

Antimicrobial Activity Test of the Combination of Pineapple Leaf Extract (*Ananas Comosus*) and Green Betel Leaf Extract (*Piper Betle L*) Against *Candida Albicans*

Adjani Buaya, Yolanda Eliza Putri Lubis, Suandy

PUI Phyto Degenerative & Lifestyle Medicine, Universitas Prima Indonesia

Email: kjani9508@gmail.com, yolandaelizaputrilubis@unprimdn.ac.id,

Suandy@unprimdn.ac.id

ABSTRACT

Candida albicans is an opportunistic fungus that can cause infections, especially in individuals with low immune systems. Efforts to control this fungal infection can be made by utilizing natural ingredients as alternative antifungal agents. Pineapple leaves (*Ananas comosus*) and green betel leaves (*Piper betle L.*) are known to contain active compounds with antimicrobial properties. This study aims to determine the inhibitory effect of a combination of pineapple leaf and green betel leaf extracts on the growth of *Candida albicans* and to compare their effectiveness at various concentrations. This research is a laboratory experimental study using the disc diffusion method. Three groups of extract concentration combinations were used: 20%, 40%, and 60%. The diameter of the inhibition zone was measured in millimeters, and the data were analyzed using a one-way ANOVA test followed by Tukey's HSD test. The results showed that the 20% concentration produced the largest average inhibition zone diameter of 38.32 mm, while the 40% and 60% concentrations produced 28.46 mm and 28.31 mm, respectively. The ANOVA test indicated a significant difference between the groups ($p < 0.05$), and the Tukey HSD test revealed that the 20% concentration differed significantly from the other two groups. The combination of pineapple leaf and green betel leaf extracts at a concentration of 20% was the most effective in inhibiting the growth of *Candida albicans* compared to the 40% and 60% concentrations.

Keywords: *Candida albicans*; Pineapple leaves; Green betel leaves; Inhibitory power; Plant extract; Natural antifungal

This article is licensed under [CC BY-SA 4.0](https://creativecommons.org/licenses/by-sa/4.0/) 

INTRODUCTION

Pathogenic fungi infect millions of people every year, with an estimated death toll of around 1.35 million per year. Several species from the genera *Candida*, *Aspergillus*, and *Cryptococcus* are the main causes of invasive infections, with *Candida* being the most common cause of invasive fungal disease in developing countries, namely candidiasis (Boral et al., 2018; Denning & Bromley, 2015; Lee et al., 2023; SHARMA et al., 2021; Tits et al., 2020). *Candida albicans* itself is a pathogenic fungus that lives commensally in the digestive and genitourinary tracts and can be found as part of the normal microflora in the oral cavity and conjunctiva. However, this fungus can cause infection if the body's immune system is weakened (Kalumpiu, 2019; Manuntung, 2020; Nina et al., 2023; Novialdi, 2019; Sari & Adelina, 2020) .

The disease caused by *Candida albicans* is known as candidiasis. This infection is the most common type of fungal infection in humans. *Candida albicans* itself is a commensal organism commonly found in the digestive tract, oral cavity, and vagina. This fungus can cause various diseases, ranging from oral thrush, vaginitis, endocarditis to septicemia. In superficial candidiasis, excessive growth of *Candida albicans* can damage skin tissue, leading to local invasion by yeast or pseudohyphae (Shahabudin et al., 2024). In systemic candidiasis, the fungus can enter the bloodstream when the immune system is weakened, then spread through circulation to infect organs such as the kidneys and heart.(Soelistijo Soebagijo Adi, 2019b) .

Candida albicans is one type of fungus that is normally found in healthy humans, for example in the mouth, esophagus, genital tract, feces, under the nails, and on the skin. The presence of this fungus generally does not cause problems, but it can develop into a pathological condition if the immune system weakens (Soelistijo Soebagijo Adi, 2019b) .

Indonesia is renowned for its cultural diversity and local wisdom, which can be seen in its communities that are closely connected to their cultural heritage. The Indonesian way of life tends to utilize various types of plants. The relationship between humans and the environment, influenced by local cultural values, is considered a tradition (Soelistijo Soebagijo Adi, 2019b) . The use of traditional medicine in Indonesia has shown significant development. People are now returning to traditional medicine as an alternative treatment option, in addition to the increasingly widespread use of modern medicine on the market. Traditional medicines made from plants and natural ingredients generally have lower risks, side effects, and levels of danger than chemical medicines (Soelistijo Soebagijo Adi, 2019b) .

Indonesia, as a country spread across islands and located along the equator, is one of the countries with the largest tropical climate and biodiversity in the world. The number of plant species in Indonesia reaches 30,000 to 40,000 medicinal plant species worldwide. In Indonesia alone, it is estimated that around 90% or approximately 9,000 plants are believed to have medicinal benefits (Soelistijo Soebagijo Adi, 2019) . The use of natural ingredients as inhibitors is one of the efforts in implementing the concept of *back to nature* (Soelistijo Soebagijo Adi, 2019) . Natural resources that are beneficial to health include pineapple leaves (*Ananas comosus*) and green betel leaves (*Piper betle L.*).

Pineapple leaves contain various phytochemical compounds that exhibit pharmacological activity (Soelistijo Soebagijo Adi, 2019b) . Crude extracts from pineapple leaves, stems, and fruits contain various secondary metabolites, including terpenoids, flavonoids, amino acids, proteins, cardiac glycosides, phytosterols, carbohydrates, alkaloids, and saponins (Soelistijo Soebagijo Adi, 2019b) . The results of pineapple leaf extraction using ethanol solvent showed the content of various metabolite compounds, such as carbohydrates, flavonoids, glycosides, tannins, phenolic compounds, and alkaloids (Soelistijo Soebagijo Adi, 2019b). Pineapple leaves are known to have various pharmacological activities, such as antidiabetic, antidyslipidemic, antioxidant, antidyspeptic, antidiarrheal, anti-inflammatory, antimicrobial, anthelmintic, vermifuge, menstrual cycle regulator, antiedema, antiparasitic, and antipyretic (Soelistijo Soebagijo Adi, 2019b) .

Green betel leaves (*Piper betle L.*), belonging to the *Piperaceae* family, are one of the beneficial plants that are easily found around residential areas and are often cultivated in home gardens. The main component of these leaves is essential oil with a content ranging from 0.7–2.6%. The essential oil consists of phenolic compounds (approximately 82.2%) and non-phenolic compounds (approximately 17.8%). In addition, green betel leaves also contain several active compounds, such as cavitol (5.40%), methyl eugenol (3.50%), eugenol (4.40%), and allyl pyrocatechol (7.5%) (Soelistijo Soebagijo Adi, 2019b). Green betel leaves contain various secondary metabolites with antibacterial activity, including flavonoids, alkaloids, phenolics, saponins, and tannins (Soelistijo Soebagijo Adi, 2019b) .

Green betel leaves contain various chemical compounds, including essential oils, saponins, polyphenols, alkaloids, and flavonoids. The most prominent main ingredient is essential oil, which consists of phenolic compounds and their derivatives, such as betelphenol

and cavicol. In addition, green betel leaves also produce alkaloids with properties similar to cocaine. The essential oil content in these leaves ranges from 0.8–1.8%, with components including cavicol, betelfenol, eugenol, allyl pyrocatechol, terpenes, cineol, caryophyllene, cadinene, and menthone. The essential oil content is generally higher in young leaves (Soelistijo Soebagijo Adi, 2019b). Green betel leaves have long been used for traditional medicine. This plant is a climbing herbaceous plant from the *Piperaceae* family. The main component of green betel leaves is tannin, which has strong antimicrobial and antifungal properties, thereby inhibiting the growth of various types of bacteria. In addition, green betel leaves also contain various secondary metabolites, such as saponins, tannins, flavonoids, terpenoids, polyphenols, and steroids, which are thought to act as antibacterial agents (Soelistijo Soebagijo Adi, 2019b).

Previous studies have demonstrated the antifungal activity of pineapple and green betel extracts against *Candida albicans*. (Soelistijo Soebagijo Adi, 2019b) reported that low concentrations (5–15%) of pineapple peel ethanol extract produced an inhibition zone against *Candida albicans*. However, different results were obtained from the study by (Soelistijo Soebagijo Adi, 2019b). The results showed that pineapple peel ethanol extract did not produce an inhibition zone against the growth of *Candida albicans*. On the other hand, (Soelistijo Soebagijo Adi, 2019b) showed that green betel leaf extract effectively inhibits the growth of *Candida albicans*. Similar results were also reported by (Soelistijo Soebagijo Adi, 2019b), who studied the combination of betel leaf extract with orange peel extract, although its effectiveness was still lower than that of high-concentration betel leaf extract alone.

However, these studies still focus on single extracts or combinations of betel leaf with other plants. There has been no research specifically combining pineapple leaf extract and green betel leaf extract in various medium concentrations (20%, 40%, 60%). In fact, these two plants contain different active compounds, namely bromelain in pineapple, which plays a role in the degradation of fungal cell wall proteins, and eugenol in green betel leaves, which can damage cell membranes. The interaction between the two has the potential to produce synergistic or antagonistic effects. Therefore, this study is novel in that it explores the combination of these two extracts in inhibiting the growth of *Candida albicans*, something that has not been reported in previous studies.

The research questions for this study are as follows: 1) Is there an inhibitory effect of the combination of pineapple leaf extract and green betel leaf extract on *Candida albicans*? 2) What is the optimal concentration of the combination of pineapple leaf and betel leaf extracts in inhibiting the growth of *Candida albicans*? The objectives of this study consist of both general and specific goals. The general objective is to analyze the inhibitory effect of the combination of pineapple leaf extract and green betel leaf extract against *Candida albicans*. The specific objectives include: 1) to analyze the inhibitory effect of the combination of pineapple leaf extract and green betel leaf extract against *Candida albicans*, and 2) to analyze the optimal concentration of these extracts in inhibiting the growth of *Candida albicans*.

The benefits of this research are both theoretical and practical. The theoretical benefits include contributing to scientific knowledge by providing new insights and concepts regarding the inhibitory effect of the combination of pineapple leaf and green betel leaf extracts against *Candida albicans*. This can later serve as a foundation for further research and in educational contexts. The practical benefits are significant for various stakeholders: for researchers, this study offers new experiences and knowledge through experimental research, while also

fulfilling the requirements for obtaining a Bachelor of Medicine degree from Prima Indonesia University. For the community, the research provides additional information about the health benefits and uses of pineapple and betel leaves, particularly in preventing the growth of *Candida albicans*. In the future, this research may enable the community to use these plants as alternative health remedies. Finally, for future researchers, this study serves as a valuable reference for those interested in exploring similar topics.

METHOD

This research is an experimental laboratory study using a Post Test Only Control Group Design. According to Sugiyono (2019), experimental research involves controlling and observing research variables to determine the effect of one variable on another under strictly controlled conditions. The research was conducted at the Prima Indonesia University Laboratory.

The population in this study refers to all elements that are the object of research and share certain characteristics, such as pineapple leaves and green betel leaves from the city of Medan (Handayani, 2020). The sample is a portion of the population selected and measured for its characteristics, which represents and estimates the characteristics of the entire population (Djaali, 2020). In this study, the samples consist of pineapple leaves and green betel leaves, each weighing 1 kg, obtained from markets in the city of Medan.

The research variables in this study include independent, dependent, and controlled variables. The independent variables are the combinations of pineapple leaf extract and green betel leaf extract at doses of 20%, 40%, and 60%. The dependent variable is the inhibitory effect on *Candida albicans*.

The research tools and materials used in this study included various laboratory equipment such as glassware (petri dishes, beakers, test tubes, etc.), aluminum foil, Bunsen burners, blenders, stirring rods, incubators, calipers, cotton, sterile gauze, wire ose, disc paper, silica gel, laminar air flow, spirit lamps, micropipettes, tweezers, spatulas, ovens, analytical balances, and rotary evaporators, among other supporting equipment. The materials used were distilled water, pineapple leaves, green betel leaves, dimethyl sulfoxide (DMSO), 96% ethanol, *Candida albicans* isolate, and ketoconazole.

The research procedure began with the preparation of pineapple leaf and green betel leaf samples. The leaves were sorted, washed, sliced, and dried under the sun before being blended into powdered simplisia, which was then sieved and stored in airtight containers. The extract was prepared through maceration with 96% ethanol, followed by a second maceration, and then concentrated using a rotary evaporator. The yield of both the simplisia and the extract was calculated using specific formulas.

The inhibition test for *Candida albicans* involved cultivating the fungus on Potato Dextrose Agar (PDA) medium, followed by an inhibition test where the combination extracts of pineapple leaf and green betel leaf at concentrations of 20%, 40%, and 60% were applied to sterile discs. These discs were placed on inoculated PDA medium, with positive and negative controls for comparison. The Petri dishes were incubated at room temperature for 24 hours, and the inhibition zone was measured using a caliper.

For data analysis, SPSS version 25 was used, starting with normality and homogeneity tests. If the data were normally distributed and homogeneous, One-Way ANOVA was applied,

while the Kruskal-Wallis test was used for non-normally distributed data. The hypothesis was considered significant if $p < 0.05$, followed by a t-test to assess the effect of the extracts on *Candida albicans*.

Ethical clearance was obtained by submitting a proposal to the Ethics Committee of the Faculty of Medicine, Prima Indonesia University, ensuring compliance with ethical standards before conducting the research.

RESULTS AND DISCUSSION

This study aimed to evaluate the effectiveness of a combination of pineapple leaf extract (*Ananas comosus*) and green betel leaf extract (*Piper betle* L) on the growth of *Candida albicans*. Observations were made by measuring the inhibition zone using the disc diffusion method in agar media, with various concentrations of the extract combination. The data obtained were presented in tables accompanied by analysis and interpretation of each observed result.

Results of the Average Diameter of the Inhibition Zone of the Combination of Pineapple Leaf and Green Betel Leaf Extracts Against the Growth of *Candida Albicans*

The following table presents the average diameter of the inhibition zone formed as an indicator of the antifungal activity of the combination of pineapple leaf and green betel leaf extracts against the growth of *Candida albicans*. The test was conducted with various concentrations of the extract combination, and the results obtained showed variations in the inhibition zone diameter, reflecting the level of effectiveness of each concentration in inhibiting fungal growth. These average values were obtained from two repetitions of the measurement to increase the accuracy of the data.

Table 1. Average Results of the Inhibitory Zone Diameter of the Combination of Pineapple Leaf and Green Betel Leaf Extracts Against the Growth of *Candida Albicans*

Group	Mean \pm SD
Combination of Extract Concentration 20%	38.82 \pm 1.619
Combination of 40% Concentration Extracts	28.46 \pm 5.168
Combination of 60% Concentration Extracts	28.31 \pm 5.847

Source: Processed Primary Data (2025)

Based on the data in Table 3, it can be seen that the combination of pineapple leaf and green betel leaf extracts at a concentration of 20% produced an average inhibition zone diameter of 38.82 mm with a standard deviation (SD) of 1.619 mm. This indicates that at a concentration of 20%, the extract combination has the highest inhibitory effect on the growth of *Candida albicans*, as indicated by the widest inhibition zone. Meanwhile, at a concentration of 40%, the average inhibition zone diameter decreased to 28.46 mm with a standard deviation of 5.168 mm, which means that the inhibitory effect is lower than at a concentration of 20%. This decrease continued at a concentration of 60%, where the average inhibition zone was recorded at 28.31 mm with a standard deviation of 5.847 mm.

Interestingly, even though the extract concentration increased, the diameter of the inhibition zone actually decreased. This shows that an increase in concentration is not always proportional to an increase in inhibitory power against *Candida albicans*. The difference in

standard deviation values at concentrations of 40% and 60% also shows that the measurement results in these groups were more varied.

Overall, these results indicate that the combination of pineapple leaf extract and green betel leaf extract is most effective in inhibiting the growth of *Candida albicans* at a concentration of 20%.

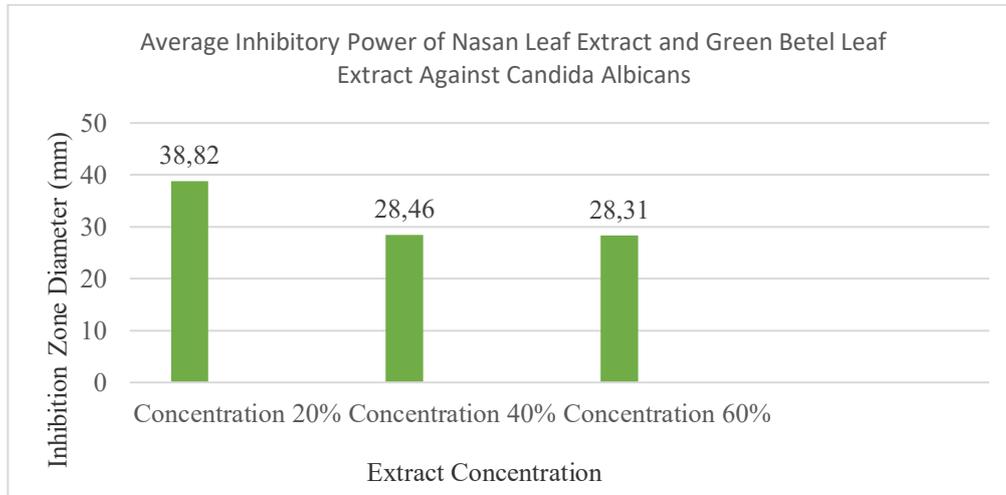


Figure 1. Average Inhibitory Power
Source: Processed Primary Data (2025)

The bar graph visualization in Figure 1 also shows the same trend, where the 20% concentration exhibits the most dominant average inhibition zone diameter compared to the 40% and 60% concentrations.

Results of Normality and Homogeneity Tests

Before conducting statistical tests, normality tests were first performed using the *Shapiro-Wilk test* and homogeneity tests using the *Levene Test*. The results can be seen in Table 2.

Table 2. Results of Normality and Homogeneity Tests

Group	Normality	Homogeneity
Combination of 20% Extract Concentration	0.991	
Combination of Extract Concentration 40%	0.998	0.318
Combination of 60% Concentration Extracts	0.999	

Source: Processed Primary Data (2025)

Table 2 presents the results of normality and homogeneity tests for the data on the inhibition zone diameter of three groups of extract concentration combinations against *Candida albicans*. The normality test aims to determine whether the data is normally distributed, while the homogeneity test is used to determine whether the variance between data groups is homogeneous (uniform). From the normality test results, the 20% concentration combination extract group had a value of 0.991, the 40% concentration group had a value of 0.998, and the 60% concentration group had a value of 0.999. These values indicate that all groups have values above 0.05, which means that the data is normally distributed, in accordance with the assumptions of parametric statistical tests.

Meanwhile, the homogeneity test results obtained a value of 0.318. Since this value is also > 0.05, it can be concluded that the data between groups is homogeneous or has uniform variance. Overall, these test results indicate that the data meets the requirements for further

statistical analysis using parametric tests, such as one-way ANOVA, because the assumptions of normality and homogeneity are met.

Statistical Test Results of the Inhibitory Effect of the Combination of Pineapple Leaf and Green Betel Leaf Extracts on the Growth of Candida Albicans

A statistical test was conducted to determine whether there was a significant difference between the average diameter of the inhibition zone in each group of extract concentration combinations (20%, 40%, and 60%) against the growth of *Candida albicans*. Based on the normality test results showing that the data were normally distributed and the homogeneity test showing that the data were homogeneous, further analysis was performed using a *one-way ANOVA* test.

The ANOVA test results showed a statistically significant difference ($p < 0.05$) between the three concentration groups. This indicates that the difference in the concentration of pineapple leaf and green betel leaf extract combinations has a significant effect on the inhibition of *Candida albicans* growth.

Table 3. Results of the *One-Way ANOVA* Test

Group	Mean \pm SD	P-Value
Combination Extract Concentration 20%	38.82 \pm 1.619	
Combination of 40% Concentration Extract	28.46 \pm 5.168	0.014*
Combination of 60% Concentration Extract	28.31 \pm 5.847	

Source: Processed Primary Data (2025)

Note: *Significant (P-Value < 0.05)

From the results of the *P-value* of the *One-way ANOVA* statistical test in Table 5, the *P-value* = 0.014 ($P < 0.05$), it can be concluded that there is a significant difference in the average inhibition zone of the combination of pineapple leaf extract (*Ananas Comosus*) and green betel leaf extract against *Candida albicans* s treated with a combination extract at concentrations of 20%, 40%, and 60% (.). From the research results, it can be stated that there is effectiveness in administering a combination of pineapple leaf extract (*Ananas comosus*) and green betel leaf extract against *Candida albicans* ($P < 0.05$).

Differences in the Effectiveness of the Combination of Pineapple Leaf and Green Betel Leaf Extracts Against Candida albicans

The following are the results of the Tukey HSD (*Honestly Significant Difference*) test conducted after the ANOVA test, with the aim of determining specifically which concentration group had a significant difference in its inhibitory effect on the growth of *Candida albicans*.

Table 4. HSD Test Results (*Honestly Significant Difference*)

Group	Subset for alpha = 0.05	
	1	2
Combination of 60% Concentration Extracts	28.31	
Combination of 40% Concentration Extracts	28.46	
Combination of 20% Concentration Extracts		38.32
Sig	0.998	1,000

Source: Processed Primary Data (2025)

Table 4 shows that the 60% and 40% concentration groups are in subset 1, with mean inhibition zone diameters of 28.31 mm and 28.46 mm, respectively. This means that the two groups do not have a statistically significant difference because they are in the same subset.

The 20% concentration group was in subset 2 alone, with an average inhibition zone diameter of 38.32 mm, indicating that this group was significantly different from the other two groups. Significance values (Sig.) of 0.998 and 1.000 indicate that the differences between the 60% and 40% concentration groups are not significant ($p > 0.05$), while the 20% group has a significant difference from both groups. Thus, it can be concluded that the combination of extracts at a concentration of 20% has the highest effectiveness in significantly inhibiting the growth of *Candida albicans* compared to concentrations of 40% and 60%.

Analyzing the Inhibitory Effect of the Combination of Pineapple Leaf and Green Betel Leaf Extracts on *Candida Albicans*

From the results of the study, it can be concluded that the combination of pineapple leaf (*Ananas comosus*) and green betel leaf (*Piper betle* L) extracts showed a significant inhibitory effect on the growth of *Candida albicans*. This was evidenced by the formation of an inhibition zone around the disc in all concentration groups tested, namely 20%, 40%, and 60%. The diameter of this inhibition zone is an important indicator in determining the antifungal activity of a test material, where the larger the inhibition zone formed, the higher the ability of the material to inhibit the growth of the target microorganism.

This inhibitory effect can be explained by the active compounds contained in both plants. Pineapple leaves are known to contain bromelain, flavonoids, saponins, and tannins, which have antimicrobial effects, including against fungi. Bromelain, as a proteolytic enzyme, has the potential to damage the cell wall structure of microorganisms, causing cell death. Meanwhile, green betel leaves contain eugenol, cavicol, tannins, flavonoids, and saponins, which have been extensively studied for their antifungal, antimicrobial, and antiseptic effects. Eugenol works by damaging the fungal cell membrane and inhibiting enzymes that are important in the metabolism of *Candida albicans*. The combination of these two extracts provides a synergistic effect, where the active compounds from each plant complement each other in inhibiting fungal growth.

Bromelain is a proteolytic enzyme complex that has been reported to have several biological activities, including immune system modulation and indirect antimicrobial/antifungal activity. Several studies have shown that bromelain can enhance phagocytosis and the killing of *Candida albicans* by phagocytic cells, as well as help destroy the structure of biofilms through its proteolytic activity against biofilm matrix proteins or fungal cell surface adhesins. Therefore, bromelain in combination formulations has the potential to increase the susceptibility of fungal cells to other components by breaking down surface proteins or biofilm matrix (Soelistijo Soebagijo Adi, 2019b).

Eugenol, one of the main phenolic components in betel leaf essential oil, reacts with fungal cell membranes due to its lipophilic nature, enabling it to penetrate and reduce the integrity of the lipid bilayer. Reported mechanisms include binding/removal of membrane ergosterol, disruption of membrane pumps (e.g., plasma membrane H⁺-ATPase), increased membrane permeability, leakage of intracellular components, increased ROS, and inhibition of lipid/ergosterol synthesis. These effects can result in fungicidal or fungistatic treatment depending on the dose (Soelistijo Soebagijo Adi, 2019b).

The results of the ANOVA statistical test showed that the difference in inhibition zone diameter between the sig concentration groups was statistically significant ($p < 0.05$), reinforcing the assumption that the combination of extracts is indeed effective as an antifungal agent. In addition, the results of the normality and homogeneity tests showed that the data were normally distributed and homogeneous, so the statistical results obtained can be considered valid and reliable.

Thus, it can be concluded that the combination of pineapple leaf and green betel leaf extracts does have antifungal activity against *Candida albicans*. This supports the use of natural ingredients as a potential alternative in the treatment of fungal infections. These findings are in line with previous studies showing that both plants contain bioactive compounds that are effective against various types of pathogenic microorganisms. The use of a combination of two plants is also a relevant strategy in enhancing the effectiveness of natural treatments and reducing the risk of microbial resistance that often occurs with the long-term use of synthetic antifungals. Research conducted by (Soelistijo Soebagijo Adi, 2019b) reported that low concentrations (5–15%) of pineapple peel ethanol extract were able to produce an inhibition zone against *Candida albicans*. However, the results differed from those of (Soelistijo Soebagijo Adi, 2019b), which found that no inhibition zone was formed when testing pineapple peel ethanol extract against *Candida albicans* fungus.

On the other hand, (Soelistijo Soebagijo Adi, 2019b) shows that green betel leaf extract is effective in inhibiting the growth of *Candida albicans*. Similar results were also reported by (Soelistijo Soebagijo Adi, 2019b) who studied the combination of betel leaf extract with orange peel, although its effectiveness was still lower than that of high-concentration betel extract alone.

This study contributes scientifically to the utilization of local herbal plants as antifungal agents, while also opening opportunities for the development of topical formulations based on a combination of pineapple leaf and green betel leaf extracts, such as ointments or gels, which can be used as alternative treatments for skin or mucosal infections caused by *Candida albicans*. However, further research is still needed to test the effectiveness of this combination in pharmaceutical preparations and *in vivo* tests to determine its systemic effects.

Analyzing the Optimal Concentration of Pineapple Leaf and Betel Leaf Extract Combination in Inhibiting the Growth of Candida Albicans

The results of the study indicate that the combination of pineapple leaf extract and green betel leaf extract at a concentration of 20% is the most effective concentration in inhibiting the growth of *Candida albicans*. This is based on the measurement of the average inhibition zone diameter, which shows that the 20% concentration group produced the largest inhibition zone diameter of 38.32 mm, compared to the 40% concentration group (28.46 mm) and the 60% concentration group (28.31 mm). This finding is quite interesting because, in general, an increase in the concentration of active ingredients is usually expected to increase the effectiveness of the inhibitory power. However, the results obtained show that optimal effectiveness is achieved at a concentration of 20% and decreases at higher concentrations.

The decrease in inhibition at concentrations of 40% and 60% may be due to several factors. One of them is the possibility of excessive interaction of active compounds in the extract combination, causing a decrease in effectiveness or even antagonistic effects. In addition, at too high a concentration, the extract may form a layer that is too thick or dense on

the test disc, which can actually inhibit the diffusion of active compounds into the agar medium, resulting in a smaller inhibition zone. This effect has been reported in several previous studies, where an increase in extract concentration beyond a certain threshold does not always correlate with an increase in its biological activity.

Statistical analysis using ANOVA showed that the differences between the three concentrations were statistically significant ($p < 0.05$). The analysis was continued with a Tukey HSD post-hoc test, which showed that the 20% concentration was significantly different from the 40% and 60% concentrations, while there was no significant difference between the 40% and 60% concentrations. This reinforces the conclusion that the 20% concentration is the most optimal concentration for inhibiting the growth of *Candida albicans* among the concentrations tested.

This condition can also be linked to the principle of dose efficiency, where the use of too high a concentration does not always provide additional benefits, and may even cause unexpected effects. Therefore, the 20% concentration can be considered an effective and efficient working dose in the context of developing an antifungal formulation based on a combination of pineapple leaf and green betel leaf extracts.

However, empirical data sometimes show a decrease in effectiveness or even antagonism in combinations at high concentrations. Possible mechanisms explaining antagonism at concentrations of 40%–60% include physical-chemical interactions between components, such as poor solubility/phase separation that reduces the bioavailability of eugenol, target saturation so that an excess of one component interferes with the penetration or target action of the other component, changes in pH or ion strength that inactivate enzymes (bromelain is a protein sensitive to environmental conditions) so that proteases lose their activity at high concentrations or in certain formulations, and the formation of inactive complexes between polyphenolic components and proteins (*protein-polyphenol binding*) that reduce antibacterial/antifungal activity. Therefore, the reduction in effectiveness at 40% and 60% can be explained by one or a combination of these mechanisms and requires further experimental testing.

These results provide important information for the development of evidence-based herbal antifungal therapy. This combination of easily obtainable local plants has the potential to become an affordable natural antifungal alternative, especially in areas with limited access to synthetic drugs. In the future, further research is needed to test the stability of the formulation, its effectiveness in topical preparations such as ointments or gels, and safety testing (toxicity) both *in vitro* and *in vivo*.

CONCLUSION

The study concludes that combining pineapple leaf and green betel leaf extracts effectively inhibits the growth of *Candida albicans*, as indicated by the formation of inhibition zones across all test concentrations (20%, 40%, and 60%). Among these, the 20% concentration demonstrated the strongest antifungal effect, producing the largest inhibition zone diameter of 38.32 mm and showing statistical significance compared to higher concentrations based on ANOVA and Tukey HSD tests. Future research should focus on identifying the active compounds responsible for this antifungal activity and evaluating their safety and efficacy in *in vivo* or clinical settings.

REFERENCES

- Djaali, H. (2020). *Metodologi penelitian kuantitatif*. Bumi Aksara.
- Handayani, R. (2020). *Metodologi penelitian sosial*. Trussmedia Grafika.
- Sugiyono. (2019). *Metodologi penelitian kuantitatif, kualitatif, dan R&D*. Alfabeta.
- Boral, H., Metin, B., Döğen, A., Seyedmousavi, S., & Ilkit, M. (2018). Overview of selected virulence attributes in *Aspergillus fumigatus*, *Candida albicans*, *Cryptococcus neoformans*, *Trichophyton rubrum*, and *Exophiala dermatitidis*. *Fungal Genetics and Biology*, *111*, 92–103. <https://doi.org/10.1016/j.fgb.2017.10.008>
- Denning, D. W., & Bromley, M. J. (2015). How to bolster the antifungal pipeline. *Science*, *347*(6229), 1414–1416. <https://doi.org/10.1126/science.aaa6097>
- Kalumpiu, J. V. (2019). Dapagliflozin: Manfaat dan risiko pada diabetes melitus tipe 2. *Jurnal Biomedika dan Kesehatan*, *2*(2), 81–89.
- Lee, Y., Robbins, N., & Cowen, L. E. (2023). Molecular mechanisms governing antifungal drug resistance. *NPJ Antimicrobials and Resistance*, *1*(1), 1–12. <https://doi.org/10.1038/s44259-023-00007-2>
- Manuntung, A. (2020). Efikasi diri dan perilaku perawatan diri pasien diabetes melitus tipe 2 di wilayah Puskesmas Pahandut. *Adi Husada Nursing Journal*, *6*(1), 45–52. <https://doi.org/10.37036/ahnj.v6i1.159>
- Nina, N., Purnama, H., Adzidzah, H. Z. N., Solihat, M., Septriani, M., & Sulistiani, S. (2023). Determinan risiko dan pencegahan terhadap kejadian penyakit diabetes melitus tipe 2 pada usia produktif di wilayah DKI Jakarta. *Journal of Public Health Education*, *2*(4), 221–230. <https://doi.org/10.53801/jphe.v2i4.148>
- Novialdi, Y. (2019). Penatalaksanaan abses submandibula pada penderita diabetes melitus tipe 2. *Bagian Telinga Hidung Tenggorok Bedah Kepala Leher (THT-KL) Fakultas Kedokteran Universitas Andalas Padang*, 1–6.
- Sari, S. W., & Adelina, R. (2020). Apakah pola makan menjadi faktor dominan kejadian diabetes melitus tipe 2 di Indonesia? (Studi literatur). *Jurnal Pangan Kesehatan dan Gizi Universitas Binawan*, *1*(1), 54–63.
- Shahabudin, S., Azmi, N. S., Lani, M. N., Mukhtar, M., & Hossain, M. S. (2024). *Candida albicans* skin infection in diabetic patients: An updated review of pathogenesis and management. *Mycoses*, *67*(6), 543–556. <https://doi.org/10.1111/myc.13753>
- Sharma, A., Tanwar, S., Asija, R., & Agarwal, R. (2021). Fungal infections associated with COVID-19. *International Journal of Pharmacy and Pharmaceutical Sciences*, *13*(10), 1–6. <https://doi.org/10.22159/ijpps.2021v13i10.42554>
- Soelistijo, S. A., et al. (2019). *Pengelolaan dan pencegahan diabetes melitus tipe 2 dewasa di Indonesia*. Perkumpulan Endokrinologi Indonesia.
- Tits, J., Cammue, B. P. A., & Thevissen, K. (2020). Combination therapy to treat fungal biofilm-based infections. *International Journal of Molecular Sciences*, *21*(22), 8873. <https://doi.org/10.3390/ijms21228873>