Original Article

The Effect of Pandan Leaf Extract on Heat-Polymerized Acrylic Resin: Alteration of Physical Properties and Reduction of *Candida albicans*

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Abstract

This study investigates the antifungal activity of a cleaning solution containing pandan leaf extract against *Candida albicans (C. albicans)* in a laboratory setting and evaluates its effect on the color and physical properties of a denture base material. Cleaning solutions with pandan leaf extract at concentrations of 8, 16, and 24 mg/ml were tested for their inhibitory effect on *C. albicans* and their impact on the physical properties of the denture base material including color change, surface hardness, surface roughness, and flexural strength. *C. albicans* counts (CFU/ml) were measured across test groups, including controls. Extracts at 16 and 24 mg/ml showed the greatest reduction in fungal presence, which is comparable to 0.5% sodium hypochlorite. After immersion in cleaning solutions for two 10-min cycles and seven days, the solution with pandan leaf extract showed no significant color change within the same group. Surface roughness and surface hardness changes were also insignificant within the same group and across groups after immersion for two 10-min cycles (t1) and seven days (t2). Similarly, flexural strength showed no significant difference among groups compared to the control group. A cleaning solution with pandan leaf extract at 16 and 24 mg/ml effectively inhibits *C. albicans* without impacting the color or physical properties of a denture base material, suggesting its potential as a safe and effective cleaning solution for denture bases.

Keyword: *C. albicans*, Cleaning solution, Denture base, Pandan leaf, Physical property

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Introduction

Polymethyl methacrylate (PMMA) has been used as a denture base material since the 1930s due to its low cost, ease of use, simple manufacturing process, and aesthetic qualities. However, PMMA is highly susceptible to colonization by *C. albicans* due to its surface porosity, roughness, and hydrophobicity, which promote fungal adhesion and biofilm formation. It lacks intrinsic antimicrobial properties and absorbs salivary proteins that facilitate fungal attachment. Over time, microcracks increase colonization sites, while oral cavity conditions further support fungal growth.[1] These factors contribute to denture-related infections, such as denture stomatitis, emphasizing

the need for improved hygiene and antifungal modifications.[2] These infections can result from inadequate cleaning by the patient or cross-contamination during dental procedures such as repairs or adjustments, which involve movement between the clinic and laboratory.[3] This creates potential for infection transmission among patients, dentists, and laboratory technicians. The presence of *C. albicans* on denture surfaces not only poses a risk for oral infections but can also lead to denture stomatitis, affecting up to 65% of denture wearers.[4] This condition can impact the quality of life and oral health of patients, making effective denture cleaning protocols crucial for both patient care and infection control.

Denture cleaning methods include mechanical and chemical approaches, with chemical solutions being particularly important for eliminating microorganisms in areas that mechanical cleaning might miss. Various chemical agents have been employed to clean dentures and prevent the spread of infections. Studies have reported that 0.5% sodium hypochlorite (NaOCl), while effective at eliminating fungi after 10 minutes of immersion,[5] may damage denture materials, depending on the immersion time and concentration. Specifically, peroxide-based cleaners have been shown to reduce the color, surface roughness,[6] and surface hardness of the material, and cause corrosion to metal components.[7] Additionally, these chemicals may cause tissue irritation if they come into direct contact with oral tissues or are left on the dentures for extended periods.[8]

Given these concerns, the research team is interested in developing a more effective cleaning solution incorporating natural herbal extracts. The growing global interest in natural products has led the research team to explore plant-based alternatives to conventional chemical cleaning agents. This approach aligns with the current trend toward sustainable and biocompatible dental materials and treatments. Medicinal plants have long been used in both medical and dental treatments due to their accessibility, cost-effectiveness, and minimal side effects compared to synthetic drugs, which can contribute to drug resistance and other complications. Many herbs have antimicrobial properties, and their local availability can reduce production costs while promoting agricultural practices and job creation within communities. One such herb is pandan (*Pandanus amaryllifolius*), which has been traditionally used for oral care. Pandan leaves contain phenolic compounds known for their antibacterial properties. Previous studies have shown that ethanol and water extract of pandan leaves (in a 70:30 ratio) exhibit inhibitory effects on various oral pathogens including *Streptococcus sanguinis, Streptococcus salivarius, Streptococcus mutans,* and Porphyromonas gingivalis, with minimum inhibitory concentrations (MIC) of 31.25, 62.5, 3.9, and 125 µg/ml, respectively.[9]

Pandanus amaryllifolius also known as pandan leaves are a plant in the Pandanaceae family that is found in Southeast Asian countries. Pandan leaves have a distinctive fragrance due to the presence of volatile compounds, 2-acetyl-1-pyrroline (2AP). This compound includes alcohols, aromatics, carboxylic acids, ketones, aldehydes, esters, hydrocarbons, furans, furanone, and terpenoids.[10] Pandan leaves are generally used in the food industry for color and aroma. The pandan leaf extract contains bioactive compounds with antioxidants, antivirals, antihyperglycemics, anticancer and antimicrobial properties, moreover, it has antifungal property in *C. albicans.* [11] However, the inhibitory effect of pandan leaf extract against *C. albicans* has shown a MIC (minimum inhibitory concentrations) of 8 mg/ml in our laboratory studies using ethanol extraction. Despite this promising result, no research has yet explored the use of pandan leaf extract as a denture cleaning solution. Pandan leaf extract offers a novel solution for denture cleaning by combining antimicrobial properties with sustainability. Understanding its effects on both *C. albicans* and denture base materials is crucial for developing evidence-based cleaning protocols. Therefore, the objective of this study is to investigate the antifungal activity of pandan leaf extract against *C. albicans* in a laboratory setting, as well as to evaluate its effect on the color and physical properties of denture base material.

Materials and Methods

Preparation of pandan leaf extract

The process begins with the preparation of fresh pandan leaves (Pandanus amaryllifolius) from plants aged

for 0-five years. The leaves are first cleaned with distilled water to remove contaminants. After cleaning, the leaves are chopped into small pieces, air-dried, and then dried further using hot air at 45-75°C for at least 30 minutes. The dried leaves are ground into a powder to increase the surface area. In the extraction process, 50 grams of the powdered leaves are mixed with ethylene glycol in concentrations of 95% (v/v). The mixture, totalling 450-750 ml, is stirred at 10-150 rpm, either manually or with a magnetic stirrer, and then filtered at least three times to remove solid residues. The filtered solution undergoes centrifugation at 7,000-15,000 rpm for 20-40 minutes to separate light precipitates, ensuring the temperature does not exceed 10°C. The clear solution is then filtered through 0.5-micron filter paper and concentrated by evaporation using a rotary evaporator at 50-100 rpm for 15-40 minutes. Finally, the extract is dried in a hot air oven at 45-75°C for 24-36 hours, maintaining a temperature below 10°C to preserve the extract. The process for producing pandan leaf extract has received a pretty patent application number from the Department of Intellectual Property of Thailand as request number1- 2403003729 by Nattapon Rotpenpian. Moreover, pandan leaf extract was used at concentrations of 8,16 and 24 mg/ml because the inhibitory effect of pandan leaf extract against *C. albicans* has shown a MIC of 8 mg/ml in our laboratory studies using ethanol extraction. However, the dose of MIC has not been elucidated in the solution for experiment and tested the dose dependence.

Preparation of specimens

Acrylic resin specimens were fabricated using Polymethyl methacrylate (PMMA) (Vertex™ Rapid Simplified, Vertex dental by 3D system; Netherlands), a material commonly used for making removable denture bases. The specimens were shaped from a wax model (dental wax, pink wax no. 1, Prominent Co., Ltd., Thailand) and cut to dimensions of 64 mm in length, 10 mm in width, and 3 mm in thickness. These were then placed into a denture flask (Hanau™ Varsity Ejector Type; Hanau Engineering Co., Inc., USA) and filled with plaster (stone-10 dental plaster type III, Knauf Plaster Co., Ltd., Thailand). After dewaxing the acrylic resin was mixed according to the specifications of the manufacturer with 0.95 grams of monomer and 2.3 grams of polymer. This mixture was poured into the mold in the denture flask and cured with heat in water at 100°C for 20 minutes After curing, the specimens were polished to remove excess material using a carbide bur and sanded with silicon carbide paper (grits 240, 600, and 1,000, respectively) using a rotary polishing machine (Buehler, Metaserve, Buehler Ltd., USA) to achieve the specified dimensions (64 mm long, 10 mm wide, and 3 mm thick).

To evaluate the antimicrobial efficacy against *C. albicans*, the specimens polished with silicon carbide paper were cut into smaller squares (10 mm × 10 mm × 3 mm) using a specimen cutting machine (Buehler, ISOMET 1000, Buehler Ltd., USA), yielding 50 pieces. These specimens were soaked in distilled water at 37°C for 12 hours to remove any residual monomer. Afterward, they were cleaned in an ultrasonic cleaner for 20 minutes and sterilized with ultraviolet radiation for another 20 minutes.[6]

For physical property tests, which included color change, surface roughness, surface hardness, and flexural strength, fifty polished specimens measuring 64 mm × 10 mm × 3 mm, conforming to ISO 1567:1999 specifications, were used. The specimens were polished with one-micron diamond paste (MetaDiTM monocrystalline diamond suspension; Buehler Ltd., USA) using a grinding and polishing machine (JeanWritz, Phoenix 4000, Germany) on one flat surface and cleaned in an ultrasonic cleaner using distilled water for 20 minutes.[12]

Antifungal testing

The *C. albicans* strain was cultured on Sabouraud dextrose agar (SDA; Becton Dickinson, Sparks, MD) and incubated at 37°C for 48 hours. Following this incubation, a suspension containing 106 *C. albicans*/ml was prepared in sterile saline using a spectrophotometer (Shimadzu Corp., Kyoto, Japan) set to a wavelength of 530 nm, with an optical density of 0.284, corresponding to an absorbance that reflects 106 CFU/ml. Each specimen was placed into a sterile tube containing 2 ml of the fungal suspension and incubated at 37°C for 90 minutes.[13] After incubation, the specimens were randomly divided into three experimental groups (n = 10) based on the concentration of the pandan

leaf extract (8, 16, 24 mg/ml) cleaning solutions, along with the negative control group (n = 10) immersed in sterile distilled water and the positive control group (n = 10) immersed in 0.5% sodium hypochlorite. The specimens were immersed at room temperature for 10 minutes for each group. Following this, each specimen was transferred to tubes containing 10 ml of sterile saline, and the adhered cells were dislodged by vertexing. Serial dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were then prepared in sterile saline, and 0.1 ml aliquots were plated onto SDA. After 48 hours of incubation at 37°C, the number of colonies were counted and expressed in CFU/ml. The reduction in microbial population was calculated by comparing the number of cells adhering to the test samples with those in the control group.[14]

Testing of Physical Properties

Fifty polished specimens measuring 64 mm ×10 mm× 3 mm were grouped into sets of ten and randomly assigned into five groups:

1. Control group (not immersed in any solution) for baseline flexural strength.
2. Test group immersed in 0.5% sodium hypochlorite.
3. Test group immersed in pandan leaf extract at 8 mg/ml.
4. Test group immersed in pandan leaf extract at 16 mg/ml.
5. Test group immersed in pandan leaf extract at 24 mg/ml.

Each specimen was labeled at its terminal end on the unpolished surface (without diamond paste polishing) for comparing physical properties before and after immersion in different solutions at the specified intervals:[15]

- **Time t0:** Specimens were immersed in distilled water at 37°C for 48 hours, simulating a first patient follow-up appointment after two days of denture use. Color, surface roughness, and surface hardness measurements were recorded.
- **Time t1:** Specimens were subjected to two cleaning cycles. Each cycle consisted of immersion in cleaning solutions for 10 minutes, rinsing with distilled water, and drying. This protocol simulated a clinical denture disinfection procedure performed when receiving dentures from patients and before delivering adjusted dentures back to patients. Color, surface roughness, and surface hardness measurements were recorded.
- **Time t2:** Specimens were immersed in cleaning solutions for seven days (168 h) and blot-dried without distilled water rinsing to assess potential long-term exposure effects on denture base material. Measurements of color, surface roughness, and surface hardness were recorded.

Color Change Testing

Color change testing was conducted using a spectrophotometer (HunterLab, ColorQuest XE, Hunter Associates Laboratory Inc., USA) to measure the color of 40 specimens in each experimental group. The polished surface of each specimen, prepared using diamond polishing paste, was positioned towards the device's measuring center with a measurement aperture size of 0.375 inches, against a black background. Color measurement was conducted using the CIELab color system, recommended by the Commission Internationale de L'Eclairage (CIE),[6] employing standard light D65, a 10-degree observer angle, and a light source covering the visible wavelength range (400 to 700 nanometers). The RSEX (reflectance specular exclude) value was recorded for each specimen. This measurement system includes the gross value of materials, similar to what the human eye sees. Materials of the same color but with different surface textures or gloss levels will yield different color measurements. Measurements were taken three times per sample at specified time intervals, both before and after immersion in various cleaning solutions. The color difference (ΔE) for each specimen was then calculated automatically by comparing the color values at time t0 with those at time t1 and at time t0 with those at time t2, within the same specimen. The color value measured at time t0 served as the baseline for determining the color difference in relation to the values at t1 and t2.

To relate color change (ΔE) to the clinical environment, the data were converted into National Bureau of Standards (NBS) units using the formula: NBS

units = ΔE × 0.92, and then categorized according to the following scale: 1) Indicial from 0.0-0.5, 2) Slight from 0.5-1.5, 3) Noticeable from 1.5-3.0, 4) Considerable from 3.0-6.0, 5) Very from 6.0-12.0, and 6) Excessive for +12.0.[6]

Surface Roughness Testing

Surface roughness was measured using a profilometer (Surfcorder, SE2300, Kosaka Laboratory Ltd., Japan) with a tracing length of 5 mm and a cutoff value of 0.8 mm. The diamond stylus moved at a speed of 0.5 mm/second. Measurements were taken on the polished surface of each specimen before and after immersion in various cleaning solutions at specified test intervals. The measurements were performed at the center of each specimen in both length and width of the sample. Three measurements were taken at positions spaced 1 mm apart across the width of each specimen.[16] The average roughness (Ra) values were calculated for each group at each testing time, and the changes in average surface roughness (ΔRa) from t0 to t1 and t0 to t2 were statistically analyzed.

Surface Hardness Testing

Surface hardness was evaluated using a micro-hardness tester (Mitutoyo, HM-211, Mitutoyo Corporation, Japan) with a square-based pyramid indenter, applying a load of 10 grams for 15 seconds. Measurements were taken on the polished surface of each specimen, marked 1.5 cm from the center along the length. Three indentations were created at different positions along each specimen, and the Vickers hardness number (VHN) was recorded for statistical analysis.[15]

Flexural Strength Testing

Flexural strength was assessed using a universal testing machine (Lloyd Instruments, LRX-Plus, AMETEK Lloyd Instruments Ltd., UK) on rectangular specimens measuring 64 mm ×10 mm × 3 mm, adhering to ISO 1567: 1999 specifications.[17] Ten specimens from the control group (not immersed in cleaning solutions) were tested, alongside 40 specimens previously immersed in different cleaning solutions. Specimens were positioned with the polished surface facing upward on a 50 mm support span, and a vertical load was applied at a rate of 5 mm/min until fracture occurred. Flexural strength was calculated using the formula $S = 3NL/2bd^2$, where N is the maximum load applied, L is the span length, b is the width, and d is the height (thickness) of the specimens. A digital vernier caliper was used to measure the dimensions of each specimen. These measurements were then applied in the formula for further calculations.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, v15.0; Chicago, IL) and are presented as means ± standard deviations (SDs). Antifungal activity between groups was determined by normality and homogeneity test by the Shapiro-Wilk test so that its normality was non-normal distribution and consequently compared using the Kruskal-Wallis test, followed by Dunn's post hoc test. The effects of different tested solutions and treatment periods on color, surface roughness, and hardness of the specimens were assessed using analysis of covariance (ANCOVA). For comparing changes in color, surface roughness (ΔRa), and Vickers hardness (ΔVHN) from t0 to t1 and from t0 to t2 across different cleaning solution groups, the t0 measurements for each group were used as covariates. The flexural strength test was analyzed using one-way ANOVA followed by Tukey's honestly significant difference (HSD) post hoc test. Differences were considered statistically significant at $p < 0.05$.

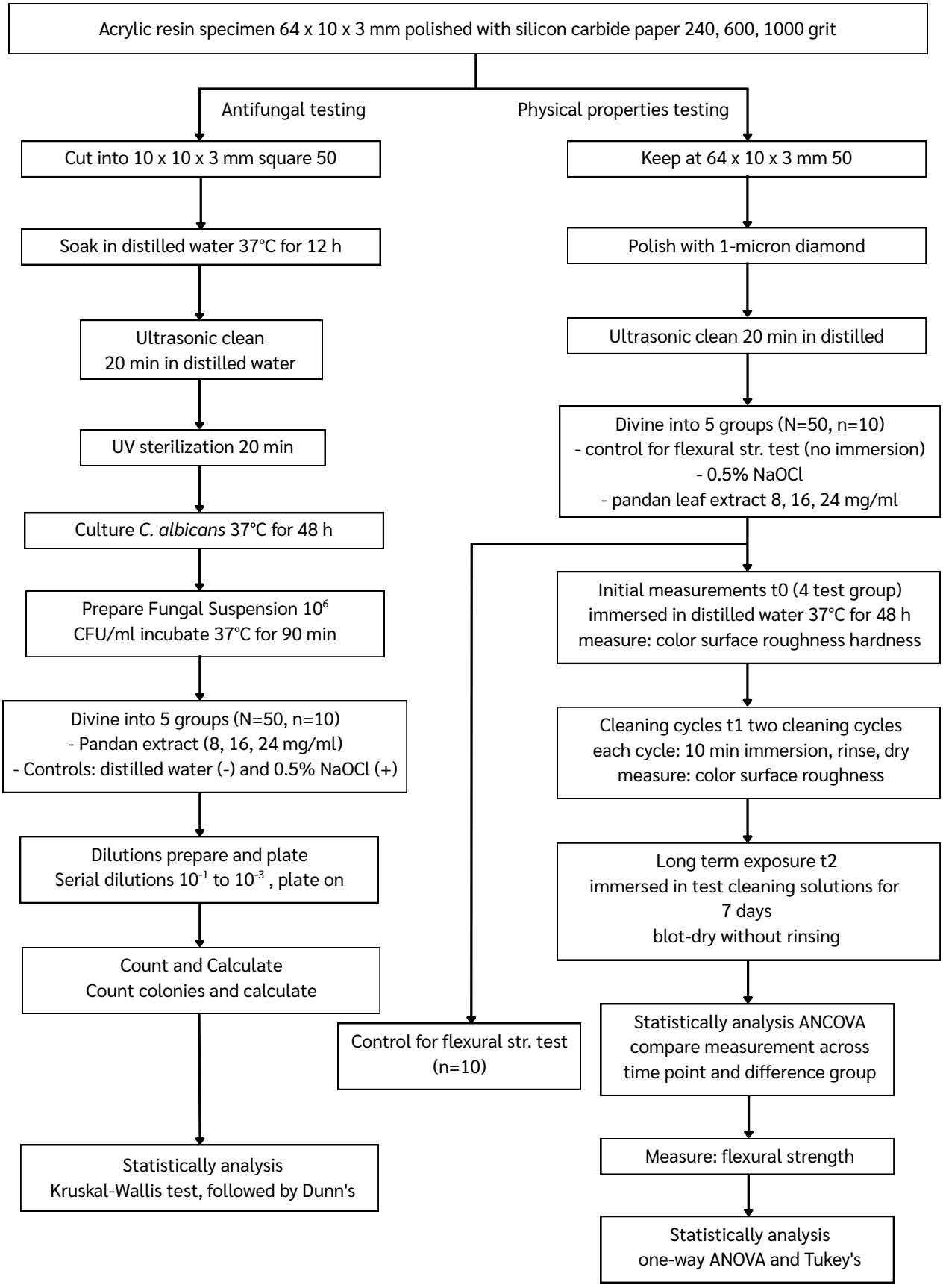

Figure 1 *Flow chart showing the steps of antifungal and physical test*

Results

Effect of cleaning solution with pandan extract as an antifungal test

The *C. albicans* count (CFU/ml) for different test groups was compared, including the control and various cleaning solutions with pandan extract. The C. albicans count (CFU/ml) of pandan leaf extract at 16 mg/ml is 0.8 CFU/ml, for pandan leaf extract at 24 mg/ml is 0.16 CFU/ml, and for 0.5% sodium hypochlorite (positive control) is 0.2 CFU/ml. The *C. albicans* count (CFU/ml) of pandan leaf extract at 8 mg/ml is 2.5 CFU/ml and for distilled water (negative control) is 3 CFU/ml. The pandan leaf extract at 16 and 24 mg/ml, exhibits antifungal properties comparable to those of 0.5% sodium hypochlorite. There was no significant difference between 0.5% sodium hypochlorite and pandan leaf extract at 16 and 24 mg/ml. The results show that 0.5% sodium hypochlorite and pandan extract at 16 and 24 mg/ml were the most effective at eliminating the fungus, while the sterile distilled water and pandan extract at 8 mg/ml groups showed varying levels of fungal presence as shown in Figure 2.

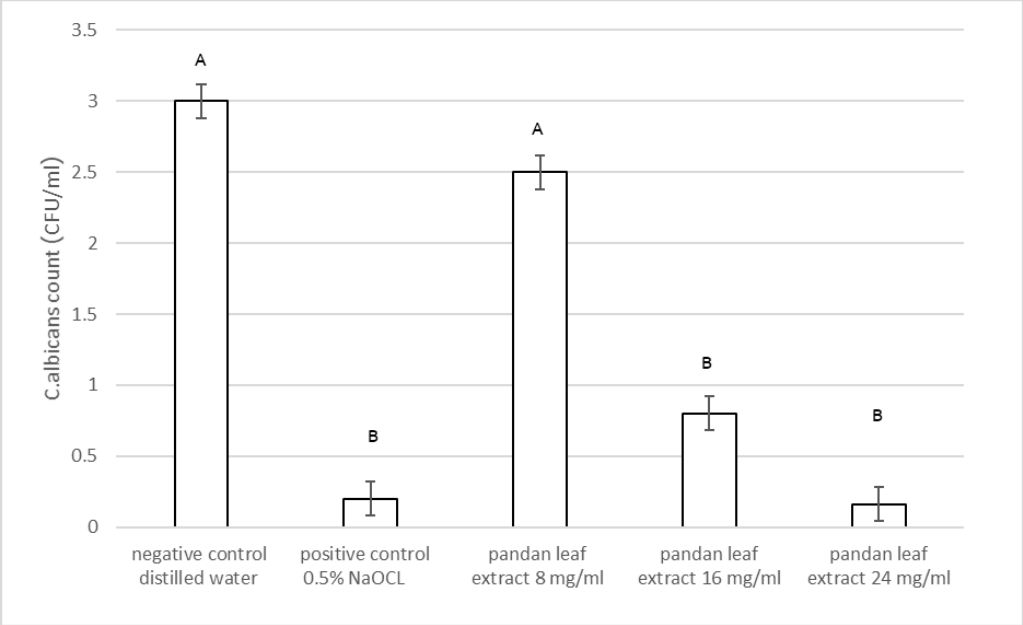

Figure 2 *Comparison of Candida albicans colony-forming units (CFU/ml) across different test groups. Uppercase letters (A,B) Indicate statistically significant differences.*

Effect of cleaning solution with pandan extract on physical properties of the denture base

After immersion in the cleaning solution for two 10-minute cycles (t1) and seven days (t2), the color change of the specimens within the same group showed no significant differences. After immersion for either two 10-minute cycles or seven days, the color change of the specimens showed significant differences between groups. The 0.5% sodium hypochlorite group showed significant differences compared to pandan leaf extract at 8 mg/ml and 24 mg/ml. The pandan leaf extract at 8 mg/ml group showed significant differences compared to the pandan leaf extract at 24 mg/ml. Meanwhile, the pandan leaf extract at 16 mg/ml showed no significant differences compared to the other groups, as shown in Table 1. However, the mean ΔE t0-t1 and ΔE t0-t2 values of all groups quantified by NBS (National Bureau of Standards) were classified as slight with 0.981 and 0.751 for the 0.5% sodium hypochlorite group, 0.802 and 0.881 for the pandan leaf extract at 8 mg/ml group, 0.914 and 0.676 for the pandan leaf extract at 16 mg/ml group, and 1.217 and 0.816 for the pandan leaf extract at 24 mg/ml group, respectively.

After immersion in distilled water at 37°C for 48 hours (t0) followed by immersion in the cleaning solution for two 10-minute cycles (t1) and seven days (t2), no significant differences ($p > 0.05$) were observed in surface roughness within the same group of cleaning solutions or among groups of different cleaning solutions as shown in Table 2.

After immersion in distilled water at 37°C for 48 h (t0) followed by immersion in the cleaning solution for two 10-minute cycles (t1) and seven days (t2), no significant differences ($p > 0.05$) were observed in surface hardness within the same group of cleaning solutions or among groups of different cleaning solutions as shown in Table 3.

After immersion in the cleaning solution for seven days (t2), flexural strength for the test group showed no significant differences ($p > 0.05$) compared to the control group as shown in Table 4.

Table 1 *Mean and standard deviation of color change (ΔE) and NBS units in denture specimens after immersion in different cleaning solutions*

Group	Mean ΔE at t0-t1 (two 10-min cycles) +SD	NBS units at t0-t1 ΔEx0.92 (category)	ΔE at t0-t2 (7 day) ±SD	NBS units at t0-t2 ΔEx0.92 (category)
0.5% sodium hypochlorite	1.066 ± 0.423[a]	0.981 (slight)	0.816± 0.416[a]	0.751 (slight)
Pandan leaf extract at 8 mg/ml	0.872±0.436[b]	0.802 (slight)	0.958±0.628[b]	0.881 (slight)
Pandan leaf extract at 16 mg/ml	0.993±0.445[abc]	0.914 (slight)	0.735±0.407[abc]	0.676 (slight)
Pandan leaf extract at 24 mg/ml	1.323±0.614[c]	1.217 (slight)	0.887±0.408[c]	0.816 (slight)

Different lowercase letters (a,b,c) indicate statistically significant difference by the Tukey's test ($p<0.05$)

Bureau of Standards (NBS) units using the formula: NBS units = ΔE × 0.92, and then categorized according to the following scale: 1) Indicial from 0.0-0.5, 2) Slight from 0.5-1.5, 3) Noticeable from 1.5-3.0, 4) Considerable from 3.0-6.0, 5) Very from 6.0-12.0, and 6) Excessive for +12.0.5

Table 2 *Mean and standard deviation of surface roughness in denture specimens after immersion in different cleaning solutions*

Group	t0 mean+SD (μm)	t1 mean+SD (μm)	t2 mean+SD (μm)
0.5% sodium hypochlorite	0.017±0.075	0.013±0.035	0.017±0.038
Pandan leaf extract at 8 mg/ml	0.013±0.035	0.013±0.035	0.01±0.030
Pandan leaf extract at 16 mg/ml	0.003±0.018	0.003±0.018	0.00+0.000
Pandan leaf extract at 24 mg/ml	0.003±0.054	0.033±0.053	0.033±0.054

Table 3 *Mean and standard deviation of surface hardness in denture specimens after immersion in different cleaning solutions for the evaluated group*

Group	t0 (VHN)	t1 (VHN)	t2 (VHN)
0.5% sodium hypochlorite	14.890± 1.260	14.533± 1.055	14.176± 1.346
Pandan leaf extract at 8 mg/ml	14.823± 1.341	14.806± 1.251	14.77± 1.318
Pandan leaf extract at 16 mg/ml	14.38± 1.3712	14.32± 1.116	14.053± 1.073
Pandan leaf extract at 24 mg/ml	16.083±0.482	15.693±0.4322	14.62±1.1966

Table 4 *Mean and standard deviation of flexural strength in denture specimens after immersion in different cleaning solutions for 7 days (t2)*

Group	flexural strength mean+SD (MPa)
Control group (not immersed)	76.09±5.1899
0.5% sodium hypochlorite	72.23±5.694
Pandan leaf extract at 8 mg/ml	73.964±4.914
Pandan leaf extract at 16 mg/ml	76.721±6.168
Pandan leaf extract at 24 mg/ml	75.311±4.854

Discussion

The current study evaluated the antifungal efficacy of pandan leaf extract against *C. albicans* and its impact on color, surface hardness, surface roughness, and flexural strength of denture base material. The findings indicate that pandan leaf extract, particularly at concentrations of 16 and 24 mg/ml, exhibits antifungal properties comparable to those of 0.5% sodium hypochlorite. Since sodium hypochlorite may cause irritation and has a strong odor[18], using pandan leaf extract, which is a natural and edible plant, is likely to be a safer alternative. This suggests its potential as a safer and more natural option for denture cleaning solutions.

The antifungal properties of pandan leaf extract against *C. albicans* are largely attributed to its bioactive compounds, including alkaloids, flavonoids, and phenolics, which have demonstrated antifungal activity in prior studies. At concentrations of 16 and 24 mg/ml the pandan leaf extract significantly reduced *C. albicans* colony-forming units (CFU/ml), achieving a comparable antifungal effect to 0.5% sodium hypochlorite. The antifungal test in pandan leaf extract solutions for 10 minutes was compared to 0.5% sodium hypochlorite, as according to dental safety goals and guidelines 2023 from the Dental Council of Thailand who recommend immersing resin acrylic dentures in 0.5% sodium hypochlorite for 10 minutes in clinical settings for disinfection.[18] Active compounds in pandan leaves, such as flavonoids, terpenoids, and phenolic compounds, may weaken the cell wall and membrane structure by altering its permeability, leading to leakage of intracellular components and cell death.[19] Secondly, pandan leaf extract may inhibit *C. albicans* biofilm formation, which contributes to its resistance against antifungal treatments, by interfering with signalling pathways or disrupting the protective matrix of the biofilm.[20] Additionally, the antioxidant properties of the extract, rich in polyphenols and flavonoids, may neutralize reactive oxygen species (ROS), reducing fungal growth and inducing oxidative stress that damages fungal cells.[21] Pandan leaf extract could also inhibit protein synthesis by binding to ribosomes or interfering with enzymes necessary for protein biosynthesis, disrupting cellular functions critical to *C. albicans* growth and reproduction.[22] Furthermore, it may alter the activity of enzymes like proteases, phospholipases, and lipases, which are essential for *C. albicans* pathogenicity, thus preventing fungal adhesion and tissue invasion.[23] Lastly, pandan leaf extract might modulate gene expression in *C. albicans*, down regulating genes involved in virulence, biofilm formation, and drug resistance, thereby reducing the overall pathogenicity.[24] The similarity in efficacy suggests that pandan leaf extract at these concentrations could serve as a natural alternative to traditional chemical disinfectants, especially given the risks associated with prolonged use of sodium hypochlorite, such as tissue irritation and denture material degradation. Interestingly, the 8 mg/ml concentration showed inconsistent antifungal results, indicating that lower concentrations may lack the potency required for effective fungal inhibition. This dose-dependent response aligns with other studies on plant-based antifungals, where effectiveness is often linked to concentration.[25]

In terms of material properties, pandan extract showed a favorable profile, with no significant impact on the color, surface roughness, or surface hardness of the denture base material. The color changes of all groups were classified as 'slight', which is undetectable to the human eye.[26] The human eye can distinguish color differences when the NBS (National Bureau of Standards) unit is greater than 1.5 (noticeable), and differences above 3.0 NBS unit are considered as clinically unacceptable. This finding is crucial for denture aesthetics, as color stability is a key consideration for denture maintenance.[6] Previous research has shown that denture cleaning agents can cause color changes over time, with sodium hypochlorite known to cause slight discoloration in certain materials due to its oxidizing properties.[27] The absence of significant color alteration with the 16 and 24 mg/ml pandan extract solutions indicate that it can maintain denture aesthetics while offering antifungal benefits. Moreover, the color stability observed with pandan extract suggests that its bioactive compounds do not interact negatively with the pigment molecules in the denture base material. This result is encouraging for patients who prioritize

both the functional and aesthetic aspects of denture cleaning solutions, as pandan extract provides a balance between antifungal efficacy and color stability.

Surface roughness is another critical property for denture cleanliness, as increased roughness can promote biofilm adherence, making the denture more susceptible to microbial colonization.[28] After immersing the denture base in distilled water for 48 hours (t0), and then in the pandan leaf extract solution for two 10-min cycles (t1) and seven days (t2), the surface roughness measurements remained comparable to the baseline (t0). This outcome suggests that pandan extract does not induce surface degradation or microscopic abrasions in the denture material. The stability in surface roughness supports the notion that pandan extract is gentle on denture materials. Maintaining smoothness on denture surfaces is essential to reduce microbial accumulation and plaque formation, which can lead to denture stomatitis and other oral health issues.[29] Therefore, using a natural extract that preserves the surface integrity of the dentures provides an advantage in terms of both functionality and hygiene.

The study also investigated surface hardness, a property critical to the ability of a denture to resist wear and deformation. Pandan leaf extract at the tested concentrations did not cause significant changes in surface hardness. This suggests that the bioactive compounds in pandan leaf extract do not compromise the structural integrity of the material. Maintaining surface hardness is important to ensure the long-term durability and resistance of the denture to daily mechanical stresses. Previous studies have shown that some natural extracts may soften polymer-based materials due to their acidic or chemical nature.[30] However, the lack of such an effect with pandan extract is promising for users seeking to avoid material degradation often associated with traditional denture cleaners. These findings align with research on plant extracts used in dental care,[31] which have shown minimal interaction with synthetic materials, making them suitable candidates for bio-friendly dental applications.

Flexural strength is essential for the functional reliability of dentures, as it determines their ability to withstand masticatory forces without fracturing. The results demonstrated that flexural strength was unaffected by immersion in pandan extract solutions, even after prolonged exposure. This is a favorable outcome, as some cleaning agents, especially those containing strong oxidizing agents, can weaken denture materials over time, increasing the risk of fractures. The stability in flexural strength aligns with the objective of developing a gentle yet effective cleaning solution.[32] By retaining flexural strength, pandan extract allows the denture to perform its intended function without compromising material integrity. This property could enhance the longevity of denture materials, reducing the need for frequent replacements and thereby providing economic benefits to denture wearers. [33] When comparing the effects of pandan leaf extract on the physical properties of the denture base in this study with previous research, no prior studies have explored this aspect. Therefore, there is no available data for direct comparison.

Sodium hypochlorite is widely used for its potent antimicrobial properties; however, its prolonged use poses risks such as material degradation and potential adverse reactions in sensitive patients.[33] Pandan leaf extract, particularly at the 16 and 24 mg/ml concentration, provides a comparable antifungal effect without these risks, suggesting its suitability as a safer alternative for regular use. The gentle action of pandan extract may also be advantageous in terms of patient compliance, as it minimizes the risks associated with daily denture cleaning. Previous studies have found that using a sodium hypochlorite denture cleaner caused color changes and reduction in surface roughness and surface hardness of denture base material when immersed for an extended period.[6,14] In contrast, this study did not find that 0.5% sodium hypochlorite altered the physical properties of the denture base, possibly due to the shorter immersion time used in the experiment.

The clinical application of pandan leaf extract could serve as an alternative to chemical denture cleansers, particularly for patients with hypersensitivity to sodium hypochlorite or concerns about its long-term effects on denture materials. Its use may include

disinfecting dentures after receiving them from the laboratory before patient try-in or after retrieving used dentures from patients before making adjustments.

Limitations of this study such as variations in pandan leaf extraction methods might influence bioactive compound concentration, the extraction process of pandan leaf which involves such as the mixture volume ranging from 450-750 ml, mixing speed, centrifugation, evaporation process, as well as the temperature and time required for drying the extract. These variations may affect the antifungal efficacy against *C. albicans.* Therefore, further research is needed to standardize the methods to ensure consistent results. Additionally, this study simulated nocturnal immersion ("overnight soaking"), where each 24-hour period corresponded to three immersions of 8-hours per day. Over a total of 21 days, dentures were continuously immersed without removal for seven days, which is equivalent to nightly 8-hour soaking for 21 days (7 days × 24 hours / 8 hours = 21 days). However, this duration is still relatively short. Extending the immersion period beyond this study, such as simulating long-term soaking for one to three years, would better reflect real-life conditions in patients who use denture soaking solutions daily. This would allow for an assessment of whether the extract affects the physical properties of the denture base over prolonged use. Therefore, further research is recommended to explore the inhibitory effects on *C. albicans* in more detail to confirm whether pandan leaf extract can effectively inhibit *C. albicans.* Additionally, studies should investigate the effects of immersing dentures in pandan leaf extract for extended periods beyond those used in this study or under conditions simulating daily use. Additionally, since this study was conducted in a controlled laboratory setting, clinical trials are needed to evaluate its effectiveness in real-world scenarios, where factors such as saliva, dietary habits, and regular wear may influence outcomes.

Conclusion

Within the limitations of this study, a cleaning solution containing pandan leaf extract at 16 and 24 mg/ml was found to be effective against *C. albicans* and did not compromise the color, surface roughness, surface hardness, or flexural strength of denture base materials. This indicates that pandan leaf extract could serve as a viable, natural alternative to sodium hypochlorite.

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Conflict of interest

The authors declare no conflict of interest. No funding was received for this study.

Data availability statement

All data generated and analyzed during this study are included in this published article.

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