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

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ABSTRACT

Background: *Candida albicans* is a fungus that often results in opportunistic infections in humans, called candidiasis. People generally practice self-medication to treat candidiasis withazole antifungals. However, this causes resistance due to inappropriate drug or dose selection. Betel leaf extract, which contains active compounds with antifungal properties such as phenols, tannins, and flavonoids, can be used as alternative medicine. The purpose of this study was to determine whether there was an effect of green betel leaf extract (*Piper betle* L.) on the growth of *Candida albicans*. **Methods:** This study is an experimental study and uses the disc diffusion method. The betel leaf was extracted using the maceration method and made into concentrations of 40%, 60%, 80%, and 100%. **Results:** The mean inhibition zone diameters were 9.12 mm, 8.79 mm, 8.18 mm, and 8.55 mm at concentrations of 40%, 60%, 80%, and 100%, sequentially. **Conclusion:** Betel leaf extract has an antifungal effect, with the most effective concentration being 40%.

1. Introduction

Fungal infection is one of the most common diseases in tropical countries such as Indonesia.^{1,2} Candidiasis is an infection caused by fungi of the *Candida*.³⁻⁵ In The last three decades, the number of candidiasis incidents continues to increase in general in all parts of the world.⁶ *Candida albicans* is one of the species in the genus *Candida* which often causes opportunistic infections in humans.^{7,8} Aspartyl proteinase is an enzyme secreted by *Candida albicans* that plays an important role in the process of invasion and colonization and causes damage to tissues in the human body as its host.^{8,9} Candidiasis can affect the

mouth, vagina, skin, nails, and bronchi and can be experienced by humans of all ages, both men and women.

In dealing with candidiasis, especially those affecting the skin, people, in general, have practiced self-medication or self-medication, namely withazole antifungals such as imidazole, ketoconazole, fluconazole, and itraconazole.¹⁰⁻¹² This is because fungal infections of the skin are considered harmless, and there is easy access to antifungal drugs. This action results in the formation of resistance due to the selection of drugs that are not in accordance with the

cause or the use of inappropriate doses.¹³ Resistance of *Candida* toazole drugs has been recorded in the range of 6-36%.¹⁴ Therefore, an alternative is needed to treat candidiasis where the shadow of antifungal resistance is getting higher today.

Green betel or *Piper betle* L. which has long been cultivated in Indonesia, is often used traditionally to treat health problems such as bad breath, boils, itching, and skin sores.^{15,16} Green betel plants, especially the leaves, contain active compounds that have antifungal properties, such as phenols, tannins, and flavonoids. Phenol compounds damage the structure of proteins that make up the fungal cell wall, tannins interfere with the physiological activity of fungi by inhibiting the work of enzymes, and flavonoids interfere with the permeability of fungal cell membranes so that their growth is inhibited.¹⁷ Green betel leaf (*Piper betle* L.) has been proven empirically effective in inhibiting the growth of *Candida albicans*.¹⁸ This study aims to conduct a study to test the effectiveness of green betel leaf extract (*Piper betle* L.) on the growth of *Candida albicans*.

2. Methods

The design of this study is an experimental study conducted at the Laboratory of the Faculty of Medicine, Universitas Prima Indonesia, Medan, from June to July 2022. The method used is the disc diffusion method, where a clear zone is formed around the paper disc that has been soaked in the leaf extract. Green betel diameter was measured using a caliper. The sample used in this study was the isolate of the fungus *Candida albicans*. 2.5 kg of betel leaves were washed and dried, then stored in a closed room that was not exposed to direct sunlight. The dried betel leaves are crushed with a blender until they are powdered, then sifted and stored in a closed container. 350 grams of simplicia powder, which was then dissolved in 4000 ml of 96% ethanol and allowed to soak for 3 days while stirring occasionally. After 3 days, it was filtered to get filtrate 1 and residue 1. Residue 1 was then soaked again in 3000 ml of 96% ethanol solvent for 3 days, then filtered again to get

filtrate 2 and residue 2, filtrate 1 and 2 were combined, and the results were as much as 6,800 ml. The liquid extract was then concentrated with a rotary evaporator and a water bath until the extract became completely viscous. The thick extract of betel leaf is diluted using dimethyl sulfoxide (DMSO) 10% to make the concentration of betel leaf extract with concentrations of 40%, 60%, 80%, and 100%. The positive control that has been proven to be effective for use in this study is Ketoconazole 2% on paper discs, while for the negative control, 10% DMSO can be used on paper discs.

Preparation of stock of *Candida albicans* is done by inoculating it on sabouraud dextrose agar media (SDA) in a petri dish, then incubated at 35°C for 24 hours in an incubator. Suspension *Candida albicans*, as much as 50 µl was applied to the surface of sabouraud dextrose agar media (SDA) evenly using sterile L rods to dry. The disc paper was then soaked in each of the existing betel leaf extract concentrations. Drain the disc paper on the edge of a sterile petri dish, then place it in the center of the SDA media that has been smeared with *Candida albicans*. The treatment was carried out on each betel leaf extract with 4 replications and at each dilution (40%, 60%, 80%, and 100% concentrations). Do the same thing using a positive control, namely 2% ketoconazole, and negative control, namely DMSO 10%. Then, incubate the petri dish for 24 hours at 35°C. Furthermore, the growth of the clear zone or inhibition zone formed was observed and measured using a ruler or caliper.

The data obtained was calculated as the average diameter to determine the average diameter of the inhibition zone formed in the concentration groups of 40%, 60%, 80%, 100%, and positive and negative controls. After that, the data were also analyzed statistically using SPSS software with the One-way ANOVA method to determine the differences in the inhibition zones formed in the media between all existing treatment groups.

3. Results

Figure 1 and Table 1 show the diameter of the inhibition zone on sabouroud dextrose agar media

(SDA) using concentrations of 40%, 60%, 80%, and 100%. The positive control used in this study was 2%

ketoconazole and 10% DMSO negative control.

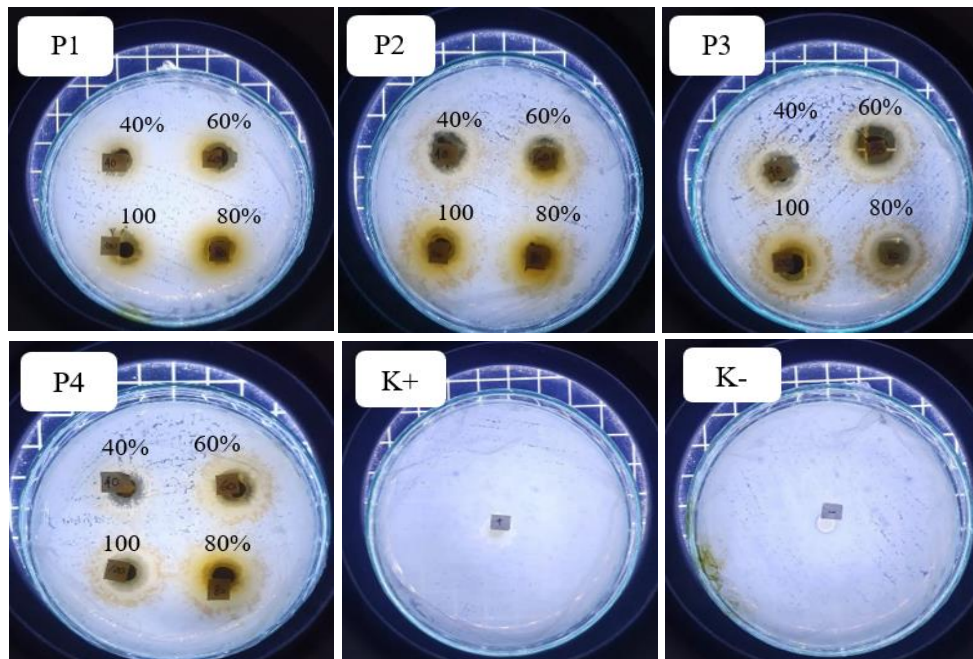


Figure 1. Effect of betel leaf extract (*Piper betle* L.) with P1 (repetition 1), P2 (repetition 2), P3 (repetition 3), P4 (repetition 4), positive control Ketoconazole 2% and negative control DMSO 10%.

Table 1. Measurement of average inhibitory zone diameter

| Concentration | Average inhibition zone diameter (mm)±SD | Inhibition |
|--------------------|--|------------|
| Control (+) | 10.35±1.12 | Moderate |
| Control (-) | - | - |
| Concentration 40% | 9.12±0.91 | Moderate |
| Concentration 60% | 8.79±0.93 | Moderate |
| Concentration 80% | 8.18±0.82 | Moderate |
| Concentration 100% | 8.55±0.82 | Moderate |

Data analysis on the diameter of the inhibition zone showed that the positive control (ketoconazole) had a diameter of the inhibition zone that was better than the treatment with concentrations of 40%, 60%, 80%, and 100%, $p < 0.05$. The diameter of the inhibition zone between treatment groups with concentrations of 40%, 60%, 80%, and 100% 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

The results of this study showed that the greatest inhibitory power was found in the 40% concentration group. Previous studies showed the opposite results, namely, the higher the concentration of green betel leaf extract, the greater the inhibition zone produced. This

difference may be caused by several things, such as incubation temperature, the thickness of the agar medium, the lack of extract diffusion power into the medium, time of disc installation, incubation time, and the reaction between the active ingredients and the medium. This is indeed a weakness of the disc diffusion method, where the clear zone formed depends on the incubation conditions and the thickness of the medium. The optimal temperature of 35°C has been applied in this study. However, plate storage stacked with more than 2 plates can cause the incubation temperature to be less than the optimal temperature so that the extract diffusion becomes less effective. Furthermore, the ideal thickness of the medium is 4 mm. The thickness of the medium of more than 4 mm can slow the diffusion of the extract. In this study, the thickness of the medium was not measured, so this can also be considered as one of the differentiating factors.¹⁹

Another differentiating factor lies in the concentration group used. This study used green betel leaf extract with relatively high concentration groups, namely 40%, 60%, 80%, and 100%. In contrast, the two previous studies used green betel leaf extract with lower concentration groups, namely 3.125%, 6.25%, 12.5%, 25%, and 50%. A previous study stated that the higher the concentration of the extract, the lower its solubility (the thicker).^{20,21} This can slow down the diffusion of the active ingredients of the extract into the media, thereby reducing the ability of extracts with high concentrations to inhibit the growth of *Candida albicans* and produce a relatively smaller zone of inhibition. Length of the incubation period of paper discs and suspension of *Candida albicans* on SDA media. The longer incubation period gave more time for the extract in the disc paper to diffuse into the SDA medium so that a larger zone of inhibition could be generated.

5. Conclusion

Green betel leaf extract (*Piper betle* L.) has antifungal activity against the fungus *Candida albicans*, where the 40% concentration has the

greatest potential to inhibit the growth of *Candida albicans*.

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