

THE EFFECTIVENESS OF KETAPANG LEAF (*Terminalia Catappa L.*) INFUSION AS A DENTURE CLEANSER FOR HEAT-CURED ACRYLIC RESIN DENTURES IN INHIBITING *CANDIDA ALBICANS* GROWTH

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ABSTRACT

Tooth loss can be managed using removable dentures. However, heat-cured acrylic resin, a material commonly used for denture bases, has a porous structure that promotes the growth of *Candida albicans*. One method of maintenance involves soaking dentures in a cleansing solution to inhibit fungal growth. This study aimed to evaluate the effectiveness of ketapang leaf infusion (*Terminalia catappa L.*) as a denture cleanser in inhibiting *Candida albicans* growth. This was a true experimental post-test-only control group design involving 30 heat-cured acrylic resin specimens contaminated with *Candida albicans*. The specimens were divided into five groups: a positive control group (0.5% sodium hypochlorite), a negative control group (sterile distilled water), and three experimental groups treated with ketapang leaf infusion at concentrations of 30%, 60%, and 90%. Each specimen was soaked in the respective solution for 15 minutes and subsequently cultured to determine fungal colony counts. Statistical analysis showed a significant difference between the infusion groups and the negative control group ($p < 0.05$). The 30% concentration group had the lowest fungal count, whereas the 90% concentration group had the highest. It can be concluded that ketapang (*Terminalia catappa L.*) leaf infusion is effective in inhibiting *Candida albicans* growth on heat-cured acrylic resin, with 30% being the most effective concentration.

Keywords: *Candida albicans*., *Terminalia catappa*., acrylic resin., denture cleanser

INTRODUCTION

Tooth loss can cause problems in both aesthetics and masticatory function, and it may also affect an individual's self-confidence. The use of dentures is a widely accepted solution for addressing tooth loss. Acrylic resin is the most commonly used material for removable denture bases. It meets the requirements for denture base materials as it is readily available, cost-effective, dimensionally stable, has good physical and esthetic properties, and is easy to manipulate and repair. Additionally, it does not irritate or dissolve in the oral cavity.¹

Despite its many advantages, acrylic resin also has drawbacks, one of which is its porosity. If dentures are not cleaned properly, food debris can become trapped in the pores of the acrylic resin, creating an ideal environment for the growth of pathogenic microorganisms.^{1,2} Prolonged use of denture also impairs natural cleansing by the tongue and saliva, resulting in the accumulation of biofilm plaque. *Candida albicans* is one of the pathogenic microorganisms responsible for denture stomatitis.³ Cleansing solutions or denture cleansers are necessary to reduce the presence of *Candida albicans* on denture surfaces. One commonly used

cleanser is sodium hypochlorite. However, this compound has limitations—it may reduce the impact strength of acrylic resin and may not be accessible to all communities.⁴

Therefore, there is a need for alternative denture cleansers that are accessible, easy to prepare by the community, and effective as antifungal agents in inhibiting *Candida albicans* growth. One such natural substance from traditional medicinal plants is the ketapang leaf (*Terminalia catappa L.*). The antifungal properties of ketapang leaves against *Candida albicans* are supported by their bioactive contents, such as phenols, flavonoids, C-glycoside flavonoids, tannins, saponins, alkaloids, steroids, and terpenoids.^{5,6,7} Previous studies have shown that extracts of ketapang leaves at concentrations of 30%, 60%, and 90% significantly inhibit *Candida albicans* growth *in vitro*.⁸ However, research on the use of ketapang leaf extract as an antifungal agent against *Candida albicans* remains limited, especially in the form of infusions. Therefore, this study aims to evaluate the effectiveness of ketapang leaf (*Terminalia catappa L.*) infusion as a cleanser for heat-cured acrylic resin dentures in inhibiting *Candida albicans* growth at concentrations of 30%, 60%, and 90%.

MATERIALS AND METHODS

The materials used in this study included ketapang leaves (*Terminalia catappa* L.), heat-cured acrylic resin plates (10×10×2 mm), sterile distilled water (aquadest), Sabouraud Dextrose Agar (SDA) powder, *Candida albicans* suspension, tryptic soy broth, 0.5% sodium hypochlorite, and 0.9% NaCl solution. The equipment used included measuring cylinders, Erlenmeyer flasks, test tube holders, Petri dishes, inoculation loops, test tubes, analytical balance, filter paper, black cardboard, fine-point black markers, incubator, autoclave, vortex mixer, micropipettes, and a magnetic stirrer hotplate.

This study used a true experimental laboratory design with a post-test-only control group approach. The research was conducted at the Phytochemistry Laboratory of the Faculty of Mathematics and Natural Sciences, and the Microbiology Laboratory of the Faculty of Medicine, Udayana University. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Udayana University (No. 1163/UN114.2.2.VII.14/LT/2022). The sample size was determined using Federer's formula, resulting in 25 samples. After correction, the final sample size was 30.

The ketapang leaf infusion was prepared at the Phytochemistry Laboratory of the Faculty of Mathematics and Natural Sciences, Udayana University. The leaves were washed, dried at 50°C for one day, crushed, and filtered. To prepare infusions of different concentrations, 30 g of leaves were infused in 100 ml of aquadest for the 30% concentration, 60 g in 100 ml for the 60% concentration, and 90 g in 100 ml for the 90% concentration. The infusion was heated at 90°C for 15 minutes with continuous stirring using a magnetic stirrer.

The fabrication of heat-cured acrylic resin plates was carried out at AO Dental Laboratory, Denpasar, Bali. The samples were soaked in sterile aquadest for 24 hours to reduce residual monomer content, followed by sterilization using an autoclave at 121°C for 15 minutes. The samples were then contaminated with *Candida albicans* and divided into five treatment groups: positive control (0.5% sodium hypochlorite), negative control (sterile aquadest), and three treatment groups using ketapang leaf infusion at concentrations of 30%, 60%, and 90%.

After treatment, each acrylic resin plate sample was immersed in 0.9% NaCl solution and agitated with a vortex mixer for 2 minutes to release *Candida albicans*. The resulting solution was serially diluted up to a 10⁻⁵ dilution. A volume of 0.2 µl from the 10⁻⁵ dilution was plated onto SDA media, spread using a sterile spreader, and incubated for 24 hours at 37°C. The resulting *Candida albicans* colonies were counted to analyze differences among the treatment groups.

RESULTS

Plant identification was conducted at the Characterization Laboratory of the "Eka Karya" Botanical Garden – BRIN,

Bedugul, Bali, using ketapang leaf samples. The results confirmed that the leaves used in this study were accurately identified as ketapang (*Terminalia catappa* L.). In addition, phytochemical analysis of the ketapang leaf infusion revealed the presence of various active compounds, including phenols, flavonoids, flavonoid glycosides, tannins, saponins, alkaloids, terpenoids, and steroids.

Fungal colony counts, as presented in Table 1, showed variation across all treatment groups. The negative control group (sterile distilled water) exhibited the highest mean fungal count (8.53×10^9 CFU/ml), while the positive control group (0.5% sodium hypochlorite) had the lowest (2.89×10^9 CFU/ml). Among the ketapang leaf infusion groups, the 30% concentration showed the lowest mean fungal count (3.2×10^9 CFU/ml).

Table 1. Fungal Count Results

Sample	K+	K-	P1	P2	P3
1	2,88	9,02	2,72	4,96	6,13
2	2,94	8,10	3,68	5,82	5,29
3	3,23	8,15	2,75	5,50	5,31
4	3,31	8,88	3,26	5,81	5,83
5	2,98	8,76	3,59	5,02	5,62
6	1,97	8,26	3,17	5,05	6,02
Mean	2,89	8,53	3,20	5,36	5,70

Description:

K+	:	Positive control (0.5% sodium hypochlorite)
K-	:	Negative control (sterile distilled water)
P1	:	30% ketapang leaf infusion
P2	:	60% ketapang leaf infusion
P3	:	90% ketapang leaf infusion

As this study used a post-test-only control group design, reductions in fungal counts were evaluated by comparing post-treatment results of the infusion groups to the negative control group.

Data analysis began with the Shapiro–Wilk test for normality (appropriate for sample sizes under 50), which indicated a p-value greater than 0.05, confirming that the data were normally distributed. Levene's test was then used to assess homogeneity of variances, also yielding a p-value greater than 0.05, indicating that the data were homogeneous.

Given the assumptions of normality and homogeneity were met, a One-way ANOVA was performed, revealing a statistically significant difference among the treatment groups ($p = 0.000$; $p < 0.05$). This result indicates that the ketapang leaf (*Terminalia catappa* L.) infusion had a significant antifungal effect in inhibiting *Candida albicans* growth on heat-cured acrylic resin denture bases.

A post hoc analysis using the Least Significant Difference (LSD) test was conducted to determine pairwise differences between groups. As shown in Table 2, the 30% infusion group differed significantly from the 60% and 90% groups. No significant difference was found between the 60% and 90% groups. Both the 60% and 90% infusion groups

showed significant differences when compared to both the positive and negative control groups. The 30% group had a p-value of 0.094 ($p > 0.05$) compared to the positive control group, indicating no significant difference, but it was significantly different from the negative control group.

Table 2. Summary of LSD Post Hoc Test

Group	K+	K-	P1	P2	P3
K+	-	0,000*	0,094	0,000*	0,000*
K-	0,000*	-	0,000*	0,000*	0,000*
P1	0,094	0,000*	-	0,000*	0,000*
P2	0,000*	0,000*	0,000*	-	0,148
P3	0,000*	0,000*	0,000*	0,148	-

Description:

K+ : Positive control treatment with 0.5% sodium hypochlorite
 K- : Negative control treatment with sterile distilled water
 P1 : Treatment with 30% ketapang leaf infusion
 P2 : Treatment with 60% ketapang leaf infusion
 P3 : Treatment with 90% ketapang leaf infusion
 (*) : Indicates a statistically significant difference

DISCUSSION

The results of this study showed differences in the mean fungal counts across all treatment groups. The highest fungal count was observed in the negative control group, which used sterile distilled water due to its neutral properties and lack of influence on *Candida albicans* growth.⁹ In contrast, the positive control group used 0.5% sodium hypochlorite, which at concentrations below the Minimum Inhibitory Concentration (MIC) acts as a fungistatic agent. It reduces the adhesion of *Candida albicans* to the acrylic resin surface and epithelial cells without altering its pathogenic characteristics.^{23,24} At the MIC level, sodium hypochlorite inhibits biofilm formation, while at the Minimum Fungicidal Concentration (MFC), it acts as a fungicidal agent.^{25,26,27}

The mean fungal counts in the treatment groups using ketapang leaf (*Terminalia catappa* L.) infusion at concentrations of 30%, 60%, and 90% were lower than those in the negative control group. This antifungal activity is believed to be due to the presence of active compounds in ketapang leaves, including flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, and steroids. Flavonoids are known to bind to ergosterol, inhibit the synthesis of beta-glucan and chitin, leading to cell shrinkage and increased membrane permeability, ultimately resulting in cell lysis.^{10,11} Phenols damage *Candida albicans* cell wall, denature enzymatic proteins, disrupt cellular metabolism, and damage mitochondria, thereby interfering with cell wall formation.^{5,12,13} Tannins interfere with C-14 demethylase, an

essential enzyme in ergosterol biosynthesis, thereby disrupting membrane structure and inducing cytoplasmic leakage that leads to cell lysis.^{14,15}

Saponins are known to inhibit the formation of hyphae and mycelia from yeast, which is crucial in preventing invasive mycosis and biofilm development at early stages of infection. They also reduce *Candida albicans* adhesion.¹⁶ Alkaloids cause mitochondrial dysfunction, increase oxidative stress, and suppress the growth of *Candida albicans*. Their alkaline nature ($pH > 7$) can inhibit fungal growth, which typically thrives at an optimal pH of 4.5–6.5.^{17,18} Terpenoids induce apoptosis and arrest the *Candida albicans* cell cycle at G1, S, and G2-M phases.¹⁹ Steroids, due to their lipophilic properties, disrupt the cytoplasmic membrane and cause leakage of intracellular contents, resulting in cell death. They also inhibit fungal spore development and maturation.^{20,21,22}

This study found that the 30% ketapang leaf infusion had a comparable antifungal effect to 0.5% sodium hypochlorite in inhibiting *Candida albicans* growth. Higher concentrations (60% and 90%) were less effective than sodium hypochlorite. This reduced efficacy at higher concentrations may be attributed to increased viscosity, which hinders the diffusion of active compounds into the pores of acrylic resin.^{7,28} The 30% infusion, with its lower viscosity, allows better diffusion and penetration of active compounds onto the surface of heat-cured acrylic resin.

Statistical analysis showed that the 30% ketapang leaf infusion group did not differ significantly from the positive control but differed significantly from the negative control. These findings indicate that a 30% concentration is the most effective in inhibiting *Candida albicans* growth on heat-cured acrylic resin denture bases.

CONCLUSION AND SUGGESTION

Based on this study, it can be concluded that the infusion of ketapang leaf (*Terminalia catappa* L.) is effective in inhibiting the growth of *Candida albicans* on heat-cured acrylic resin denture bases. The most effective concentration for inhibiting *Candida albicans* growth was found to be 30%.

Suggestions for future research include exploring the antifungal effects of ketapang leaves using alternative extraction methods, evaluating the biocompatibility of the infusion, assessing its impact on color stability and dimensional changes of acrylic resin, and identifying the most active antifungal compounds responsible for its inhibitory effect.

REFERENCES

1. Anusavice KJ, Shen C, Rawls HR. *Phillips' Science of Dental Materials*. 13th ed. Philadelphia: Elsevier; 2021.
2. Anggoro GP. Surface roughness differences of two types of resin after immersion in denture cleansers [dissertation]. Bandung: Universitas Padjadjaran; 2018.

3. Gad MM, Al-Thobity AM, Shahin SY, Alsaqer BT, Ali AA. Inhibitory effect of zirconium oxide nanoparticles on *Candida albicans* adhesion to repaired polymethyl methacrylate denture bases and interim removable prostheses: A new approach for denture stomatitis prevention. *Int J Nanomedicine*. 2017;12:5409–5419.
4. Rahmayani L, Fitriyani S, Andriany P, Dumna R. Effect of sodium hypochlorite concentration as a disinfectant on the impact strength of acrylic resin denture bases. *Dentika Dent J*. 2014;27.
5. Teodoro GR, Ellepola K, Seneviratne CJ, Koga-Ito CY. Potential use of phenolic acids as anti-*Candida* agents: a review. *Front Microbiol*. 2015.
6. Terças AG, Monteiro AS, Moffa EB, dos Santos JRA, de Sousa EM, Pinto ARB, et al. Phytochemical characterization of *Terminalia catappa* Linn. extracts and their antifungal activities against *Candida* spp. *Front Microbiol*. 2017.
7. Triana E, Nurhidayat N. Water extract of ketapang leaves (*Terminalia catappa* L.) as natural cleanser using CIP method. Malang: Universitas Muhammadiyah Malang; 2016.
8. Mikahab ARH. The effect of ketapang leaf (*Terminalia catappa*) extract on *Candida albicans* growth: An in vitro study. Semarang: UNISSULA; 2017.
9. Henaulu AH, Kaihena M. Antibacterial potential of ethanol extract of winged bean leaves (*Psophocarpus tetragonolobus* (L.) DC) against *Escherichia coli* and *Staphylococcus aureus* in vitro. *OJS UNPATI*. 2020.
10. Aboody MSA, Mickymaray S. Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics*. 2020;9(2):3–42.
11. Nurhafani F. Antimicrobial comparison of *Moringa oleifera* n-hexane leaf extract and cashew nut (*Anacardium occidentale*) pericarp extract against *Pseudomonas aeruginosa*. Malang: Universitas Brawijaya; 2012.
12. Ansari MA, Anirag A, Fatima Z, Hameed S. Natural phenolic compounds: a potential antifungal agent. In: *Formatex*. 2013. p. 1189–95.
13. Kolondra A, et al. The transcriptome of *Candida albicans* mitochondria and the evolution of organellar transcription units in yeasts. *BMC Genomics*. 2015;16(827):2–22.
14. Kurniawan D. Antifungal activity of ethanol extract of *Moringa oleifera* leaves against *Candida albicans* in vitro. Pontianak: Universitas Tanjungpura; 2015.
15. Wibowo A, Widjiastuti I, Saraswati W, Setyowati L. Minimum fungicidal concentration of Lawang propolis extract against *Candida albicans*. *Conservative Dent J*. 2017;7(1):37–42.
16. Sadowska B, et al. New pharmacological properties of *Medicago sativa* and *Saponaria officinalis* saponin-rich fractions against *Candida albicans*. *J Med Microbiol*. 2014;63(8):1076–1086.
17. Dhamgaye S, et al. Molecular mechanisms of herbal antifungal alkaloid berberine in *Candida albicans*. *PLoS One*. 2014.
18. Lutfiyanti R, Ma'ruf WF, Dewi EN. Antifungal activity of bioactive compounds from *Gelidium latifolium* extract against *Candida albicans*. *J Fish Process Biotechnol*. 2012;1(1):1–8.
19. Zore GB, et al. Terpenoids inhibit *Candida albicans* growth by affecting membrane integrity and arresting the cell cycle. *Phytomedicine*. 2012;18(13):1181–1190.
20. Febriani TH. Antifungal effect of bitter melon (*Momordica charantia* L.) juice against *Candida albicans* growth in vitro. Surakarta: Universitas Muhammadiyah Surakarta; 2014.
21. Freiesleben S, Jäger A. Correlation between plant secondary metabolites and their antifungal mechanisms: a review. *Med Aromat Plants*. 2014;3(2):1.
22. Makhfirah N, Fatimatuzzahra C, Mardina V, Hakim RF. Utilization of natural substances to inhibit *Candida albicans* in the oral cavity. *J Jeumpa*. 2020;7(2):400–413.
23. Sari WP, Surya LS, Pratiwi CA. Difference in *Candida albicans* adhesion on non dental glass fiber and e-glass fiber reinforced polymer after 0.5% NaOCl immersion. *Padjadjaran J Dent Res Students*. 2021;5(1):64–70.
24. Singh M, et al. In vitro comparative evaluation of antimicrobial efficacy of propolis, *Morinda citrifolia* juice, sodium hypochlorite, and chlorhexidine on *Enterococcus faecalis* and *Candida albicans*. *J Contemp Dent Pract*. 2019;20(1):40–45.
25. Syaula Y, Antari AL, Purbaningrum DA. Effect of *Hibiscus rosa-sinensis* extract immersion on *Candida albicans* growth on acrylic resin plates. *e-GiGi*. 2019;9(2):159–166.
26. Dahar E, Chandra D. Effect of denture cleansers on *Candida albicans* growth on polished and unpolished heat-polymerized acrylic denture base. *Dentika Dent J*. 2014;18(1):75–79.
27. Sedigh-Shams M, et al. In vitro comparison of the antimicrobial effect of sodium hypochlorite solution and *Zataria multiflora* essential oil as irrigants in root canals contaminated with *Candida albicans*. *J Conserv Dent*. 2016;10(1):101–105.
28. Allo MB. Antibacterial activity test of water extract from green Ambon banana peel (*Musa acuminata* Colla) against *Staphylococcus aureus* growth. Yogyakarta: Universitas Sanata Dharma; 2016.

