

Comparison of the Effectiveness of 32% and 40% Ethanol Extracts of Areca Nut (*Areca catechu L.*) in Inhibiting the Growth of *Candida albicans* on Heat-Cured Acrylic Resin Dentures

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Abstract

Complete dentures made from heat-polymerized acrylic resin are widely used for their good esthetics and biocompatibility, but their porous nature can promote the growth of *Candida albicans* and lead to denture stomatitis. While chemical cleansers such as alkaline peroxide are effective, long-term use may damage the denture material, highlighting the need for safer natural alternatives, including the ethanolic extract of areca nut (*Areca catechu L.*) with antimicrobial properties. This study aimed to evaluate and compare the inhibitory effectiveness of 32% and 40% areca nut ethanolic extracts against the growth of *Candida albicans* on heat-polymerized acrylic resin denture bases. This research was an in vitro experimental laboratory study with a post-test only control group design. A total of 24 acrylic resin specimens were divided into four groups: 32% areca nut extract, 40% areca nut extract, alkaline peroxide as a positive control, and distilled water as a negative control. After treatment, the number of *Candida albicans* colonies was counted and statistically analyzed. The results showed a significant difference among groups ($p < 0.05$). Both concentrations reduced fungal colonies, with the 40% extract showing greater inhibitory effectiveness than the 32% extract, although its antifungal activity remained lower than alkaline peroxide. In conclusion, the ethanolic extract of areca nut, especially at a 40% concentration, shows potential as a natural denture cleanser to inhibit *Candida albicans* growth.

Keywords: *Areca nut, Alkaline peroxide, Candida albicans, Heat-cured acrylic resin*

INTRODUCTION

Complete dentures are used to replace all missing teeth in edentulous patients in order to restore masticatory, phonetic, and aesthetic functions. One of the main components of complete dentures is the denture base, which supports all denture components and directly adapts to the oral mucosa (Alqutaibi et al., 2023). The most commonly used denture base material is heat-polymerized acrylic resin (HPAR) due to its good biocompatibility, low density, acceptable esthetic properties, color stability, and ability to produce a relatively smooth surface after finishing and polishing (Dimitrova et al., 2022). However, HPAR also presents limitations such as low thermal conductivity, insufficient impact and flexural strength, and relatively high fluid absorption and porosity (Adhershitha & Viswambharan, 2024; Ahmed et al., 2024; Babu et al., 2024).

The porosity and fluid absorption of HPAR facilitate the penetration of food debris, contaminants, and microorganisms into the denture base, promoting biofilm formation on and within the material (Ismiyati & Ali Ahasyimi, 2023). Inadequate denture hygiene and continuous use during sleep create a humid environment that supports the growth of *Candida albicans*, contributing to denture stomatitis (Bacali et al., 2021). Denture cleaning is commonly performed using chemical immersion techniques, including agents containing alkaline compounds and sodium perborate, which releases oxygen to help remove stains (Siyulan & Yuliarsi, 2022). However, certain chemical cleansers have limitations, such as discoloration caused by sodium hypochlorite and the limited ability of alkaline perborate to remove calculus deposits (Siyulan & Yuliarsi, 2022).

Indonesia's high biodiversity provides opportunities for the use of herbal materials as alternative denture cleaners that are safer, environmentally friendly, affordable, and easily accessible. Areca nut (*Areca catechu* L.) contains active compounds such as tannins, flavonoids, and alkaloids with antimicrobial and antifungal properties. Previous studies have reported that areca nut ethanol extract can inhibit *Candida albicans* growth by damaging fungal cell walls and membranes (Naid) et al., 2025; Sun et al., 2024; Taufik, 2020). However, studies evaluating its effect specifically on HPAR denture bases remain limited. Therefore, this study aims to compare the inhibitory effectiveness of 32% and 40% areca nut ethanol extract against *Candida albicans* on HPAR denture bases and to determine the most effective concentration by comparison with alkaline peroxide and distilled water. This research is expected to contribute theoretically to the development of natural antifungal denture cleansers and practically to provide an effective, safe, and economical alternative for denture care in Indonesia.

RESEARCH METHOD

Types of Research

This study is a laboratory experimental research with a design *post-test only control group design*. This study involved four treatment groups, namely two groups of areca nut extract with different concentrations, one positive control, and one negative control, to compare the effectiveness of inhibition against *Candida albicans* on the base of heat polymerized acrylic resin.

Location and Time

Microbiology Laboratory, Faculty of Pharmacy, University of North Sumatra. Seven Indo Dental Lab and Prima Indonesia University laboratory. will be held in August – September 2025.

Sample

In this study, four treatment groups were established: Group I received ethanol extract of areca nut seeds (*Areca catechu* L.) at a concentration of 32%, Group II received ethanol extract of areca nut seeds (*Areca catechu* L.) at a concentration of 40%, Group III received alkaline peroxide, and Group IV received distilled water. The sample size and number of repetitions were determined using Federer's formula to ensure that the collected data were meaningful and statistically reliable. With the number of treatments (k) set at four, the calculation followed the formula $(k-1)(n-1) \geq 15$, resulting in $(4-1)(n-1) \geq 15$. Based on this calculation, each treatment group required six replications to minimize bias. Consequently, the total number of samples used in this study was 24, consisting of four groups with six replications each.

Research Variables

Bound Variables

Antifungal activity against *Candida albicans*, which is measured based on the inhibition or growth of colonies on the base surface of the dentures.

Independent Variables

The concentration of areca nut extract given to the base of dentures made of heat-polymerized acrylic resin measured 10x10x2mm.

Research Tools and Materials

Research Tools

Blenders, Digital Scales, Ose, Test Tubes, Maceration Containers, Rotary Evaporators, Erlenmeyer Flasks, Cuvettes, Becker Glasses, Measuring Cups, Brushes, Spatulas, Autoclaves,

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Petri Cups, Bowls, Vortexes, Press Tools, Water Baths, Spectrophotometers, Sebofan Plastics, Sandpaper nos. 240, 400, 1000, and Strainers.

Research Materials

Ethanol extract of areca nut seeds (*Semen areca catechu*L.), NaOH, Hydrochloric Acid (HCl), NaCl 0.9%, Sabouraud Dextrose Agar (SDA), Distilled Water, Culture *Candida albicans*, Cold Mold Seal (CMS), Polymer and Heat Polymer Acrylic Monomers, as well as Aluminum Foil.

Research Procedure

Making Areca Nut Extract

In this study, four treatment groups were established: Group I received ethanol extract of areca nut seeds (*Areca catechu L.*) at a concentration of 32%, Group II received ethanol extract of areca nut seeds (*Areca catechu L.*) at a concentration of 40%, Group III received alkaline peroxide, and Group IV received distilled water. The sample size and number of repetitions were determined using Federer's formula to ensure that the collected data were meaningful and statistically reliable. With the number of treatments (k) set at four, the calculation followed the formula $(k-1)(n-1) \geq 15$, resulting in $(4-1)(n-1) \geq 15$. Based on this calculation, each treatment group required six replications to minimize bias. Consequently, the total number of samples used in this study was 24, consisting of four groups with six replications each.

Manufacture of Sabouraud Dextrose Agar Media (SDA)

The manufacture of Sabouraud Dextrose Agar (SDA) media is carried out before the inoculation process. A total of 6.5 grams of natural resources powder is dissolved in sterile distilled water through heating until thoroughly mixed. The homogeneous solution is then covered with aluminum foil and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the media is poured into a petri dish and left at room temperature until it hardens (Sofya *et al.*, 2021).

Manufacture of Heat Polymerized Acrylic Specimens

The manufacture of a specimen of a heat polymerized acrylic resin begins by applying a Cold Mold Seal to the mold chamber and allowing it to dry. A mixture of polymers and monomers with a ratio of 23 mg: 10 mL is prepared in a porcelain cup until it reaches a consistency like wet sand. The mixture is put into the mold space, covered with cellophane plastic, and pressed in a cuvette until it reaches the metal-to-metal position while removing the remaining material. After there is no excess acrylic, the final pressure is carried out and the curing process is carried out in water at 100°C for 45 minutes. The specimens that have been removed from the cuvette are then flattened using sandpaper No. 240, 400, and 1000 until they reach a size of 10 × 10 × 2 mm (Mallombassang *et al.*, 2024).

***Candida albicans* Suspension Manufacturing**

One loopful culture *Candida albicans* 24 hours old is transferred to a test tube containing 10 mL of 0.9% NaCl solution, then is vortexed for about 15 seconds until a homogeneous suspension is formed. A total of 7 mL of the suspension was then inserted into another tube and measured the level of turbidity using a spectrophotometer. The turbidity value obtained is then calibrated to the 0.5 McFarland standard, which is equivalent to a concentration of approximately 1.5×10^8 CFU/mL (Sofya *et al.*, 2021).

Sample Immersion

The sample immersion stage begins by preparing 24 acrylic plates as test samples. The entire sample is then put into a test tube containing the suspension *Candida albicans* with a concentration according to the McFarland standard of 1.5×10^8 CFU/mL. Initial incubation is carried out for 24 hours at a certain temperature so that the fungus can adhere to the surface of the sample. After the incubation period, the samples were divided into four groups, each containing 6 samples. The immersion was then carried out by giving different treatments to the four groups for 8 hours (Sofya *et al.*, 2021).

***Candida albicans* Colony Counting Procedure**

The acrylic specimen is removed from the vial after immersion, then rinsed with a saline buffered phosphate solution. Each specimen is placed into a tube containing 10 mL of 0.9% NaCl solution and is divorced for about 30 seconds. A total of 0.1 mL of the formed suspension was inoculated in Sabouraud Dextrose agar media, then incubated for 48 hours at 37°C. Colony *Candida albicans* which grows next is calculated using the colony counter (Sofya *et al.*, 2021).

Data Analysis

Growth inhibition zone diameter data *Candida albicans* From the negative control group (distilled water), positive control (alkaline peroxide), and ethanol extract concentrations of 32% and 40% were analyzed to determine the difference in inhibition between treatment groups. The selection of analysis methods is adjusted to the characteristics of the data obtained. Furthermore, comparisons were made between groups to assess the effectiveness of areca nut ethanol extract in inhibiting growth of *Candida albicans*.

RESULTS AND DISCUSSION

1. Mean Number of *Candida albicans* Colonies

This study used four treatment groups, each consisting of six samples. The treatment groups included soaking the base of heat-polymerized acrylic resin dentures in areca nut ethanol extract with a concentration of 32%, ethanol extract with a concentration of 40%, alkaline peroxide solution as a positive group, and distilled water as a negative control.

The number of *Candida albicans* colonies was calculated after all samples were treated according to their respective groups. Results of the calculation of the average number of colonies *Candida albicans* presented in Table 3.1.

Table 1. Average Number of *Candida albicans* Colonies

Treatment Groups	Number of Samples (n)	Mean ± SD
Areca Nut Ethanol Extract 32%	6	1276.33 ± 23.57
Ethanol Extract of Areca Nut 40%	6	1156.83 ± 12.59
Alkaline Peroxide	6	990.17 ± 46.83
Distilled Water	6	2686.67 ± 222.90

Based on Table 3.1, the average number of colonies *Candida albicans* in the areca nut ethanol extract group, the concentration of 32% was 1276.33 ± 23.57, while in the 40% concentration it was 1156.83 ± 12.59. The alkaline peroxide positive control group showed a lower average number of colonies, which was 990.17 ± 46.83. Meanwhile, the distilled water negative control group had the highest average number of colonies, which was 2686.67 ± 222.90.

Differences in the number of colonies *Candida albicans* Between treatment groups was statistically analyzed. Comparison of the average number of colonies in the four treatment groups was conducted using the Welch ANOVA test. Pairwise comparisons between two treatment groups were performed using an unpaired t-test with Welch's correction. Before the difference analysis is carried out, the normality test and the homogeneity test are first carried out as a prerequisite test to determine the distribution of data and the similarity of variance between groups.

Table 2. Normality Test Results

Treatment Groups	p-value	Remarks
Areca Nut Ethanol Extract 32%	0,400	Normal
Ethanol Extract of Areca Nut 40%	0,433	Normal
Alkaline Peroxide	0,163	Normal

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Distilled Water	0,635	Normal
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Based on the results of the normality test using the Shapiro-Wilk test presented in Table 3.2, a p value of > 0.05 was obtained in all treatment groups. This shows that the data on the number of colonies *Candida albicans* in all groups of normally distributed treatments.

Table 3. Homogeneity Test Results

Test	p-value	Remarks
Levene Test	< 0.001	Not Homogeneous

Furthermore, the homogeneity test was carried out using the Levene test to determine the similarity of data variance between treatment groups. Based on the results of the homogeneity test presented in (Table 3.3), a p value of $p < 0.05$ was obtained. This shows that the variance of the number of colonies data *Candida albicans* between treatment groups is not homogeneous.

With the fulfillment of the assumption of normality but the unfulfilled of the assumption of homogeneity, the analysis of the difference in the average number of colonies *Candida albicans* The treatment groups in this study were continued using the Welch ANOVA test.

Results of Inhibition Effectiveness of Ethanol Extract of Areca Nut (*Areca catechu L.*) 32% and 40% Concentrations of *Candida albicans* Growth on Heat Polymerized Acrylic Resin Denture Base

Analysis of the effectiveness of ethanol extract on growth of *Candida albicans* On the base of dentures, the acrylic resin heat polymerization was carried out by comparing the average number of colonies between treatment groups. This analysis was performed using the Welch ANOVA test because the data were normally distributed but the variance between treatment groups is not homogeneous.

Table 4. Analysis of the Average Difference in the Number of *Candida albicans* Colonies Between Treatment Groups

Analysis	p-value	Remarks
The average difference in the number of <i>Candida albicans</i> colonies between the groups:		
(1) Ethanol Extract of Areca Nut 32%,(2) Ethanol Extract of Areca Nut 40%,(3) Alkaline Peroxide, and (4) Distilled Water	< 0.001	Significant

The results of the analysis in Table 3.4 show that there is a difference in the average number of colonies *Candida albicans* which is statistically significant between treatment groups, with a p value of < 0.05 . These results showed that the administration of ethanol extract of areca nut seeds with a concentration of 32% and 40%, alkaline peroxide, and distilled water had a different effect on the number of colonies *Candida albicans* on the base of the dentures are heat-polymerized acrylic resins.

Difference in Effectiveness of Ethanol Extract of Areca Nut Concentration 32% and 40% on the Growth of *Candida albicans*

Analysis of the difference in the inhibition effectiveness of ethanol extract of areca nut extract concentrations of 32% and 40% on growth of *Candida albicans* This was done by comparing the average number of colonies between the two treatment groups. This analysis was performed using a non-paired t-test with a Welch correction, because the variance of data between treatment groups was not homogeneous.

Table 5. The results of the analysis showed that the difference in the average number of *Candida albicans* colonies between concentrations of 32% and 40%

Analysis	p-value	Remarks
The difference in the average number of <i>Candida albicans</i> colonies between Ethanol Extract of Areca Nut Concentration of 32% and 40%	< 0.001	Significant

Number of colonies *Candida albicans* in the areca nut ethanol extract group, concentrations of 32% and 40% showed a significant average difference (Table 3.5). The results of the analysis showed a p value < 0.001, which indicates that the difference is statistically significant. The average number of colonies at a concentration of 40% is lower than the concentration of 32%, so that ethanol extract of areca nut at a concentration of 40% has a greater inhibitive effectiveness against growth of *Candida albicans* on the base of the dentures are heat-polymerized acrylic resins.

Difference in the Effectiveness of Ethanol Extract of Areca Nut Concentration of 32% compared to Alkaline Peroxide and Distilled water on Growth of *Candida albicans*

Analysis of the difference in the inhibition effectiveness of ethanol extract of areca nut with a concentration of 32% on growth of *Candida albicans*. It was carried out by comparing the average number of colonies between the ethanol extract group of areca nut seed with a concentration of 32%, the positive control group of alkaline peroxide, and the negative control group of Distilled water. This analysis was carried out using unpaired t-test with the Welch correction.

Table 6. Average Difference in Number of *Candida albicans* Colonies between Ethanol Extract of Areca Nut 32% and Alkaline Peroxide

Analysis	p-value	Remarks
The average difference in the number of <i>Candida albicans</i> colonies between the groups: (1) Ethanol Extract of Areca Nut 32% and (2) Alkaline Peroxide	< 0.001	Significant

Based on the results of the analysis in Table 3.6, a p value of < 0.001 was obtained which indicates a difference in the average number of colonies *Candida albicans*. Significant differences between the areca nut ethanol extract group with a concentration of 32% and the alkaline peroxide group. The average number of colonies in the extract group with a concentration of 32% was higher than that of alkaline peroxide, indicating that the inhibition effectiveness of the extract concentration of 32% was still lower than that of alkaline peroxide.

Table 7. Average Difference in Number of *Candida albicans* Colonies between Areca Nut Ethanol Extract 32% and Distilled water

Analysis	p-value	Remarks
The average difference in the number of <i>Candida albicans</i> colonies between groups: (1) Areca Nut Ethanol Extract 32% and (2) Distilled water	< 0.001	Significant

The results of the analysis in Table 3.7 show a p value of < 0.001, which indicates a difference in the average number of colonies *Candida albicans*. The significant difference between the areca nut ethanol extract group with a concentration of 32% and the distilled water

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group. The average number of colonies in the concentration extract group was 32% lower than that of distilled water, indicating the effectiveness of growth inhibition *Candida albicans* .

Difference in the Effectiveness of Ethanol Extract of Areca Nut Concentration of 40% compared to Alkaline Peroxide and Distilled water on the Growth of *Candida albicans*

Analysis of the difference in the effectiveness of ethanol extract of areca nut extract with a concentration of 40% on growth of *Candida albicans* It was carried out by comparing the average number of colonies between the 40% concentration of areca nut ethanol extract, the alkaline peroxide positive control group, and the distilled water negative control group. Pairwise comparisons were performed using an unpaired t-test with Welch’s correction.

Table 8. Average Difference in the Number of *Candida albicans* Colonies between Ethanol Extract of Areca Nut 40% and Alkaline Peroxide

Analysis	p-value	Remarks
The average difference in the number of <i>Candida albicans</i> colonies between groups: (1) Ethanol Extract of Areca Nut 40% and (2) Alkaline Peroxide	< 0.001	Significant

Based on the results of the analysis in Table 3.8, a p value of < 0.001 was obtained which indicates a difference in the average number of colonies *Candida albicans* Significant between the ethanol extract group of areca nut with a concentration of 40% and the alkaline peroxide group. The average number of colonies in the ethanol extract group of areca nut is 40% higher than that of alkaline peroxide, thus showing that the inhibition effectiveness of 40% concentration extract is still lower than that of alkaline peroxide on the base of heat-polymerized acrylic resin dentures.

Table 9. Average Difference in Number of *Candida albicans* Colonies between Ethanol Extract of Areca Nut 40% and Distilled water

Analysis	p-value	Remarks
Average difference in the number of <i>Candida albicans</i> colonies between groups:(1) Ethanol Extract of Areca Nut 40% and (2) Distilled water	< 0.001	Significant

Based on the results of the analysis in Table 3.9, a p value of p<0.001 was obtained which showed a significant difference in the average number of *Candida albicans* colonies between the 40% concentration of areca nut ethanol extract group and the distilled water group. The average number of colonies in the ethanol extract group of areca nut is 40% lower than that of distilled water, which indicates that the ethanol extract concentration of 40% has an effective inhibition against the growth of *Candida albicans* .

Discussion

Candida albicans is a microorganism normally found in the oral cavity; however, an increase in its number may lead to pathogenic changes, particularly in conditions of poor oral hygiene, and contributes to the occurrence of denture stomatitis in users of complete dentures made of heat-polymerized acrylic resin (HPAR) (The Bar et al., 2022). Complete denture bases are commonly fabricated from HPAR, a material with relatively high porosity and fluid absorption, which allows food debris, contaminants, and microorganisms to adhere to and penetrate the denture surface. These properties facilitate the attachment of *Candida albicans* and support biofilm formation, making microorganisms more difficult to eliminate when denture cleaning is not performed optimally (Alajami, 2025).

Biofilm accumulation of *Candida albicans* on denture bases is closely associated with denture stomatitis. Long-term denture use, especially without regular cleaning and when worn during sleep, creates a humid environment that promotes the growth of *Candida albicans* on HPAR surfaces and increases the risk of inflammation of the oral mucosa in contact with the denture base (McReynolds et al., 2023; Pearl et al., 2024). Therefore, denture cleaning is an essential component of complete denture treatment, as inadequate hygiene can lead to continuous microbial accumulation and increased *Candida albicans* colony counts, emphasizing the need for effective denture cleansers that do not cause adverse effects on denture bases or oral tissues (Abuhajar et al., 2023; Ferro et al., 2023).

Administration of areca nut ethanolic extract has been shown to reduce *Candida albicans* colonies on HPAR denture bases due to its active compounds, particularly tannins, flavonoids, and alkaloids, which possess antifungal properties (Taufik, 2020). Tannins inhibit fungal growth by interacting with cell wall and membrane proteins, causing structural alterations and reducing fungal adhesion, while flavonoids disrupt fungal cell membranes and impair cell function (Dawn et al., 2021; Nguyen et al., 2021). Higher extract concentrations provide greater antifungal effectiveness, with a 40% concentration showing stronger inhibition of *Candida albicans* growth than 32%, consistent with previous findings (Sun et al., 2024; Taufik, 2020).

CONCLUSION

Based on the results of the research and discussions that have been conducted, it can be concluded that areca nut ethanol extract (*Areca catechu* L.) at concentrations of 32% and 40% is proven to be able to reduce the number of *Candida albicans* colonies on the base of heat-polymerized acrylic resin dentures, with a concentration of 40% showing a higher inhibition effectiveness compared to the concentration of 32%, so it has the potential as an alternative to denture cleaning agents natural. In this regard, it is suggested that 40% concentration of areca nut ethanol extract can be considered in the development of natural denture cleaners, and it is necessary to conduct further research to determine the most optimal concentration and duration of soaking, develop other forms of preparations such as gels to improve ease of use, and evaluate their effect on the physical properties of the base of heat-polymerized acrylic resins, including surface roughness and color stability.

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