Biofarmasetikal Tropis
(The Tropical Journal of Biopharmaceutical) 2025, 8(2), 10-15
e-ISSN 2685-3167
p-ISSN 2828-6685

Antibacterial Activity Of Green Sirih Leaves (*Piper betle* Linn) Against *Propionibacterium Acnes*

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Accepted: 26 September 2025; Approved : 16 Oktober 2025

ABSTRAK

Sirih termasuk salah satu tanaman obat yang sering ditemukan di sekitar kita. Karena sifatnya sebagai antiseptik dan obat luka, tanaman ini dapat dibudidayakan. Tujuan Penelitian ini untuk mengetahui aktivitas antibakteri minyak atsiri daun sirih hijau terhadap Propionibacterium acnes. Penelitian eksperimental laboratorium ini menggunakan Rancangan Acak Lengkap dengan tiga kali pengulangan pada lima perlakuan konsentrasi, satu kontrol positif dan satu kontrol negatif untuk bakteri Propionibacterium acnes. Metode uji yang dipakai adalah metode difusi agar. Pengumpulan data dilakukan dengan mengamati dan mengukur zona hambat pada setiap cawan petri kemudian ditabulasi. Data yang diperoleh dianalisis secara deskriptif. Pada konsentrasi 10µl minyak atsiri daun sirih hijau memiliki kemampuan sebagai agen antibakteri terhadap Propionibacterium acnes, sesuai dengan hasil pengukuran diameter zona hambat yang dihasilkan.

Kata kunci: Tanaman Obat, *Piper betle* Linn, *Propionibacterium acnes*.

ABSTRACT

Betel is one of the medicinal plants often found around us. Because of its properties as an antiseptic and wound medicine, this plant can be cultivated. This research aimed to determine the antibacterial activity of green betel leaf essential oil against Propionibacterium acnes. This laboratory experimental research used a completely randomized design with three repetitions in five concentration treatments, one positive control and one negative control for Propionibacterium acnes bacteria. The test method used was the agar diffusion method. Data was collected by observing and measuring the inhibition zone on each Petri dish, which was then tabulated. The data obtained were analyzed descriptively. At a concentration of 10µl, green betel leaf essential oil can be an antibacterial agent against Propionibacterium acnes, according to the results of measuring the diameter of the inhibition zone produced.

Keywords: *Medicinal plants, Piper betle* Linn, *Propionibacterium acnes.*

1. INTRODUCTION

A medicinal plant is a type of plant that is often used as a medicine, substance, or herb. Betel is one of the medicinal plants found in the surrounding environment. Because this plant can be utilized as a wound medication and antiseptic, it has the potential to be grown[1]. Liquid, or essential oils, is produced using fragrant plant parts in an extraction procedure. These materials are a source of ingredients for products in the culinary, cosmetics, and pharmaceutical industries[2]. Green betel leaves contain saponins, tannins, flavonoids, polyphenols, and essential oils as antibacterial[3]. Empirically, in Kiawa village, the community

e-ISSN 2685-3167
p-ISSN 2828-6685

uses two green betel leaves to treat wounds and mouth ulcers.

It should also be noted that green betel leaf essential oil is only known to have activity as an antibacterial against *Staphylococcus epidermidis*[4] and can inhibit the growth of *MRSA*[5] as an antifungal *Candida albicans*[6] as an antioxidant[7].

Gram-positive, one of the common bacteria found on human skin, in the mouth cavity, colon, conjunctiva, and external ear canal, is *Propionibacterium acnes*. These bacteria dominate in the follicular region of the skin and can cause acne when infecting the skin[8]. *Propionibacterium acnes* are commonly used as target bacteria in bacterial growth inhibition research; these bacteria include pathogenic bacteria that cause disease. Therefore, it is necessary to extract natural ingredients that can be used to kill these pathogenic bacteria[9].

Many bacteria that cause disease (pathogens) in humans have shown drug resistance due to inappropriate antibiotic use. Therefore, the search for antibacterial compounds of natural origin continues[10-19].

2. RESEARCH METHODS

Place and Time of Research

This research was conducted at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, Indonesian Christian University Tomohon. The time of research implementation was in November 2023 - February 2024.

Tools and Materials

The tools used in this research are a set of distillation tools, rotary evaporator, Laminar Air Flow (LAF), Incubator, autoclave, heating mantle, 500mL round bottom flask, 500mL Erlenmeyer, 250mL measuring cup, Petri dish, analytical balance, aluminum foil, filter paper, scissors, separating funnel, label paper, pipette, test tube, bunsen, stirring rod, beaker glass, vernier, ose needle.

The materials used are essential oil from *Piper betle* Linn, *P. acnes* bacteria distilled water, clindamycin 300mg, nutrient agar (N.A.), and nutrient broad (N.B.).

Research Type and Research Design

This research employs a completely randomized design (C.R.D.) in a laboratory setting. This kind of research uses an entirely randomized design (C.R.D.) in a laboratory setting by doing three repetitions on five concentration treatments, one positive control, and one negative control for one type of bacteria, particularly *Propionibacterium acnes.* The diffusion method with the Kirby Bauer Technique and the agar diffusion method with paper discs were the antibacterial test methods employed in this investigation. The concentrations of green betel leaf essential oil (*Piper Betle* Linn) used were 2µL/disc, 4µL/disc, 6µL/disc, 8µL/disc, and 10µL/disc. The positive control was clindamycin antibiotic 2µL/disc, and the negative control was sterile distilled water.

Description:
I : First Repetition
Ii : Second Repetition
Iii : Third Repetition
A : Positive Control Clindamycin 2µl/Disc
B : Negative Control Sterile Aquadest
C : Green Betel Leaf Essential Oil 2µl/Disc
D : Green Betel Leaf Essential Oil 4µl/Disc
E : Green Betel Leaf Essential Oil 6µl/Disc
F : Green Betel Leaf Essential Oil 8µl/Disc
G : Green Betel Leaf Essential Oil 10µl/Disc

Sample Preparation

Green betel leaves were collected in Taas Village, Tikala District, Manado. The obtained green betel leaves were wet sorted, cleaned, chopped, and dried under indirect sunlight until dry. The sample was then cut into small pieces, after which it was weighed.

Preparation of Green Betel Leaf Essential Oil

1 kg of fresh green betel leaves are washed and dried under the sun for 15 days, obtained betel leaves that have been dried and cut into small pieces using scissors, then put into a distillation flask. Next, the distillation tool is pressed the on button and waited until the water boils. When the water boils, water vapor will carry particles of betel leaf essential oil to the condenser. Furthermore, water vapor and oil are accommodated in a closed container. Put the essential oil obtained from distillation into a separating funnel to separate the essential oil with water; distillation is carried out until the essential oil droplets in the container are no longer dripping.

e-ISSN 2685-3167
p-ISSN 2828-6685

Preparation of Test Bacteria Suspension

Test bacteria were taken, stirred, and then suspended in an Erlenmeyer containing 100 mL of distilled water with 0.8g of sterilized N.B. The bacterial suspension was then incubated at 30ºC for 24 hours. Bacterial growth is characterized by turbidity in the media. After incubating for 24 hours, it was taken and shaken until homogeneous. Then, the turbidity level was seen, namely, compared visually to the McFarland 0.5 standard solution.

Antibacterial Testing

The antibacterial activity of green betel leaf *(Piper betle* Linn.) against *P. acnes* was tested using the Kirby Bauer method with the agar diffusion method with paper discs. The suspension of *P. acnes* bacteria was taken as much as 1 mL using a micropipette, then put into an Erlenmeyer containing 90 mL NA and stirred until homogeneous. Next, the media was poured into each Petri dish as much as 30 mL, slowly shaking the Petri dish again in a circular motion without being lifted from the table surface and then left to solidify. After that, the solidified media is affixed to paper discs saturated with test solutions with several concentrations.

Each repetition contained 30 µL of test solution extract with a concentration of 2 - 10 µL, with the positive control used, namely clindamycin 2µL/disc, and negative control. Namely, Aquadest was then dripped onto sterile disc paper and allowed to dry; after the sterile disc paper was dry, it was placed on solid agar media that already contained test bacteria. Petri dishes were tightly wrapped and incubated at 30°C for 1x24 hours. Antibacterial activity was observed by measuring the diameter of the clear zone around the disk using a calliper[20]. The inhibition zone formed indicates the level of sensitivity of the test bacteria to the antibacterial material. The test was conducted three times[21].

Data Analysis

After the data was collected, the results of observations and measurements were tabulated in tables and figures Calculation of inhibition zone diameter according to :

Formula: d = (A+B+C)/3

Description:
D : diameter of inhibition zone
A : vertical diameter
B : horizontal diameter
C : diagonal diameter

3. RESULTS AND DISCUSSION

***Propionibacterium acnes* Bacterial Suspension**

The results of bacterial suspensions incubated for 24 hours at 30°C show turbidity in Nutrient Broth (NB) media, which means there is bacterial growth in the media. Based on these results, bacterial suspensions can be mixed with Nutrient Agar (NA) media, and antibacterial tests can be conducted.

Green Betel Leaf Essential Oil

The sample used in this research is 0.3 ml of green betel leaf essential oil obtained from the distillation of yellowish-white betel leaves as large as 1kg.

Antibacterial Activity Test

The results of the antibacterial activity test of Chinese betel leaf juice against *Propionibacterium acnes* bacteria showed positive results. The antibacterial test uses the disc diffusion method, which is an 8 mm disc paper saturated with test compounds with various concentrations using seven treatments, including concentrations of 2µL/disc, 4µL/disc, 6µL/disc, 8µL/disc and 10µL/disc of green betel leaf essential oil each pipetted in a disc paper, negative control which is sterile distilled water as much as 2µL, and positive control clindamycin pipetted as much as 2µL.

Furthermore, the disc paper is affixed to NA media filled with bacteria in a 10cm petri dish. After incubating for 24 hours, the petri dish press shows an inhibition zone formed around the disc paper.

The zone of inhibition that has formed indicates the presence of antibacterial activity produced by green betel leaf essential oil, meaning that green betel leaf essential oil has the potential as an antibacterial agent in inhibiting the growth of *Propionibacterium acnes*. Table 1 and Figure 1 show the average diameter of the inhibition of each concentration obtained by measuring the zone of inhibition using a caliper.

Table 1. Results of antibacterial activity test of green betel leaf essential oil against *Propionibacterium Acnes*

Concentration	Description	P.I	P.II	P.III	Average
(1)	(2)	(3)	(4)	(5)	(6)
2µL/disc		11.60 mm	11.73 mm	11.43 mm	11.58 mm
4µL/disc		11.50 mm	12.40 mm	12.03 mm	11.97 mm
6µL/disc	M.A Green betel leaf	12.30 mm	12.00 mm	12.03 mm	12.11 mm
8µL/disc		12.66 mm	12.26 mm	12.73 mm	12.55 mm
10µL/disc		13.10 mm	12.76 mm	12.93 mm	12.93 mm
2µL/disc	Control (+)	26.40 mm	29.26 mm	28.46 mm	28.04 mm
2µL/disc	Control (-)	0	0	0	0

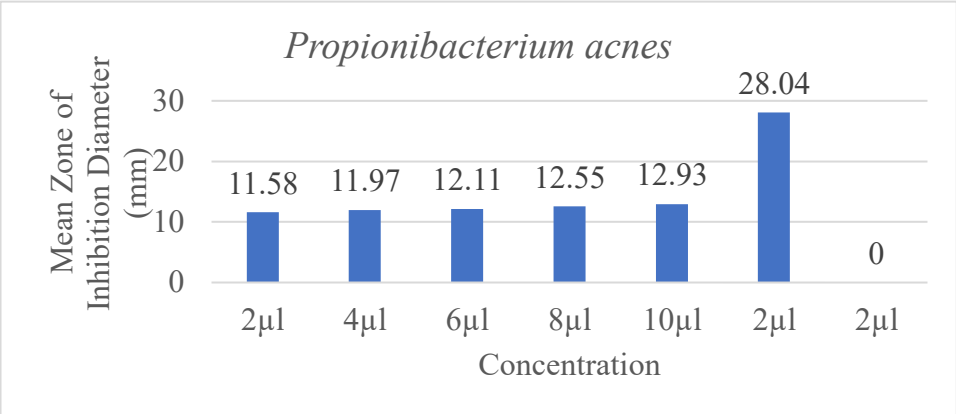

Figure 1. Graph of Antibacterial Activity Testing on *P.acnes* Bacteria

Based on the results of measuring the average diameter of the inhibition zone in Table 1 and Figure 1, green betel leaf essential oil shows that all concentrations of green betel leaf essential oil have antibacterial activity against *Propionibacterium acnes*. Concentrations of green betel leaf essential oil from 2 - 10µL have inhibition zone values that are classified as vital, with an average of 11.58 mm for 2µL, 11.97 mm for 4µL, 12.11 mm for 6µL, 12.55 mm for 8µL and 12.93 mm for 10µL concentration. The positive control clindamycin with a concentration of 2µL had a solid inhibition zone value with an average of 28.04 mm. Meanwhile, the negative control aqua dest showed no inhibition zone against *Propionibacterium acnes*.

4. CONCLUSION

Based on the results obtained, it can be concluded that green betel leaf essential oil (*Piper betle* Linn) has antibacterial activity against *Propionibacterium acnes* at a concentration of 2µL —10µL, with the average diameter of the inhibition zone obtained classified as vital.

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