COMPARISON OF ANTIMICROBIAL ACTIVITY OF CRUDE ETHANOLIC EXTRACTS OF FIVE DIFFERENT PLANTS AGAINST ACNE-INDUCING BACTERIA

By

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celcius
%	Percentages
g	Gram
L	Liter
m	Meter
µ	Micro
mg	Miligram
ml	Mililiter
mm	Milimeter
µg	Microgram
µl	Microliter
CFU	Colony-forming unit
DMSO	Dimethyl Sulfoxide
HUSM	Hospital Universiti Sains Malaysia
MIC	Minimum Inhibitory Concentration
MHA	Muellen-Hinton agar
MHB	Muellen-Hinton broth
PPSP	Faculty of Medical Science

ABSTRACT

Acne vulgaris is defined as a typical skin disorder among adolescence, causing inflammation of pilosebaceous follicle which is characterized by comedones, papules, pustules, cysts, nodules and often scars in face, neck, upper trunk and also arms. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized as a causal factor in acne formation. This study was conducted to compare the antimicrobial activity of five plant extracts of *Piper betle*, *Aloe vera*, *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* against *P. acnes* and *S. epidermidis.* The antimicrobial activity of five ethanolic plant extracts were investigated against the two acne-inducing bacteria using well diffusion and minimum inhibitory concentration (MIC) method. The well diffusion method result revealed that the ethanolic extracts of *Piper betle*, *Aloe vera*, *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* showed mean of inhibition zone diameter of 29.0 mm, 7.7 mm, 10.7 mm, 13.9 mm and 11.5 mm respectively against *P. acnes* bacteria and mean of inhibition zone diameter of 32.0 mm, 30.7 mm, 13.0 mm, 14.3 mm and 15.3 mm respectively against *S. epidermidis* bacteria. The mean value of MIC against *P. acnes* for *Piper betle* extract and *Cinnamomum zeylanicum* extract showed 39.06 mg/ml and 2.45 mg/ml respectively. Whereas the mean value of MIC of all five ethanolic extracts of *Piper betle* and *Aloe vera*, showed 2.45 mg/ml dan 78.13 mg/ml respectively. Meanwhile, the other ethanolic extracts of *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* against *S. epidermidis* bacteria showed 39.06 mg/ml. This suggests that *Piper betle* and *Cinnamomum zeylanicum* extracts have showed the most effective antimicrobial effects against acne-inducing bacteria. However, all of the ethanolic extracts were found to have potency against acne-inducing bacteria which can be further evaluated in animal and clinical research that can be used as an alternative treatment of acne commercially.

ABSTRAK

Jerawat ditakrifkan sebagai penyakit kulit yang sering dihadapi khususnya oleh para remaja. Penyakit ini menyebabkan keradangan pada folikel rambut dan kelenjar minyak dan dicirikan kepada komedo, bintil merah, sista, nodul yang sering meninggalkan kesan parut di bahagian muka, leher, badan dan juga lengan. *Propionibacterium acnes* dan *Staphylococcus epidermidis* telah dikenalpasti sebagai penyebab dalam pembentukan jerawat. Kajian ini dijalankan untuk membandingkan aktiviti antimikrob daripada lima jenis ekstrak tumbuhan iaitu *Piper betle, Aloe vera, Solanum lycopersicum, Cinnamomum zeylanicum* dan *Cucumis sativus* terhadap *P. acnes* dan *S. epidermidis*. Aktiviti antimikrob daripada lima jenis ekstrak etanol tumbuhan telah dikaji terhadap dua bakteria berkenaan dengan menggunakan dua kaedah iaitu 'well-diffusion' dan kepekatan perencatan minima (MIC). Hasil keputusan 'well-diffusion' menunjukkan ekstrak etanol daripada *Piper betle, Aloe vera, Solanum lycopersicum, Cinnamomum zeylanicum* dan *Cucumis sativus* telah menunjukkan purata diameter zon adalah 29.0 mm, 7.7 mm, 10.7 mm, 13.9 mm dan 11.5 mm masing-masing terhadap bakteria *P. acnes*, dan purata diameter zon adalah 32.0 mm, 30.7 mm, 13.0 mm, 14.3 mm dan 15.3 mm masing-masing terhadap bakteria *S. epidermidis*. Nilai MIC terhadap bakteria *P. acnes* untuk ekstrak etanol *Piper betle* ialah 39.06 mg/ml dan nilai MIC bagi *Cinnamomum zeylanicum* ialah 2.45 mg/ml. Nilai MIC bagi ekstrak etanol *Piper betle* dan *Aloe vera* terhadap bakteria *S. epidermidis* ialah 2.45 mg/ml dan 78.13 mg/ml masing-masing.. Manakala, nilai MIC bagi ketiga-tiga ekstrak etanol *Solanum lycopersicum, Cinnamomum zeylanicum* dan *Cucumis sativus* terhadap bakteria *S. epidermidis* ialah 39.06 mg/ml. Ini menunjukkan bahawa ekstrak etanol *Piper betle* dan *Cinnamomum zeylanicum* mempunyai kesan antimikrobial yang paling berkesan terhadap bakteria yang menyebabkan jerawat. Walau bagaimanapun, kesemua ekstrak etanol tumbuhan didapati mempunyai potensi dalam

mencegah jerawat. Justeru, mendorong pengkaji untuk melakukan kajian secara klinikal dan haiwan dan seterusnya boleh digunakan sebagai rawatan alternatif jerawat secara komersial.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Acne vulgaris is a common chronic skin disease causing blockage and inflammation of pilosebaceous units such as hair follicles and the accompanying sebaceous gland. Formation of acne can present as inflammatory and non-inflammatory lesions, which are normally affecting face, back and also chest (Rao, 2014). This disorder is most prevalence in adolescence and causes some psycho-social changes for them such as depression and low self-esteem. Over 80% of teenagers suffer acne vulgaris, persists beyond the age of 25 years in 3% of men and 12% of women (Amrita *et al.*, 2012). There are four main pathogenic components which involved in the acne development including the increased production of sebum, hypercornification of the pilosebaceous duct, abnormality of the microflora and inflammation (Chaudhary *et al.*, 2013).

The accumulation of sebum in the pilosebaceous channel aids the proliferation of skin bacteria such as *Propionibacterium acnes (P. acnes)* and *Staphylococcus epidermidis (S. epidermidis)*. *P. acnes* is a Gram-positive anaerobic microorganism that resides beneath the surface of human skin. It plays a vital role in pathogenesis of acne. It is concerned in acne inflammation by its aptitude to activate complements and by its capability to metabolize sebaceous triglycerides into fatty acids which chemotactically attract neutrophils (Gurjar *et al.*, 2012). According to Chaudhary *et al.*, (2013), another acne-causing bacteria is *S. epidermidis,* the aerobic Gram-positive organism which is also a resident of human skin flora. This aerobic bacteria is associated with superficial infections within the sebaceous unit.

Over the past 20 years, many concerns increased in the prevalence of antibiotic-resistant *P. acnes* strains. Topical antibiotics like erythromycin, tetracycline and clindamycin have been used. These topical antibiotics are known as the first line treatment for acne vulgaris. However, there are risks for the usage of antimicrobial agents. For example, the colonies production of antibiotic resistant organisms. Moreover, medicines such as retinoids and isoretinoin produced side effects such as erythema, burning, scaling, pruritus and dryness (Chaudhary *et al.*, 2013).

Since the development of antibiotic resistance is multifactorial and hard to prevent, in overcoming the problem, medicinal plants have been used as alternative medicines for acne disorder (Gurjar *et al.*, 2012). In addition, traditional medicines especially plants have been found to have major role in the treatment of skin disorders.

In the this study, five plants which are *Piper betle*, *Aloe vera*, *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* have been chosen to investigate their antimicrobial activities against two acne-inducing bacteria, the *P. acnes* and *S. epidermidis*.

1.2 Significance of Study

Acne is a common problem among adolescents and young adults. There are different beliefs as to what causes acne especially in a multiracial country with different cultural practices. As acne is a medical disease, medical treatment by healthcare providers is required. If left untreated, acne may have a profound psychological and emotional impact. Acne presents with different spectrums of disease severity and there are numerous treatment options currently available. All these factors along with variable exposure to dermatology in medical schools result in a wide variation in prescribing patterns. Hence, there is a necessity to assess acne and its treatment options in a more objective manner.

Throughout this study, the relationship between the antibacterial activity of plant crude extract of interests against the inflammatory effects of acne-inducing bacteria, *P. acnes* and *S. epidermidis* will be determined. The plants were used in this study because they are safe and less adverse effects compared to modern medicine. In addition, this study may contribute to the development of prevention methods of acne vulgaris. It will reduce the rate of patients from getting acne vulgaris. In the other way round, it will reduce the medical cost. It also can improve the individual self-esteem as individual perceives acne may have a profound psychological and emotional impact.

1.3 Objectives of Study

1.3.1 General Objective

1) To compare the antimicrobial activity of five extract plants against *P. acnes* and *S. epidermidis.*

1.3.2 Specific Objectives

1) To extract *Piper betle* leaves, *Aloe vera* plants, *Solanum lycopersicum* fruit, *Cinnamomum zeylanicum* and *Cucumis sativus* fruits using an ethanolic extraction method.
2) To identify the plant extract that exhibit the antibacterial activity against *P. acnes* and *S. epidermidis.*
3) To determine the inhibition zone of *P. acnes* and *S. epidermidis* by well diffusion method.
4) To determine the minimum inhibitory concentration (MIC) of *P. acnes* and *S. epidermidis* by the presence of turbidity and colour changes on the medium.

1.4 Conceptional Framework

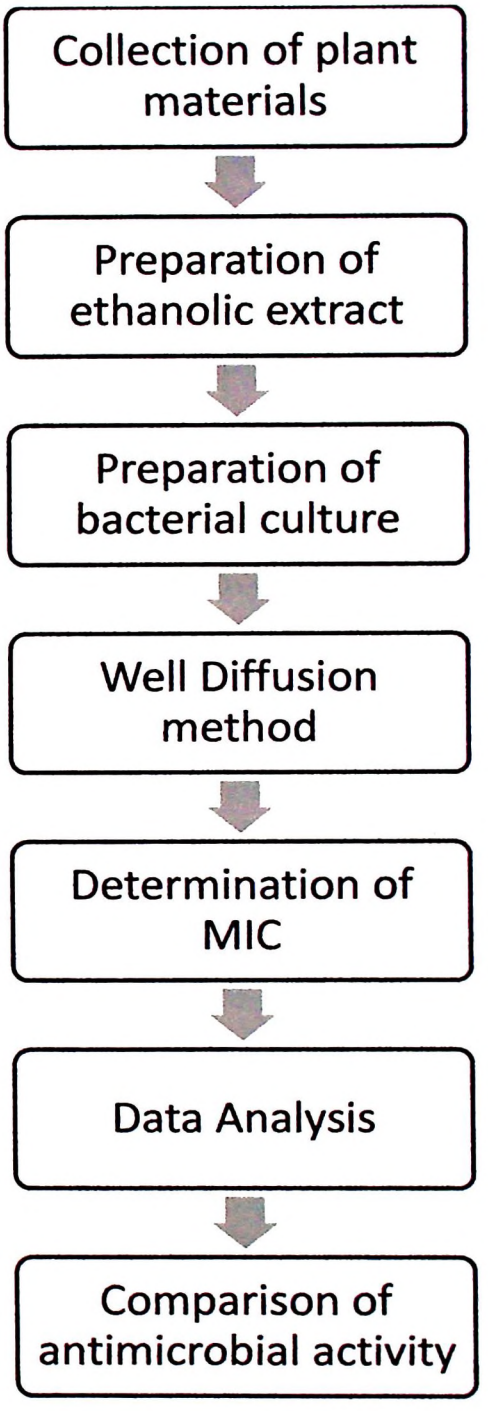

Figure 1.1: Research framework for this study

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of acne vulgaris

Acne vulgaris is a common skin condition with substantial cutaneous and psychological disease burden which is accounts for substantial societal and health care burden. Americans use more than 5 million physician visits for acne each year, leading to annual direct costs in excess of $2 billion (Yazici *et al.*, 2004). According to Singh *et al.* (2011), acne vulgaris is defined as a chronic inflammatory disease of the pilosebaceous follicle, characterized by comedones, papules, pustules, cysts, nodules and often scars in certain sites for examples face, neck, upper trunk and also arms. The formation of comedones essential to the diagnosis of acne vulgaris. There are several disease that mimic acne such as rosacea, folliculitis, angiofibromas, perioral dermatitis and keratosis pilaris. However, those disorders occur vary with the patient's age. Keratosis pilaris and perioral dermatitis usually present in childhood, whereas rosacea tends to affect older adults.

Generally, acne is characterized into mild, moderate and severe forms. Mild acne is commonly limited to the face and categorized by the presence of non-inflammatory closed and open comedones with few inflammatory lesions. Whereas moderate acne is characterized by major presence of inflammatory papules and pustules on the face and often mild truncal disease. Severe acne occurred is when typically nodules and cysts are presence (Dawson & Dellavalle, 2013). The pathogenesis and existing treatment strategies for acne are complex. This disease commonly affects young people at a time when they are undergoing maximum psychological, social and physical changes because facial appearance always represents an important aspect of one's perception of body

image. Emotional stress can exacerbate acne, and patients with acne develop psychiatric problems as a consequence of their condition such as decreased self-esteem or self-confidence, interpersonal difficulties, unemployment and increased prevalence of depression and anxiety. Thus the influence of acne on body image is believed to be the main factor associated with depression and anxiety (Yazici *et al.*, 2004).

2.2 Pathogenicity of acne vulgaris

Acne is an inflammatory disorder of the pilosebaceous duct which results from four major pathophysiologic process includes abnormal keratinocytes proliferation and desquamation that leads to ductal obstruction, increased androgen hormone in sebum production, proliferation of *Propionibacterium acnes* and inflammation (Dawson & Dellavalle, 2013).

A study by Truter (2009) reported that acne arises in the pilosebaceous units in the dermis that consist of a hair follicle and associated with sebaceous gland. The pilosebaceous gland produces sebum in response to increased testosterone levels, which is a mixture of fats and waxes that protect the skin and hair by retarding water loss and forming a barrier against external agents. The hair follicle is lined with epithelial cells and become keratinised as they mature.

Abnormal epithelial desquamation that causes by increasing of androgen production during puberty may lead to primary precursor lesion in acne which is the microcomedone. Microcomedones are not visible to the naked eye since it is a pathological structures but then evolve into the visible lesions. Besides that, a rising of circulating androgens may enhance sebum production and cause the obstructed follicles to fill with lipid rich material and form visible open and closed comedones that known as blackhead and whitehead respectively (Dawson & Dellavalle, 2013).

The blackhead as the keratinous material darkens in contact with the air. Hence, this material can escape, the comedone does not become inflamed. If the follicular orifice does not open sufficiently, a closed comedone (whitehead) results, within which inflammation can occur (Truter, 2009). Sebum acts as a substrate for bacterial growth that lead to proliferation of *P. acnes*. This bacteria cause the follicular wall of closed comedones to disrupt and collapse, spilling their contents into the surrounding tissue and provoking an inflammatory response. Moreover, bacterial enzymes decompose triglycerides in the sebum to produce free fatty acids, which also cause inflammation (Dawson & Dellavalle, 2013).

The inflammation is propagated by traumatic rupture of comedones into the surrounding of the dermis and manifests through the development of inflammatory papules, pastules, nodules and cysts (Dawson & Dellavalle, 2013). *P. acnes* proliferate in the stagnant oil and stimulating cytokine production, which produces the local inflammation leading to the appearance of a spot. White blood cells infiltrate the area in response to the proliferation of bacteria and kill the bacteria and in turn die leading to pus formation. The pustule eventually bursts on the skin surface, carrying the plug away (Truter, 2009).

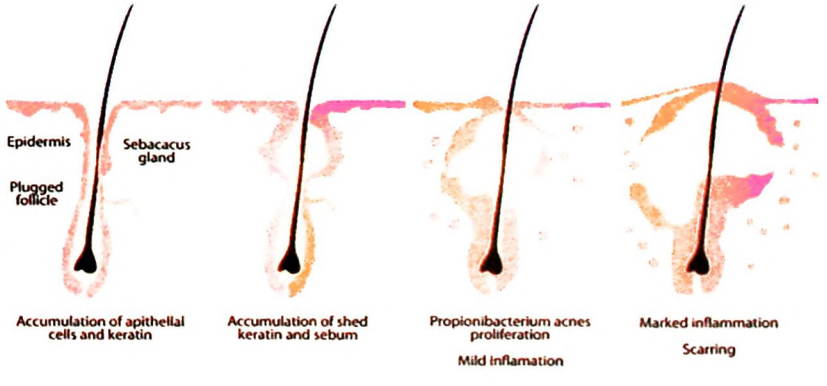

Figure 2.1: The stage development of acne pathogenesis. Figure was taken from Skin Image (2013).

2.3 Epidemiology

Approximately about 80% of people aged 11 to 30 years are affected by acne disorder. Commonly, acne lesion occurs at the onset of puberty therefore girls tend to develop acne at earlier age than boys. The incidence peak for girls is between 14 and 17 years while for boys is between 15 to 19 years old. Besides, there may be a familial tendency to acne and it is slightly typical to boys who experience with more severe acne development. A common misconception by the medical and lay community is that acne is a self-limited teenage disease and, thus, does not warrant attention as a chronic disease. Nevertheless, the chronicity of many cases of acne as well as the well documented psychologic effects of chronic acne contributes to the burden of the disease (Siri Knutsen-Larson, 2012).

During adolescence, acne is more common in males than in females but is more typical in women during adulthood than in men (Truter, 2009). A study by Lucky *et al.* (1991), found that severity of acne among boys correlated with pubertal maturation and that 50% of 10 – 11 years old boys had more than 10 comedones. In addition, another study by the same team showed that 78% of girls between aged 8 and 12 years had acne. Hence, the severity of acne increased with advancing maturity and prepubertal girls with severe acne had notably higher dehydroepiandrosterone sulphate levels.

In the U.K., France and the U.S.A, acne consistently represents the top three most prevalent skin conditions in the general population that found as a large studies (Bhate & Williams, 2012). In South Africa, dermatologists found lower rates of acne among the Bantu than among whites residing in Pretoria. Bantu adolescents (aged 15 – 19 years) maintained a 16% incidence rate of acne which is lower than the white adolescents who maintained a 45% incidence. Meanwhile for the entire sample of Bantus of all ages, the overall occurrence of acne was 2%, very much less than total white sample across all

ages, which had acne incidence of 10%. Thus it conclude that the prevalence of acne is lower among rural, non-westernized people than in fully modernized Western societies (Siri Knutsen-Larson, 2012). Figure 2.2 showed the severity according to grade of acne with respect to age and sex in 2012.

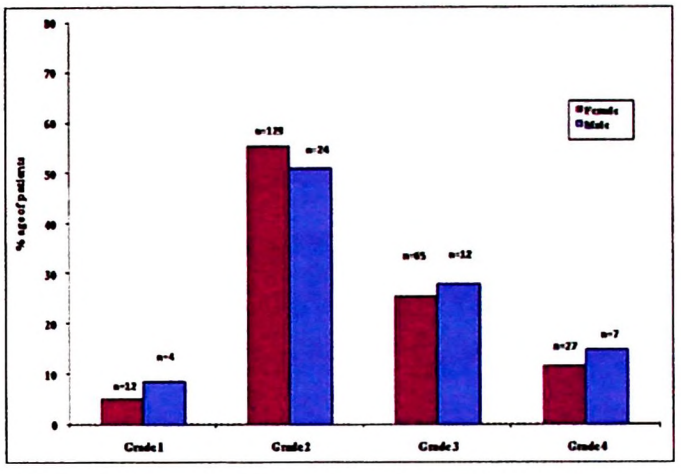

Figure 2.2: The severity according to grade of acne with respect to age and sex in 2012. Figure was taken from Khunger & Kumar (2012).

2.4 Treatment of acne

According to Truter (2009), the main objectives of acne management includes to reduce the number of lesions, to improve the skin condition, to eliminate pathogens, to reduce the impact of psychological stress and to prevent scarring. A structured approach to the acne management needs an understanding of the cause, severity, type of lesions present and also the treatment options available. For preventing a scar formation, a therapy should be started at an early stage. However, the choice of medicine depends on previous treatment, patient acceptability and the type of lesion as either comedonal or inflammatory. An ideal effective anti-acne treatment should decrease the number of both types of lesion. Generally, selection of treatment is based on acne severity. It is vital to show understanding and empathy when counselling patients since acne is predominantly

a condition that affects among adolescents which is a time when appearance is very important.

The pharmacological treatment of acne can be divided into topical and systemic treatment. Topical treatment are best managed with milder cases whereas systemic drugs are indicated in more severe cases (Dawson & Dellavalle, 2013). Retinoids and benzoyl peroxide topicals are the therapy foundation for both comedonal and inflammatory acne, regardless of severity. Both of these topical treatments are recommended by the American Academy of Dermatology (AAD). However, evidence suggests that only 55% of dermatologists and 10% of primary care providers recommend them (Nguyen, 2013).

Isotretinoin is well-recognized as a successful therapy for acne vulgaris. In the last 10 years it has transformed the lives of many young people afflicted with severe and or prolonged acne (Cunliffe *et al.,* 1993). Isotretinoin causes normalization of epidermal differentiation, depresses sebum excretion by 70%. It also functions as an anti-inflammatory agemt, and even able to reduces the presence of *P acnes.* However, it is a teratogen, so pregnancy must be avoided while on prescription of isoretinoin. Contraception counseling is mandatory, and negative pregnancy test results are required prior to the initiation of therapy in women of childbearing potential. The baseline laboratory examination should also include cholesterol and triglyceride assessment, hepatic transaminase levels, and a complete blood count (CBC). Pregnancy tests and laboratory examinations should be repeated monthly during treatment (Lee *et al.*, 2010).

Besides, photodynamic therapy (PDT) with topical aminolevulinic acid (ALA) is known as an emerging modality for the treatment of acne vulgaris. Topically applied ALA is taken up by epithelial cells and metabolized via the porphyrin pathway to protoporphyrin IX (PpIX), which is a photosensitizer that accumulates in the

pilosebaceous units and causing membrane damage and cell destruction. Thus, ALA-mediated photodynamic reaction can directly kills the pathogenic *P. acnes* and specifically damages the pilosebaceous glands and leads to clinical improvement of acne lesions (Ma, et al., 2013).

Systemic antibiotics used in acne vulgaris have both antimicrobial and anti-inflammatory properties. They reduce *P. acnes* within follicles, thereby inhibiting production of bacterial-induced inflammatory cytokines. The main systemic antibiotics used in acne vulgaris are tetracycline, doxycycline, minocycline, and erythromycin (Haider, 2004). Tetracycline and erythromycin suppress leukocyte chemotaxis and bacterial lipase activity while minocycline and doxycycline inhibit cytokines and matrix metalloproteinase which contribute to inflammation and tissue breakdown. A study by Haider (2004) showed that in a randomized controlled trial study in which 51 patients received either a submicrobicidal doseof doxycycline for 6 months or placebo. The mean reduction of total lesion was 52 % with doxycycline and 18 % with placebo. Even low doses of doxycycline may be effective via inhibition of collagenases including matrix metalloproteinases.

2.5 Acne-inducing bacteria

2.5.1 *Propionibacterium acnes*

Propionibacterium acnes is an anaerobic and Gram-positive opportunistic pathogen that forms part of the normal microbiota on human skin and also surface of mucosal (McDowell *et al.*, 2013).

Researcher in the University of Texas Medical Branch at Galveston reported that this bacteria have their own mechanism in developing resistance to new antimicrobial agents. There are a number of ways in which bacteria can become resistant to

antimicrobial agents. The basic mechanisms by which a microorganism can resist an antimicrobial agent are (1) to alter the receptor for the drug; (2) to decrease the amount of drug that reaches the receptor by altering entry or increasing removal of the drug; (3) to destroy or inactivate the drug; and (4) to develop resistant metabolic pathways (Neu & Gootz, 1996). This pathogen colonizes and grows optimally in the presence of lipids and in the oxygen absence. Sebum secretion is increased during puberty and other hormonally active periods. Due to the increasing in sebum production, it provides an ideal growth environment for *P. acnes* and this attracts the bacteria into the hair follicles, which are located deeper within the skin. Additional genetic factors may be also contributed (Selak, 2013).

The role of *P. acnes* in acne pathogenesis was shown in Figure 2.3. Firstly, sebum secreted and clogged pores limit access to oxygen. Then, high lipid content and low oxygen concentration creates optimal growth environment for *P. acnes* which is residing on the skin surface. They are attracted by the lipid-rich, optimal environment inside the hair follicles. At here, the bacteria rapidly multiply inducing a local inflammatory response. If the immune system is not able to efficiently kill and remove the bacteria, the inflammatory reaction persists leading to the creation of cysts and pustules, ultimately leading to the creation of scars (Selak, 2013).

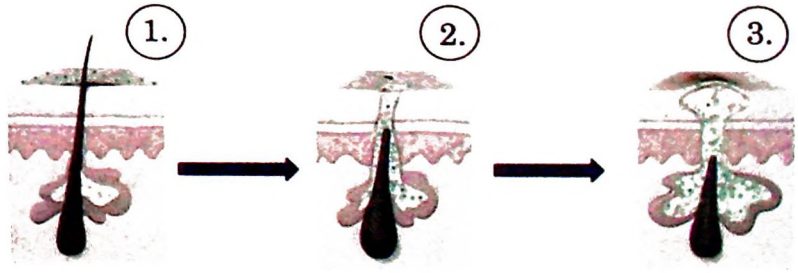

Figure 2.3: The role of *P. acnes* in acne pathogenesis. Figure was taken from Selak (2013).

2.5.2 *Staphylococcus epidermidis*

According to Otto (2009), the family of Staphylococci are Gram-positive microorganism which are common bacterial colonizers of the skin and mucous membranes of humans and mammals. *S. epidermidis* is the most typical Staphylococci bacteria isolated species from human epithelia where infects mainly the axillae, head and nares. Analysis of this bacterium showed that this species able to contribute protection from the harsh environments in its natural habitat such as being able to survive with extremes of salt concentration and osmotic pressure.

Biofilm is mainly composed of polysaccharide component that is the vital factor by which *S. epidermidis* adheres to and colonizes artificial materials in patients. Besides biofilm is believed to make clinical *S. epidermidis* strains more resistant to administered antibiotics and to host defense mechanism (Cabrera-Contreras *et al.*, 2012).

2.6 Traditional medicine plants

According to WHO (2000), traditional medicine is the total of the understanding, expertises, and practices based on the theories, beliefs, and experiences natives to different cultures and used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. In addition, herbal medicines known as herbs, herbal materials, herbal preparations and finished herbal products that consist active ingredients parts of plants, or other plant materials, or combinations. Traditional use of herbal medicines basically refers to the long historical use of these medicines. Their use is well established and widely acknowledged to be safe and effective, and may be accepted by national authorities.

2.6.1 *Piper betle*

The genus *Piper* is one of Piperaceae family which belonging to superorder Nymphaeiflloraea, order Piperales that typically known as sireh composes over thousand species. Over 700 species is predominantly distributed in tropical and subtropical regions of the world. *Piper betle* have been cultivated in India, Sri Lanka, Malaysia, Indonesia, Philippine Islands and East Africa. The parts of this plant which usually used are leaves, roots, stems, stalks and fruits. Commonly, *Piper betle* is used to cure alcoholism, bronchitis, asthma, leprosy and dyspepsia (Chakraborty & Shah, 2011).

The *Piper betle* leaf has a significant antimicrobial activity against microorganism such as *Streptococcus pyrogen, Staphylococcus aureus, Proteus vulgaris, E.coli, Pseudomonas aeruginosa*. The active ingredient found in this *Piper betle* leaf which act as antimicrobial activity which know as sterol. The mode of action may be due to surface interaction of sterol molecule present in the extracts with the bacterial cell wall and membrane leading to alteration in the primary structure of cell wall, ultimately lead to pore formation and degradation of the bacterial components. It is reported that sterol act through the disruption of the permeability barrier of microbial membrane structures. Gram-positive bacteria were more susceptible to the inhibitory effects of the plant extract because of single layer and lack the natural sieve effect against large molecules, whereas gram negative bacteria are multi layered and complex cell wall structure (Pradhan *et al.*, 2013).

Figure 2.4: *Piper betle* leaves. Figure was taken from Natureloveyou (2014).

2.6.2 *Aloe vera*

Aloe vera is a traditional medicinal plant that belonging to the family Liliaceae which is reported to cultivate in the islands of Cyprus, Malta, Sicily, Canary cape, Cape Verde and arid tracts of India. This plant has been widely used to cure various skin conditions such as cuts, burns and eczema. Besides that, the sap from *Aloe vera* can reduce pain and inflammation. *Aloe vera* consists antiseptic and antibiotic properties, thus important in treating cuts and abrasions (Rajeswari *et al.*, 2012). This plant are popular throughout history and has been used as a folk medicine which effectively in treating stomach ailments, gastrointestinal problems, skin disease and as an anti-ulcer. Currently, this plant is widely used in skin care, cosmetics and as nutraceuticals (Arunkumar & Muthuselvam, 2009).

Active ingredient found in aloe vera that poses strong antibacterial characteristic which is aloin and aloe – emodin. Aloin and aloeemodin are the major anthraquinones in aloe plants, and their levels range between 0.1% and 25.5% dry weight in the leaf exudates of 68 Aloe species. Aloin and aloe-emodin have polyphenolic structures which

play role in antimicrobial activity by inhibit protein synthesis by bacterial cells (Kohanteb, 2012).

Amrita *et al.* (2012) examined on anti-acne potential of medicinal plant such as *Aloe vera* against acne-causing bacteria which is *Propionibacterium acnes.* Topical herbal gel were developed for the treatment of mild *acne vulgaris*, aqueous extract of Garcinia *mangostana* and *Aloe vera* were formulated in an aqueous based carbopol-934 (1% w/w) gel system. The microbial assay of all the formulations demonstrated inhibitory activity against *Propionibacterium acnes* and *Staphylococcus* epidermis compared to the marketed Clindamycin phosphate gel. From their studies, they concluded that aqueous extract of *Garcinia mangostana* and *Aloe vera* can be formulated in an aqueous based gel system for topical therapy of mild acne vulgaris.

Figure 2.5: *Aloe vera* leaves. Figure was taken from Púštna ľalia (2012).

2.6.3 *Solanum lycopersicum*

Solanum lycopersicum is a red plant belongs to family Solanaceae and genus Solanum which is commonly called as tomato. Tomato is the plant that mainly consumes worldwide and consist large content of health related components which used as juice, soup, puree, ketchup or paste. Therefore, this plant is the most important vegetables

around the world as people believe tomatoes can give benefit to heart among other organs. Tomatoes consist one of the most powerful natural antioxidants which is carotene lycopene. The flavour properties of tomatoes are possess a lot of beneficial nutritional and bioactive components that may also advantage to human health (AL-Oqaili *et al.*, 2014).

This fruit consists the antioxidant lycopene that protects against cancer, facial diseases and also as anti-ageing agent. Several investigations showed the effect of dietary lycopene in decreasing the risk of chronic diseases such as cancer and coronary heart disease. Even though the antioxidant properties of lycopene are thought to be primarily responsible for its beneficial properties, the evidences are accumulating to suggest other mechanisms such as intercellular gap junction communication, hormonal and immune system modulation and metabolic pathways may also be involved ((Sharma *et al.*, 2009).

Figure 2.6: *Solanum lycopersicum* fruits. Figure was taken from Ethno-botanic (n.d.).

2.6.4 *Cinnamomum zeylanicum*

Cinnamon or its scientific name, *Cinnamomum zeylanicum* is a type of spice acquired from the inner bark of some trees which is comes from family of Lauraceae and Cinnamomum genus. This spice usually used in both sweet and savoury foods. In addition, in Unani classical texts cinnamon act as a potent drug in acne treatment, melasma, abdominal pain, hiccups, headache, jaundice, vomiting and diarrhoea. Meanwhile, modern scientific reported its role in antifungal, antibacterial, antiulcer and immunomodulatory activities (Vangalapati *et al.*, 2012). In addition, *Cinnamomum zeylanicum* contains of active ingeredient such as phenolic compound called cinnamaldehyde, acetic acid 1 - octyl acetate and eugenol (Chaudhary *et al.*, 2013).

Based on classical Unani literature, *Cinnamomum zeylanicum* is mentioned in the treatment of acne. Acne reducing activity may be due to its action of *mufatteh* (deobstruent), *muhallil* (anti-inflammatory) and *jazib* (absorbefacient) and is also claimed that it corrects all types of *ufoonat* (infection) of *akhlat* (humor) (Chaudhary *et al.*, 2013).

Figure 2.7: *Cinnamomum zeylanicum* spices. Figure was taken from GranNutrille Empresa (2011).

2.6.5 *Cucumis sativus*

Cucumis sativus is one of Cucurbitaceae family typically known as cucumber and found wildly in the Himalayan regions and cultivated throughout India. Cucumber is used as folk medicines to treat headaches, the seeds are cooling and diuretic meanwhile the fruit juice of cucumber usually used as a nutritive and a demulcent in anti-acne lotions (Kumar *et al.*, 2010).

This cucumber contains an enzyme, erepsin, Vitamin B1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases. Meanwhile, the seeds contain α- and β-amyrin, sitosterols and cucurbitasides, whereas, the leaves contain free cucurbitasides B and C and ferredoxin. Based on its traditional use and phytoconstituents, the fruit of the plant was selected and screened for free radical scavenging and analgesic activities using *in-vitro* and *in-vivo* models (Kumar *et al.*, 2010).

Figure 2.8: *Cucumis sativus* fruits. Figure was taken from Crops (n.d.).

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Material

3.1.1 Traditional Plants

Five types of traditional plant materials were used in this study. The plant materials were collected from various locations in Kelantan and Penang, Malaysia specifically nearby in Kelantan and Penang. All five different plant materials used are shown in Table 3.1.

Table 3.1: Five traditional plants and collected locations

Scientific Name & Family	Part used	Collected Location
Piper betle (Piperaceae)	Leaves	Kota Bharu, Kelantan
Aloe vera (Liliaceae)	Leaves	Kg.Selamat, Penang
Solanum lycopersicum (Solanaceae)	Fruits	Kubang Kerian, Kelantan
Cinnamomum zeylanicum (Lauraceae)	Barks	Kubang Kerian, Kelantan
Cucumis sativus (Cucurbitaceae)	Fruits	Kubang Kerian, Kelantan

3.1.2 Microorganisms and media

ATCC strain for both *Propionibacterium acnes* and *Staphylococcus epidermidis* were obtained from Laboratory of Microbiology and Medical Parasitology of Hospital University Science Malaysia (HUSM). Meanwhile, media used in this study were Mueller-Hinton agar (Oxoid™, England), Mueller-Hinton broth (Oxoid™, England) and blood agar (Oxoid™, England),. All of these media were obtained from Microbiology Laboratory or Mycology Laboratory. Both of bacteria *P. acnes* and *S. epidermidis* was cultured several times on blood agar before used to obtain new colonies.

3.1.3 Chemicals and Reagents

The solvent used in this study during extraction method was absolute ethanol which was provided by Pharmacology Laboratory of HUSM. Dimethyl Sulfoxide (DMSO) was used to dissolve ethanolic extracts before proceeding with well diffusion method. Doxycycline was used as a positive control and was provided by Microbiology Laboratory of HUSM. Resazurin powder which was used for minimal inhibitory concentration (MIC) was provided also by Microbiology Laboratory.

3.1.4 Commercial kits and Consumable items

Commercial kits and consumable items are shown in Table 3.2 and Table 3.3

3.1.5 Laboratory Equipment

Laboratory equipment are shown in Table 3.4

3.1.6 Media and Reagent Preparation

3.1.6.1 Mueller-Hinton agar media

About 38 g of the Mueller-Hinton agar was suspended in one liter of distilled water. Then, the mixture were mixed well and heated with frequent agitation. Next, the

media was boiled for one minute and sterilized at 121° C for 15 minutes. The media then was cooled to 40-45° C and poured into the Petri dishes and was harden in incubator.

3.1.6.2 Resazurin solution

Resazurin solution was prepared by dissolving 0.1 g of resazurin powder in 10 ml of distilled water. The diluted resazurin was stored at room temperature.

Table 3.2: List of commercial kits used in this study

Commercial kits	Supplier
Anaerogen™ Compact	Oxoid Microbiology Products, UK

Table 3.3: List of consumable items used in this study

Consumable items	Suppliers
Pipette tips	Thermo Fisher Scientific, Inc. USA
Mycology Pipette	Eppendorf Asia Pacific Sdn Bhd
96-well culture plate	BD Falcon

Table 3.4: List of laboratory equipment used in this study

Laboratory Equipment	Suppliers
Biological Safety Cabinet type II	NuAire, Inc., USA
Calliper	Mycology Laboratory
Hot Air Oven	Memmert, German
Incubator	Thermo Fisher Scientific, Inc. USA
Nephelometer	PhoenixSpec, USA
Rotary Evaporator R-114	Buchi Rotavapor, Switzerland
Soxhlet extractor	Pharmacology Laboratory
Pasteur pipette	Sigma Aldrich, USA
Weighing Balance	Precisa, Switzerland