatality after being recognized wih STEC infection. Individual that faced conditions with bloody diarrhoea or severe cramps at abdominal area should concern to seek for clinical treatment. Antibiotics however is not an option to overcome diseases with STEC may possibly increase the risk of the diseases itself.

Annual outbreak reported that asscociated with *E. coli O157:H7* was identified it transmission to human body system through consumption of contaminated foods, or raw or undercooked food product. Cross contamination during preparation of food due to faecal contamination of water also able to lead to infection (WHO, 2018).

2.4.3 *Staphylococcus aureus* (*S.aureus*)

Parte (2018) stated in research that futher subdivided the genus staphylococcus was into 46 species and 24 subspecies. Tham (2014) describe that the cells of staphylococcus are spherical, diameter (0.5 -10 µm), arrangement either of single, paired, clusters (grape-like) . Colonies are more smooth, raised, glistening and translucent and a single colonies able to reach a size of 6-8mm on non-selective media. Growth range of staphylococci or recognized as mesophilic bacteria was between 6 –

48 °C, and 37 °C for the optimal temperature . Staphylococci are growth best with the presence of oxygen as it facultative anaerobic bacteria (Hennekinne *et al.*, 2012). Other species of staphylococci besides *S.aureus* are still identified as pathogenic and able to transmitted toxins infection, for instance, coagulase positive staphylococci (CPS) and coagulase negative staphylococci (CNS) (Tham, 2014).

2.4.4 Staphylococcal Enterotoxins

Wide array of toxins produces by *S.aureus* become human concern towards pathogenic issues. Consume of contaminated food which lead to various diseases such as gastroenteritis are due to Staphylococcal enterotoxins (SEs), family of serological types enterotoxins, family of pyrogenic toxins, originated from saphylococcus dan streptococcus species (Balaban & Rasooly, 2000). Ses are the medium that bring *S. aureus* become causative agents to staphylococcal food poisonings. *S. aureus* not forming a spores. Thus, required heat supply of food can avoid *S. aureus* contamination. Despite that, *S.aureus* contaminate food product during preparation and processing with its toxins remained as a major foodborne diseases (Le Loir *et al.*, 2003).

Enterotoxigenic strains of *S.aureus* and sometimes produced by other *staphylococcus* species that caused staphylococcal enterotoxins that which if it been ingested could lead to most foodborne diseases such as staphylococcal food poisoning (Danielsson-Tham, 2014).

2.4.5 *S.aureus* Infection Outbreak

Gram positive bacteria *S. aureus* carried out responsibilities one third of the general population towards serious diseases and infection. *S. aureus* are believe able to produced toxins which lead to these diseases that is food poisoning and Toxic Shock Syndrome (TSS) (Pinchuk *et al.*, 2010). Intoxication of Staphylococcal food poisoning are caused by consumption of certain amount of enterotoxins (Rusnak *et al.*, 2004).

Toxic compound that initiates from actively growth bacteria causes some of food poisoning through invasion of inside human body system. Therefore, eventough a consumed of food that free from infection of staphylococci could yet involved in infection outbreak of food poisoning. It was been recorded (1µg/g) minimal level of enterotoxins *S. aureus* lowest cell that required to able to cause gastrointestinal syndrome in humans appears to differ among substrates and for particular enterotoxins (Danielsson-Tham, 2014). It takes less than 1 µg to determine total amount of toxins that able to caused diseases (Pinchuk *et al.*, 2010). Histamine that can causes vomiting is one of the effect of SEs when SEs able to penetrated into body's system and activated inflammatory mediators (Argudín *et al.*, 2010).

Staphylococci commonly able to withstand to growth in over wide range of temperatures and pH. The pathogen also able to grow well in short assortment of food supply and heat resistant. It needed a higher conditions of heat temperature as it cannot

be destroyed by cooking under low temperature of heat (Pinchuk *et al.*, 2010). Several outbreak associated with staphylococcal illness include inadequate refrigeration, poor personal hygiene and inadequate heat temperature in food serving (Montville & Matthews, 2007). Thus, main source of food contaminated outbreak are commonly due to food that being allow to kept at temperature where rapid growth of bacteria is possible (Pinchuk *et al.*, 2010).

2.5 Pathogen In Mollusc Species

According to the Graczyk *et al.* (2003) stated that Zebra mussels (*Dreissena polymorpha)* and Asian freshwater clams (*Corbicula fluminea)* are non- indigenous bivalve mollusc species, usually in North American fresh waters and have been reported contaminated with human enteric parasites. It is believed that correlation exist between number of parasites in this bivalve tissue as it related with concentration of contaminated waterborne. These contamination in mollusc species can causes severe adverse impact towards exposure individual that consumed mollusc species as food serve. (Defer *et al.*, 2009) stated that the antimicrobial activities present in the hemolymph of bivalve species against selected panel of bacteria, three Gram-positive and seven Gram-negative pathogen and some of it involve in aqualculture-pathogenic marine strains. From the journal stated that *C. edule* showed broadest antimicrobial activity however in gills and mantle of *O. edulis* showed the highest activities. Therefore, it must aware that pathogenic bacteria existed in mollusc species can be contributed to bacterial infection.

2.6 Foodborne Diseases

Foodborne diseases have been on rise reported as an emerging infectious disease. Classical enteropathogens like *S.typhi* and *Vibrio cholerae* (*V. cholerae*) have shown decreases currently. Developed countries, the new enteropathogens have been rise emerged, such as *E. coli 0:157* or the enterohaemorrhagic *E. coli, Vibrio vulnifikus, Listeria monocytogenes, Salmonella serotype Typhimururium*, parasite *Cyclmpora cayetanensis*. 76 million diseases estimated every year in United States associated with foodborne diseases including severe cases of fatality. However, Malaysia still lack of awareness towards hygienic concern especially on food processing. The standard of hygienic is still lower to be applied in Malaysia, and infection issues accosiated with bacterial pathogen is owing at it risk. In addition with the official reported figures for food-borne infections are in control and not a very serious problem (Soon *et al.*, 2011).

Technologies of food intervention include chemical, physical and biological such as bacterial phage treatment method been effectively investigated able to inhibit human pathogens on foods. In addition, conditions for thermal treatment need to be considered to maintain the quality of fresh product in reducing of foodborne pathogens (Fan *et al.*, 2009). Foodborne diseases associated with microbial pathogen are an important cause of human illness. Tham (2014) states that foodborne infection increased as number of people susceptible to food infection is high reported, especially among the population age over 65 years. It have been concerned that lifestyles and eating habits actually affecting the possibility transmission of pathogenic bacteria in foods. Beside that, excessive consumption of raw food also contribute to the risk of

food infection. However, some food borne pathogens able of being active even at refrigeration freeze temperature, for instance refrigerated ready-to-eat food.

Ashraf A Mostafa *et al.* (2017) stated that usually food preservatives contain chemical that have negative effect towards human health. Food preservatives are being used to prevent food damage and food poisoning pathogen. Natural alternative preservatives that potentially safe and effective nowdays are demandly required due to these distress. Ávila-Quezada *et al.* (2010) in research review presents reportly diseases outbreak in the human population associated with *E. coli* O157: H7 which is associated with the use of fresh raw product.

Le Loir *et al.* (2003) states that food-borne infections is due to many different diseases-causing pathogen that contaminated food, whereas foodborne- poisoning are due to poisonous or harmful chemical or other harmful substances that present. It capability to emitted toxins after ingestion, in human digestive system, lead to pathogenesis of bacteria that caused foodborne poisoning.

Infection due to foodborne bacterial pathogens continue to be major public health around the world. In developing country, the ingestion of pathogenic virulent are rise affecting the population. Worldwide incidence and prevalence of foodborne diseases are unable to estimated. However, it have been reported in 2005, more than 2.8 million cases mortality involved of diarrhoeal diseases and these attributed from contamination of food and drinking water (WHO, 2018).

2.7 Pharmacological Studies

2.7.1 Antibacterial activity

Plant extracts that potentially effective can be used as a natural alternative to preventing food poisoning and preserving food stuffs preventing the harmful effects of chemical antimicrobic agent applications (Ashraf A Mostafa *et al.*, 2017). According to Rahman and Islam (2013) stated that the methanolic extract *L. zeylanica* and 80% ethanol extract of *Leucas aspera (L. aspera)* showed a strong inhibition activity against pathogenic bacteria, *S. aureus* and *B. subtilis.*

The efficacy of antimicrobial treatment depends on it chemical, physical and mechanical action that been applied. Concentration, time, temperature, pH, organic material build-up, target pathogen and level of microorganism are the component involved that act as parameter. These combination of factor able to reduced microbial populations in effectiveness of microbial treatment (Herdt & Feng, 2009).

The traditional therapeutic claims that these herbs of the aqueous extract exhibits genuine high degree of antibacterial activity. (Ashraf A. Mostafa *et al.*, 2018) Based on research, the extract from the plant that exhibited a marked significant activity, especially toward the Gram negative bacteria, *Proteus vulgaris* (*P. vulgaris*) are one of the suitable plant because it shows that the presence of good antibacterial potency or contain high of concentration of an active principle in the extract. For the last decades, medicinal plants have been a precious natural sources as it being linked

with human health, added with intensive studies for natural therapies. Pharmaceutical purposes by used of plant compunds has gradually increased especially in Brazil.

States by World Health Organization (WHO), medicinal plants become one of the best source to obtain a variety of medicine. 80% of individuals from developed countries use traditional medicine, which has natural compounds that derived from medicinal plants. Therefore, such plants that being used as traditional medicine should be investigated through further research, to provide more understand about their properties, safety and efficiency and toxicity towards consumers (Nascimento *et al.*, 2000).

Negi (2012) state that antimicrobial properties of plants commonly have elements and valuable source of new and biological molecules. Standardized extract or pure compund of constituents in medicinal plant provide unlimited chances for the inhibition of microbial growth owing to their chemical diversity. Antimicrobial activity against a range of bacteria possess from various plant extracts. Antimicrobial activity of *L. aspera* flowers, genus species with *L. zeylanica* showed good antibacterial activity for methanolic extract and methanol fraction with maximum activity for the alkaloidal residue with the methanolic extract of *L. aspera* flowers, it fractions, alkaloidal residue and the expressed flower juice (Prajapati *et al.*, 2010).

2.7.2 Antibiotic agent

Antibiotics or antibacterials are a type of antimicrobial agents that been used to against pathogen and currently being worldwide used for medical treatment against bacterial infections and certain parasitic infection. The aminoglycoside antibiotic are potent bactericidal agents that are rapidly lethal to bacteria in a concentration-dependent manner, their therapeutic potential for widespread use antibiotics are been limited by by ototoxicity and nephrotoxicity. Aminoglucosides have a broader spectrum of activity, widely been used in treatment of many Gram negative and Gram positive bacterial infections. The percentage of Gram negative bacteria *E.coli* resistant to antibiotic in Table 2.2. Aminogylcosides antibiotics sometimes used in combination with *B*-lactam antibiotics. Aminoglucoside antibiotics include gentamicin, neomycin, streptomycin, tobramycin and amikacin that being commonlu used clinically (Anderson et al., 2012).

Table 2.2 : Percentage of *E. coli* resistant to antibiotics.

Antibiotic	2011 % R [no. Tested]	2012 % R [no. Tested]	2013 % R [no. Tested]
Ampicillin	67.1 [27496]	69.1 [27784]	68.9[28720]
Ampicillin/sulbactam	22.1 [11837]	24.5 [14780]	23.2[16979]
Cefotaxime	15.8 [22524]	20.2 [24880]	22.9[27020]
Ceftazidime	11.7 [26967]	14.8 [28418]	17.1[29824]
Cefoperazone/sulbactam	1.8 [9063]	2.5 [6664]	-
Ciprofloxacin	21.2 [24473]	23 [27168]	23.4[29400]
Gentamicin	11.8 [27843]	12.3 [28041]	12.8[28888]
Imipenem	0.2 [25456]	0.2 [26978]	0.3[28696]
Meropenem	0.3 [24351]	0.3 [26510]	0.3[27759]
Trimethoprim/sulphamethox	43.4 [24967]	43.8 [26672]	41.5[27963]
Piperacillin/tazobactum	2.6[14035]	3.1[20301]	2.9[23202]

Sources : (Kumar *et al.*, 2014)

In advanced of create new antimicrobial drug, the future however faced the problem with the increases of antibiotic's bacteria resistant. Appearance of bacteria that resistant to antibacterial agents is a serious concern to worldwide countries in aspect of health. Discovery of new drug to overcome this issues are crucial but the time to counter the medicine is running out. Antimicrobial drug or antibiotic are produced based on their capability inhibition of bacteria multiplication (Anderson *et al.*, 2012).

The efficacy of present time medicine is limited by the world concern issues of antibacterial resistance. Due to natural constituents of bacterium, effect of mutation or gene crossing become main reason of antimicrobial's resistance. As a result of such irresponsible action, the effectiveness of antibiotics for treating illness or infection is decreasing because bacterial infection often failed to respond to standard treatment. Excessive usage of antibiotic also contributes to the enhancement of immune bacterial cases such as Methicillin Resistant Staphylococcus Aureus (MRSA) as well as Vancomycin-Resistant Staphylococcus aureus (VRSA) (Coates *et al.*, 2002).

Antimicrobial drug could be origin from microbial itself or synthetically manufactured. Antimicrobial drug well-known essentially good for health for both human and animals applied. Component of the drug have the capacity to selectivity inhibit growth of pathogen. Different mechanisms inhibition depends on antimicrobial drug.

Antimicrobial drug named as bactericidal or bacteriostatic depends on it inhibition of pathogenic micrrorganisms. Table 2.3 shows therapeutic indications for the aminoglycoside antibiotics. Range of bacterial are classified on different spectrum, broad spectrum, intermediate spectrum and narrow spectrum depends on it susceptibility to these agents. Through the biochemical pathways that involved in the biosynthesis of essential components bacterial cell, antimicrobial drug act inhibiting through it. Antimicrobial drug main target of bacterial components are inhibition of cell wall synthesis, damage functionallity to cell membrane, inhibition nucleic acid synthesis, inhibition of protein synthesis and metabolic processes (Munita & Arias, 2016).

Table 2.3: Therapeutic indications for the aminoglycoside antibiotics

Aminoglycoside antibiotic	**Indications**
Gentamycin	Septicaemia, burns, endocarditis, used topically to treat eye and ear infections, pneumonia, Listeria meningitis
Neomycin	Suppression of intestinal bacteria prior to colonic surgery
Streptomycin	M. tuberculosis
Amikacin	Serious infections caused by Gram negative bacteria resistant to gentamicin.

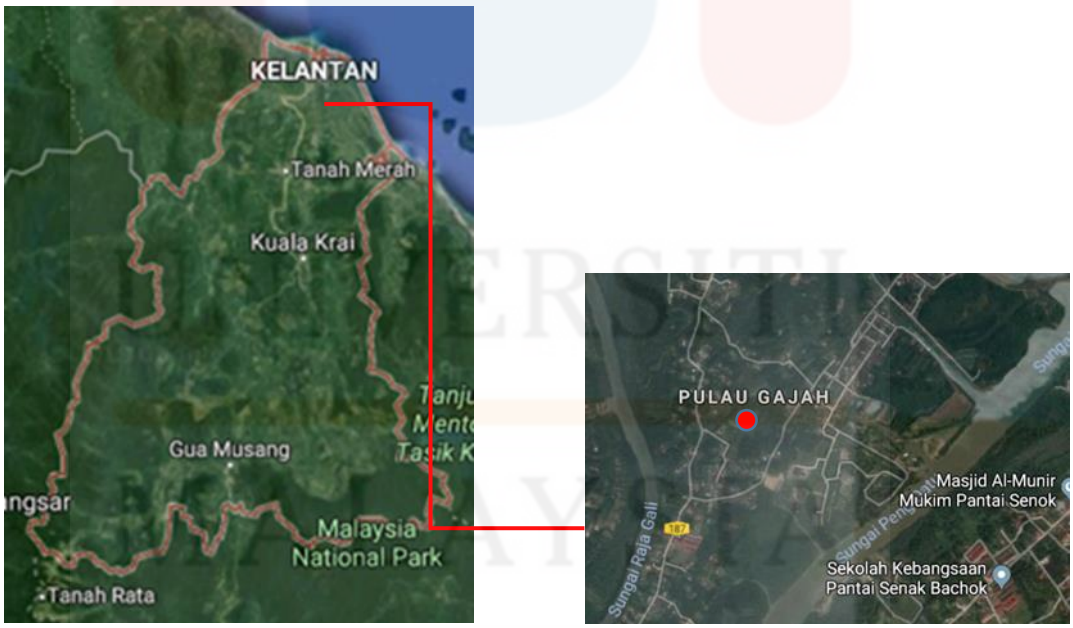
(Anderson *et al.*, 2012)

CHAPTER 3

MATERIALS AND METHOD

3.1 Study area

The sample of *L. zeylanica* were used in this study. The plant material were collected from Pulau Gajah, Pengkalan Chepa, Kelantan (6˚09'40.9"N). Figure 3.1 showed location of plant sample was collected. *L. zeylanica* plant are found abundantly distributed in the bushes area.



(Google Maps)

Figure 3.1: Location of Study Area

3.2 Plants Materials and Extraction

3.2.1 Preparation of Plant Sample

The leaves of plant sample were cut, clean wash with distilled water, dried in the oven at 38 ◦C (Figure3.1). The plant sample leaves were grinded and blend into smaller pieces, until become powdered texture and being stored at 4 ◦C**.** Next, 50 g of shade dried pulverized plant leaves were placed inside a thimble filter paper, which then used Soxhlet extractor. Plant sample goes through extraction by Soxhlet apparatus used of 350 ml methanol (Merck, 70%). The methanolic extract obtained then was filtered using filter paper. The extraction then concentrated more under vacuum rotary evaporator (80 °C, 110 RPM) and been stored at 4°C until next use (Babu *et al.*, 2016).

Figure 3.2: Dried *L.zeylanica* leaves after oven at 38◦C

3.2.2 Different Concentration of Plant Extract

Different concentration of methanolic leaves extract were used from 100 µg/µL to 600 µg/µL. 20 g of crude extract with 10 ml of methanol solvent to produced stock solution of extract. The concentration of plant extract were determined by using equation :

$$M_1V_1 = M_2V_2$$

3.3 Bacterial Preparation

3.3.1 Bacteria Gram-strain

Two bacterial strains were used for the antibacterial test *(E.coli and S. aureus).* All of the bacteria strain have been identified, tested and characterized by culturing in the specific appropriate media of rapid testing gram's staining. Stock cultures of both bacteria were obtained from Microbe Technology Laboratory, Universiti Malaysia Kelantan.

A technique to differentiate variety group type of bacteria based in their cell wall constituents called Gram stainning were tested. The Gram stain procedure differentiate between Gram positive and Gram negative groups by observed different coloured cell red or violet. Three processes involved with staining with water-soluble dye crystal violet, decolorization, and counterstaining, with safanin. Thickness of peptidoglycan in cell membrane that differentiated both between Gram positive and Gram negative bacteria. Retained with crystal violet stain is a Gram positive bacteria due to thicker peptidoglycan layer while Gram negative bacteria decolourise violet stain and instead stained by the safranin colour during staining process (Bruckner, 2016).

3.3.2 Serial Dilution of Bacterial Culture

The cultures were streaked upon Nutrient Agar Medium (NAM) and incubated overnight. One colony was inoculated into 3 ml of nutrient broth and incubated until turbid. The test microbe was taken from the broth culture with an inoculating loop and transferred serial bottle for serial dilution of bacteria. 10×6 dilution factor were used and 100µL of each factor spread to agar**.** Plates were incubated at 37℃ for 24 hours to observe nutrient agar plate to recognize the best colonies formation of bacterial growth**.**

3.3.3 Media Preparation for Antibacterial Activity

Bacterial strains preserved in nutrient agar at 4°C were revived in nutrient broth (liquid medium) and incubated at 37±1°C overnight. For testing of the anti-bacterial activity, NAM medium was used. NAM were prepared by dissolving 38 g agar powder in 1000 ml of distilled water and brought to boil to completely dissolve The medium was autoclave at 15 lbs for 20 min at 121ºC. Furthermore, to test the antimicrobial activity on agar plates, the agar test plates were prepared by pouring about 15 ml of the medium into 10 cm Petri dishes under aseptic condition and left for 2 hours to solidify the medium.

3.4 Antibacterial Activity of the Leaves Extract

3.4.1 Antibacterial screening by Disc Diffusion technique

The disc diffusion assay methods (Gebreyohannes *et al.*, 2013) were used to determine bacterial growth inhibition of leaves extracts. In disc-diffusion method, Whatman No.1 filter paper were used for making the dics. This experiment is carried out in the laminar flow to avoid contamination as it could easily to occur. Filter paper disc of 6 mm diameter using Whatman No.1 filter paper will be prepared and sterilized. Paper discs impregnated with different concentration of plant leaves extraction are placed on the surface of the nutrient agar medium. Diluted bacterial culture of *E.coli and S. aureus* were spread over nutrient agar plates with a sterile glass L-rod. 20 µl of the each extract were applied to each filter paper disc of 6 mm diameter Whatman No.1 filter paper and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate of 3 discs plate and the plates were inoculated at 37±1°C for 24 hours. The standard well of 10 mm diameter injected with antibiotics Gentamicin (10µg/disc) was used as positive control for antibacterial activity and methanol solvent as negative control.

After incubation, the diameter of the inhibition zones was measured with digital calliper. The zone of inhibition is a parameter to determine a particular bacterium whether it susceptible to the action of a particular antimicrobial agent that is plant extract (Babu *et al.*, 2016).

3.5 Extraction

The Soxhlet apparatus as in Figure 3.3 was followed according to the type of solvent used,and depedns on polarity of solvent and sample. For example, methanol as solvent was used to extract the plant leaves . The extraction solvent was placed in the boiling flask. The mantle was heated up with solvent in the bottom flask. When the methanolic solvent was boiling, the vapour produced rises through the tube that vertically into the top of condenser. In the other hand, the vapour then produce liquid condensate drip straight into thimble filter paper located at centre of the soxhlet. The thimble filter paper contains powdered plants samples to extracted. The powdered plant extract absorbed through the pores of thimble and fills up the siphon tube, where it then flows back down into the round bottom flask. This process was continuous and carried out until the plant sample was done extracted (Yusup *et al.*, 2015).

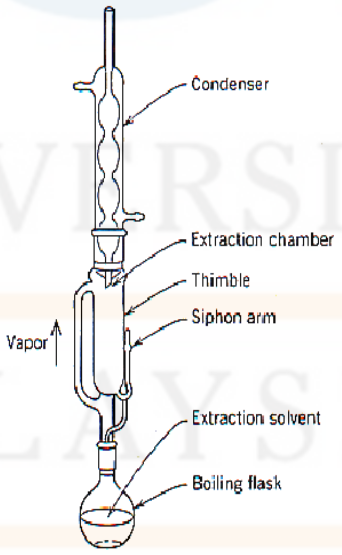


Figure 3.3: Soxhlet extractor (Yusup *et al.*, 2015)

3.6 Data analysis.

Statistical analysis was obtained by using SPSS 15.0 version. Post hoc tests and One way ANOVAwas carried out. Data from zone of inhibition of antibacterial activity were expressed as a mean (±SD) followed by (Tukey) a pair wise comparison of means for all statistical analysis. Comparative statistical analysis between means was calculated and *p* value less than 0.05 was considered as significantly different.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Antibacterial Susceptibility Test

4.1.1 Disc Diffusion Assay

L..zeylanica leaves extract of antibacterial activities are summarised in Table 4.1. The results showed that methanol solvent in leaves extract affecting in different concentration in against both bacterial strain. From the results, the highest antibacterial activities leaves extracts against both bacterial strains *E. coli* and *S. aureus* was 600 µg/µL, with zones of inhibition of (12.1±0.5mm) and (16.3±0.9) mm respectively. The results for the antibacterial actvities of plant extracts were presented in Figure 4.1. The results specify that concentration 100, 200, 300 µg/µL showed a significant difference for mean for *E. coli* as *p* value less than 0.05. While, for *S. aureus* the result indicated significant differences at concentration of 100 and 400 µg/µL. However, the methanolic leaf extracts at concentrations of 100 µg/µL showed lowest antibacterial activity for both bacteria activity.

Table 4.1: Inhibition zone of *L. zeylanica* leaves extract

		Zone of Inhibition (mm)	
Extract	**Concentration (µg/µL)**	*E. coli*	*S.aureus*
Leaves	100	10.7 ± 0.9	10.7 ± 0.4
	200	11.0 ± 0.6	12.4 ± 0.4
	300	11.3 ± 0.6	12.6 ± 0.8
	400	11.9 ± 0.8	14.9 ± 0.8
	500	12.0 ± 0.4	16.2 ± 0.7
	600	12.1 ± 0.5	16.3 ± 0.9
	Methanol	14.2 ± 0.3	17.5 ± 0.4
	Gentamicin	21.7 ± 0.6	25.3 ± 0.3

*Zone of mean ± SD for n=2. Statistical analysis to be significant p <0.05

After incubation for 24 hours, leaves extract antibacterial activity was compared with positive control, Gentamicin (10µg/disc). The mean zone of inhibition produced by the standard drug, Gentamicin, was (21.7 ±0.6mm) for *E.coli* and (25.3±0.3mm) for *S. aureus*. Gentamicin showed larger inhibition compared with inhibition produced by methanol extracts which was between (14.2±0.3 mm) for *E.coli* and *S.aureus* (17.5±0.4mm). Overall from the data showed, Gentamicin and methanol as positve and negative control showed the highest antibacterial activity compared to all the extract leaves..

The result are simplified and ilustrated as bar graph shown in Figure 4.2 and Figure 4.3. The result from figure was obtained by bar graph plot mean bacteria against concentration. Based on the results, highest zone of inhibition compared with all the extracts was showed at concentration 600 ug/uL.

All the selected leaves extract *L.zeylanica* shown antibacterial activity against bacterial strain *E. coli* and *S. aureus.* The diameter zone of inhibition increasing with increase of concentration which described it mean directly proportional.

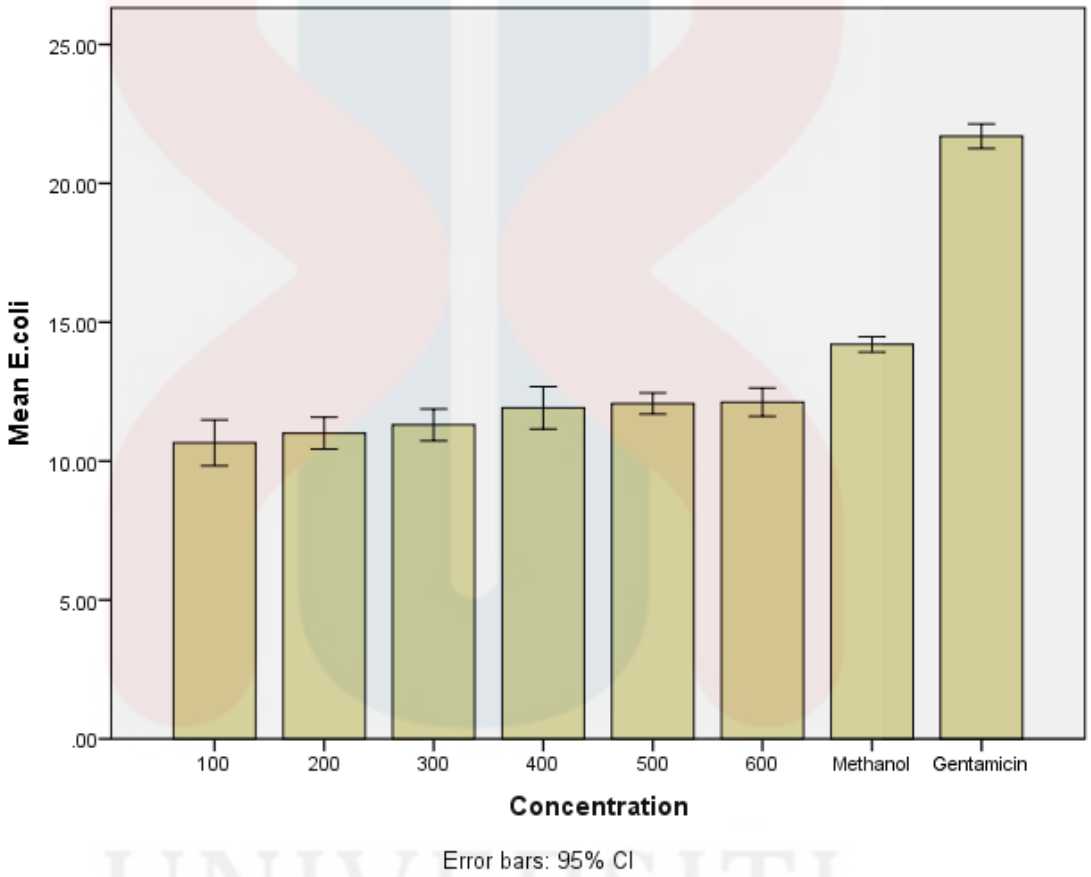


Figure 4.1: Antibacterial activity of leaves extract against *E.coli*

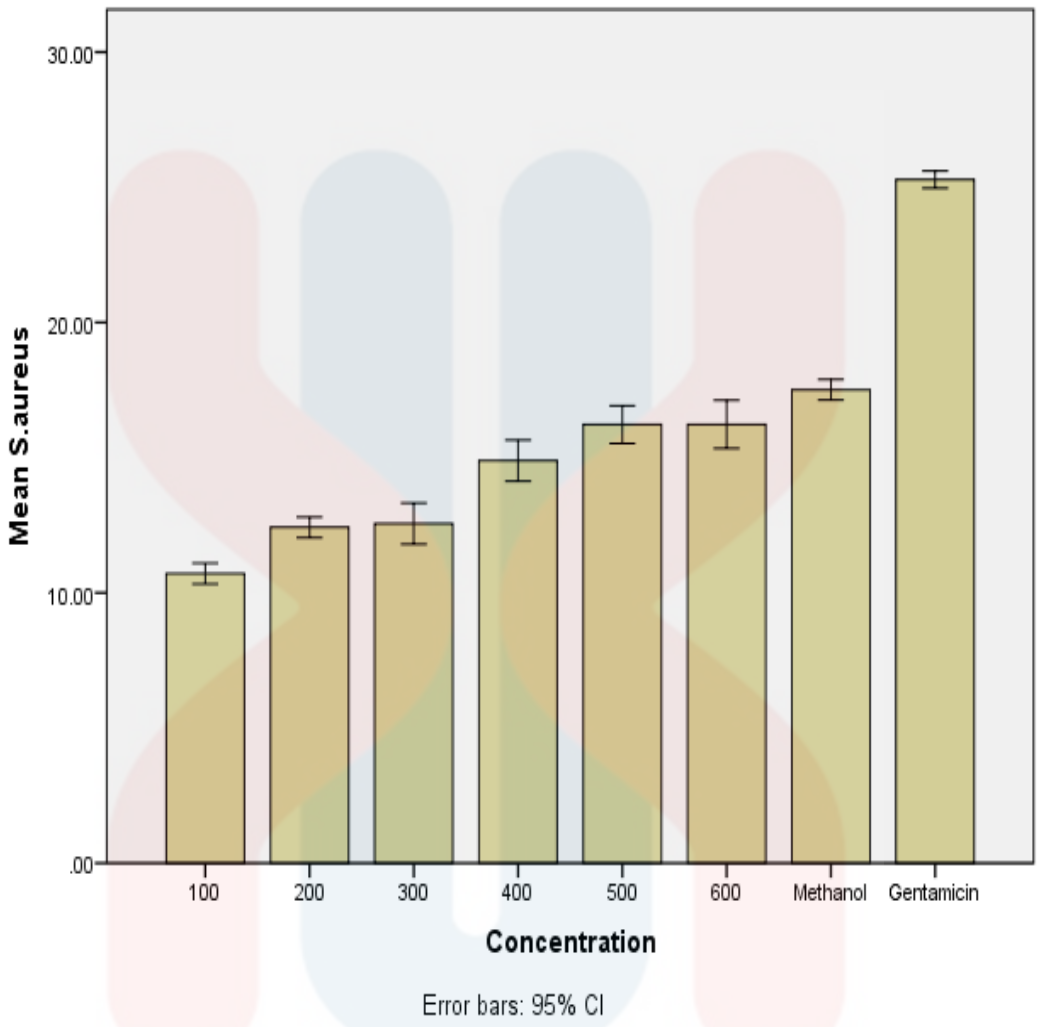


Figure 4.2 : Antibacterial activity of leaves extract against *S. aureus*

Methanolic extract of *L. zeylanica* leaves that showed antibacterial activity were assessed by agar disc diffusion method . The activity was investigated against two bacteria strains. The concentrations were 100 to 600 µg/µL leaves extract. Results revealed that the leaves extract showed vital antibacterial characteristics. All the concentrations of leaf extract inhibited the bacterial, eventhough with different ranges of inhibition.

From the result, we can say that all concentration shown inhibition against bacteria, leaves extract plants might contain compounds with therapeutic activity that able leaves extract to act as antibacterial as this proved by (Aboaba & Efuwape, 2001) stated that constituents in plant that contain non toxic glycosides which able to hydrolyzed and emitted phenolics which able to give toxicity to microbial pathogens. phytochemical properties that found in most common plants extracts showed the presence of tannin, glycoside and flavonoid.

Both Gram negative pathogen , *E. coli* and Gram positive strain, *S. aureus* was chosen in these research as these pathogens are increasingly potential to be antibiotic resistance pathogens. This is due increased of pathogen antibiotic resistance as antibiotic uses are at rise. From the result it showed that inhibition of *S.aureus,* Gram positive strain showed larger inhibition zone compared to *E.coli,* Gram negative strain. This was being stated by Parekh *et al.* (2006) in his research by 12 plant species, the extract (aqueous and methanol) of plant *Caesalpinia pulcherrima Swartz* were more active inhibit or showed antibacterial activity against the Gram-positive microorganisms than against the Gram-negative microorganisms.

On the other hand, Rabe and Van Staden (2000) stated methanol extract of *Warburgia salutaris* was the only one that showed antibacterial activity against *E. coli,* a Gram-negative bacterium while other extract does not showed any activity against *Klebsiella pneumonia*, Gram-negative strain. Thus, in general, it showed that Gram negative bacteria have higher resistant than Gram-positive ones.

In this research, Gentamicin produced the largest ranges. The mean zone of inhibition produced by the Gentamicin, was largest compared to those produced by leaves extracts and methanol. This is due to the fact that bioactive components in commercial antibiotics contain higher concentration comapred to plant extract that might have amount of bioactive compound (Anderson *et al.*, 2012).

However, from the result shown concentration 600 µg/µL for both strain were shown largest diameter zone of inhibition. This is due to methanolic components that contain in leaves plant were the highest among all of the extract concentration affecting the antibacterial activity. Besides, there are several factor that influence antibacterial activity. Research done by Soberón *et al.* (2007) stated that antibacterial activity possible to differ according to the applied extractive method and possibly influence by glycoflavonoid contain in plant.

In addition, growth area is another factor that affecting bioactive components of the plants and effect the antibacterial activity difference. Proved in research done by (Liu *et al.*, 2016) stated that environmental factors affect the types and active components and antioxidant activity of *Potentilla fruticosa L.* from different regions of China. Another limitation of antimicrobial activity may be attributed by method extraction method the use of crude extract. Another extraction method to be considered was percolation extraction, subfraction, semipure compound, or pure compounds isolated are believe to exhibit antibacterial activity more efficiently (Bishnu P.Marasini *et al.*, 2015).

Next, according to (Arunbala *et al.*, 2014) the active ingredients parts of plant such as leaves are better efficacy extract with methanol. Plant leaves with methanolic extract posses antimicrobial activity in only duration of 2 days at dose ranges of 50 – 100 mg/ml agaisnt bacteria *E.coli, S.sureus, Bacillus* and *Salmonella.*

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, these study determine the antibacterial activity of *L. zeylanica* leaves extract against two bacteria. The results indicate that the leaves extract possess antibacterial activity as concentration increases. However, methanolic extract are one of the factor affecting the antibacterial activity. From the result 600 µg/µL the highest concentration of leaves extract indicated showed the highest inhibition against both bacteria. Overall, all the concentration of *L. zeylanica* methanolic leaves extract showed good inhibition antibacterial activity against the tested pathogenic bacteria species causing infections, even shows inhibition at different ranges. This revealed that these extracts contained active compounds which were able to inhibit the growth of bacteria.

5.2 Recommendation

Based on medicinal plant traditional knowledge, the leaves extract able to become medicinal medicine plant- based and show better efficacy and found suitable drug than low spectrum antibiotics like penicillin, amoxicillin or amoxyclav. The ability of bacteria to become antibiotic pathogen resistence to antimicrobial agents has

become a significant problem are continue to develop. Thus, continues research and development of new natural antibiotics are vital.

The leaves extract shown able to be potential antibacterial based on the antibacterial activity susceptibility test. However, further pharmacological and studies are vital to assured this recommendation. Further analysis of phytochemicals needed to carried out as to identify characterization of the compounds contain *in L.zeylanica* that believe able to act as antibacterial agents. This studies denoted that *L. zeylanica* potentially to be a natural antibiotics or natural- based medicine for human health, and as antibacterial agent.

Results that shows therapeutic potency of selected plant been used in natural medicine and potentially to further in pharmaceutical field within future research. In addition, further phytochemical and pharmacological investigation are required for the medicinal plant in treat various diseases. Overall, the results of research support natural alternative of plant extract that possess compounds with antibacterial properties againts several pathogen. New drug or medicine based on natural alternatives required to undergo further pharmacological evaluation.

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APPENDIX A

Preparation of Plant Extract

Figure1: *L.zeylanica* methanolic leaves using soxhlet extractor

Figure 2*: L.zeylanica* methanolic leaves using rotary evaporator

APPENDIX B

Preparation of Bacterial Culture

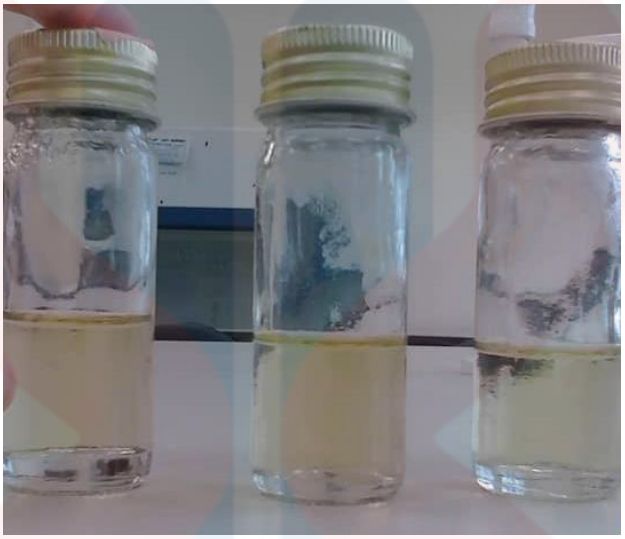

Figure 1: Bacterial culture in universal bottle after incubate at 37◦C

Figure 2: Cultured *E. coli*

APPENDIX C

Preparation of Antibacterial Susceptibility Test

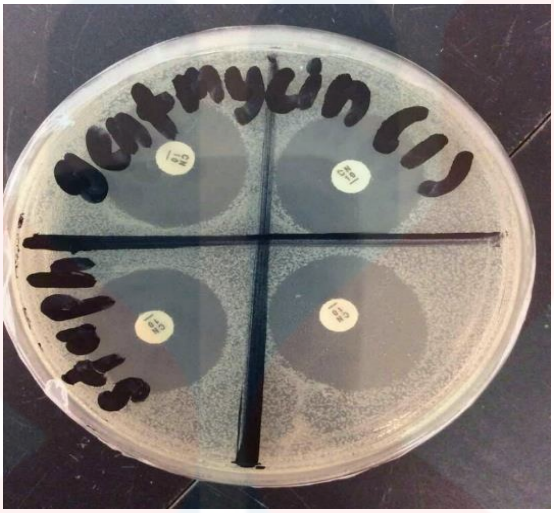

Figure 1: Inhibition zone of antibiotic Gentamicin against *S.aureus*

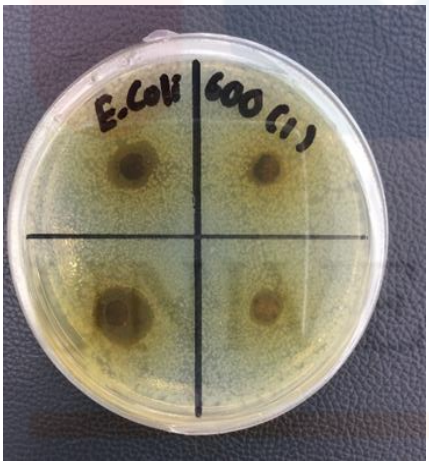

Figure 2: Inhibition zone of plant extract 600 µg/ µL against *E. coli*

Figure 3: Inhibition zone of plant extract 600 µg/ µL against *S .aureus*