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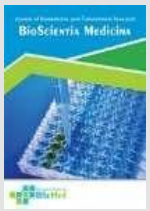

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Effectiveness Test of Green Betel Leaf Extract (*Piper betle L.*) on the Growth of *Staphylococcus aureus*

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A B S T R A C T

Background: Diseases due to infection where one of the causes is *Staphylococcus aureus* is one of the biggest problems faced today. Treatment using penicillin antibiotics tends to cause resistance and side effects, so people prefer to overcome them by means of self-medication or the use of traditional ingredients such as betel leaf. The purpose of the study was to examine the effect of green betel leaf extract (*Piper betle* L.) on the growth of *Staphylococcus aureus* on the antibacterial activity. **Methods:** This study is experimental research in vitro using a well diffusion method. The betel leaf was extracted using the maceration method and made into concentrations of 5%, 10%, 15%, and 20%. **Results:** The average diameter of the inhibition zones was 9.82 mm, 9.11 mm, 9.28 mm, and 9.01 mm at concentrations of 5%, 10%, 15%, and 20%, sequentially. **Conclusion:** The antibacterial activity of betel leaf extract in the overall concentration and the most optimal concentration was 5% at 9.82 mm.

1. Introduction

Infectious diseases are one of the biggest problems facing the world of health in Indonesia.[1,2] This refers to fairly high mortality and morbidity rate caused by infection, especially *Staphylococcus aureus*.[3] *Staphylococcus aureus* is a type of facultative anaerobic microorganism and is generally the most common bacterial contributor to skin infections, such as folliculitis, furuncles, and carbuncles that occur around hair follicles and more severe infections such as endocarditis, meningitis and pneumonia.[4,5]

The available treatment to treat Staphylococcus aureus is the use of antibiotics, especially the penicillin group.[4,5] Penicillin drugs work by directly inhibiting the synthesis of cell walls and diffusing well in tissues and body fluids. However, the use of antibiotics tends to lead to resistance due to the irrational use of antibiotics.[6] Resistance to penicillin is 25-50% in various countries.[7] In addition to the problem of resistance to antibiotics, the most dominant side effect of using antibiotics is allergic reactions. According to research, beta-lactam antibiotics are one of the most common causes of allergies, with a prevalence of 11.11%.[8,9]

Indonesia is a country with the second largest biodiversity in the world. The potential for biological wealth gives Indonesia the potential to be developed

into a new therapeutic modality based on natural ingredients. One type of plant that has been proven to be useful as a good antibacterial is a betel. These plants already exist and have been known by the public for a long time. Since ancient times, betel has been known as a plant with a million properties, especially in traditional medicine.[10-12] Every part of the betel plant has various uses that play a role in traditional medicine, one of which is the betel leaf.[12-15] Apart from essential oils, other elements such as saponins, flavonoids, and tannins in betel leaf also have a strong effect on *Staphylococcus aureus*.[15,16] This study aims to explore the potential of green betel leaf extract (*Piper betle L.*) in the growth of *Staphylococcus aureus* against the antibacterial activity.

2. Methods

This study is a true experimental study with a posttest-only control group design conducted at the Laboratory of the Faculty of Medicine, Universitas Prima Indonesia, from May 2022 to June 2022. The method used is the well diffusion method with a bacterial population. The sample is *Staphylococcus aureus*. Betel leaves of 2.5 kg, clean thoroughly and dry, then dry the betel leaves to dry in the room for 3 weeks. Blend the dried betel leaves until later in the form of powder, then sieve and store in a container, then cover. Take 360 grams of betel leaf simplicia and put it in a jar, then take 4000 ml of 96% ethanol, leave it for 3 days, then stir every day, then strain. Later this filtering produces filtrate 1 and residue 1. Maceration for 3 days residue 1 with 3000 ml of 96% ethanol and stir every day, then filter to get filtrate 2 and residue 2, then mix and get 6,800 ml of filtering results. Then concentrate the extract using a rotary evaporator as much as 500 ml first for 20-30 minutes and repeat until all of the extracts are concentrated. Then thicken the extract using a water bath and stir once every 10 minutes until it becomes a thick extract. Weigh the previously viscous extract and then dilute it with 10% DMSO which is filled to a volume of 10 ml so that 5%, 10%, 15%, and 20% concentrations are formed.[9] Stir well until the extract becomes homogeneous.

Weigh 7.6 grams of MHA and then dissolve it with 200 ml of aquadest, then cover it with cotton. Wrap the MHA media solution with a petri dish with heat-resistant plastic and then put it in the autoclave for 15 minutes until the temperature is 121°C. Take the sterilized MHA media solution and place it in the incubator. Pour the MHA media solution into each of the petri dishes that have been provided, then turn on the Bunsen fire and let it sit until the media solidifies. Label each petri dish according to the concentration of 5%, 10%, 15%, and 20%. Sterilize the cotton bud and then insert it into 0.9% NaCl and the bacteria, and then scratch it in a circular motion from corner to corner on all available MHA media three times at an angle of approximately 60°C. Make holes with a diameter of 6 mm with yellow tips on MHA media according to the concentrations that have been labeled. Take as much as 50 µl of extract from each concentration that has been made and then injection into the hole in the petri dish.[10] Repeat this 4 times and then take the amoxicillin blank discs as a positive control and place it in a petri dish and then label it. Take the paper discs, dissolve them in 10% DMSO as a negative control and place them in a petri dish and then label them. Incubate for 24 hours at 37°C and then see and measure the inhibition zone from the clear zone that occurs in the well area vertically and horizontally using a caliper and then take the average value obtained. Then classify according to the strength of the resistance.

After being classified, the data were analyzed statistically using SPSS Statistics 26 software. Initially, the Shapiro-Wilk normality was tested from the data and seen whether the data were normally distributed or not, then Levene's homogeneity was tested from the data and seen whether the data was homogeneous or not. If later the data is normally or homogeneously distributed, then One Way ANOVA is carried out, but for the example, later the data is not normally distributed or not homogeneous, then after that, the Kruskal-Wallis test is carried out. Then tested, the Post Hoc test using the Games Howell test.

3. Results

Figure 1 and Table 1 show the average diameter of the inhibition zone of betel leaf extract at concentrations of 5%, 10%, 15%, and 20%, positive control Amoxicillin and 10% DMSO negative control. The media used is MHA (Muller Hinton Agar) with the well method. At all concentrations, it can form a clear or inhibited zone.

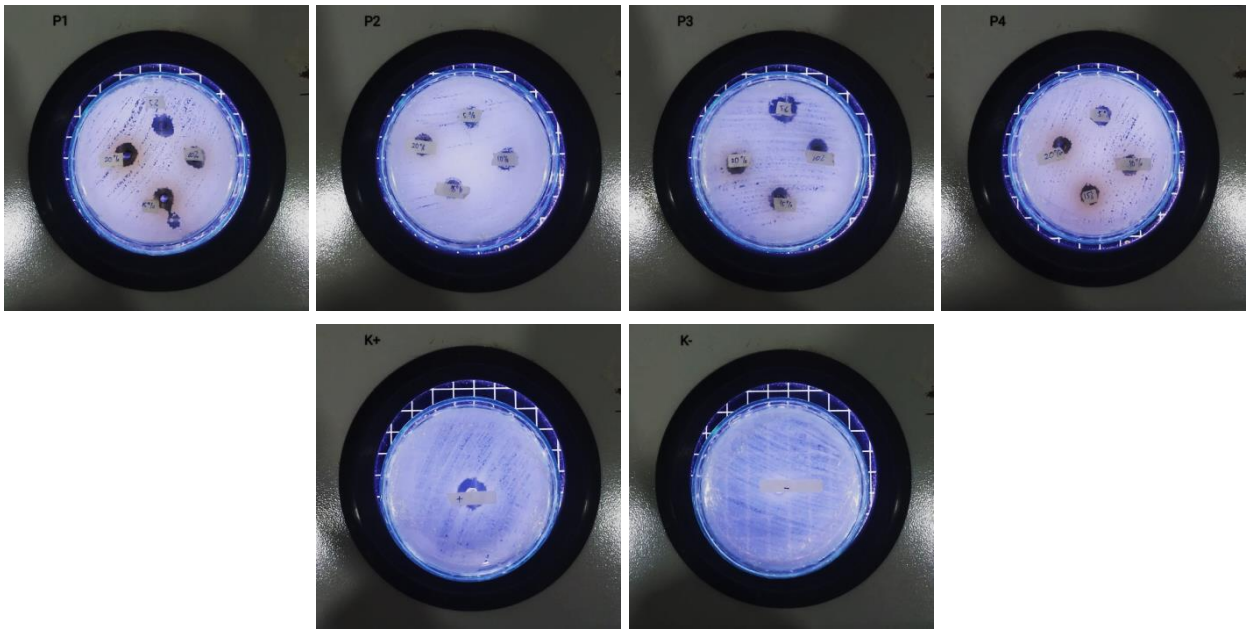

Figure 1. Effect of green betel leaf extract (*Piper betle L.)* on *Staphylococcus aureus.*

Table 1. Average inhibitory zone diameter and interpretation

No	Concentration or control	The average diameter of inhibition zone±SD	Interpretation of average inhibitory strength
1	Control (+)	15.15± 1.64	Strong
2	Control (-)	7.00±1.01	Moderate
3	5%	9.82±1.02	Moderate
4	10%	9.11±0.92	Moderate
5	15%	9.28±0.93	Moderate
6	20%	9.01±0.91	Moderate

Analysis of the data on the diameter of the inhibition zone showed that the positive control (Amoxicillin) had a better diameter of the inhibition zone than the treatment with concentrations of 5%, 10%, 15%, and 20%, $p<0,05$. The diameter of the inhibition zone between treatment groups with concentrations of 5%, 10%, 15%, and 20% did not show a significant difference, where the increase in concentration was not directly proportional to the increase in the diameter of the inhibition zone, $p>0.05$. The treatment of betel leaf extract with various concentrations showed a better diameter of the inhibition zone than the negative control, $p<0.05$.

4. Discussion

Another study showed betel leaf extract with a concentration of 5% with 5 repetitions, and a negative control showed that the formation of inhibition zones did not occur.[16,17] There are differences in the results obtained with the research that the researchers did where at a concentration of 5% the researchers found an inhibition zone that occurred with an average

diameter of 9.82 mm. This condition may occur due to differences in solvent dilution at concentrations where the researcher used 10% DMSO and the previous researcher used aquadest. The formation of a clear zone or inhibition zone can be influenced by the dilution solvent, where the wider the inhibition zone created by the low concentration is said to have optimal potential as an antibacterial compound.[15] In addition to the difference in the dilution solvent used, this can also be caused by the different antibacterial testing methods where the researchers used the disc method while this study used the well method, where the drawback of the well method is in the way of making wells. The media has been given a hole and then removed so that it tends not to be attracted and can cause the area next to the hole to break. Another study used light green and dark green betel leaves in the extract. The study showed that at a concentration of 1.25 mg/disk, the inhibition zones of young and old green betel leaves were 7.08 mm and 7.22 mm, and at a concentration of 5 mg/disk found 8.2 mm of inhibition zone for young leaves and 11 mm for old leaves.[16.17]

A previous study has shown differences in the clear zone that forms dark green betel leaves and light green betel leaves. Dark green betel leaves had a greater inhibition zone than light green betel leaves, but there were no significantly different results.[17] In this study, old and young leaves were combined into a single unit without distinguishing the age of each leaf so that there were differences in the diameter of the inhibition zone obtained. Another study used the betel leaf infusion method and showed that the higher the concentration, the wider the inhibition zone. Meanwhile, the results of research conducted by researchers illustrate that the lowest concentration of 5% is the most optimal concentration. At this concentration, it was found that the average diameter of the formation of clear or inhibited zones was higher than the others, namely 9.82 mm. This may be due to the incubation temperature of the bacteria, the storage method of the plate, the thickness of the agar medium, and the time required for incubation of the bacteria.

When viewed from the temperature, there is no difference, but when viewed from the storage method of the plate, this may be the cause of the difference in results where there is a buildup of plates during incubation which makes it difficult for bacteria to grow. The thickness of the agar media can also affect it, but in this study, no measurements were made of the thickness of the agar media, which should have a thickness of 4 mm or more. The time required for incubation can affect, so it should be necessary to compare the time at the time of incubation.

From several studies that have been carried out on the same topic, the most different thing in this study lies in the concentration used, which is a low concentration the reason that researchers want to know whether a low concentration of green betel leaf extract (*Piper betle L.*) can also inhibit growth Staphylococcus aureus as well as at high concentrations that have been studied by several previous researchers.[17] The results obtained that low concentrations can also inhibit the growth of these bacteria so that the betel leaf extract is effective as a natural antibacterial.

5. Conclusion

Green betel leaf extract (*Piper betle L.*) with all concentrations showed antibacterial activity, and the most optimal concentration to inhibit the growth of *Staphylococcus aureus* was a concentration of 5%.

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