EVALUATION OF ANTIMICROBIAL ACTIVITY OF *ECLIPTA ALBA* LEAF EXTRACT FOR FOOD PRESERVATION

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by

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LIST OF SYMBOLS

P	Level of Significance
Mg	Microgram
G	Gram
%	Percentage
Mg	Milligram
^{o}C	Celsius

LIST OF ABBREVIATIONS

AEEA	Alcoholic Extract of *Eclipta alba*
AKI	acute kidney injury
$AlCl_3$	Aluminum chloride
DMSO	Dimethyl sulfoxide
EMJH	Ellinghausen-McCullough-Johnson-Harris
FAO	Food and Agriculture Organization
HCl	Hydrochloric acid
INCI	International Nomenclature for Cosmetic Ingredients
LERG	Leptospirosis Burden Epidemiology Reference Group
LPS	Lipopolysaccharide
$NaNO_3$	Sodium nitrate
NaOH	Sodium hydroxide
NCCLS	National Committee for Clinical Laboratory Standards
OMPs	Outer Membrane Proteins
QTOF-MS	Quadruple time of flight mass spectrometer
RRLC	Rapid Resolution liquid chromatography
RS	rabbit serum
RTE	Ready-to-eat
SEM	Scanning Electron Microscopy
SPSS	Statistical Package for the Social Sciences
TCM	Traditional Chinese Medicine
TEM	Transmission Electron Microscopy
TNF-α	Tumor necrosis factor-alpha
WHO	World Health Organization

PENILAIAN AKTIVITI ANTIMIKROBIAL EKSTRAK DAUN *ECLIPTA ALBA* DALAM PENGAWETAN MAKANAN

ABSTRAK

Leptospirosis yang disebabkan oleh leptospira, sejenis bakteria spirochete, adalah sejenis penyakit zoonotik dan mudah dijangkiti, terutamanya pada manusia, di seluruh dunia. Penyakit ini terjadi apabila manusia terdedah kepada air atau persekitaran yang dicemari oleh patogen yang dikenali sebagai *Leptospira interrogans (L. interrogans),* terutamanya semasa banjir. Di Malaysia, kejadian penyakit ini dilihat meningkat semasa musim banjir kerana mangsa terambil makanan yang tercemar dengan *L. interrogans* atau terdedah kepada tisu, darah atau air kencing haiwan yang telah dijangkiti Leptospira. Terdapat banyak tumbuhan herba di Asia yang menunjukkan aktiviti antileptospira dan salah satu tumbuhan yang dikatakan memiliki aktiviti antileptospira ialah *Eclipta alba (E. alba)* ataupun lebih dikenali sebagai 'false daisy'. Kajian ini telah dilakukan untuk menyiasat aktiviti antimikrob *Eclipta alba* terhadap *L. interrogans* dan menerapkan polifenol antimikrob *Eclipta alba* dalam penyediaan makanan untuk mencegah jangkitan Leptospira. Dalam kajian ini, sampel *E. alba* yang diambil dari Taiping (Perak), Segamat (Johor) and Pulau Pinang telah diuji untuk membuktikan aktiviti antileptospira *E. alba.* Sejumlah 96.7 ± 0.9 mg/g flavonoid telah diekstrak daripada sampel *E. alba* dan kandungannya didapati sama dengan jumlah rutin bagi polifenol tumbuhan tersebut. Seterusnya, melalui teknik *disc diffusion assay* serta *broth dilution assay,* aktiviti antileptospira telah dibuktikan namun kajian ini mendapati bahawa tahap aktiviti antileptospira *E. alba* adalah lebih rendah berbanding disk antibiotik Penicillin G. Penemuan ini ditunjukkan oleh z*one of clearance* di mana

ukuran zon tersebut lebih luas bagi disk antibiotik Penicillin iaitu 14 ± 0.8 mm berbanding dengan luas zon bagi disk ekstrak polifenol daun *E. alba* leaves iaitu 10 ± 0.9 mm. Melalui *broth dilution assay*, didapati bahawa kepekatan minimum polifenol *E. alba* untuk menghalang pertumbuhan *L. interrogans* adalah 1250 µg/ml dan patogen tersebut kehilangan bentuk *cork-screw* dan *hook* sepenuhnya selepas pendedahan selama 14 hari kepada polifenol daun *E. alba.* Bagi pengesahan lanjut tentang keberkesanan aktiviti antileptospira *E. alba* dalam makanan, nasi yang dimasak dan teh yang diokulasi dengan *L.interrogans* telah dirawat dengan ekstrak *E. alba.* Hasilnya, bacaan OD bagi makanan yang telah dirawat dengan ektrak *E. alba* adalah lebih rendah daripada bacaaan OD bagi sampel yang tidak dirawat dengan ekstrak *E. alba.* Dalam erti kata lain, kebolehan *L.interrogans* untuk hidup dalam makanan adalah terjejas dengan kehadiran ekstrak *E. alba.* Kesimpulannya, kajian ini jelas menunjukkan dan membuktikan tumbuhan *E. alba* mempunyai aktiviti antileptospira yang mampu mengelakkan serta mengurangkan kejadian penyakit leptospirosis semasa musim banjir.

EVALUATION OF ANTIMICROBIAL ACTIVITY OF *Eclipta Alba* LEAF EXTRACT FOR FOOD PRESERVATION

ABSTRACT

Leptospirosis caused by Leptospira, a spirochete bacterium, is a zoonotic and highly contagious disease, especially in humans, worldwide. The disease occurs when humans are exposed to water or the environment polluted by pathogens known as *Leptospira interrogans (L. interrogans*), especially during floods. In Malaysia, the incidence of this disease is seen to increase during the flood season because the victims take food contaminated with *L. interrogans* or exposed to the tissues, blood or urine of animals that have been infected with Leptospira. There are many herbal plants in Asia that show antileptospira activity and one of the plants that is said to have antileptospira activity is *Eclipta alba* (*E. alba*) or better known as 'false daisy'. This study was conducted to investigate the antimicrobial activity of *Eclipta alba* against *Leptospira interrogans* and to apply the *Eclipta alba* antimicrobial polyphenol in food preparation to prevent the Leptospira infection. In this study, *E. alba* samples collected from Taiping (Perak), Segamat (Johor) and Penang were tested to prove the antileptospira activity of *E. alba*. A total of 96.7 ± 0.9 mg/g flavonoid were extracted and this is equivalent to rutin for *E. alba* leaves polyphenol. Furthermore, through the disc diffusion assay and broth dilution assay, the antileptospira activity has been proven but this study found that the level of antileptospira *E. alba* activity is lower than Penicillin G antibiotic. This finding is indicated by the zone of clearance where the zone size is wider for Penicillin antibiotic which is 14 ± 0.8 mm compared to the zone area for *E. alba* leaves polyphenol extract which is 10 ± 0.9 mm. Through the broth dilution assay, it was

found that the minimum concentration of *E. alba* polyphenols to inhibit the growth of *L. interrogans* was 1250 μg/ml and the pathogen lost its cork-screw and hook form completely after 14 days of exposure to *E. alba* leaf polyphenols. For further confirmation of the effectiveness of antileptospira *E. alba* activity in food, cooked rice and tea coated with *L.interrogans* were treated with E. alba extract. As a result, the OD readings for foods treated with *E. alba* extract were lower than the OD readings for samples not treated with *E. alba* extract. In other words, the ability of *L.interrogans* to survive in food is impaired by the presence of *E. alba* extract. In conclusion, this study clearly shows and proves th*at E. alba* plant has antileptospira activity that can prevent and reduce the incidence of leptospirosis during the flood season.

CHAPTER 1

INTRODUCTION

1.1 Leptospirosis infection in Malaysia

Food contaminated with pathogenic microbes is a common and worrying problem when flood happens and it is usually attributed to poor hygiene by food handlers. During flood, there is a rise in pathogenic infections such as *Leptospira interrogans* when the flood victims consume the contaminated water and food polluted without proper hygienic food handling and this is because the flood water may contain raw sewage, human and infected animal faeces as well as chemical wastes. Food that are ready to eat (RTE) during and after the flood can be contaminated with bacteria as well as when it is not handled hygienically thus our ambient temperature is ideal for the microorganism to multiply fast.

Leptospirosis is from the genus *Leptospira* and known as a global zoonotic disease caused by spirochetes. The pathogenic strains of *Leptospira interrogans* has several serovars and known to cause leptospirosis (Benacer *et al*., 2013). This disease transmission shoots up during rainy season and as the water accumulates and stays stagnant during flood (Seguro & Andrade, 2013). The downpour moves the leptospires to the surface of the soil and water bodies because accumulation of water tends to clean up rat holes.

The first report of leptospirosis in Malaysia was in 1925 (Yaakob *et al*., 2015). Most studies reported that leptospirosis in Malaysia as endemic where the high-risk affected human group are sewage workers, cleaners, military personnel, abattoir workers and recently individuals involved in recreational activities that are involved in social activities (Garba *et al*., 2017). However, it is a seasonal epidemic where the incidents of leptospirosis cases increase tremendously following massive floods in the year 2014 (Garba *et al*., 2017). There were ninety-four (94) leptospirosis cases reported after flood that hit the state of Kelantan, Malaysia and it was only reported to have twenty to twenty-five cases (20 - 25) before the flood (Sasidharan *et al*., 2015). There is a great need for improvement in the surveillance which will then help to identify control and prevention mechanism for morbidity and mortality related to leptospirosis. In light of this, under the Prevention and Control of Infectious Disease Act 1988, leptospirosis has been gazetted as a notifiable disease in December 2010 (Yaakob *et al*., 2015).

Human acquire leptospirosis when come into direct contact with tissues, blood, organs or urine of infected animals. Leptospires indirect portal entry is through cuts or abrasions in the skin when it gets in touch with polluted water (Seguro & Andrade, 2013). Leptospirosis in human to human transmission is rare but it still can be transmitted through sexual intercourse and breastfeeding (Yaakob *et al*., 2015). At its onset, leptospirosis is often misdiagnosed as influenza, aseptic meningitis, hepatic disease or fever (pyrexia) of unknown origin unless the patient shows symptoms of Weil’s disease such as fever plus jaundice, pulmonary haemorrhage and renal failure which can leads to death as well (Budihal and Perwez, 2014).

Domestic animals and wild rodents are carrier hosts of leptospires and natural reservoirs which occasionally act as maintenance and accidental hosts. They usually develop the shed leptospiral cells and chronic renal infection and can continue to survive in their urine for a prolonged period of time. It could possibly invade a new host (human and other animals) through contaminated water or soil (Garba *et al*., 2017).

1.2 *Eclipta alba* leaf extract as natural food preservative/ antimicrobial agent

Numerous plant species extracts have become prominent in present years, and attempts to characterize their bioactive compounds have been accelerated for many pharmaceutical and food processing applications. Followed by the current issues which is associated with the use of chemical preservatives, the antimicrobial properties of plants have gained attention. Due to consumers negative perspectives about synthetic preservatives, the focus has been diverted towards natural alternatives.

The objective of food preservation is the capability to produce safe food product with extended storage life which is acceptable to the consumer according to the relevant food standard guidelines. The processed food with limited refrigerated shelf-life has got high demand among the consumers. Researches has improved processing technologies which lowers the risk of microbial poisoning such as *Leptospira* poisoning.

Therefore, in preventing strategy in safe food preparation during and after flood, the findings suggest that *Eclipta alba* extracts and their polyphenol compounds have good potential as natural antimicrobial substances against *L. interrogans* poisoning. To the best of our knowledge, most of the recorded studies have looked at the effectiveness of the plant extracts against food related pathogenic bacteria which causes food spoilage. There is no any literature review about the screening for antimicrobial action against native microbiota of the food or microbiota at inconsistent time of the storage period, and changes during processing and packaging. Therefore, this thesis would be one of the initial research working in this field.

1.3 Objectives of research

1. To investigate the antimicrobial activity of *Eclipta alba* against *Leptospira interrogans;*
2. To investigate the mode of action of *Eclipta alba* against *Leptospira interrogans* by electron microscopy observation; and
3. To apply the *Eclipta alba* antimicrobial polyphenol in food preparation to prevent the Leptospira infection

1.4 Study Scope

The early step of the study was the collection of *E. alba* leaves from various parts of Malaysia followed by washing, drying and grinding into fine powder. The powdered *E. alba* leaves extraction was carried out as characterized by Crozier and colleagues (1997) with some alteration. The total flavonoids content of leaves was evaluated by the method described by Zhishen and friends (1999).

The chromatogram of *E. alba* leaf extract was then analyzed for standardization purpose. Quantification of rutin was then conducted using the Agilent 1200 series Rapid Resolution liquid chromatography (RRLC) system tandem with an Agilent 6520 Accurate-Mass quadruple time of flight mass spectrometer (QTOF-MS) (Agilent Technologies, USA).

In order to investigate the role of *E. alba* polyphenol to prevent leptospirosis, few methods were performed; antimicrobial activity (determination of microbial inhibitory concentration and growth profile) evaluation, study of the mode of action of *E. alba* polyphenol against *L. interrogans* by electron microscopy observation and analysis of survivability of *L. interrogans* in cooked rice and tea.

Statistical analysis was carried out by using the Statistical Package for the Social Sciences (SPSS). Data was given as the Mean ± SD. The statistics was performed using t-tests and one-way analysis of variance where the p-value less than 5% was considered statistically significant ($P<0.05$).

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal plants

Medicinal plants by definition, may afford health-promoting properties including antioxidant, anti-inflammatory action, sunlight protection, antimicrobial activity or wound healing activity (Schmidt, 2012). Medicinal plants exhibit a broad scope of biological properties which is useful in fighting human diseases. A vast number of medicinal plants are currently in use all over the world for curing diseases. Granting to the estimation done by World Health Organization (WHO), around 80% of population worldwide use plant-derived medications in treating several types of diseases, including cancer as per the easy accessibility, safe methods of treatment and cost effectiveness of the natural resources (Yadav *et al*., 2017). Furthermore, medicinal plants given more importance owing to the growing need to induce affordable health care despite of the belief that natural cures are more trustworthy and its higher efficacy compared to the conventional drugs (Alsarhan *et al*., 2014).

The intellect and practice about certain plants had the healing power sparked far in the past, even before man discovered the existence of germs. Indeed, the current studies about plant secondary metabolites as antimicrobial principles were also easily taken since then. People of ancient times have been using plants to heal common transmissible diseases and even traditionally some of these medications are even admitted as component of the regular treatment for various types of ailments (Rios & Recio, 2005). Yet, few innovative drugs such as aspirin, digitoxin, tubocurarine, ephedrine reserpine, ergometrine, atropine, and vinblastine and had their roots in traditional herbal medicines (Alsarhan *et al*., 2014) where traditionally the crude plant extract was in use (Khan *et al*., 2013).

All through human history, the famous and established categories of traditional medicines are from Asia, especially those from India (Ayurvedic, Unani, Siddha),Japan (Kampo) and China (Wu-Hsing). Even for thousands of years the concepts of diagnosis and remedy which have been recognized and nevertheless adopted by current practitioners for the same purpose alongside conventional medicines (Alsarhan *et al*., 2014).

2.1.1 Secondary metabolites in medicinal plants and the uses

The bioactive compounds exist in plants are synthesized during secondary vegetal metabolism and known as secondary metabolites (Silva & Fernandes Junior, 2010). Plant secondary metabolites can be split into three chemically distinct groups: terpenoids, phenols (flavonoids) and alkaloids (composed of nitrogen) which are brought out with a vast range of chemical, physical and biological activities (Irchhaiya *et al*., 2014). The majority of bioactive compounds belong to a group of families, which each of it has particular characteristics structure arising from the way in which they are built up in nature (biosynthesis) (Azmir *et al*., 2013).

The major four pathways in the bioactive compounds or in the synthesis of secondary metabolites are: (1) Shikimic acid pathway, (2) Malonic acid pathway, (3) Mevalonic acid pathway and (4) Non-mevalonate (MEP) pathway (Azmir *et al*., 2013). Three major groups of plant bioactive compounds are shown in a simplified illustration of different pathways for the production in Figure 2.1. Of these the bioactive compounds widely dispersed in the plant kingdom are phenolics and polyphenols (Irchhaiya *et al*., 2014).

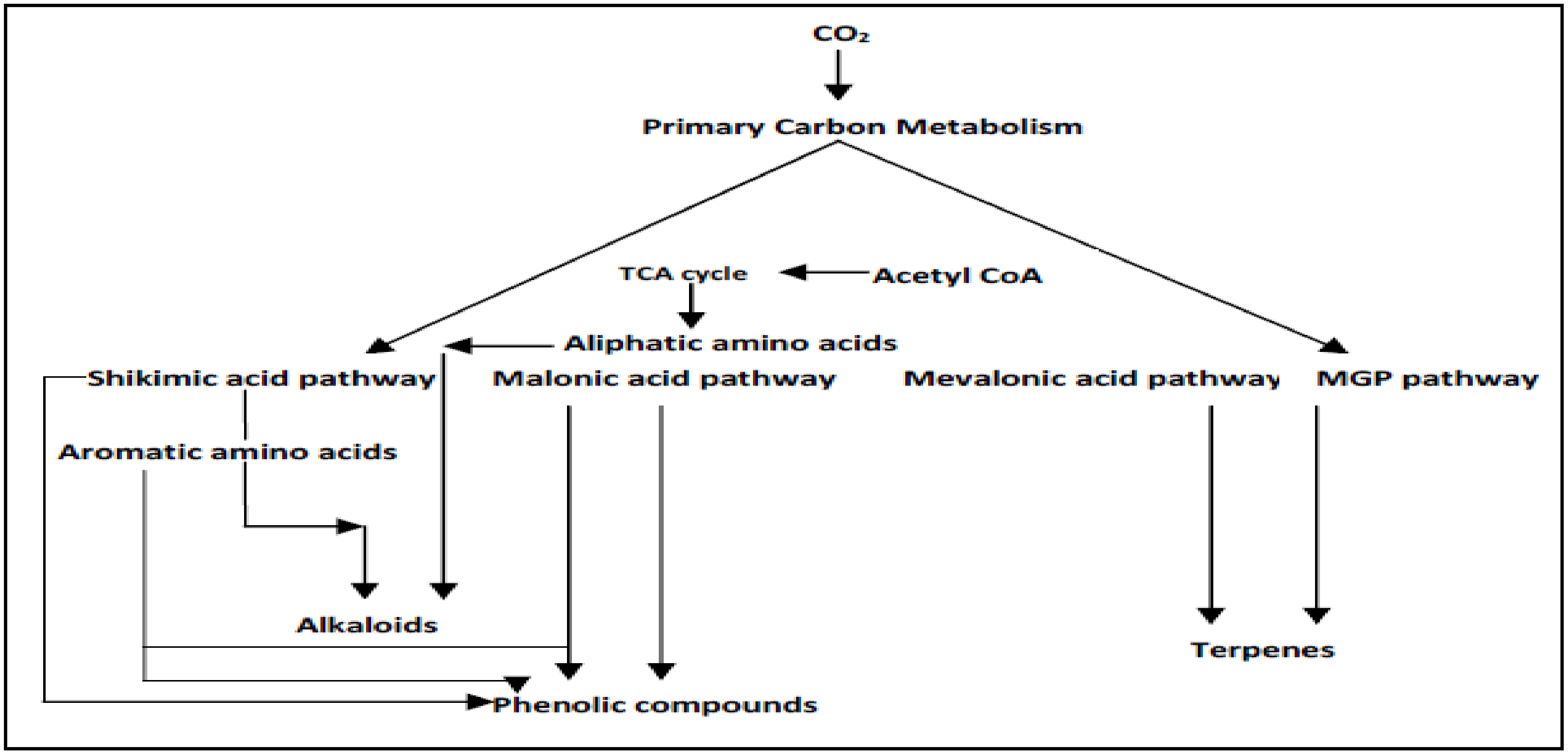

Figure 2.1: A simplified view of pathways for production of three major groups of bioactive compounds of plant resources (Azmir *et al*., 2013)

Both in the past and present years, the medicinal plants has been invariably used as an antibiotic, antimicrobial agents, anti-infection medium, as preservatives, in cosmetics, as drugs in the medical sector and the most important as healing agents in pharmaceutical industries. The increasing demand presently, is gaining momentum for more researches and investigations of the medicinal properties of plants (Rios & Recio, 2005).

2.1.1(a) In Pharmaceutical industry

Lately, the related toxicity issues limit the use of antimicrobial agents and development of antibiotic resistance. Therefore, a forward step taken in reviving the researches on the antimicrobial role of plants against resistant strains in order to maintain the safety and efficacy. The extracts or phytochemicals is found in plant components and acts through a different mechanism than that of antibiotics (Gyawali *et al*., 2015).

In Malaysia, dengue cases have been on the rise since 2002 and currently there is no specific antiviral drug or vaccines available for the prevention and treatment of dengue infection (a viral disease caused by *Aedes aegypti*) (Ahmad *et al*., 2011). Studies were conducted with *Carica papaya leaf extracts* on patients who suffers from dengue fever and dengue haemorrhagic fever; increased the platelet count (Subenthiran *et al*., 2013).

Azadirachta indica (Neem) also acts as an important character every bit ace of the healing agents in disease management. A double blinded clinical drug test was examine directed to check the efficacy of the drug (aqueous extract of neem leaves) in fifty cases of uncomplicated psoriasis taking conventional coal tar regime. The patients portray results of taking drugs in addition to coal tar had a more immediate and better response compared to the placebo results (Alzohairy, 2016).

A recent survey conducted by Khan and his colleagues (2013) reported that, medicinal plants such as *Bergenia ciliate* (roots), *Santalum album* (wood) and *Jasminum officinale* (leaves) performed possible antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris*, and *Bacillus subtilis* the human pathogenic bacteria. Also, on that point is a study delineated the antimicrobial effect of pomegranate extract on antibiotic-resistant forms of *Staphylococcus aureus*; the interactive effect of pomegranate extract with selected antibiotics gave an idea to create new antimicrobial drugs by adding the plant products in order to combat antibiotic-resistant microorganisms (Gyawali *et al*., 2015).

2.1.1(b) In Food industry

Thither is a trend, so-called "functional food" and "functional beverages" which denotes food as a source of nutrition at the same time functions to prevent and heal various diseases (Shikov *et al*., 2017). They may be sorted according to the potential medical benefits and attributes of their constituents. Phytochemicals and antioxidants are one of the classes which is likewise owned by medicinal plants (Rincon-Leon, 2003).

Food preservation is another major topic to be equalled in the food industry, which associated with medicinal plants. The combination of essential oils with electrolyzed sodium chloride for fish (carp fillet) preservation showed that technological advances have far improved (Mahmoud *et al*., 2006). On the other hand, Hoque and the co-workers (2007) have experimented the antibacterial activity of neem (*Azadirachta indica*) and Guava (*Psidium guajava*) extracts against 21 strains of foodborne pathogens which showed a successful result in controlling those spoilage organisms. (In detail in section 2.2)

By the same token, natural food additives such as flavours and colours also have been used for centuries. For instance, the compounds in spices and produced from herbs are the natural agent of self-defense in plants against infectious organisms. Thus in India, food and traditional medicine, many spices are used which performs dual roles as flavouring additive and as bio-preservatives (Hintz *et al*., 2015). The study conducted by Agaoglu and his co-researchers (2007) on antimicrobial studies of some food additives used in meat products such as cloves, cumin, fennel, cinnamon, crushed red pepper, and anise opposing some microorganisms has proved that cinnamon found to be the most effective. Nevertheless, there is a strict protocol on the habit of food additives in the sense of safety, the permitted concentrations and specific permitted food uses.

2.1.1(c) In Cosmetics industry

Cosmetics industries as well use plants as bioactive raw material in personal care products such as personal hygiene products, shampoos, shower products, soaps, sunscreens, skin and hair care products, deodorants, make up, hair dye, lipsticks, toothpastes, dental care products, fragrance and many others). Plant based cosmetics are addressed as cosmeceutical.

The plants use in cosmetics is mainly ascribable to the significant importance given to the safety of natural source and the effectiveness as well as economical purposes. There is competition for market share among the cosmetics companies. Therefore, more new molecules derived from nature and standardized extracts has been introduced. The International Nomenclature for Cosmetic Ingredients (INCI) system incorporates over 800 new ingredients added every year. Currently the trend in Western cosmetics is diverted into Traditional Chinese Medicine (TCM) or Ayurvedic Medicine which may contain extracts or compounds from plant origin (Schmidt, 2012).

Currently, there are many interesting studies of medicinal plants in cosmetics such as use of lemongrass in shampoos to treat *Malassezia furfur* (a yeast associated with dandruff) (Wuthi-Udomlert *et al*., 2011), *Orthosiphon stamineus* extraction of leaf which is an ingredient to reduce oily appearance of skin (Vogelgesang *et al*., 2010), *in vivo* and *in vitro* skin anti-aging activities increased with the addition of gallic acid from *Terminalia chebula* galls (Manosroi *et al*., 2011) and etc.

2.2 Food preservation with bio-preservatives

Literally, from bacteria and fungi antimicrobial agents have been isolated and either produced chemically or made through fermentation (Hintz *et al*., 2015). The high utilization of chemical preservatives in food has imparted significantly to antibiotic resistance. Due to the existence of antibiotic-resistant pathogens, antimicrobial agents could have derived from plants and be an effective and safe alternative, especially to combat pathogen associated with foodborne illnesses (Gyawali *et al*., 2015). To boot, it is a major challenge for food scientist in the assurance of food safety and avoid food quality decay parallel to consumers concern and demand for adding natural additives to reach quality and safe food (Ortega-Ramirez *et al*., 2014).

The bearing of both antimicrobial and antioxidant components in a single molecule make the natural resources to be more efficient and better suited as food preservatives (Hayek *et al*., 2013). The antimicrobial components present in the foods can hold out the shelf life of crude or by reducing the microbial growth rate or viability in processed foods (Tiwari *et al*., 2009). Natural commodities are seen as a fortunate group of structures which have developed to cooperate with a different type of protein targets for particular purposes. The protein structure which is same with little or no variation serves different functions in distinctive beings (plants, humans and microorganisms). In detail, the same protein binding target in various organisms can perform distinct functions in different organisms. Thus, natural products in the angle of molecular evolution have preserved the capability to preferentially bind to this target (Ngwoke *et al*., 2015).

Although there were thousands of plants and herbs used traditionally as food preservatives, there is still less comprehensive research on the implementation of antimicrobial compounds from derivatives other than bacteria. In lodge to satisfy consumer need for natural conservatives and the speedy rate of herbal plant species extinction particularly, it is crucial to conduct more researches on the implementation of plant antimicrobials to safety in food (Hintz *et al*., 2015).

2.3 Biodiversity prospect of medicinal plants in Malaysia

Malaysia has been classed as one of the twelve mega diverse countries in the globe. This sets the luxuriance of variation of wild natural resources in Malaysia's tropical rainforest and is edible by human beings. The plants in tropical rain forest are considered biologically and chemically diverse resource as they produce various chemicals as defence agents against diseases, pest and predators. The Food and Agriculture Organization (FAO) has given a statistic that a total of 15, 500 plants in Malaysia are naturally rich in medicinal properties and yet, there were only 7.7% of the plants are exploited globally (Aziz and Zakaria, 2013).

Traditional medical common practices are among all ethnics in Malaysia including Malays, Chinese, Indians and aborigines. Although thousands of medicinal plants have been in use since time immemorial, the indigenous uses of plants were largely passed along by word of mouth from one generation to another and has largely remained undocumented. Lately during the medicinal plant research by natural products chemist and botanists, there is a systematic record together with voucher specimens at various herbariums including Herbarium of the Forest Research Institute of Malaysia, the country's national herbarium (Jantan, 2004).

In the past six decades, the medicinal plant research in Malaysia has been carried out mainly by government-funded universities and research institutes with partial support of industries and multinationals. While lots of active researches and studies on natural products carried out by the institutions, there is nonetheless a serious effort to embark on a systematic drug discovery programme at a national level. The drug development approach is nevertheless in a poor province in Malaysia like other growing countries because of limitation in funds, facilities, qualified research personnel and lack of collaborations with the institutions (Jantan, 2004).

Some of the selected local medicinal plants used by the traditional medicine companies in Peninsular Malaysia; Pegaga (*Cantella asiatica*), Tongkat Ali (*Eurycoma longifolia*), Ganoderma (*Ganoderma lucidum*), Serai wangi (*Cymbopogon nardus*), Kacip Fatimah (*Labisia pumila*), Cekur/Kecur (*Kaempfera galanga*), Pala (*Myristica fragrans*), Hempedu bumi (*Andrographis paniculata*), Asam jawa (*Tamarindus indica*), Kunyit (*Curcuma domestica*), Halia putih/bara (*Zingiber officinale*), Teja sarsi (*Cinnamomum* sp.), Mengkudu (*Morinda citrifolia*), Pakma (*Rafflesia hasseltii*), Lengkuas (*Languas conchigera*). The resources of some plants such as pegaga, tongkat ali, cekur, kunyit and mengkudu are still imported from other countries even though can be found locally. This is mainly due to the high demand of consumers and inconsistent local supply (Mohd Satefarzi and Mansor 2001).

2.3.1 Medicinal plants in Malaysia and the uses

The awareness of the significance of local plants has created the botanical garden in metropolitan regions for both plant conservation and edible purposes. Malaysia has a pproximately 70 species of edible herbs called ' ulam ' such as ' daun tenggek burung ' (*Euodia ridleyi*), betel leaf (*Piper sarmentosum*), 'pokok ketumpangan air' or shining bush (*Pepromia pellucid*), 'pegaga' (*Cantella asiatica*) and many more (Adnan & Othman, 2012).

Tongkat Ali or scientifically named as *Eurycoma longifolia* is well known for its medicinal value in all parts of the plant. Beside for its broad scope of traditional uses known, it is mostly renowned for its aphrodisiac property. The active compound of this plant can increase the testosterone level in blood (proandrogenic effect) which consequently helps in bone fracture healing process (Jalil *et al*., 2012). A survey conducted by Al-Adhroey *et al*. (2010), on the plants which has been used traditionally in Malaysia to treat malaria. The only species which has been mentioned by all respondent groups is *E. longifolia*.

Kacip Fatimah (Labisia pumila) is traditionally eaten by Malaysian women to treat menstrual irregularities and painful menstruation, assist contract birth after delivery, and encourage the function of sexual health. This plant extract's antioxidant and anti-inflammatory characteristics efficiently treat multiple illnesses such as osteoporosis, rheumatism and sexual function of females (Jalil *et al*., 2012).

Misai Kucing (Orthosiphon stamineus) is also a well-known local medicinal plant with a multitude of pharmacological characteristics, including anti-allergic, anti-hypertensive, diuretic, antioxidant, anti-diabetic, anti-inflammatory, mild antiseptic, antifungal and even significant antibacterial and antiviral activity. A research conducted by Azizan and colleagues (2017) showed *O.stamineus* had moderate to powerful antibacterial characteristics against seven pathogenic oral bacterial strains.

A review paper has presented about diabetes which is a major public health concern in Malaysia can be cured using traditional plants and herbs. Hempedu bumi (*Andrographis paniculata*), Sambung Nyawa (*Gynura procumbens*), Mas Cotek (*Ficus deltoidea*), Ulam Raja (*Cosmos caudatus*), Dukung Anak (*Phyllanthus niruri*), Pokok Gajus (*Anacardium occidentale*), Belimbing Buluh (*Averrhoa bilimbi*), Pokok Bunga Raya (*Hibiscus rosa-sinensis*), Misai Kucing (*Orthosiphon stamineus*) and Sireh (*Piper sarmentosum*) are reported as the common medicinal plants in Malaysia used for the treatment of diabetes (Sekar *et al*., 2014).

It was covered by Al-Adhroey *et al*. (2010) in a community based survey, that most of the identified plant remedies are used for therapeutic purposes to cure malaria. Table 2.1 shows the list of species of plants that are practiced to treat malaria plus with its local names, parts used and the preparation. Several sections of the plants were applied in the formulation of anti-malarial plant remedies. Withal, the remedies were obtained from the leaves and roots in most of the species (63%).

Table 2.1: Local names, part used and preparation of species of plants that are used to treat malaria (Al-Adhroey *et al*., 2010)

Species/Application	Local name	Part used	Preparation
	Plants used orally		
Azadirachta indica	Margosa	Leaves	Decoction
Brucea javanica	Lada pahit	Fruits, Leaves	Infusion
Cassia siamea	Johor	Stem bark	Decoction
Cocos nucifera	Kelapa	Flesh	Infusion
Eurycoma longifolia	Tongkat Ali	Root	Decoction
Labisia pumila	Kacip Fatimah	Leaves	Decoction
Languas galanga	Lengkuas	Rhizomes	Decoction
Lansium domesticum	Langsat	Peel and bark	Infusion
Morinda citrifolia	Peremuh/ Mengkudu	Leaves and fruits	Decoction
Nigella sativa	Jintan hitam/ Habatulsawda	Seeds	Infusion
Ocimum tenuiflorum	Tulsi	Leaves and seed	Decoction
Phyllanthus niruri	Dukung anak	Whole plants	Decoction
Piper betle	Sirih/Serih	Leaves	Chewing
H. rosa-sinensis	Bunga Raya	Flowers	Infusion
Tinospora crispa	Putarwali/ Batang Wali	Stems, leaves or roots	Decoction
	Plants used externally		
Aeschynanthus sp.	Sambuk	Leaves	Decoction
Alstonia angustiloba	Pulai getah	Gum	Decoction
Curcuma domestica	Kunyit	Root	Decoction
Elateriospermum tapos	Perah/parah	Leaves	Decoction

2.4 Human immunodeficiency of bacteria

Bacteria are the microorganisms or infectious agent that most often cause infections in man. The results can range from no disease to an acute or chronic disease when an infectious agent invades a host. There are few variables that lead clinically obvious infection to evolve; it relies on the virulence (capacity to cause severe disease) and the dose of the invading microorganism, the route through which the microorganism enters the body and the capacity of the host to withstand infection. The capacity of the host is determined by age, gender, inheritance, earlier gained immunity, existence or lack of other mechanisms of disease and general dietary status.

As the protection mechanism against the infectious agents, immune system plays a role which includes the natural barriers against infection, innate and adaptive immunity. It is the main impediment against the occurrence of disseminated infections that are normally connected with high death rate. In fact, the number of individuals exposed to infection is much higher compared to those who are suffering from the disease. This indicates that most people fight and able to destroy the microorganisms so that the progression of an infection is prevented (Machado & Carvalho, 2004).

In contrast, immune deficiencies whether of innate immunity or adaptive immunity are strongly related with increased susceptibility to infections. Depends on the severity of the infection, many non-specific host defence mechanisms involve in the battle. Yet, the weakening of immunological and non-specific defences can lead, in turn, to a more severe illness or diseases.

Food-borne microbial illness is a significant issue in infection. Some of the bacteria which cause food-borne infections are *Salmonella* sp., *Shigella* sp., *Campylobacter, Escherichia coli, Vibrio parahemolyticus, Listeria monocytogenes* and *Yersinia* sp. Gastrointestinal symptoms are the most common clinical presentation of food-borne diseases. However, such diseases can also have other symptoms in the aspects of neurological, gynaecological and immunological. Multiorgan failure and even cancer may result consequent of the illnesses, thus representing a considerable burden of disability as well as mortality (WHO 2018, Foodborne diseases).

Leptospirosis includes a wide range of human and animal clinical and subclinical diseases. Nearly every mammal can serve as a leptospire carrier, harbouring the spirochetes in the kidney's proximal renal tubules, leading to urinary shedding. Rats serve as the main reservoirs in most human leptospirosis, excreting high leptospiral concentration (107 organisms per ml) even after months of initial infection. At the same time, people are regarded as incidental hosts with acute but sometimes deadly diseases (Evangelista & Coburn, 2010).

Like other bacterial diseases, the growth of leptospirosis and progression of illness are affected by host susceptibility variables, inoculum infection dose, and infection strain virulence features. Leptospira selected species and serovars are found to cause serious disease in humans more frequently than others. However, the knowledge of acquired and innate host factors that affect infection and disease progression is still lacking. A study of a triathlon-related outbreak investigation has shown that the human leukocyte antigen HLA-DQ6 serotype is the first and only genetic susceptibility factor for leptospirosis to date (Ko *et al*., 2009).

2.4.1. Overview of the epidemic of Leptospirosis

Leptospirosis is an endemic disease in South East Asia's tropical and subtropical nations. It is suitable for Leptospira to survive lengthy periods in the setting due to the elevated humidity and hot temperature (Benacer *et al*., 2013). Due to flooding and outdoor recreational activities, there has been an increase in epidemic events. According to the World Health Organization (WHO), the global incidence of leptospirosis is more than one million severe human cases per year. The Leptospirosis Burden Epidemiology Reference Group (LERG) was created by the WHO to create right estimates of the disease burden so that better intervention, control and avoidance can be achieved (Yaakob *et al*., 2015).

The symptoms of leptospirosis, which differ from asymptomatic to fatal, indicate the infection stage. The anicteric and icteric leptospirosis is the two stages of leptospirosis (Benacer *et al.*, 2013). Leptospirosis clinical indication ranges from mild symptoms to serious jaundice, acute renal injury (AKI) and lung haemorrhage. The early stage of manifestations of leptospirosis extends from 3 to 7 days, including fever, headaches, myalgia (particularly in calves), nausea, vomiting, malaise, and hyperaemia to the conjunctival. 80-90% of patients are symptom-free after this phase. The second stage, which is the Weil syndrome, progresses by about 10 percent. For more serious symptoms such as jaundice, meningitis, pulmonary haemorrhage and AKI, the stage lasts from 4 to 30 days. Antibodies of immunoglobulin M can generally be discovered at this point (Seguro & Andrade, 2013).

2.5 *Leptospira interrogans*

Leptospirosis is an emerging disease induced by spirochetes from the *Leptospira* genus. It is generally helical in the form of motile spirochetes belonging to the Spirochaetales family of Leptospiraceae (Budihal *et al.*, 2014). Leptospira can be categorised as pathogenic (*Leptospira interrogans*) and non-pathogenic (*Leptospira biflexa*) strains depending on their metabolism (Mohammed *et al.*, 2011). In environmental water and soil, the saprophytic strains are naturally present and rarely cause disease Ironically, pathogenic strains trigger leptospirosis by getting transmitted straight to human bloodstream through direct contact with contaminated urine in animal reservoirs or indirectly through contaminated water and soil (Benacer *et al*., 2013).

In the presence of homologous antigens, these two species were further split into serogroups, serovars and strains identified by agglutination methods; cross-reaction of cell antigens using the cross-agglutinin absorption test (Mohammed et al., 2011). To date, 21 species, 25 serogroups and 250 serovars have been identified as pathogenic leptospires. Alongside the phenotypic classification, genotyping classification of *Leptospira* also done through genome sequencing. Two *L. interrogans* isolates were published. They were serovar Lai and Copenhageni, two isolates of *L. borgpetersenii* (serovar Hardjo) and two isolates of *L. biflexa* (serovar Patoc) (Yaakob *et al*., 2015; Budihal *et al*., 2014). On the other hand, Leptospira's serovar classification is based on the expression of surface-exposed epitopes in a mosaic of lipopolysaccharide antigens, and the specificity of epitopes depends on their sugar structure and orientation. (Adler & Alejandro, 2010). The seven of the species which are the primary agents of leptospirosis are *L. interrogans*, *L. borgpetersenii*, *L. santarosai*, *L. noguchii*, *L. weilli*, *L. kirschneri* and *L. alexanderi* (Evangelista & Coburn, 2010).

Leptospires are corkscrew-shaped bacteria that vary by the existence of end hooks (like a question mark in form) from other spirochetes. They have two sub terminally connected periplasmic flagella that confer translational as well as non-translational movement types. They are thin, with a diameter of about 0.1 μm and a length of 6-20 μm. Because they are mobile and their bodies are tiny in diameter, observation requires the use of dark field microscopy or stage contrast. Leptospires are slow increasing compulsory aerobes with an ideal growth temperature of 28^{o}C -30^{o}C and are not resistant to drought or hypertonicity but promote alkalinisation at pH 7.8. (Yaakob *et al*., 2015; Mohammed *et al*., 2011). In the laboratory, they grow in vitamin B1 and B12, long-chain fatty acids and ammonium salts enriched with easy media. The long-chain fatty acids are used as the sole source of carbon and metabolized through β-oxidation (Adler & Alejandro, 2010).

The leptospires have surface structures that share Gram-positive and Gram-negative bacteria's characteristics. They have a typical double membrane structure (like Gram-negative bacteria) in which the cytoplasmic membrane and peptidoglycan cell wall are tightly related (as Gram-positive bacteria) and coated by an outer membrane. The primary elements of the outer membrane are phospholipids, outer membrane proteins and lipopolysaccharide (LPS). The lipopolysaccharide is the primary antigen in the outer membrane for *Leptospira*, and is structurally, chemically and immunologically similar to lipopolysaccharide from Gram negative bacteria with decreased biological activity in endotoxic testing. The cell wall of leptospires is as seen in Figure 2.2 (Evangelista & Coburn, 2010; Mohammed *et al*., 2011; Yaakob *et al*., 2015).

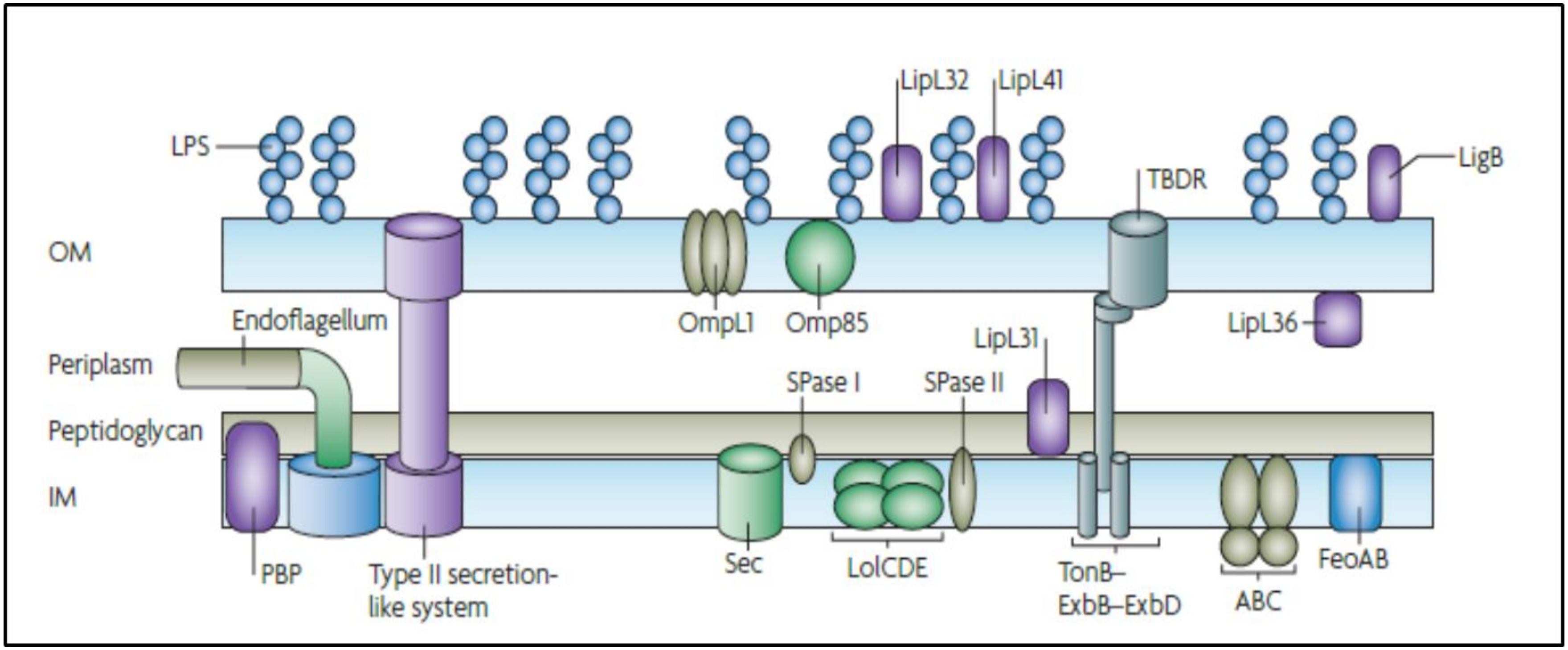

Figure 2.2: The cell walls of leptospires. Leptospires have an inner membrane (IM), and an outer membrane (OM) which contains lipopolysaccharides (LPS). They have a typical double membrane structure (like Gram-negative bacteria) in which the cytoplasmic membrane and peptidoglycan cell wall are tightly related (as Gram-positive bacteria) and coated by an outer membrane. (Ko *et al*., 2009).