

Inhibition Zone of Soapnuts Extract (*Sapindus rarak*) Against *Candida albicans*

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Abstract: *Candida albicans* infection is a common health problem in Indonesia and other tropical countries. Synthetic antifungals have been shown to develop resistance against *Candida albicans*. Therefore, it is necessary to explore potential natural ingredients as alternatives to synthetic antifungals. Soapnut fruit contains secondary metabolites that can inhibit or kill *Candida albicans*. The purpose of this study was to test the antifungal activity of Soapnuts fruit by examining the inhibition zone. The inhibition zone was tested using the well diffusion method. In this study, Soapnuts extract was used with concentrations of 25%, 50%, 75%, and 100%. The results showed a probability value (p) of $0.008 < 0.05$, indicating a significant difference between each concentration of soapnut extract and the inhibition zone diameter of *Candida albicans*. The best results were shown by the 100% concentration, which produced an average inhibition zone of 27.50 mm, categorized as very strong inhibition. Soapnut fruit extract exhibits a potent inhibitory effect against *Candida albicans*. This study can serve as a reference in the development of antifungal agents from Soapnut fruit extract.

Keywords: Antifungal activity; *Candida albicans*; Inhibition Zone; Resistance; Soapnuts Fruit Extract.

Introduction

Candida albicans infection is a common health problem in Indonesia and other tropical countries [1]. *Candida albicans* is a type of opportunistic fungus, a microorganism that normally lives as normal flora on the skin, mouth, digestive tract, and genitals without causing symptoms [2]. However, when the immune system is weakened or the microflora is imbalanced, this fungus can overgrow and cause an infection known as candidiasis [3].

In Indonesia, the prevalence of candidiasis is estimated at 20–25%, with a tendency to increase every year [4]. This figure encompasses various forms of infection, including both superficial and systemic infections. Factors that increase the risk of candidiasis include long-term use of broad-spectrum antibiotics that can disrupt the balance of the body's normal flora, decreased immunity due to chronic diseases such as diabetes mellitus or HIV/AIDS, and poor personal hygiene [5]. In addition, in women, hormonal changes due to pregnancy or the use of hormonal contraceptives can also trigger excessive growth of *Candida albicans* [6].

Candidiasis is generally managed with synthetic antifungals, such as ketoconazole, fluconazole, itraconazole, and miconazole [7]. These drugs work by inhibiting the synthesis of ergosterol in the fungal cell membrane, a process that plays a crucial role in maintaining the integrity and function of the cell membrane. However, long-term use of synthetic antifungals is not free from problems, such as the emergence of fungal resistance, toxic effects on the liver, gastrointestinal disorders, and drug interactions with other compounds [8]. This resistance phenomenon is increasingly being reported, particularly in immunocompromised patients who receive repeated antifungal therapy.

Therefore, alternative treatments are needed that are safer, more effective, and have minimal side effects. One approach

currently being widely developed is the use of natural ingredients from medicinal plants as a source of natural antifungal compounds [9]. One of the plants that has the potential to be a source of natural antifungal ingredients is the Soapnut (*Sapindus rarak* DC.). [10].

Soapnuts are widely known in Indonesia as a natural cleaning agent due to their saponin content, a secondary metabolite compound with surfactant and antimicrobial activity [11]. In addition to saponins, soap nuts also contain flavonoids, tannins, and alkaloids, which are known to have the potential to inhibit the growth of microorganisms, including pathogenic fungi [12]. Several studies have shown that soap nut extract can inhibit the growth of fungi such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton* sp [13].

The use of soap nuts as a natural antifungal agent not only supports the development of safe and effective herbal products but also has the potential to reduce dependence on synthetic chemicals. Therefore, research on the antifungal activity of soap nut extract is crucial to determine its effectiveness in inhibiting fungal growth and its potential as a natural antifungal agent.

Research Methods

This research is a qualitative experimental research with a design (post-test only control group design) where the well diffusion method was used to see the inhibition zone at the end of the study. The research stages include: determination of the plants used, extraction of Soapnut fruit simplicia using the maceration method, phytochemical screening of the extract, and an antifungal activity test (inhibition zone) of the Soapnut fruit extract against *Candida albicans*. The data obtained from the inhibition zone measurements were analyzed using a one-way ANOVA statistical test to determine the significance of differences

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between treatment groups and to assess the effectiveness of the inhibition zone of Soapnuts fruit ethanol extract against *Candida albicans*.

Extraction Preparation

Extraction was carried out using the maceration method at a ratio of 1:5, with 200 grams of the dried Soapnut fruit sample being weighed. After weighing the sample, it was transferred to a vessel, and 1000 mL of ethanol solvent was added. Gently stirred with a stirring rod until the mixture was homogeneous. The container was then covered with aluminium foil and stored at room temperature. The maceration process was carried out for 3 days, with the solvent changed every 24 hours and stirring repeated. The extract was then filtered through filter paper. The resulting extract was concentrated using a rotary evaporator until a thick extract was obtained.

Phytochemical Screening

Alkaloids: 1 mg of extract is taken, placed in a test tube, and added to 0.5 ml of 2% HCl. The solution is then divided into two tubes. Tube 1 is added with 2-3 drops of Dragendorff's reagent, and tube 2 is added with 2-3 drops of Mayer's reagent. A positive result for alkaloids is if a brick red, red, or orange precipitate is formed with Dragendorff's reagent and a white or yellowish precipitate with Mayer's reagent, indicating the presence of alkaloids.

Flavonoids: Add 0.5 grams of extract to 10 ml of distilled water and boil for 5 minutes. Then, add 0.05 grams of Mg powder and 1 ml of concentrated HCl. A red to purple color indicates the presence of flavonoids.

Tannins: Dissolve 0.5 g of each sample in distilled water and then add FeCl_3 solution. A positive result is indicated by the formation of a blue-black or green-brown color.

Saponins: Weigh 0.5 g of the sample into a test tube, add 10 mL of hot water, cool, and shake vigorously for 10 seconds. The presence of saponins is indicated by the formation of a foam, 1–10 cm high, that lasts for at least 10 minutes.

Preparation of the test solution

Preparation of a 1% ketoconazole positive control solution: The positive control solution used was ketoconazole at a concentration of 1%, using 50 grams (0.5). This solution was prepared by crushing 2.5 ketoconazole tablets, weighing the equivalent of 0.5 grams of ketoconazole, and dissolving them in 49.5 grams of ethyl acetate, resulting in a total mixture of 50 grams at a concentration of 1%.

Making Soapnuts fruit extract solution: Test solutions were prepared at concentrations of 25%, 50%, 75%, and 100% by weighing 0.25 g, 0.50 g, 0.75 g, and 1 g of concentrated Soapnuts extract, then dissolving each in 1 ml of ethanol solution.

Inhibition Zone Test

The antifungal activity of Soapnuts ethanol extract against *Candida albicans* was tested using the well diffusion

method with a spread plate inoculation technique. Prepare *Candida albicans* fungal suspensions by pouring each suspension into a Petri dish containing solid media. Then, dip the short end of the spreader rod into a beaker containing alcohol. Place the suspension over a Bunsen burner with the short end upright. Cool for 10-15 seconds. Then, spread the fungal suspension evenly over the surface of the media with the short end of the spreader rod. The inoculation was then incubated for 24 hours at 37°C. Afterward, make holes in the media with a cork punch no. 4 and remove the contents of the perforated media, in one media containing 6 wells, Fill each well with a concentration of ethanol extract of Soapnuts fruit 25%, 50%, 75% and 100% respectively positive control (+) ketoconazole, and negative control (-) sterile distilled water that has been determined, then the petri dish is incubated at a temperature of 350-370 for 24 hours, measuring the diameter of the inhibition zone which is marked by a clear zone formed around the well hole using a caliper, antifungal activity testing is carried out 3 times.

Data analysis

All antifungal activity test results (inhibition zone diameter) were analyzed using one-way ANOVA if the data distribution was normal, using SPSS software.

Results and Discussion

Candida albicans typically exists as a harmless member of the human microbiota but can shift to a disease-causing state when the balance of the host's immune defences, hormonal milieu, or resident microbiome is disturbed. This species is clinically significant due to its involvement in both mucosal infections (such as oral and vulvovaginal candidiasis) and severe systemic infections in individuals with weakened immunity. Its capacity to swiftly modify its physiology and morphology in response to environmental stresses and antifungal treatment contributes to the ongoing difficulty in effectively managing infections caused by this pathogen [14].

Candida albicans has a complex and adaptive cell structure, rendering many antifungals less effective against it. One key factor is its thick, layered cell wall, composed of β -1,3-glucan, β -1,6-glucan, chitin, and mannoproteins in the outermost layer. This structure serves as both a mechanical and physical barrier, limiting the penetration of antifungal drugs into the cell. Furthermore, *Candida albicans* can modify the composition of its cell wall, for example, by increasing the chitin content, thereby making certain drug targets less sensitive [15].

Candida albicans' ability to undergo dimorphism, changing from a yeast form to a hyphal or pseudohyphal form, also contributes to its resistance. The hyphal form has a thicker cell wall and different surface protein expression, allowing for altered or shielded antifungal targets. Furthermore, its cell membrane is equipped with efflux pumps that actively remove antifungal molecules from the cell, reducing intracellular drug levels. This combination of cell wall structure, membrane, biofilm, and transport systems makes *Candida albicans* difficult to kill with many antifungals [15].

Extraction

The maceration extraction method was chosen due to its simplicity and minimal equipment requirements; it does not involve heating, thereby preventing the decomposition of active substances contained in the sample that can be caused by high temperatures and unstable compounds. The working principle of maceration is based on the ability of a solvent solution to penetrate cell walls and enter the cell cavities containing various active components [16].

The maceration method was chosen based on its advantages, namely a relatively simple procedure, easy-to-use equipment, and the absence of heating. The absence of a heating process makes this method suitable for extracting thermolabile compounds, because it can prevent degradation or changes in the chemical structure of active compounds due to the influence of high temperatures. Furthermore, the success of the maceration process is influenced by several factors, including the type and polarity of the solvent, the particle size of the material, the length of soaking time, and the intensity of stirring, which play a role in increasing contact between the solvent and the material matrix so that the extraction process takes place optimally.

The extraction process of Soapnut extract simplicia powder was carried out using the maceration method with a solvent ratio of 1:5, which can be achieved by soaking 200 grams of Soapnut simplicia powder. The solvent used in this study was 1000 mL of ethanol. The maceration process of Soapnuts extract was carried out for 3 days with the aim of maximizing the process of extracting chemical compounds contained in the sample. The results of this process yielded 89 grams of thick extract, corresponding to a yield of 44.5%. These results indicate that the extraction process was successful.

Phytochemical Test

A qualitative phytochemical screening test was conducted to identify the secondary metabolite compounds present in natural ingredients. Qualitative phytochemical screening tests are the initial stage in natural product research, aiming to qualitatively identify the presence of secondary metabolites in a material, particularly those derived from plants. The principle of qualitative phytochemical tests is to detect the presence of secondary metabolites in natural materials through specific reactions between the test compound and certain reagents, characterized by color changes, the formation of sediment, or the appearance of foam, thus indicating the presence or absence of certain compound groups. The results of the phytochemical screening test on the ethanol extract of Soapnuts fruit and white frangipani flower acetate are shown in Table 1. Based on the results obtained, five secondary metabolites were identified in the Soapnut fruit extract.

Based on phytochemical tests, it was found that Soapnuts fruit extract contains secondary metabolites in the form of flavonoids, tannins, saponins, and alkaloids. The antifungal properties of *Sapindus rarak* are mainly due to its high triterpenoid saponin content, which has been well documented for its antimicrobial and surfactant properties [17][18].

Table 1. Phytochemical screening test results

Secondary metabolites	Test method	Standard color	Result
Flavonoids	Mg powder + concentrated HCl	Redish orange	+
Alkaloid	2% HCl + 5 drops of mayers	White precipitate	+
Tannin	FeCl ₃	Blue-black	+
Alkaloid	2% HCl + 3 drops of dragendorph reagent	Red	+
Saponin	10 ml aquadest	Foam	+

Inhibition Zone Test

The antifungal inhibition zone test was used with ethanol extracts of Soapnut fruit at concentrations of 25% (P1), 50% (P2), 75% (P3), and 100% (P4). Increasing the percentage of extract simplifies the observation process. After that, the antifungal inhibition zone test of the ethanol extract from Soapnuts fruit was carried out using the well method, as this method allows for a more even and efficient osmosis process, thereby increasing the effectiveness in inhibiting fungal growth. The well method was chosen because of its advantages over the disc diffusion method, particularly in terms of the volume and concentration of the test compound. This method allows for a larger volume of test solution to be introduced into the well, making it suitable for testing extracts, secondary metabolites, or viscous solutions that are difficult to absorb with paper discs. Furthermore, the absence of a carrier material, such as filter paper, minimizes the possibility of interactions that could affect the activity of the test compound. Furthermore, the well diffusion method can make the inhibition zone clearer than the disc diffusion [19]. The test results are presented in Figure 1 and Table 2.



Figure 1. Bacterial inhibition zone with concentrations of 25%, 50%, 75%, 100%, positive control and negative control

The antifungal activity shown in Figure 1 indicates that *Sapindus rarak* effectively interferes with fungal growth

mechanisms. The clear inhibition zones obtained indicate efficient diffusion of the active compounds into the agar medium, allowing direct interaction with fungal cells. This supports the rationale for selecting the well diffusion method, which facilitates better diffusion of liquid extracts compared to disc diffusion, especially for viscous or complex phytochemical preparations. Unlike the disc diffusion method, which relies on the passive absorption of the test substance onto filter paper discs, the well diffusion method allows for the direct application of liquid extracts into wells, enabling more uniform and efficient diffusion into the agar matrix. This is particularly important for plant extracts containing high-molecular-weight compounds such as saponins, which may diffuse poorly from impregnated discs. The clearer inhibition zones obtained through the well diffusion method facilitate more accurate measurement and interpretation of antifungal activity.

Table 2. Results of the inhibition zone of Soapnuts fruit extract against *Candida albicans*

Repl.	Inhibition Zone Diameter (mm)					
	C(-)	25%	50%	75%	100%	C(+)
R1	0	25.5	26	26.5	27.5	24
R2	0	24	26.5	27	28	25
R3	0	24.5	27	26.5	27	25.5
Average	0	24.7	26.1	26.7	27.5	24.8

An analysis results study using the SPSS program at a 95% confidence level. The normality test at a 95% confidence level ($\alpha = 0.05$) aims to determine whether the data in the study are normally distributed. The normality test used is the Shapiro-Wilk. The results of the normality test showed that one of the test groups was not normally distributed, namely at a concentration of 75% which had a probability value (p) <0.05 , so the normality assumption was not met. Because the data were not normally distributed, the analysis continued with non-parametric tests, specifically the Kruskal-Wallis test.

Table 3. Results of the Kruskal-Wallis Test of Soapnuts Fruit Extract Against *Candida albicans* Based on the Diameter of the Inhibition Zone

		Mean	SD	P-value
Inhibition Zone	C (-)	0.00	0.00	
Diameter	C (+)	24.8	0.76	
	25%	24.7	0.76	0.008*
	50%	26.1	0.50	
	75%	26.7	0.28	
	100%	27.5	0.50	

Based on Table 3, the results show that the probability value (p) is $0.008 <0.05$. This indicates a significant difference in the diameter of the *Candida albicans* inhibition zone between each concentration of soapnut extract. The best results were obtained with a concentration of 100%, exhibiting a very strong inhibition zone diameter.

In this study, the method used to test bacterial inhibition zones was the well diffusion method. The well diffusion method was chosen because the inhibition zones formed would be more clearly visible compared to the disc diffusion [20] [21]. With the well diffusion method, bacteria are active not only on the surface of the nutrient agar but also

throughout the substrate. Furthermore, the test material can come into direct contact with the medium without the intermediary of a paper disc, as is the case in the disc diffusion method.

Based on antifungal test results, the ethanol extract of Soapnuts fruit has activity that can inhibit the growth of *Candida albicans*. This is evidenced by the formation of a zone of inhibition, characterized by the appearance of a clear zone around the well. Antibacterial activity strength has 4 categories based on the inhibition zone: <5 mm (Weak), 5-10 mm (Moderate), $>10-20$ mm (Strong), and $>20-30$ mm (Very Strong) [22]. The size of the clear zone varied among the different treatment groups. These results indicate an increase in the diameter of the inhibition zone as the concentration of the extract increases, suggesting that the antifungal inhibitory power is stronger at higher concentrations. The best results were obtained at a concentration of 100%, which produced an average inhibition zone of 27.50 mm, categorized as very strong inhibition. This result was superior to the positive control, which yielded a zone of only 24.83%.

Based on phytochemical tests, it was found that Soapnuts fruit extract contains secondary metabolites in the form of flavonoids, tannins, saponins, and alkaloids. This research is in line with that conducted by Putri et al., that the inhibitory power of Soapnuts fruit extract certainly comes from its secondary metabolites, namely: flavonoids, tannins, saponins, and alkaloids [23]. The current investigation reveals that the extract derived from *Sapindus rarak* fruit demonstrates significant antifungal effects against *Candida albicans*, as shown by distinct inhibition zones produced in the agar well diffusion assay. These results align with contemporary evidence that *Sapindus rarak*, commonly referred to as soapnut or lerak, exhibits pronounced antimicrobial activity largely attributable to its abundant saponin constituents [17].

The mechanism of action of flavonoid compounds in inhibiting the growth of *Candida albicans* is by causing permeability disorders, thereby damaging the structure and function of the fungal cell membrane [24]. The mechanism of action of tannin compounds in inhibiting the growth of *Candida albicans* is by inhibiting the synthesis of chitin used for the formation of fungal cell walls [25]. The mechanism of action of saponin compounds in inhibiting the growth of *Candida albicans* is by causing microbial cell lysis, namely by disrupting the stability of the cell membrane and inhibiting the formation and degradation of *Candida albicans* biofilms [26]. The mechanism of action of alkaloid compounds in inhibiting the growth of *Candida albicans* is by inserting between the cell wall and fungal DNA, thereby inhibiting the DNA replication process and disrupting fungal growth [27].

Soapnuts extract is known to contain high concentrations of triterpenoid saponins, bioactive compounds with amphiphilic properties that enable them to interact effectively with the lipid structure of fungal cell membranes. The surface-active properties of saponins enable these compounds to lower the surface tension of membranes, facilitating penetration and binding to essential lipid components. In *Candida albicans*, the ergosterol-rich cell membrane is a primary target, as this sterol plays a crucial role in maintaining membrane fluidity and stability [28]. This mode of action is particularly relevant to *Candida*

albicans, as the integrity of its plasma membrane is crucial for osmotic regulation and cellular homeostasis. Membrane destabilization results not only in the efflux of intracellular components but also in the disruption of essential physiological functions, including nutrient transport, enzymatic activity, and intracellular signalling pathways [18].

The interaction between triterpenoid saponins and ergosterol causes the formation of pores or disorganization of the fungal cell membrane's lipid bilayer. This condition results in a significant increase in membrane permeability, allowing ions, metabolites, and other essential molecules to escape uncontrolled from the cell. This loss of membrane integrity triggers osmotic imbalance and internal cell pressure, ultimately leading to lysis and death of *Candida albicans* cells.

In addition to causing physical damage to cell membranes, saponin-induced permeability disruption also disrupts various physiological functions of the fungus. Nutrient transport becomes ineffective, membrane enzyme activity is disrupted, and intracellular signalling pathways essential for *Candida albicans* growth and biofilm formation are inhibited. This condition accelerates the decline in fungal cell viability and hinders the cell's ability to adapt to its environment.

Furthermore, sustained membrane damage can trigger cellular stress responses in *Candida albicans*, including mitochondrial dysfunction and an increase in the production of reactive oxygen species (ROS). The accumulation of ROS exacerbates damage to cellular components such as proteins, lipids, and nucleic acids, thus accelerating cell death. Thus, the antifungal activity of soapberry fruit extract is not only fungistatic but also fungicidal through a mechanism of widespread and irreversible membrane damage.

Candida albicans is a major opportunistic fungal pathogen implicated in a broad spectrum of clinical diseases, ranging from superficial mucocutaneous infections to severe, potentially fatal systemic candidiasis. The escalating incidence of antifungal resistance, particularly in strains capable of forming biofilms, has intensified the need to identify novel antifungal strategies, including agents derived from natural resources. Within this framework, the antifungal activity of *Sapindus rarak* extract is of particular interest, as it offers a locally sourced, environmentally friendly, and potentially safer alternative to conventional synthetic antifungal therapies [29].

Conventional antifungal drug classes, including azoles, polyenes, and echinocandins, remain the mainstay of antifungal therapy; nevertheless, their clinical utility is increasingly limited by the emergence of resistant strains, dose-related toxicity, and undesirable side effects. In contrast, antifungal compounds derived from plants, such as *Sapindus rarak*, are characterized by a multitarget mode of action that diminishes the probability of resistance development. Unlike synthetic antifungals that typically inhibit a single molecular pathway, saponins exert antifungal activity primarily through direct disruption of fungal cell membranes while simultaneously impairing multiple intracellular functions, thereby enhancing their overall antifungal efficacy [30].

Although the inhibition zones documented in the present work cannot be quantitatively equated with those

produced by standard antifungal agents in the absence of complementary assays such as minimum inhibitory concentration (MIC) determination, they nonetheless reflect a considerable antifungal capacity. The best results were obtained with a concentration of 100%, exhibiting a very strong inhibition zone diameter. Moreover, the well-defined and uniform nature of these inhibition zones indicates consistent and reproducible antifungal activity, a fundamental prerequisite for advancing a bioactive compound toward further pharmaceutical evaluation and development. In this research, 3 replications were used to ensure the reliability and validity of the research results.

The results of this study further strengthen the scientific evidence showing that natural products have significant potential as alternative antifungal agents. In recent decades, the increasing resistance of fungi to synthetic antifungal drugs and the side effects associated with long-term use have prompted the search for new, safer, and more sustainable antifungal sources. The findings of this study contribute to the growing body of literature emphasizing that bioactive compounds from plants, particularly those rich in secondary metabolites such as saponins, flavonoids, and polyphenols, may be effective candidates for controlling fungal infections.

The use of *Sapindus rarak* aligns with global initiatives to develop sustainable, environmentally friendly, and socially and culturally acceptable therapeutic resources. This plant has long been used in various regions as a natural cleaning agent and traditional medicine, thus providing a strong ethnopharmacological basis. This traditional use not only reflects empirical efficacy but also demonstrates a relatively good safety profile and high level of acceptance among local communities, as reported in various previous studies [31].

From a pharmaceutical perspective, *Sapindus rarak* extract holds great promise for development into various topical antifungal formulations. Dosage forms such as vaginal cleansers, sprays, and gels have the potential to provide a direct therapeutic effect at the site of infection while minimizing systemic side effects. Its high saponin content provides natural surfactant properties, which can enhance the spreadability and adhesion of preparations to mucosal surfaces. Furthermore, these characteristics have the potential to enhance penetration of active ingredients and maintain longer contact with target tissues, thereby enhancing therapeutic efficacy.

Furthermore, utilizing locally available plant materials such as *Sapindus rarak* provides added value in the context of self-sufficiency and resilience of health systems. This approach not only encourages the development of locally resource-based health solutions but can also reduce dependence on imported synthetic antifungal drugs, which are often expensive and difficult to access. This is particularly relevant in resource-limited regions, where the availability of conventional antifungal therapies remains a major challenge [32]. Thus, the development of antifungal products based on *Sapindus rarak* has the potential to provide significant clinical and socio-economic benefits.

Conclusion

The fruit extract of soapnut (*Sapindus rarak*) shows potential as a natural antifungal agent against *C. albicans*,

considering its hierarchical categorization in the very strong inhibition zone group. This high antifungal activity suggests that the bioactive components of *S. rarak*, notably saponin and other secondary metabolites based on their polarity, play a significant role in disrupting fungal cell walls and inhibiting the development of fungi. This possibility of *Sapindus rarak* extract as an alternative to synthetic fungicides, especially during periods of great concern about the use of synthetic chemicals.

Author's Contribution

S. Y. Kurniawan: As the first researcher, she was responsible for antibacterial testing. Novitarini: assisted in preparing samples and conducting phytochemical testing of the extracts.

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