Original Article

Comparison of antimicrobial activity of crude extracts of *Piper betle, Aloe vera, Solanum lycopersicum, Cinnamomum zeylanicum* and *Cucumis sativus* against acne inducing bacteria

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Abstract

Acne vulgaris is a typical skin disorder among adolescence, causing inflammation of pilosebaceous follicle which characterized by comedones, papules, pustules, cysts, nodules and often scars in face, neck, upper trunk and arms. *Propionibacterium acnes* and *Staphylococcus epidermidis* have been recognized that play as a major role in acne formation. This study was conducted to compare the antimicrobial activity of five plant extracts namely *Piper betle*, *Aloe vera*, *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* against *P. acnes* and *S. epidermidis*. The well diffusion assay was used to determine the sensitivity of the samples, while the liquid dilution method was used for the determination of the minimal inhibition concentration (MIC). The result showed a remarkable antibacterial activity of *Piper betle* extract compared to other plant extracts and Doxycycline (positive control) against both of acne-inducing bacteria, *P. acnes* and *S. epidermidis*.

Keywords: **:** Acne-inducing bacteria; antimicrobial activity; sensitivity
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Introduction

Acne vulgaris is a common chronic skin disease causing blockage and inflammation of pilosebaceous units such as hair follicles and the accompanying sebaceous gland [1]. Formation of acne can present as inflammatory and non-inflammatory lesions, which are normally affecting face, back and chest [2]. The accumulation of sebum in the pilosebaceous channel aids the proliferation of skin bacteria such as *Propionibacterium acnes* and *Staphylococcus epidermidis* [3-5]. *P. acnes* is a gram-positive anaerobic microorganism that resides beneath the surface of human skin [3, 6]. Another acne-causing bacteria *S. epidermidis*, the aerobic gram-positive organism is also a resident of human skin flora [3-6]. This aerobic bacterium is associated with superficial infections within the sebaceous unit.

Many topical antibiotics formulations for the management of acne are available, either alone or in combination. They inhibit the growth of *P. acne* as well as *Staphylococcus epidermidis* and reduce inflammation. Topical antibiotics such as erythromycin and clindamycin are the most popular antibiotics [7]. Combination therapy, which containing clindamycin and benzoyl peroxide, has become the standard for the management of acne [8]. However, the major limitation is irritation and dryness from higher concentrations of benzoyl peroxide [9]. Over the past 20 years, many concerns increased in the prevalence of antibiotic-resistant *P. acnes* strains [10]. The development of antibiotic resistance is multifactorial and hard to prevent. To overcome this problem, medicinal plants have been used as alternative medicines for acne disorder [11-13].

Piper betle from the genus of Piper is one of Piperaceae family which belongs to super order Nymphaeiflloraea, order Piperales [14]. It is known as sireh in Malaysia and composes over thousand species. *Piper betle* have been cultivated in India, Sri Lanka, Malaysia, Indonesia, Philippine Islands and East Africa [15, 16]. The parts of this plant which usually used are leaves, roots, stems, stalks and fruits [17]. *Piper betle* leaf has a significant antimicrobial activity against microorganism such as *Streptococcus pyogenes*, *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* [18].

Aloe vera is a traditional medicinal plant that belongs to the family Liliaceae that originated in South Africa. It has remained an important component in the traditional medicine China, India, the West Indies, the southern USA and Japan [19, 20]. It also can be found in Malaysia and also known as "lidah buaya". This plant has been widely used to cure various skin conditions such as cuts, burns and eczema. *Aloe vera* consists of antiseptic and antibiotic properties, thus important in treating cuts and abrasions [21].

Solanum lycopersicum is a red plant belongs to family Solanaceae and genus Solanum which is commonly called as tomato [22]. 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. Some reports have shown that tomato fruit extracts inhibited the growth of pathogens such as *E. coli*, *Salmonella Typhimurium*, *Staphylococcus aureus* and *Listeria ivanovii* [23, 24].

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 [25, 26]. Cinnamon is a common spice used by different cultures around the world for several centuries. This spice usually used in both sweet and savoury foods. In Unani classical texts cinnamon act as a potent drug in acne treatment, melasma, abdominal pain, hiccups, headache, jaundice, vomiting and diarrhoea. It has a role in antifungal, antibacterial, antiulcer and immunomodulatory activities [27, 28].

Cucumis sativus is one of Cucurbitaceae family and typically known as cucumber [29]. Cucumber is used as folk medicines to treat headache, the seeds are cooling and diuretic [30]. The fruit juice of cucumber usually used as nutritive and demulcent in anti-acne lotion [31].

In this study, *Piper betle*, *Aloe vera*, *Solanum lycopersicum*, *Cinnamomum zeylanicum* and *Cucumis sativus* were investigated on their antibacterial activities against two acne-inducing bacteria, *P. acnes* and *S. Epidermidis.*

Materials and Methods

Plant material

The plant materials were collected from various locations in Kelantan, Malaysia. All five different plant materials used are shown in Table 1. All the plant materials were obtained gradually because to maintain their freshness ingredients before used in the study. After the material was acquired, the leaves or fruits were cut thinly and incubated in hot air oven of 50^{o}C for overnight before proceed with methanol extraction method.

Preparation of the extract

All dried plant materials were successively extracted using a Soxhlet apparatus with 95 % methanol as solvent, at 55°C for 3 hrs. The methanolic extracts were then concentrated to dryness in a rotary evaporator under

reduced pressure at a temperature of 50°C for 72 hrs. Each of the methanolic extracts were weighed for 1 g and dissolved with 0.1 % of DMSO to obtain concentration of 500 mg/ml each.

Microorganisms Studied
ATCC strain for both *P. acnes* and *S. epidermidis* were obtained from Laboratory of Microbiology and Medical Parasitology of Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia.

Antimicrobial Activity Test
The antimicrobial activity of methanolic extracts of plants against *P. acnes* and *S. epidermidis* were detected by well diffusion method. The Mueller-Hinton agar plates were used to determine the susceptibility of *S. epidermidis*, while blood agar was used to detect the antimicrobial activity against *P. acnes*.

By using a sterile cotton swab, the top of colonies were transferred into a tube containing 0.85 % of sterile saline and the turbidity of the actively growing sterile saline was adjusted. The sterile cotton swab was dipped and touched the bottom of inoculum. The turbidity was adjusted to obtain a yield of 10^8 CFU/ml comparable to 0.5 McFarland of turbidity standard. Then, the inoculum of the bacteria was swabbed uniformly with sterile cotton on the agar.

Each of the plates was punched to make 4 mm holes using sterile rod in each plates. Hundred µl of methanolic extracts were dropped into the appropriate wells and doxycycline (50 mg/ml) was used as a reference standard. *P. acnes* was incubated for 48 hours in anaerobic condition. On the other hand, *S. epidermidis* was incubated for 24 hours. Each experiment was repeated at least three times and the mean of the diameter of the inhibition zones was calculated. Antimicrobial activities were indicated by clear zones of growth inhibition.

Determination of Minimum Inhibitory Concentration (MIC)
A microdilution method was used to determine MIC of the plant extracts against *P. acnes* and *S. epidermidis.*

A two-fold serial dilution was used in microdilution method. This serial dilution was used to reduce the concentration of extract by a factor of two that reduces the original concentration by one half. The plant extracts that have been diluted in DMSO with concentration of 1.0 g/ml were diluted in Mueller-Hinton broth to the concentration of 0.5 g/ml. The MIC test was carried out on 96-well plate. The serial two- fold dilution was prepared on 20 wells for each selected extracts directly. The well plates were labelled to represent each of selected extracts to easily distinguish them and easily to be recorded as well for the positive and negative controls. Two hundred µl of Mueller-Hinton broth was pipetted into each well (well 2 – 20) except for well 1 for each selected extracts. Two hundred µl of selected plant extracts were pipetted into well 1 and 200 µl was added into well 2 that consisted of Mueller-Hinton broth. After thorough mixing, 200 µl of mixture of Mueller-Hinton broth and plant extract was transferred from well 2 to well 3. This process was continued until the last well (well 20) which from there 200 µl was removed and discarded. As for the controls, one of selected extracts was serial diluted in Mueller-Hinton broth and used as a control negative. Doxycycline was used as positive control.

Resazurin dye assay
The lowest concentration that does not showed any growth of the bacteria was taken as MIC and confirmed with Resazurin dye as an indicator. After the incubation, 25 µl of Resazurin dye was dropped into each well including the controls and then incubated for 5 – 10 hours. The colour of the culture that was growth changed into pink whereas the culture that does not show any growth maintain as blue colour.

Data analysis
Data points were represented by the mean of the measured values. Statistical analysis was carried out using IBM SPSS Statistics. One-way ANOVA was used to compare mean differences of five different groups of plant extracts in *P. acnes* and *S. epidermidis.*

Results and discussion
In this study, five plants including *Piper betle, Aloe vera, Solanum lycopersicum, Cinnamomum zeylanicum* and *Cucumis sativus* as shown in Table 1 were used to investigate the antimicrobial activities against typical acne-inducing bacteria, namely *P. acnes* and *S. epidermidis.*

All the plant materials were extracted via Soxhlet extractor with absolute methanol. The alcoholic extracts (methanol) showed greater activity compared to aqueous and hexane extracts due to the large quantity of active substances that were precipitated during the extraction process [32- 34]. Antibacterial activities of alcoholic extracts may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall [35, 36]. They may attach to enzyme sites rendering them inactive.

Table 1 Five plants used in the study

Scientific Name & Family Name	Part used
Piper betle (Piperaceae)	Leaves
Aloe vera (Liliaceae)	Leaves
Solanum lycopersicum (Solanaceae)	Fruits
Cinnamomum zeylanicum (Lauraceae)	Barks
Cucumis sativus (Cucurbitaceae)	Fruits

The results of the antimicrobial activity tests of the methanolic extracts of the five traditional plants (Fig. 1 and Fig. 2) revealed that all extracts of the plants showed the antimicrobial activities against *P. acnes* and *S. epidermidis*, respectively. Table 2 showed the result of the mean of inhibition zone of five methanolic plant extracts (500 mg/ml) against *P. acnes and S. epidermidis*. Doxycycline was used as a positive control. The diameters of growth inhibition area of extracts studied were in the range 7.7 – 29.0 mm and 13.0 – 32 mm for *P. acnes* and *S. epidermidis*, respectively.

Figure 1. Antimicrobial activities of the methanolic extracts against *P. Acnes.* Represents: (1) *Aloe vera*, (2) *Solanum lycopersicum*, (3) *Cucumis sativirus*, (4) *Piper betle*, (5) *Cinnamomum zeylanicum* and (6) Doxycycline as control

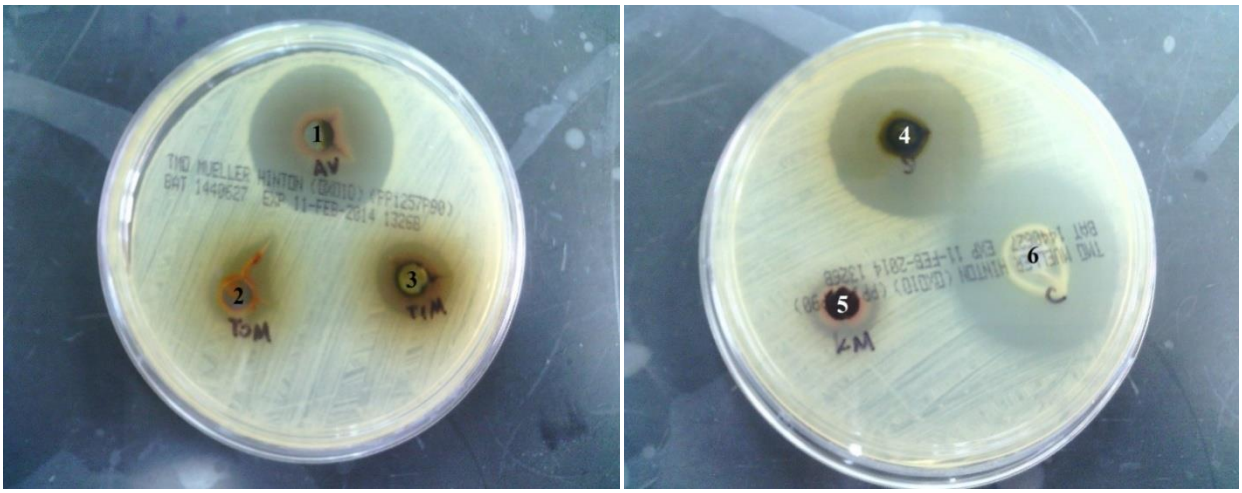

Figure 2. Antimicrobial activities of the methanolic extracts against *S. Epidermidis.* Represents: (1) *Aloe vera,* (2) *Solanum lycopersicum*, (3) *Cucumis sativus*, (4) *Piper betle*, (5) *Cinnamomum zeylanicum* (6) Doxycycline (Control)

Table 2. Mean of inhibition zone of five ethanolic plant extracts (500 mg/ml) and doxycycline

No	Ethanolic extracts of plant	Mean of inhibition zone (mm)	
		P. acnes	***S. epidermidis***
1	*Piper betle*	29.0	32.0
2	*Aloe vera*	7.7	30.7
3	*Solanum lycopersicum*	10.7	13.0
4	*Cinnamomum zeylanicum*	13.9	14.3
5	*Cucumis sativus*	11.5	15.3
6	Doxycycline	15.2	31.3

Our results showed a remarkable antibacterial activity of *Piper betle* extract compared to other plant extracts and doxycycline. *Piper betle* showed the strongest antimicrobial activity against both of acne-inducing bacteria of *P. acnes* and *S. epidermidis*. According to Chakraborty and Shah [17] ethanol extract of *Piper betle* consisted high concentration of fatty acids like palmitic acid, stearic acid and hydroxy fatty acid esters that showed potent antimicrobial activity against diverse pathogenic microorganism [17]. *Aloe vera* extract and *Solanum lycopersicum* extract showed the smallest diameter of inhibition zone against *P. acnes* and *S. epidermidis*, respectively.

All the plant extracts were subsequently subjected to MIC determination. MIC was described as the lowest antimicrobial drug that inhibits the visible microorganism growth after incubation [37]. The MIC determination of the antimicrobial drug is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth. In this study, a microdilution method was used to determine MIC of *Piper betle* extract against *P. acnes* and *S. epidermidis*.

Table 3 The MIC values of ethanol plant extracts against *S. Epidermidis*

Ethanol extracts of plant	Mean of MIC value (mg/ml)
Piper betle	3.67
Aloe vera	78.13
Solanum lycopersicum	332.03
Cinnamomum zeylanicum	58.60
Cucumis sativus	175.78

According to Eloff [38], a micro-dilution technique is robust, quick and not expensive. It gives reproducible results which is 30 times more sensitive than other method for example agar and broth dilution methods. The micro-dilution method requires a small quantity of sample. One or two of the series of wells should be used with a known antibiotic to provide reference MIC values for the test organism.

The MIC values against *S. epidermidis* showed that *Piper betle* extract obtained the lowest concentration of MIC values compared to the other plant extracts which concluded that this extract only need the smallest amount to inhibit the *S. epidermidis* bacteria growth (Table 3). This indicated that the *Piper betle* was the most effective antimicrobial agent compared to other plants that inhibits the growth of *S. epidermidis.* The less effective antimicrobial agent that prevents the *S. epidermidis* bacteria growth was *Solanum lycopersicum* extract which showed a highest concentration of MIC values.

Conclusion

The present study represents evidence based on local remedy in providing effective treatment against infection caused by acne inducing bacteria.

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