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Effectiveness of Chinese Betel Leaf Extract (*Peperomia pellucida* L) on the Growth of *Propionibacterium acnes Bacteria*

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Abstract

Propionibacterium acnes bacteria cause acne by producing lipase enzymes that break down free fatty acids in skin lipids. This study aimed to determine whether or not there is antibacterial activity from the extract of the Chinese betel leaf plant (*Peperomia pellucida* L) against Propionibacterium acnes and to determine the concentration of the inhibition zone. The study was experimental with 25%, 50%, 75% and 100% extract concentrations. The method used to test antibacterial activity in this study was the disc diffusion method, namely using discs planted on Nutrient Agar (NA) media. The maceration of Chinese betel leaves (Peperomia pellucida L) is the Chinese betel leaf extraction method. The results showed that at a concentration of 25% Chinese betel leaf extract, the results were 22.5 mm; at a concentration of 50% extract, the inhibition zone was 24 mm; at a concentration of 75% Chinese betel leaf extract, the inhibition zone was 30 mm, at a concentration of 100% Chinese betel leaf extract, the inhibition zone was 20 mm. The inhibition zone of the positive control using the antibiotic chloramphenicol was obtained at 10 mm; in the negative control using sterile distilled water, there was no inhibition zone. The results of the significance test of the Chinese betel leaf extract (*Peperomia pellucida*) from the Greenwood classification results in the Post hoc test show that the difference between concentrations can be stated as significant at each concentration.

Keywords: Chinese betel leaf extract, *Propionibacterium acnes*, Inhibition zone

Introduction

Acne is a chronic inflammatory disease of the pilosebaceous follicles characterized by the appearance of blackheads, papules, pustules, and nodules. Acne occurs on skin that contains many sebaceous glands such as the face, chest, and back. The number of acne cases in Indonesia is quite high, ranging from 85-100% of people, while according to the records of the Indonesian cosmetic dermatology study group, it shows that there were 60% of acne sufferers in 2006 and 80% in 2007 (Aida et al., 2016).

Generally, acne is found on the face, chest, neck, and back skin. Genetic factors, hormones, psychology, food, bacterial infections, sebaceous gland activity, cosmetics, and other chemicals trigger acne. In addition, excessive oil gland activity accompanied by bacterial infection can worsen acne (Meilina and Nur Hasanah, 2018). Propionibacterium acnes bacteria cause acne by producing lipase enzymes that break down free fatty acids in skin lipids. These fatty acids can cause tissue inflammation when related to the immune system, and acne can occur (Miratunnisa et al., 2015). *Propionibacteriium acnes* is a normal flora of the Pilosebaceous glands of human skin, this bacteria causes acne by producing Lipase which breaks down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when in contact with the immune system and support the occurrence of acne. This bacteria is a type of Gram Positive anaerobic bacteria that is tolerant to air (Tunnisa, et al. 2015).

One of the efforts to reduce cases of antibiotic resistance is to develop drugs derived from natural ingredients to treat acne. One of the natural ingredients that can be used is the Chinese betel plant. The Chinese betel plant is one of the plants that grows in tropical areas. Chinese betel is one of the traditional plants that can be used as a treatment. Indonesia has a variety of biodiversity so that Indonesia is rich in natural and traditional medicinal ingredients used for traditional herbal medicine from generation to generation (Rahayu et al., 2024). The World Health Organization (WHO) states that up to 65% of the population of developed countries have used traditional medicine (Ullah et al., 2023). The Chinese betel leaf plant (*Peperomia pellucida* L. Kunth) is a plant that originates from South America but is generally found in Southeast Asia. Traditionally, Chinese betel leaves (Peperomia pellucida) are used as a medicine for abscesses, boils, acne, skin diseases, headaches, reducing pain in rheumatism and gout rheumatism (Angelina et al., 2015) Based on the description above, this study was conducted to determine whether the Chinese betel plant (*Peperomia pellucida* L.) has antibacterial power against Propionibacterium acnes bacteria.

Literature Review

Morphology and classification

Chinese betel plant is a herbal plant that originates from the United States but grows wild and is easily obtained in Indonesia. We often find plants in yards, ditch edges, in damp places. This plant has a height of 10-20 cm with an upright, soft and light green stem, young stems are green, old stems are light brown, single leaves with a spiral position, oval shape, 1-4 cm long, leaf width 1.5-2 cm. pointed tip, notched base, flat edge, curved veins, smooth surface, soft, and green. Compound flowers, in the form of grains, are located at the stem's end or in the leaf axils 2-3 mm long. However, some types of betel can grow to a height of more than 1,000 meters above sea level (Widiyastuti, et al. 2014).

According to Putra (2015) the taxonomy of Chinese betel is as follows:

Kingdom : Plantae

Subkingdom : Tracheobionta

Super Division: Spermatophyta

Division : Magnoliophyta

Class : Magnoliopsida

Sub Class : Magnoliidae

Order : Piperales

Family : Piperaceae

Genus : *Piperomia*

Species : *Piperomia pellucida* L

Figure 1: Chinese betel plant (*Peperomia pellucida* L) (Personal documentation source)

Chemical Compound Content of Chinese Betel Leaves

In the study (Majumder, 2011) this plant has steroid compounds, flavonoids, carbohydrates. Alkaloids, flavonoids, saponins, tannins, and titerpenoids. From the phytochemical results conducted by Angelina et al. (2015) the Chinese betel plant (*Peperomia pellucida* L.) contains alkaloids, flavonoids, saponins, tannins and triterpenoids. With the compounds contained in the suruhan plant (*Peperomia pellucida* L.) it can be assumed that this plant can inhibit bacterial growth.

In the study (Nwokocha et al., 2012) stated that Tannin and Flavonoid compounds have antiseptic and antimicrobial activities. Tannin acts as an antibacterial through the formation of a complex with microbial enzymes or substrates, entering through its cell membrane. Flavonoids work as antimicrobials by forming a complex of extracellular proteins and cell walls. Flavonoids are lipophilic, meaning they can damage cell membranes.

a) Antibacterial Mechanism of Action of Flavonoids

The mechanism of action of flavonoids as antimicrobials can be divided into 3, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism. The antibacterial mechanism of flavonoids inhibits nucleic acid synthesis, rings A and B which play an important role in the process of intercalation or

hydrogen bonding by accumulating nucleic acid bases that inhibit the formation of DNA and RNA. Flavonoids inhibit cytochrome C reductase so that the formation of metabolism is inhibited. Energy is needed by bacteria for macromolecule biosynthesis (Rijayanti et al., 2014)

b) Mechanism of Action of Antibacterial Phenol

The antibacterial mechanism of phenol compounds in killing microorganisms is by denaturing cell proteins. Hydrogen bonds formed between phenol and protein cause the protein structure to be damaged. These hydrogen bonds will affect the permeability of cell walls and cytoplasmic membranes because both are composed of proteins. Disrupted permeability of cell walls and cytoplasmic membranes can cause an imbalance of macromolecules and ions in cells so that cells become lysed (Luliana et al., 2014).

c) Mechanism of Action of Alkaloid Antibacterials

The mechanism of alkaloids as antibacterials is disrupting the peptidoglycan components in bacterial cells so that the cell wall layer is not formed completely, causing cell death. Another mechanism of alkaloid antibacterials is that alkaloid components are known as DNA interchelators and inhibit bacterial cell topoisomerase enzymes (Rijayanti et al., 2014).

d) Mechanism of Action of Antibacterial Steroids

The mechanism of steroids as antibacterials is related to lipid membranes and sensitivity to steroid components that cause leakage in liposomes. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and changes in cell membrane morphology that cause cells to become fragile and lyse (Rijayanti et al. 2014).

Benefits of Betel Leaves

Betel leaves are known by the public as a plant that has many benefits, one of which is used as a traditional medicine. For the public, betel leaves are usually used as a medicine for gout, hemorrhoids, whooping cough, dysentery, heart, vaginal discharge, colds, stomach aches, smooth blood, muscle and joint pain, internal heat, and stroke. Other benefits of the Chinese betel plant (Peperomia pellucida L.) include as a medicine for headaches, fever (Ningtias, et al., 2014).

Betel leaves are widely known to have antifungal, antioxidant, antiplatelet, antipyretic, anti-inflammatory, antithrombotic, antimicrobial, anticancer, antibacterial, antihypertensive and depressant properties. Eugenol found in the leaves is useful as an anticonvulsant, analgesic, anesthetic, smooth muscle spasm reliever, and motor control suppressant. Tannins also found in the leaves are useful as astringents (reducing vaginal secretions) so that betel can function to treat vaginal discharge (Ningtias, et al. 2014).

Extraction and Extraction

a. Extraction

Extraction is a technique for separating a compound based on the difference in solute between two mixed solvents. Generally, the extracted solvent is insoluble or slightly soluble in a solvent but easily soluble in another. (Ministry of Health of the Republic of Indonesia, 2000) Extraction is the activity of withdrawing the content of a soluble chemical compound so that it is separated from the material that cannot be dissolved with a liquid solvent. Extraction can be done using various methods depending on the purpose of extraction, the type of solvent used, and the desired compound. The extraction methods used include:

(1) Maceration

Maceration is a process of filtering simple drugs by soaking them using a solvent with stirring at room temperature. Maceration that is carried out by stirring continuously is called kinetic maceration, while the repeated addition of solvent after filtering the first macerate and so on is called remaceration. In this study, the maceration method was used because it is simpler. This method can attract heat-resistant compounds and those that are not heat-resistant (Ministry of Health of the Republic of Indonesia, 2000)

b. extract

Extracts are dry, thick and liquid preparations, made by filtering the simplicia, outside the influence of direct sunlight. Extracts are grouped based on their properties, namely:

1) Dilute Extract
2) Thick Extract
3) Dry Extract
4) Sterilization

Sterilization is an effort to free tools or materials from all unwanted microorganisms. Sterilization is carried out by:

1) Sterilization by incandescence, this method is used to sterilize inoculation wires (Ose needles), the method is to burn the tool over a spirit lamp until it glows.
2) Sterilization with hot air (Dry) This method is used to sterilize glassware. The tool used is an oven with a temperature of 170°C - 180°C for 2 hours.
3) Sterilization with pressurized steam (Wet) This method is used to sterilize tools and materials that are resistant to high pressure temperatures. The tool used is an autoclave with a temperature of 110°C - 121°C (Kristanti, 2014).

Propionibacterium Acnes bacteria

Propionibacterium acnes bacteria are normal flora in the pilosebaceous glands. These bacteria are a group of gram-positive bacteria that are anaerobic and aerotolerant. P. acnes bacteria cause acne by producing lipase enzymes that break down free fatty acids in skin lipids. These fatty acids can cause tissue inflammation when related to the immune system, and acne can occur (Miratunnisa et al., 2015).

The following is a classification of the bacteria *Propionibacterium acnes* (Jawetz, et al. 2015):

Kingdom : Bacteria

Phylum : Actinobacteria

Class : Actinobacteria

Order : Actinomycetales

Family : Propionibacteriaceae

Genus : *Propionibacterium*

Species : *Propionibacterium acnes*

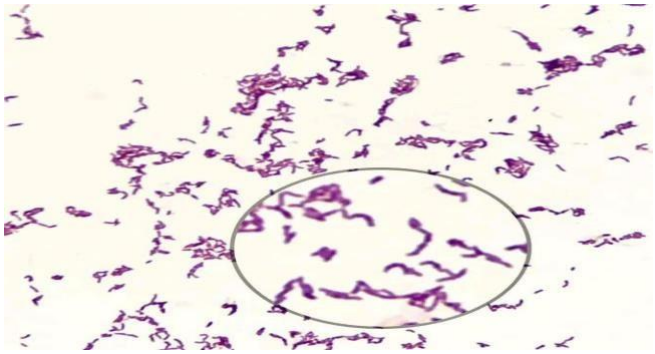

Figure 2: *Propionibacterium acnes bacteria*.
Source: Afriyanti, 2015

In general, antibiotic-resistant *P. acnes* varies across countries. High rates of *P. acnes* occur in Europe, with erythromycin resistance of 45-91% and tetracycline resistance of 26.4%. There are large differences in the number of antibiotic-resistant *P. acnes* across Asian countries. Research in Korea found 30% of cases of resistance to clindamycin and 26.7% of cases of resistance to erythromycin. Based on a study conducted at Hasan Sadikin Hospital Bandung, 12.9% of acne cases were found to be resistant to tetracycline, 45.2% were resistant to erythromycin, and 61.3% were resistant to clindamycin (Madelina and Sulistiyaningsih, 2018).

Propionibacterium acnes participates in the pathogenesis of acne by producing lipase, which breaks down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation and contribute to acne. P. acnes sometimes causes prosthetic heart valve infections and cerebrospinal fluid shunts (Jawetz et al., 2015). *P. acnes bacteria* show characteristics that are irregular rod-shaped and seen in gram staining showing purple bacteria indicating that these bacteria are gram-positive. P. acnes bacteria have characteristics of small colonies, white in color, smooth surfaces and solid consistency on Blood Agar plate (BAP) media (Lestari et al., 2015).

Pathogenicity and Symptoms of the Disease

Acne is a chronic inflammatory disease of the pilosebaceous glands characterized by comedones, papules, pustules, nodules, cysts, and scars. Acne is one of the most common skin diseases and affects 80-100% of the population. The prevalence of acne in Southeast Asia is 40-80% of cases (Afriyanti et al., 2015). According to a 2019 study of 66 acne patients at Abdul Moeloek Hospital, women (69.7%) experienced more acne than men (30.3%). In addition, the appearance of acne can reduce a person's self-confidence. As many as 30% -50% of people with acne tend to be less confident and experience psychological disorders due to the appearance of acne (Sibero et al., 2019).

Generally, acne is found on the skin of the face, chest, neck, and back. Genetic factors, hormones, psychology, food, bacterial infections, sebaceous gland activity, cosmetics, and other chemicals are triggers for acne. In addition, excessive oil gland activity accompanied by bacterial infections can worsen acne (Meilina et al. 2018).

Acne occurs due to several factors, namely excessive sebum production, abnormal hyperkeratinization of follicles, hyperkeratinocytes, colonization of P. acnes bacteria, and inflammation. Acne occurs when the duct to the skin's surface to remove sebum produced by the hair oil glands in the dermis layer is blocked. Under normal circumstances, hair follicle cells can come out. In some individuals, acne can develop into nodular cystic acne, which is characterized by the formation of nodules or scar tissue due to inflammation, excessive oil gland activity accompanied by bacterial infection can worsen acne (Meilina et al., 2018)

Acne occurs when the duct to the skin's surface to remove sebum produced by the hair oil glands in the dermis layer is blocked. Under normal circumstances, hair follicle cells can come out. However, if acne occurs, hair follicle cells together with sebum will clump and block the hair follicle duct in the epidermis layer of the skin, forming blackheads that protrude on the skin's surface. These blackheads will develop and become inflammatory acne if infected by bacteria, especially *P. acnes bacteria* (Radji, 2010).

Antibacterial Testing Method

One of the antibacterial tests can be done by the diffusion method (Disk diffusion test), which is done by measuring the diameter of the clear zone (Clear zone), which indicates the presence of a bacterial growth inhibition response by antibacterial compounds in the extract. The diffusion method is one of the methods that is often used. This method can be done in 3 ways: the cylinder, hole/well, and disc (Eli, 2017).

According to the general standards of plant-based drugs of the Indonesian Ministry of Health (2000), bacteria are said to be sensitive to plant-based antibacterials if they have an inhibition zone of 12-24 mm. Meanwhile, according to Greenwood (1995, Source: Ibrahim (2013), the effectiveness of antibiotics can be classified in the following table:

Table 1: Inhibition Zone Diameter Categories

Diameter Inhibition Zone	Response Obstacle Growth
< 0 – 5 mm	No There is
6 - 15 mm	Weak
16 - 20 mm	Currently
> 20mm	Strong

Source: Ibrahim (2013)

Research Method

1. Research Stage

   The research was conducted using a laboratory experimental method. Antibacterial tests were conducted using the Agar diffusion method using disc paper (Blank disk) to determine the diameter of the inhibition zone of Chinese betel leaf extract in inhibiting the growth of Propionibacterium Acnes bacteria.

2. Place and Time of Research

   This research will be conducted in August 2024 at the FKIP Chemistry Laboratory, Syiah Kuala University and the Research Laboratory, Faculty of Veterinary Medicine, FKH), Syiah Kuala University (USK) Banda Aceh.

3. Sample

   Samples were taken in Gue Gajah village, Cot Rangkang hamlet as much as ±3 kilograms. The bacterial culture used was obtained from the Research Laboratory, Fkh, USK.

4. Tools and materials

   The tools used to conduct this research include: petri dishes, dropper pipettes, micro pipettes, petri disks, microscopes, funnels, plastic, tissue, 250 ml Elenmeyer flasks, 100 ml measuring cups, stirring rods, filter paper, ose needles, sterile cotton swabs, label paper, aluminum foil, plastic wrapping, autoclave, water bath, Bunsen. The materials used are Chinese betel plants (*Peperomia pellucida* L.). The chemicals used in this study are 70% ethanol (technical), distilled water, spirits, Mc. Farland standard solution, chloramphenicol antibiotics and blank disks. The media used in this study are Mouler Histone Agar (MHA) media, and the bacteria used in the study are *Propionibacterium acnes bacterial cultures* .

5. Research Procedures

   The working procedures used in this research consist of several stages, namely:

   a. Sample Preparation

      Sampling was taken in the village of Gue Gajah, Cot Rangkang hamlet as much as ±3 kilograms. The parts of the plant used (leaves, stems and flowers) are then dried in room

temperature conditions (should not be exposed to direct sunlight) until the water content is reduced by 10% (±5 days).

b. Making Chinese Betel Plant Extract

The dried and ground plant parts using a mortar into powder form, weighed 200g, then macerated using 70% ethanol solvent (Technical) as much as 1200 ml (1:6) left for 3x24 hours with solvent changes every 24 hours. Then filtered using filter paper until the filtrate is obtained. The results in the form of filtrate are evaporated using a vacuum rotary evaporator until a thick extract is obtained and evaporated using a water bath at a temperature of 70oC - 80oC to evaporate the ethanol solvent. Extraction can be done using various methods depending on the purpose of extraction, the type of solvent used, and the desired compound. The extraction methods used include: Then the pure extract *of Peperomia pellucida* L. will be obtained (Mulyani et al., 2017)

c. Sterilization of tools

Sterilization of the tool is done by the dry heat method using an oven, while media sterilization is done by moist heat using an autoclave. The remaining test before being disposed of is carried out inactive process using the moist heat method which is then disposed of at the waste processing site.

d. Variable Concentration Stock Creation

The variables used in this study were 4 variables, negative control in the form of distilled water, positive control using chloramphenicol discs.

Table 2: Concentration of Chinese betel leaf extract (*Peperomia Pellucida* L)

Concentration Extract	**Sample (g)**	**Amount Aquadest (ml)**
25%	0.25	9.75
50%	0.50	9.5
75%	0.75	9.25
100%	0.5	-

e. Rejuvenation of Pure Culture of Test Bacteria

One colony of pure culture of test bacteria obtained from the Research Laboratory, FKH, USK was taken using a sterile loop from its pure culture, and then inoculated in Nutrient Agar (NA) media, then incubated in an incubator at 37°C for 1x24 hours. Test bacteria were observed, including colony morphology and gram staining (Kristanti, 2014).

f. Making Bacterial Suspension

Pure culture of test bacteria that has been propagated in Nutrient Agar (NA) media for 24 hours at a temperature of 25-30°C. Bacterial culture is taken 1 loop then transferred

in 0.9% NaCl solution. Bacterial suspension is equalized using a nephelometer (BD Phoenix) with a standard of 0.5 Mc Farland (estimated 1.5x108 bacterial cells/mL).

g. Antibacterial Testing

Antibacterial activity test was conducted using the paper disc method. Paper discs with a diameter of 6 mm were soaked in Chinese betel extract (*Peperomia pellucida* L. Kunth) for 15 minutes according to the treatment. The soaked paper discs were drained until no solution dripped. Effective testing of antibacterials was carried out using a method with several concentrations, the concentrations used were: 25%, 50%, 75%, 100%. The test was carried out by preparing a suspension of test bacteria. Then, prepare the Mueller Hinton Agar (MHA) media to use. A total of 10 mL was added to the Mueller Hinton Agar (MHA) media, put into a petri dish and then allowed to solidify. After solidifying, 1 loop of bacteria was taken that had been measured based on the Mc.Farland standard 108CFU/ml, then smeared using a cotton bud evenly on the surface of the Mueller Hinton Agar (MHA) media that had been solidified. Then Blank disk that has been given extract using micropipette with determined concentration is inserted into the surface of the media with a distance of one disk to another 1-2 cm on the edge of the petri dish. As a positive control (+), chloramphenicol and aquadest are negative controls (-). Then incubated at a temperature of 44 ° C for 1x24 hours. Furthermore, the formed inhibition zone is observed, and the diameter of the inhibition zone is measured with a caliper. Do 4 repetitions at each extract concentration.

Using a ruler, the inhibition zone formed around the disc paper is measured in vertical and horizontal diameter with mm units. The diameter of the inhibition zone is measured using the following formula (Kristanti, 2014)

$$\frac{(D_1 - D_C) + (D_2 - D_C)}{2}$$

Information:

D1 : Vertical Diameter D2 : Horizontal Diameter DC : Disc Diameter

Results

Based on the results of research that has been conducted on the antibacterial activity test of Chinese betel extract (*Peperomia pellucida* L. Kunth) against Propionibacterium acnes bacteria. After gram staining, an antibacterial effectiveness test was continued, namely by looking at the clear zone formed around the paper disk in each concentration treatment group.

Table 3: Test results of Chinese Betel Leaves (*Peperomia Pellucida* L) on the inhibition zone of *Propionibacterium acnes bacteria*

Concentration	Diameter (mm) D1	D2	Zone Index Resistor	Zone Response Resistor
K +	16	16	10mm	Weak
K -	0	0	0	No There is
25%	27	30	22.5mm	Strong
50%	30	30	24mm	Strong
75%	36	36	30mm	Strong
100%	26	26	20mm	Currently

Information:

K+ : Using Chloramphenicol

K - : Using Aquades

25% : Chinese betel extract with a concentration of 25%

50% : Chinese betel extract with a concentration of 50%

75% : Chinese betel extract with a concentration of 75%

100% : Chinese betel extract with 100% concentration

A total of ±3 kilograms of Chinese betel leaves that have been collected are then washed clean and dried until dry so that 300 g of dried Chinese betel leaves are obtained which have been dried and ground using a mortar/blender into powder and then weighed 200 g, then macerated using ethanol solvent (Technical) 70% as much as 1200 ml (1:6) left for 3x24 hours with solvent changes every 24 hours. Then filtered using filter paper until the filtrate is obtained. The results in the form of filtrate are evaporated using a vacuum rotary evaporator until a thick extract is obtained and evaporated using a water bath at a temperature of 70oC-80oC to evaporate the ethanol solvent.

The results of the significance test of Chinese betel leaf extract (*Peperomia pellucida* L) from the Greenwood classification results in the Post hoc test showed that the difference between concentrations was stated to have a strong response at 25% and 50%. Meanwhile, the Chinese betel leaf extract concentration of 75% and 100% had a strong response to Propionibacterium acnes bacteria. In the positive control test using chloramphenicol antibiotics, there was a moderate response to the growth of Propionibacterium acnes. In the negative control test using sterile distilled water, no inhibition zone was formed, which means that there was no inhibition of the growth of Propionibacterium acnes bacteria.

Discussion

The Chinese betel plant (*Peperomia pellucida* L.) has traditionally been used by the community to treat several diseases. The ability of the Chinese betel plant (*Peperomia pellucida* L.) as a medicinal plant is thought to be related to the antioxidant content of the plant.

Effectiveness of Chinese Betel Leaf Extract (Peperomia pellucida L) on the Growth of Propionibacterium acnes Bacteria

The Chinese betel plant (*Peperomia pellucida* L.) contains alkaloids, flavonoids, saponins, tannins and triterpenoids. The compounds contained in the Chinese betel plant (*Peperomia pellucida* L.) can inhibit bacterial growth.

The results of the inhibition zone of Chinese betel leaf extract at concentrations of 25% and 50% obtained results of 22.5 mm and 24 mm strong responses. The results of the Chinese betel leaf inhibition zone based on Greenwood's classification showed the presence of an inhibition zone at concentrations of 25% and 50%. A strong inhibition response was obtained at 75% and 100%. This is in accordance with the study of the inhibition zone activity test where the higher the concentration, the larger the resulting inhibition zone formed around the disc.

From the results of research conducted by Umi Julaika (2022), it was found that Chinese betel extract (*Piperomia pellucida* L. Kunth) with concentrations of 20%, 40%, 60%, and 80% on the growth of Shigella dysenteriae bacteria in this study showed the effect of each different treatment on the diameter of the inhibition zone produced, this can be seen from the formation of a clear zone around the disc paper.

In the study of Hikmah et al., (2023). it shows that each cotton leaf extract concentration group can form an inhibition zone of Propionibacterium acnes bacteria. A concentration of 60% can form an inhibition zone with an average of 9.5 mm. A concentration of 70% can form an inhibition zone with an average of 11 mm. A concentration of 80% can form an inhibition zone with an average of 11.5 mm. A concentration of 90% can form an inhibition zone with an average of 12 mm. A concentration of 100% can form an inhibition zone of 13.6 mm. In the positive control of Tetracycline, an inhibition zone of 17.6 mm was formed.

From here it can be concluded that Chinese Betel Leaf Extract (*Peperomia pellucida* L) has a stronger ability to form a bacterial growth inhibition zone than the cotton extract concentration (Gossypium hirsutun). The optimum extra strength at the concentration of Chinese betel leaf extract is 75% when the extract concentration is too high to a concentration of 100% then the optimum preparation strength falls, each extract has a different preparation strength. So the optimum strength of the concentration of Chinese betel leaf extract (Peperomia pellucida L) is 75%.

Conclusion

Based on the results of research on the effectiveness of Chinese betel leaf extract on the growth of Propionibacterium acnes bacteria:

1. Chinese Betel Leaves (*Peperomia pellucida* L) have antibacterial properties against the growth of Propionibacterium acnes bacteria.
2. The smallest inhibition zone at a concentration of 25% with an inhibition zone strength of 22.5 mm and the largest inhibition zone at a concentration of 75% obtained an inhibition zone of 30 mm which had a strong response.

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