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Research Report

Mechanical cleansing effect using purple leaf denture cleanser on *Candida albicans*

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ABSTRACT

Background: Denture stomatitis is experienced by 65% of denture wearers and is generally caused by *Candida albicans* colonization on the porous surface of acrylic resin. The use of chemical denture cleansers can increase surface roughness and discoloration of the resin, so a natural alternative is needed. Purple leaf extract (*Graptophyllum pictum*) contains flavonoids that have the potential to be used as a natural denture cleanser with antifungal activity against *Candida albicans*. **Purpose:** This study aims to investigate the antifungal potential of purple leaf extract (*G. pictum*) in the form of denture cleaning soap that effectively inhibits the growth of *C. albicans*, the cause of denture stomatitis. **Methods:** This true experimental study used a post-test-only control group design with three groups: K(-) aquades, K(+) baby soap, and P (50% *G. pictum* extract soap), each consisting of 9 samples. Acrylic resin plates were brushed for 5 seconds per side, rinsed with PBS, incubated on SDA at 37°C for 24 hours, and the number *of C. albicans* colonies was counted. Data were analyzed using the Kruskal–Wallis and Mann–Whitney tests ($p<0.05$). **Results:** Mean *C. albicans* growth was 90.55 ± 8,54 (K-), 10.55 ± 1.58 (K+), and 20.22 ± 4.05 (P). Significant differences were found among all groups ($p<0.05$). **Conclusion:** Denture cleanser containing *G. pictum* extract reduced *C. albicans* growth significantly, the extract shows potential as a natural antifungal ingredient for denture cleansers.

Keywords: *Candida albicans*; Denture cleanser; Denture stomatitis; *Graptophyllum pictum*; Medicine

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INTRODUCTION

Denture stomatitis (DS) is a common oral disease suffered by patients with complete or partial removable acrylic dentures.[1] Denture stomatitis has an incidence of 15% to 70% among denture users and is associated with trauma and poor oral hygiene.[2] Denture stomatitis is a condition of diffuse or localized erythematous inflammation in the denture-bearing area that has multifactorial etiology, such as poor denture hygiene, continuous use of dentures, especially at night, trauma to the denture-bearing area, and *Candida* biofilm.[3]

Dentures have porosity and roughness that can provide an aerobic and acidic environment for microorganisms to colonize, grow, and multiply.[3] The fungus *Candida albicans* is an important factor that triggers denture stomatitis.[4] Not only does *C. albicans* adhere to the oral mucosa, it can also colonize the uncleaned acrylic resin surface of dentures.[5] The accumulation of *C. albicans* biofilm on the base of dentures can increase the pathogenicity of fungi that play a role in the development of denture stomatitis.[2] Carbohydrate fermentation by *C. albicans* colonization on denture plaque can cause saliva pH to become more acidic. This can have a toxic effect on the epithelium, leading to inflammation of the oral mucosa.[5,6]

The use of dentures must be accompanied by regular cleaning to increase the lifespan of the dentures and improve the patient's oral health.[7] Denture stomatitis can be prevented by routinely cleaning dentures to reduce the amount of *C. albicans*.[8] Denture cleaning can be done mechanically by brushing with a soft-bristled toothbrush, chemically by soaking the dentures in denture cleanser, or a combination of both. Immersing dentures in denture cleanser significantly increases the roughness of the acrylic resin surface compared to brushing with a soft-bristled toothbrush without toothpaste, thereby increasing microbial retention on the surface.[7] The use of chemical denture cleansers has been reported to increase surface roughness and discoloration of acrylic resin. An ideal denture cleanser must meet several requirements, namely being non-toxic,

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non-irritating, not dissolving organic and inorganic denture materials, not damaging denture materials, stable in storage, and being bactericidal and fungicidal.[9] Currently, various studies are being conducted to find alternative denture cleanser ingredients from traditional plants because they are easily available, inexpensive, and relatively safer than chemical denture cleansers.[6]

Purple leaf (*G. pictum*) is one of the plant species in Indonesia that has potential as a medicine.[10] These leaves contain flavonoids, alkaloids, and saponins that can be used as antifungal agents.[11] Flavonoids are the largest group of phenolic compounds that have the ability to inhibit microorganisms by forming complex compounds with proteins.[6] A 50% concentration of purple leaf extract has effective fungicidal activity against *Candida albicans*.[12] These findings indicate that the antifungal potential of purple leaf extract can be used as an herbal denture cleanser to overcome the shortcomings of chemical denture cleansers using the soaking method, which can increase surface roughness and discoloration of acrylic resin. Therefore, the author investigated the antifungal potential of purple leaf extract (*G. pictum*) as a herbal denture cleanser in the form of soap through a brushing method to assess its effectiveness in inhibiting the growth of *C. albicans*, the cause of denture stomatitis.

MATERIALS AND METHODS

The Airlangga University Faculty of Dental Medicine Health Research Ethical Clearance Commission approved the research through Ethical Clearance Certificate number 0602/HRECC.FODM/VI/2024. This research is a true experimental design study, with a post-test only control design, where measurements are only taken after treatment is given. In this study, the parameter observed is the difference in the number of *C. albicans* colonies on the surface of acrylic resin after the treatment was administered.

This research was conducted over a period of four months (May to August 2024) offline at several different locations, including the Surabaya Pharmacy Academy Laboratory for purple leaf extraction, formulation of 50% purple leaf extract denture cleaning soap, and the Airlangga University Faculty of Dentistry Research Center for *C. albicans* growth testing.

Purple leaf extraction was carried out by soaking 150 grams of purple leaf powder in 1500 ml of 70% ethanol acidified with 1% HCl. The ratio of solvent to sample used was 1:10. After the first 6 hours of soaking, stirring was carried out occasionally, then left to stand again for 18 hours. The extract is filtered using Whatman No. 41 filter paper, while the residue is macerated again using the same amount and type of solvent 4 times. The solution was evaporated using a rotary vacuum evaporator at 60°C until a thick extract was obtained. The thick extract was weighed to calculate the yield using the following equation.[13]

After the purple leaf extraction process, soap is made by mixing a formulation consisting of purple leaf extract with a base of 2.5 g stearic acid, adeps ianae 0.5 g, triethanolamine 0.15 g, glycerin 0.7 g, peppermint perfume 0.05 ml, and distilled water up to 100 ml.[8] Soap production was carried out by mixing mixture 1 by combining stearic acid and adeps lanae in a container, then stirring until completely mixed. Next, mixture 2 was made by combining triethanolamine and glycerin in a container, stirring until dissolved. Mixture 2 is added to mixture 1 in the container little by little, then stirred until dissolved. Then, distilled water is added little by little to the container, then stirred until homogeneous. Next, add the purple leaf extract (*G. pictum*) to the container, then stir until homogeneous. Add the peppermint perfume to the container, then stir until homogeneous.[14,15]

The *C. albicans* growth test was performed using sterile acrylic resin plates immersed in artificial saliva, then immersed in a suspension of *C. albicans* ATCC 10231 with a standard McFarland turbidity of 0.5, which is approximately equivalent to 1.5 x 10^{-3} CFU/ml of bacterial suspension, and then incubated for 24 hours. After incubation, the acrylic resin plates were divided into 3 groups: K- (negative control group), K+ (positive control group), and P (treatment group), with each group consisting of at least 9 samples based on Federer's formula (t−1) (n−1) ≥15, resulting in a total of 27 samples.

Brushing was performed using a soft-bristled toothbrush with controlled manual pressure. Soft-bristled toothbrushes are the standard recommendation for cleaning dentures because the hardness of the toothbrush can affect the roughness of the denture surface.[7] Brushing for 5 seconds per side was performed following a standardized method described in earlier research.[8] The brushing time was chosen to ensure a uniform brushing duration for each sample so that the effectiveness of the cleaning agent against *C. albicans* colonization could be assessed while minimizing variations due to operator technique. In group K-, brushing was performed with 0.1 ml of distilled water for 5 seconds per side. In group K+, brushing was performed with baby bottle soap for 5 seconds per side. In group P, brushing was performed with 0.1 ml of 50% purple leaf extract soap formulation for 5 seconds per side.

After brushing, each sample was rinsed twice with PBS, then placed in Sabouraud Dextrose Broth and vortexed for 30 seconds. Next, 0.1 ml of each sample was taken, spread on SDA media, and incubated for 24 hours at 37°C. Next, the number of *C. albicans* colonies was counted using a colony counter.[8]

The data obtained through testing was processed and analyzed using the Statistical Package for the Social Sciences (SPSS) to determine the normality of the data distribution. If normality was met, the data was then analyzed using the One-Way ANOVA statistical test, followed by Tukey's Post Hoc significance test. If the normality assumption is not met, the Kruskal-Wallis alternative test is used, followed by a Post Hoc test.[8]

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RESULTS

The mean results of *C. albicans* growth in each treatment group are presented in Figure 1. The negative control (K–) showed the highest number of colonies (90.55 ± 8.54 CFU). The positive control (K+) showed the strongest inhibitory effect, with the lowest colony count (10.55 ± 1.58 CFU). The treatment group using 50% purple leaf extract soap (P) showed a reduction in fungal growth (20.22 ± 4.05 CFU), although the effect was weaker than the positive control.

The Shapiro–Wilk normality test showed that data from the negative control group (aquades) and treatment group (soap with *G. pictum* extract) were normally distributed ($p>0.05$), while the positive control group (baby soap) was not ($p<0.05$). Because not all data were normally distributed, the non-parametric Kruskal–Wallis test was performed to determine differences among groups. The test revealed a significant difference between all groups ($p>0.05$). Further analysis using the Mann–Whitney test confirmed significant differences between each pair of groups ($p>0.05$).

Based on these results, the denture cleanser soap containing *G. pictum* extract showed lower *C. albicans* growth compared to the negative control (aquades), but higher than the baby soap group. This finding suggests that *G. pictum* extract possesses antifungal properties, though less potent than the positive control. Therefore, *G. pictum* extract has potential as a natural alternative ingredient for denture cleansers with antifungal effects against *C. albicans.*

DISCUSSION

The results of this study showed a clear quantitative difference in the antifungal efficacy among the three groups. The negative control group (aquadest) showed the highest mean *C. albicans* count of 90.56 ± 8.55 CFU, confirming that without any cleaning agent, fungal growth remained high. In contrast, the baby soap group exhibited the lowest mean colony count of 10.56 ± 1.59 CFU, indicating the strongest antifungal effect. Meanwhile, the purple leaf extract (*G. pictum*) group showed a mean colony count of 20.11 ± 3.89 CFU, which was significantly lower than the negative control but still higher than the baby soap group.[16]

Flavonoids are one of the compounds in purple leaf extract that can function as antifungals through various mechanisms. Flavonoids are able to suppress fungal growth by inhibiting the growth of fungal hyphae and mycelium. Flavonoid compounds enter fungal cells through holes in the cell membrane formed through hydrogen bonds of phenolic compounds that can degrade cell membrane lipids. Flavonoids are able to bind to proteins that cause cell wall formation to be inhibited, so that hyphae growth can also be inhibited due to incomplete cell wall composition.[10,17] However, in the present study, the observed reduction of *C. albicans* colonies should be primarily interpreted as the result of the combined effect of mechanical brushing and the chemical activity of the extract, rather than direct intracellular antifungal mechanisms.[18] Brushing procedure applied in this study plays a crucial role in physically removing fungal cells and disrupting early biofilm formation on the acrylic resin surface.[19] The presence of flavonoids is thought to enhance this mechanical effect by weakening the fungal cell membrane and increasing membrane permeability, thereby facilitating easier detachment of *C. albicans* during brushing.[10,17] This interpretation is showing that mechanical cleansing remains the determinant in fungal reduction on denture surfaces.[19]

Several studies have reported that natural and herbal agents used as denture cleansers effectively reduce *C.*

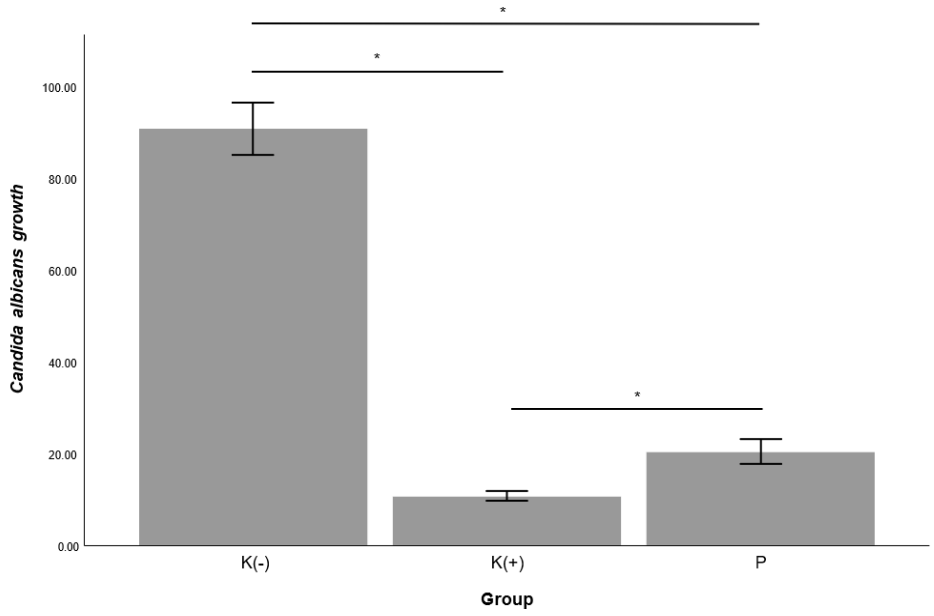


Figure 1. Comparison of colony counts among the groups, including error bars representing standard deviations and significance markers showing statistically significant differences between all groups.

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albicans. Herbal options such as *Nigella sativa* have also showed antifungal activity. At higher concentrations, *N. sativa* cleansers were capable of visibly reducing fungal plaque accumulation on acrylic denture plates, showed antifungal activity comparable in scale to the reduction observed in the present extract group.[20] Other study using citric acid as a denture cleanser showed a reduction in *C. albicans* biofilm formation on denture surfaces. In that study, the denture specimens were first coated with an artificial saliva layer before being exposed to *C. albicans*, simulating the oral environment prior to microbial adhesion.[21] Similarly, in the present study, all acrylic resin specimens were immersed in artificial saliva before *C. albicans* induction to allow the formation of a salivary pellicle like layer, which facilitates fungal adherence and better reflects clinical conditions. The present study focused specifically on evaluating the antifungal effectiveness of denture cleansing agents following mechanical brushing, without assessing recolonization potential. Therefore, the reduction in fungal colonies observed after treatment reflects the direct antifungal and cleansing efficacy of the tested agents under saliva conditions.[22] In relation to previous findings, the antifungal activity observed in the purple leaf extract group is in accordance with evidence showing that *G. pictum* possesses measurable inhibitory effects against *C. albicans* at moderate to high concentrations, confirming that the 50% extract used here within the effective range.[16]

The weaker antifungal effect of the *G. pictum* extract soap compared with baby soap can be explained by the different mechanisms of action between plant derived bioactive compounds and commercial antiseptic soaps. The antifungal activity of purple leaf extract is flavonoids, saponins, and tannins, which act by disrupting fungal cell membranes, inhibiting hyphal and mycelial growth, interfering with ergosterol synthesis, and inducing oxidative stress through the generation of reactive oxygen species. Although these mechanisms are effective in suppressing *C. albicans* growth, their activity depends on gradual biochemical interference with cellular processes.[23,24] In contrast, antiseptic soaps such as baby soap have been shown to reduce *C. albicans* by directly decreasing colony forming units and suppressing cellular metabolism through strong chemical disinfection on acrylic resin surfaces.[25] Previous studies comparing commercial cleansers and herbal agents such as thyme essential oil and *N. sativa* exhibit significant antifungal activity but their effectiveness remains inferior to commercial formulations.[26] Nevertheless, *G. pictum* extract still effective reduce the count of candidal cell on the surface of the acrylic base and showed good biocompatibility and low toxicity. Indicating its clinical relevance as a safer natural alternative denture cleanser for long-term maintenance of denture hygiene.[12] One limitation of this study lies in the formation of experimental biofilms under in vitro conditions. While in vitro models are cost effective and appropriate for antifungal assessments.[20] Future studies should quantify the total flavonoid, phenolic, and saponin contents of *G. pictum* extract before and after soap formulation to evaluate the stability of its bioactive compounds.[27]

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