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Comparison of the Effectiveness of Passion Fruit Extract Soaking and Alkaline Peroxide on *Candida albicans* Adhesion as Denture Cleaning Agents

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Abstract: Heat-cured acrylic resin dentures are widely used to restore mastication and aesthetics in individuals with tooth loss; however, their surface characteristics may facilitate *Candida albicans* adhesion, increasing the risk of denture stomatitis. Therefore, effective denture cleansers are required to inhibit fungal colonization. Purple passion fruit (Passiflora edulis Sims) contains bioactive compounds with antifungal potential and may serve as a natural alternative denture cleanser. This study aimed to compare the effectiveness of 15% purple passion fruit peel and pulp extracts with alkaline peroxide in reducing *Candida albicans* adhesion on heat-cured acrylic resin surfaces. This laboratory experimental study employed a posttest-only control group design using pure *Candida albicans* isolates. Heat-cured acrylic resin specimens (10 × 10 × 2 mm) were prepared and randomly divided into four groups (n = 6): 15% peel extract, 15% pulp extract, alkaline peroxide (positive control), and distilled water (negative control). All specimens were immersed for 15 minutes in the respective solutions. Fungal adhesion was quantified using a colony counter, and the data were analyzed using the Kruskal–Wallis test followed by the Mann–Whitney post hoc test. The results showed mean colony counts of 832.17 ± 34.79 CFU/mL for the peel extract group, 172.83 ± 35.02 CFU/mL for the pulp extract group, 1,006.50 ± 80.36 CFU/mL for the alkaline peroxide group, and 3,307.33 ± 114.38 CFU/mL for the distilled water group. Statistical analysis revealed significant differences among all groups ($p < 0.05$). The 15% purple passion fruit pulp extract demonstrated the greatest antifungal effectiveness, which may be attributed to its higher concentration of antifungal phytochemicals compared to the peel extract and the conventional cleanser. In conclusion, both 15% purple passion fruit peel and pulp extracts are more effective than alkaline peroxide in inhibiting *Candida albicans* adhesion on denture surfaces, with the pulp extract showing the strongest antifungal activity.

Keywords: Adhesion; Alkaline peroxide; *Candida albicans*; Denture cleanser; Passion fruit extract

Introduction

Tooth loss (edentulism) that is not immediately rehabilitated can trigger alveolar bone resorption in the edentulous area, resulting in decreased chewing function, mastication, speech, and facial aesthetics (Andrian et al., 2025). Rehabilitation using denture prostheses serves as a solution to restore these functions while also improving patients' quality of life (Adjani et al., 2023). One of the essential components of a prosthesis is the denture base, which functions as the attachment site for artificial teeth and comes into direct contact with the oral mucosal tissues (Rahmawati et al., 2021).

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To date, acrylic resin remains the primary material of choice for denture bases, with a usage rate of approximately 95% in the field of dentistry (Yusrini et al., 2024). The most commonly used type of acrylic resin in Indonesia is the heat-cured variety, which is a polymethyl methacrylate resin polymerized through a heating process (Pertiwisari, 2023). This material offers several advantages, including good biocompatibility, non-inflammatory properties, ease of polishing, aesthetic qualities, and relatively affordable cost. However, heat-cured acrylic resin also has several limitations, such as low mechanical strength, residual monomer content, poor thermal conductivity, porosity, fluid absorption, and susceptibility to abrasion from mechanical cleaning procedures. These conditions can increase the surface roughness of the denture base (Rahmawati et al., 2021).

An increase in surface roughness contributes to the ease with which microorganisms adhere, including *Candida albicans*, which is one of the primary causes of denture plaque formation (Vendela et al., 2022). *Candida albicans* is actually a normal flora of the oral cavity but can become pathogenic when the oral environment supports its colonization. The growth of this fungal colony can trigger an inflammatory response through the release of antigens, toxins, and irritants derived from plaque accumulation, leading to denture stomatitis (Oktaria, 2022). *Candida albicans* is the fungal species most frequently isolated from the human body and is commonly found in the oral cavity as part of the normal microbial flora alongside other microorganisms. Under normal conditions, the concentration of *C. albicans* in saliva is approximately 200 cells/mL; however, under pathological conditions, its prevalence may increase significantly, reaching up to 50% (Junuda et al., 2023).

One of the efforts to prevent denture stomatitis is through proper denture cleaning. Cleaning methods can be performed mechanically or chemically. However, mechanical cleaning using a brush often causes surface abrasion if not done correctly, which may instead increase the risk of plaque accumulation (Jubhari et al., 2024). Therefore, chemical cleaning is more recommended. One commonly used agent is alkaline peroxide, which is widely available in effervescent tablet form because it is practical and easily dissolves in water (Amiruddin et al., 2025). Although effective, this material has several drawbacks, such as being relatively expensive and having the potential to cause physical changes to the denture base if used long-term, including alterations in hardness and surface roughness (Aly, 2021).

On the other hand, the use of natural ingredients has increasingly been investigated as an alternative denture cleanser. One ingredient that has attracted attention is the passion fruit (Passiflora). Passion fruit is a tropical fruit consisting of various species that have been widely studied for their phytochemical characteristics and bioactive potential. Several commonly found varieties include Passiflora edulis, Passiflora edulis f. flavicarpa, Passiflora quadrangularis, and Passiflora ligularis. These varieties are known to contain rich and diverse secondary metabolites, making them potential antimicrobial and antifungal agents. Information on the diversity of Passiflora species has been described in an extensive review by Dhawan et al. (2004) which states that the Passiflora genus comprises more than 500 species with varying distributions of bioactive properties, including antimicrobial and anti-inflammatory activities. This fruit is known to possess anti-inflammatory, antioxidant, antimicrobial, and antifungal activities (García-villegas et al., 2022). In Indonesia, purple, yellow, and red passion fruit are the most widely cultivated varieties (Fitria et al., 2022), whereas in the North Sumatra region, purple passion fruit is the dominant variety (Widiatmika, 2015).

Purple passion fruit extract is known to contain various bioactive compounds such as flavonoids, tannins, saponins, steroids, and alkaloids (Septiningrum et al., 2024). Several of these compounds play a role in antifungal activity: flavonoids act by denaturing cell proteins; tannins inhibit ergosterol biosynthesis in the fungal cell wall; while saponins cause damage to the cell membrane, resulting in leakage of essential components such as proteins and nucleic acids, thereby triggering fungal cell death (Taufiqurrahman et al., 2023).

Several previous studies have supported the antimicrobial potential of passion fruit. Purple passion fruit peel extract has been shown to exhibit strong inhibitory activity against Staphylococcus aureus (Anabel et al., 2020). Another study by Nugraha et al. (2018) reported that purple passion fruit peel extract also demonstrates strong inhibitory effects against Propionibacterium. Furthermore, Taufiqurrahman et al. (2023) found that shampoo preparations containing purple passion fruit peel extract were able to inhibit the growth of *Candida albicans* at concentrations of 5%, 10%, and 15%, with the highest effectiveness observed at the 15% concentration.

The method for evaluating antifungal effectiveness includes measuring the number of *Candida albicans* colonies on the surface of heat-cured acrylic resin, which can be quantified using a colony counter. This device functions by detecting microbial colonies on Petri dishes and automatically counting them using an integrated sensor system (Fitriani et al., 2023, 2023). Although numerous studies have investigated the antimicrobial activity of passion fruit, research specifically comparing the effectiveness of passion fruit extract with alkaline peroxide against the adhesion of *Candida albicans* on heat-cured acrylic resin, particularly as a denture cleanser, remains very limited. Most previous studies have focused only on the peel of the fruit rather than the pulp, and have primarily evaluated inhibition zones rather than direct adhesion on denture base materials.

Therefore, this study presents a novelty in the use of passion fruit extract at a specific concentration as a soaking agent, directly compared with alkaline peroxide in the context of denture cleaning. Based on this background, the present study aims to compare the effectiveness of soaking passion fruit extract and alkaline peroxide on the adhesion of *Candida albicans* as denture cleansing agents. This research is expected to provide a natural alternative material that is safer, more economical, and effective in preventing fungal colonization on denture bases, thereby contributing to the prevention of denture stomatitis and encouraging innovation in denture care.

Method

This study was laboratory experimental research employing a posttest-only control group design, which enabled the evaluation of treatment effects after the intervention without baseline measurements. The overall research design followed a systematic experimental flow consisting of material preparation, sample fabrication, treatment application, microbiological testing, and data analysis.

The study was conducted from May to June 2025 across several laboratories in accordance with each procedural stage. The preparation of the 15% purple passion fruit (Passiflora edulis Sims) peel and pulp extracts was carried out at the ASPETRI Laboratory in Medan. Phytochemical screening to identify active antifungal compounds was performed at the Integrated Laboratory of Universitas Prima Indonesia. The fabrication of heat-cured acrylic resin plates measuring 10 × 10 × 2 mm, following ISO/DIS 1567:1997 standards, was completed at the Dental Laboratory of Medan Sei Sikambing. Subsequently, the preparation of *Candida albicans* suspension, contamination of acrylic plate surfaces, soaking procedures, and colony counting were conducted at the Microbiology Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara.

Research Flow and Experimental Design

The research flow began with the selection and preparation of research materials, including fresh purple passion fruit and standardized heat-cured acrylic resin plates. The fruit was processed into peel and pulp extracts, which were then subjected to phytochemical screening to confirm the presence of bioactive compounds with antifungal potential. The extracts were diluted to obtain a final concentration of 15%.

In parallel, heat-cured acrylic resin plates were fabricated, polished, and inspected to ensure compliance with dimensional and surface quality standards. A standardized *Candida albicans* suspension was prepared by adjusting turbidity to ensure uniform inoculum concentration across all samples. Each acrylic resin plate was then contaminated with the fungal suspension to allow microbial adhesion.

Following contamination, the samples were allocated into four experimental groups and immersed in their respective treatment solutions. After the soaking period, the plates were processed for microbiological analysis, and the number of adherent *Candida albicans* colonies was quantified using a Colony Counter (Interscience Scan® 300). The results were expressed as colony-forming units per milliliter (CFU/mL) and served as the posttest outcome measure.

Sample Size and Grouping

A total of 24 samples were included in the study, determined using the Federer formula. The samples were divided into four groups, each consisting of six specimens: 15% purple passion fruit pulp extract, 15% purple passion fruit peel extract, positive control using alkaline peroxide, negative control using distilled water.

The selection of research materials followed specific inclusion criteria, which required fresh, intact purple passion fruit and polished heat-cured acrylic resin plates with standardized dimensions. Materials that did not meet these criteria, such as rotten or wrinkled fruit and resin plates with uneven or porous surfaces, were excluded.

Data Analysis

The collected data were processed using SPSS version 24. Statistical analysis began with the Shapiro–Wilk test to assess data normality and the Levene test to evaluate variance homogeneity. For normally distributed data, a One-Way ANOVA was performed, followed by a Post-Hoc test to determine intergroup differences. If the data were not normally distributed, a Kruskal–Wallis test was applied, followed by a Mann–Whitney test to identify statistically significant differences between specific groups.

Result and Discussion

Result

The enumeration of *Candida albicans* colonies in this study was conducted using a colony counter (Interscience Scan® 300) and expressed in colony-forming units (CFU/mL). The mean analysis of *Candida* *albicans* colony counts observed across all treatments of purple passion fruit (Passiflora edulis Sims) peel and pulp extracts is presented in Table 1.

Table 1. Average Number of *Candida albicans* Colonies in Purple Passion Fruit Peel and Flesh Extracts

Treatment Group	Colony Count (CFU/mL)						Mean ± SD
	Replication						
	1	2	3	4	5	6	
Purple passion fruit peel extract 15%	802	830	879	871	808	803	832.17±34.79
Purple passion fruit pulp extract 15%	206	138	136	209	198	150	172.83±35.02
Alkaline peroxide	918	911	985	1086	1087	1052	1,006.50±80.36
Distilled water	3372	3385	3207	3122	3395	3363	3,307.33±114.38

The measurement results of *Candida albicans* colony counts after soaking in denture cleaning agents showed significant differences among the treatment groups. In the group treated with 15% purple passion fruit peel extract, the mean colony count was 832.17 ± 34.79 CFU/mL, indicating that substantial *Candida albicans* growth remained after soaking. In contrast, the group treated with 15% purple passion fruit pulp extract showed a much lower colony count of 172.83 ± 35.02 CFU/mL, suggesting that the pulp possesses stronger antifungal activity than the peel extract in reducing *Candida albicans* adhesion.

In the comparison group, alkaline peroxide, a commercial denture cleanser, showed an average colony count of 1,006.50 ± 80.36 CFU/mL. This value was higher than that of the purple passion fruit pulp extract but lower than that of the peel extract, indicating a moderate level of effectiveness in inhibiting *Candida albicans*. Meanwhile, the negative control group using distilled water exhibited the highest colony count of 3,307.33 ± 114.38 CFU/mL, representing untreated conditions and serving as a reference for optimal *Candida albicans* growth.

Overall, these results indicate that 15% purple passion fruit pulp extract was the most effective treatment in reducing *Candida albicans* colony counts compared to the peel extract and the commercial denture cleanser, alkaline peroxide. This effectiveness highlights the potential of passion fruit pulp extract as an alternative denture cleaning agent capable of significantly reducing *Candida albicans* adhesion.

Subsequently, a statistical analysis was performed on the *Candida albicans* colony count data, beginning with tests for normality and homogeneity. The normality assumption was assessed using the Shapiro-Wilk test, while homogeneity was evaluated with Levene's test. The results of the Shapiro-Wilk normality test and Levene's homogeneity test showed values of 0.041 and 0.002 ($p < 0.05$), respectively, indicating that the data were not normally distributed and not homogeneous. Therefore, non-parametric tests were conducted, starting with the Kruskal-Wallis test followed by the Mann-Whitney test. In this study, the Kruskal-Wallis test was used to determine whether there were differences in the effectiveness of purple passion fruit extract compared to alkaline peroxide and distilled water in inhibiting *Candida albicans* adhesion on heat-cured denture base materials. The results of the Kruskal-Wallis analysis are presented in Table 2.

Table 2. Comparison of the Effectiveness of 15% Purple Passion Fruit Extract on *Candida albicans* Adhesion on Heat-Cured Acrylic Resin

Test Groups	Mean ± SD	p-value
Purple passion fruit peel extract 15%	832.17±34.79	0.000
Purple passion fruit pulp extract 15%	172.83±35.02	
Alkaline peroxide	1,006.50±80.36	
Distilled water	3,307.33±114.38	

Table 2 presents the comparison of the effectiveness of soaking in 15% purple passion fruit extract on *Candida albicans* adhesion on heat-cured acrylic resin using the Kruskal–Wallis test. Based on the mean values, 15% purple passion fruit pulp extract showed the lowest colony count (172.83 ± 35.02 CFU/mL), indicating the highest antifungal activity among all treatment groups. Meanwhile, the peel extract resulted in a colony count of 832.17 ± 34.79 CFU/mL, and alkaline peroxide showed a colony count of 1,006.50 ± 80.36 CFU/mL, meaning its effectiveness was lower than the pulp extract but still better than the negative control. The distilled water group, as the control, exhibited the highest colony count (3,307.33 ± 114.38 CFU/mL), representing conditions without antifungal activity.

The Kruskal–Wallis test yielded a p-value of 0.000, indicating a statistically significant difference among all treatment groups. Thus, it can be concluded that soaking with purple passion fruit extract, particularly the pulp extract, has a significantly different effectiveness in reducing *Candida albicans* adhesion compared to alkaline peroxide and the negative control. Post-hoc comparisons in this study were conducted using the Mann-Whitney test to specifically evaluate the comparative effectiveness of soaking with 15% peel and pulp extracts versus alkaline peroxide. The results of the Mann-Whitney analysis are presented in Table 3.

Table 3. Mann–Whitney Test Results for the Comparison of the Effectiveness of 15% Purple Passion Fruit Extract, Alkaline Peroxide, and Distilled Water on *Candida albicans* Adhesion on Heat-Cured Acrylic Resin

Test Groups	Purple passion fruit peel extract 15%	Purple passion fruit pulp extract 15%	Alkaline peroxide	Distilled water
Purple passion fruit peel extract 15%	-	0.004	0.004	0.004
Purple passion fruit pulp extract 15%	[redacted]	-	0.004	0.004
Alkaline peroxide	[redacted]	[redacted]	-	0.004
Distilled water	[redacted]	[redacted]	[redacted]	-

The Mann-Whitney test results above indicate a significant difference in effectiveness between the 15% purple passion fruit peel extract group and the 15% pulp extract group, alkaline peroxide, and distilled water, with p-values < 0.05. Additionally, there was a significant difference in effectiveness between the 15% purple passion fruit pulp extract group and both the alkaline peroxide and distilled water groups, also with p-values < 0.05.

Discussion

This study focused on analyzing the effectiveness of soaking in 15% purple passion fruit peel and pulp extracts compared to alkaline peroxide and distilled water in inhibiting *Candida albicans* adhesion on heat-cured acrylic resin plates used as denture cleaning materials. Statistical analysis indicated significant differences among the treatment groups, suggesting that each solution possesses distinct antifungal capabilities. Although the quantitative data have been presented in the results section, this discussion aims to explain the underlying biological mechanisms, support from the literature, and the position of these findings within the fields of prosthodontics and oral microbiology, without reiterating the statistical figures in detail.

The difference in effectiveness between purple passion fruit extract, alkaline peroxide, and distilled water indicates that the bioactive compounds in the plant contribute significantly to inhibiting *Candida albicans* adhesion. The 15% purple passion fruit peel extract was shown to be more effective than both alkaline peroxide and distilled water. This finding is consistent with the study by Taufiqurrahman et al. (2023) which demonstrated that increasing the concentration of passion fruit peel extract from 5% to 15% resulted in progressively stronger inhibitory effects against *Candida albicans*. This effectiveness is related to the phytochemical composition of purple passion fruit, which is rich in alkaloids, flavonoids, terpenoids, tannins, and saponins, each exerting different mechanisms in disrupting fungal cell structures. The presence of these diverse phytochemicals produces a synergistic effect, resulting in antifungal activity that is more optimal compared to certain commercial denture cleaning solutions.

Alkaloids, as one of the main components in both the peel and pulp extracts of passion fruit, play a crucial role in inhibiting peptidoglycan synthesis in the fungal cell wall. This inhibition disrupts cell wall integrity, making the fungi more susceptible to lysis (Kumalasari et al., 2021). In addition, terpenoids present in the passion fruit extract can penetrate the fungal cell membrane, disrupting lipid synthesis. Imbalances in membrane lipid components compromise membrane stability, increase permeability, and cause leakage of cellular contents, thereby inhibiting fungal growth (Nazzaro et al., 2013). Flavonoids, as polyphenolic compounds, possess lipophilic properties that allow them to interact directly with membrane phospholipids. This interaction leads to protein denaturation and changes in membrane permeability, resulting in the release of intracellular fluids and fungal cell death. Tannins inhibit the biosynthesis of ergosterol, a critical component of the fungal cell membrane, which impairs membrane function. Saponins, as natural surfactants, can disrupt membrane stability by forming complexes with membrane sterols, leading to leakage of cellular contents. Collectively, these mechanisms explain why passion fruit peel and pulp extracts exhibit strong antifungal activity, consistent with findings from previous studies.

Another notable finding is that the purple passion fruit pulp extract also demonstrated high effectiveness in inhibiting *Candida albicans* adhesion. The phytochemicals present in the pulp have a composition nearly similar to that of the peel; however, several studies indicate that the pulp contains higher concentrations of flavonoids and vitamin C compared to the peel. The abundant flavonoids enhance the extract's ability to damage membranes and denature proteins. In addition, vitamin C acts as a strong antioxidant that can reduce oxidative stress on the fungal cell surface, inhibit hyphal formation, and slow the colonization process. Harefa et al. (2023) reported that the combination of flavonoids and tannins can enhance antifungal activity because the two compounds act at different sites within the fungal cell structure. Therefore, it is not surprising that the purple passion fruit pulp extract produced excellent inhibition of *Candida albicans* colonies, even outperforming alkaline peroxide in this study.

Alkaline peroxide, used as a positive control, is expected to exhibit good antifungal activity because it contains hydrogen peroxide, which produces active oxygen bubbles. These bubbles function to clean denture surfaces through oxidation and disinfection mechanisms, which can disrupt microbial biofilms on the acrylic surface (Pellizzaro et al., 2012). However, the results of this study show that the effectiveness of alkaline peroxide was still lower than that of purple passion fruit extract. One possible reason is that the oxidative activity of hydrogen peroxide occurs primarily on the outer surface and does not deeply destroy the fungal cell structure. Additionally, *Candida albicans* is known for its high adaptability to oxidative environments and can increase catalase enzyme production to neutralize the effects of hydrogen peroxide. This limits the ability of alkaline peroxide to achieve maximal inhibition of fungal growth, particularly at certain concentrations and soaking durations.

Distilled water, used as the negative control, exhibited the highest fungal colony adhesion, consistent with its lack of active antifungal compounds or oxidative properties. This condition also emphasizes that the inhibition of fungal growth observed in the passion fruit extract and alkaline peroxide groups was not merely due to the soaking process itself, but rather resulted from the presence of phytochemicals or active compounds in the solutions used.

Although the results of this study indicate that the peel and pulp extracts of purple passion fruit have great potential as alternative denture cleaning agents, there are several limitations. One limitation is that the testing was conducted in vitro, which does not fully represent the complex conditions of the oral cavity. In denture users, various factors such as dietary habits, age, saliva composition, microbial colonization levels, and oral hygiene practices can influence *Candida albicans* growth. In addition, this study did not control for the surface roughness of the acrylic resin prior to treatment, so there may have been variations in roughness between samples that could affect the number of adhering colonies. Surface roughness is an important factor in fungal adhesion, as rougher surfaces provide protective areas for microorganisms to colonize.

The use of digital colony counters has been proven to enhance the accuracy and consistency of microbial colony enumeration. These digital devices can count bacterial colonies consistently, with results not significantly different from conventional colony counters or manual counting, making them a reliable alternative in microbiology laboratories (Kurniawan et al., 2023). Therefore, the use of digital colony counters or similar automated systems provides a basis for the reliability of the colony count data in this study, offering greater consistency compared to traditional manual counting methods.

Overall, this study reinforces the understanding that plant extracts, particularly purple passion fruit extract, have great potential to be developed as natural-based denture cleaning agents. The diverse phytochemical content and complementary mechanisms of action provide advantages over some commercial cleaning agents that rely on a single mechanism, such as oxidation. Additionally, the growing public interest in natural products and concerns about chemical safety in health-related products present significant opportunities for developing denture cleaning products based on purple passion fruit extract. For clinical application, further studies are needed to evaluate effectiveness under in vivo conditions, assess biocompatibility, long-term material stability, and potential irritation to oral mucosal tissues. Consequently, the findings of this study can serve as a foundational basis for developing safer, more effective, and natural-based denture cleaning products.

Conclusion

The mean *Candida albicans* colony counts for 15% purple passion fruit peel extract, 15% pulp extract, alkaline peroxide, and distilled water were 832.17 ± 34.79; 172.83 ± 35.02; 1,006.50 ± 80.36; and 3,307.33 ± 114.38 CFU/mL, respectively. Statistical analysis showed a significant difference in effectiveness between soaking in 15% peel or pulp extracts and alkaline peroxide or distilled water in inhibiting *Candida albicans* adhesion on heat-cured acrylic resin ($p = 0.01$; $p < 0.05$). Furthermore, there was a significant difference in effectiveness between the 15% peel and pulp extracts ($p = 0.000$; $p < 0.05$), with the 15% pulp extract proving more effective in inhibiting *Candida albicans* adhesion ($p = 0.004$; $p < 0.05$), indicating its potential as an alternative cleaning agent for heat-cured acrylic resin dentures.

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Author Contributions
C.D.W: Conceptualization; methodology; formal analysis; investigation; data curation; writing—original draft preparation; project administration; S.T: Conceptualization; validation; resources; supervision; writing—review and editing; funding acquisition; S.A.S: Software; investigation;

data curation; visualization; validation; I.M.B: Validation; resources; writing—review and editing.

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No conflict of interest.

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