ORIGINAL ARTICLE

Role of cassava starch (*Manihot esculenta Crantz*) in reducing candida albicans adhesion on soft denture liners: an experimental study

Afif Surya Adena[1]*
Amiyatun Naini[1]
FX Ady Soesetijo[1]
Dewi Kristiana[1]
Rahardyan Parnaadji[1]
Ardhianing Hardita[1]

[1]Departement of prosthodontic, Faculty of Dentistry, Universitas Jember,Indonesia

* Correspondence:
suryaadena@unej.ac.id

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ABSTRACT

Introduction: Soft denture liners can be an alternative solution for various removable denture problems, but they still have several limitations, such as promoting *Candida albicans* adhesion to dentures. Modifications incorporate cassava starch. The aim of this result is toanalyze the role of cassava starch in reducing *Candida albicans* adhesion on soft denture liners. **Methods**: This laboratory experimental study used twenty-seven disc-shaped heat-cured acrylic plates with a diameter of 10 mm and a thickness of 2 mm divided into three groups: acrylic plate without a coating (negative control group), acrylic plate coated with soft denture liners (positive control group), and acrylic plate coated with a soft denture liner containing cassava starch 15% (treatment group). The samples were soaked in artificial saliva for 1 hour, then contaminated with *Candida albicans* by placing them in a test tube containing a *Candida albicans* suspension, and incubated for 24 hours at 37°C. Samples were rinsed twice with PBS, then placed in a test tube containing 10 mL of Sabouraud's broth and vortexed for 30 seconds. The turbidity of the released *Candida albicans* was measured using a spectrophotometer. Absorbance data were analyzed using one-way ANOVA and post hoc LSD. **Results**: Turbidity testing using a spectrophotometer at 600 nm showed the average absorbance values were 0.013 for negative control group, 0.028 for the positive control group, and 0.019 treatment group. The results of the one-way ANOVA test showed a p-value of 0.004 (<0.05), indicating a significant difference between the groups. Further post hoc LSD tests showed that the positive control group had the highest absorbance value. At the same time, there was no significant difference between the negative control group and treatment group. **Conclusion**: Cassava starch inhibits *Candida albicans* adhesion to the soft denture liners.

KEYWORDS
Cassava starch, soft denture liner, *candida albicans*, acrylic

INTRODUCTION

Edentulousness remains a common oral problem. According to the WHO[1], the estimated global average prevalence of edentulousness is approximately 7% in people aged 20 years and older. The 2023 Indonesian Health Survey reported a 21% prevalence of tooth loss.[2] Conventional removable denture is one treatment option for tooth loss, but their comfort remains a concern. Various factors can affect the comfort of conventional removable denture wearers. Issues such as alveolar bone resorption, sharp ridges, or flabby tissue result in uneven pressure distribution on the denture-supporting tissues, leading to tissue injury, pain, patient discomfort, and changes in prosthesis fit.[3] Soft denture liners materials can be an alternative solution to these problems.

Soft denture liners materials can help address several problems that occur during removable denture wear. Alqutaibi et al,[4] explained that soft denture liners can improve biting force and the quality of life of denture wearers. Soft denture liners materials have several benefits, including reducing aging and pathological changes; reducing local pressure; reducing occlusal loads; increasing retention; and preventing bone resorption.[5] Soft denture liners materials can adapt their shape to the mucosal tissue and follow its contours, allowing optimal contact at the fitting surface and improving denture retention.[6]

On the other hand, problems also arise after the application of soft denture liners materials to removable dentures. Fungal adhesion to dentures lined with soft denture liners materials is one such problem. The growth of fungi such as *Candida albicans* is a problem that occurs after the application of soft denture liners materials.[7] Adhesion of *C. albicans* leads to the formation of biofilms, which make the organism resistant to antimicrobial and antifungal drugs.[8] Denture-related stomatitis is the most common disease caused by *C. albicans*.[9,10] Therefore, researchers have begun modifying soft denture liners materials to address their limitations.

One potential modification to improve the quality of soft denture liners is the addition of herbal materials. Memon et al,[3,11] demonstrated that chitosan and aloe vera powder are potent antifungals, and their incorporation into soft denture liners materials can enhance antifungal activity against Candida. Alamen and Naji[12] also confirmed that herbal materials such as virgin coconut oil were successfully incorporated into soft denture liners materials and acted as potential antifungal drugs with a sustained drug delivery system against *Candida albicans*. This statement is supported by Sousa et al,[13], who stated that the use of natural antimicrobials is considered safer and has fewer side effects than synthetic chemicals.

Another herbal material that may have a positive effect when combined with soft denture liners materials is cassava starch. Cassava is an herbal product with the potential to inhibit *Candida albicans* adhesion. Cassava is commonly harvested at around 8–9 months after planting because starch content increases with plant age up to an optimal point, while early harvesting results in low starch yield and delayed harvesting leads to increased fiber content and reduced starch quality and extractability.[14] Cassava contains saponins, flavonoids, and tannins, which may act as antifungal agents[15]. The potential of soft denture liners combined with cassava starch is not yet fully understood.

Cassava has potential as an antifungal, but the role of adding cassava to soft denture liners in inhibiting the attachment of Candida albicans is not yet fully understood. This study introduces a novel bio-based approach by incorporating cassava starch into soft denture liner materials to reduce *Candida albicans* adhesion. It provides new insights into the use of natural, biocompatible additives as sustainable alternatives to conventional antifungal agents. The aim of this result is to analyze the role of cassava starch reducing Candida albicans adhesion on soft denture liners.

METHODS

This study was a laboratory experimental study with a post-test control group design. Based on the calculation of the number of samples using the Federer formula, the minimum number of samples was 8.5. In this study, 9 samples were used in each group. Twenty-seven disc-shaped heat-cured acrylic (Acrylic Denture Materials, England) plates with a diameter of 10 mm and a thickness of 2 mm were divided into three groups: negative control, positive control, and treatment. The negative control group was an acrylic plate without a coating, the positive control group was an acrylic plate coated with a soft denture liner, and the treatment group was an acrylic plate coated with a soft denture liner containing cassava starch.

Cassava (*Manihot esculenta Crantz*) at 8-9 months of age was selected and peeled.[16] The cassava was cut into four pieces and weighed 250 grams. The cassava was soaked in water at a ratio of 250 g cassava to 750 ml water. 15% sodium chloride (NaCl) was added to reduce cyanide levels. The soaking process lasted for 7 hours. The cassava was finely grated, and then water was added at a ratio of 250 g cassava: 1,000 ml water.[16]

The grated cassava was squeezed and filtered through filter paper. The filtered cassava was left to settle for 8 hours to precipitate the starch. The water at the top was discarded. The white starch which precipitated at the bottom was dried in an oven at 50°C for 6 hours to reduce its water content. It is then ground using a mortar and sieved to obtain cassava starch. Soft denture liners modification was performed by adding 0.3 g of cassava starch (15% of the total weight of the soft denture liner powder) to the soft denture liner powder; the addition must not change the powder-to-liquid ratio.

The soft denture liner used an acrylic-based, chemically polymerized soft denture liner (MAARC, India). The soft denture liner was manipulated according to the manufacturer's recommendations at a ratio of 2.2g/0.8ml (powder/liquid). The soft liner was applied to one side of the acrylic plate that had not been previously polished.

The samples were soaked in artificial saliva (containing NaCl, KCl, $CaCl_2 \cdot H_2O$, NaH_2PO_4, $Na_2S \cdot 9H_2O$, and 0.5 grams of urea mixed into 500 mL of sterile distilled water) for 1 hour to allow the formation of a salivary pellicle layer. The soft denture liner was then contaminated with *Candida albicans* by placing them in a test tube containing a *Candida albicans* suspension at a standard of 0.5 McFarland (equivalent to 1.5×10^8CFU/mL) and incubated for 24 hours at 37°C to allow microbial adhesion to the soft denture liner surface. Samples soaked in *Candida albicans* suspension were rinsed twice with PBS to remove loosely adhered Candida, then placed in a test tube containing 10 mL of Sabouraud's broth (Himedia, Germany).

Afterward, the samples were vortexed for 30 seconds using a Thermolyne vortex to dislodge any *Candida albicans* attached to them. The turbidity of the released *Candida albicans* was measured using a spectrophotometer. The spectrophotometric measurements were conducted under blinded conditions to reduce the risk of observer bias. The absorbance of *Candida albicans* was measured at 600 nm using McFarland solution no. 0.5 as a blank solution.[17,18]

The absorbance data were tabulated and tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. Data analysis was continued with the one-way ANOVA and post hoc LSD tests.

RESULTS

The results of the sample creation showed a uniform sample size of 10 mm in diameter and 2 mm in thickness. The samples in the treatment group showed no noticeable color change in the soft denture liner and remained adherent to the surface of the acrylic plate (Figure 1). However, further research is needed on the adhesive strength of the modified soft denture liner.

Figure 1. Disc-shaped acrylic plate sample. A. acrylic plate without a coating (negative control group). B. acrylic plate coated with a soft denture liner (positive control group). C. acrylic plate coated with a soft denture liner containing cassava starch (treatment group)

Light microscopic observations revealed spherical to subspherical colonies with purple buds, pseudohyphae, and blastoconidia (Figure 2).

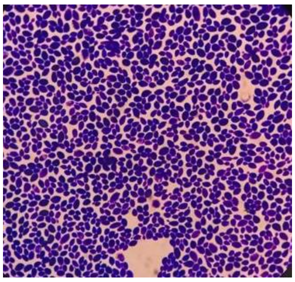

Figure 2. Results of observations of *Candida albicans* under a light microscope

Turbidity testing using a spectrophotometer at 600 nm shows the absorbance values for each group (Table 1). The average absorbance value is 0.013 for the acrylic plate without coating (negative control group), 0.028 for the acrylic plate coated with soft denture liner (positive control group), and 0.019 for the acrylic plate coated with soft denture liner containing 15% *Manihot esculenta Crantz* (treatment group). In the statistical test, the data were normally distributed and homogeneous (p>0.05).

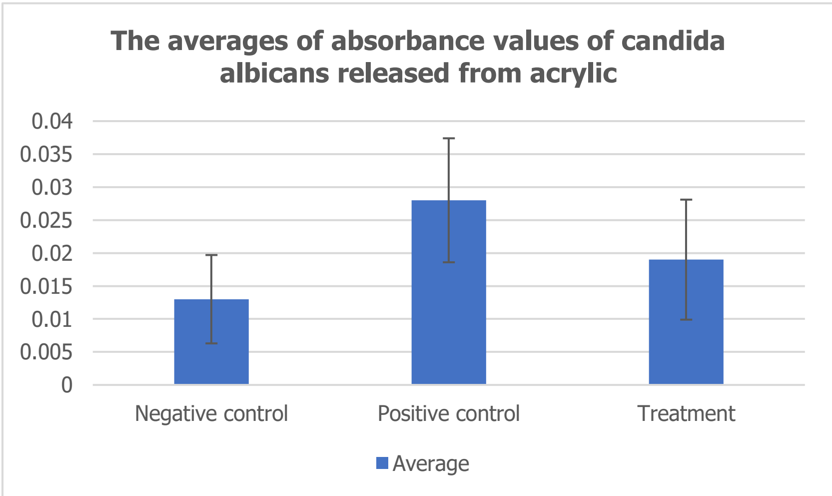

Figure 3. Averages of absorbance values of candida albicans released from acrylic

The results of the one-way ANOVA test showed a p-value of 0.004 (<0.05), indicating a significant difference between the groups (Table 1). Further post hoc LSD tests showed that the positive control group had the highest absorbance value. At the same time, there was no significant difference between the negative control group and treatment group (Table 2).

Table 1. ANOVA test result

Group	X ± SD	F	P Value
Negative control	0.013 + 0.0067		
Positive control	0.028 + 0.0094	7.045	0.004
Treatment	0.019 + 0.0091		

Table 2. LSD post hoc test results

Testing	Group	Comparison	Mean difference	p-value	95% confidence intervals	
					Lower Bound	Upper Bound
LSD	Negative control	Positive control	-0.015000*	0.001	-0.02329	-0.00671
		Treatment	-0.006222	0.134	-0.01451	0.00207
	Positive control	Negative control	0.015000*	0.001	0.00671	0.02329
		Treatment	0.008778*	0.039	0.00049	0.01707
	Treatment	Negative control	0.006222	0.134	-0.00207	0.01451
		Positive control	-0.008778*	0.039	-0.01707	-0.00049

*= significance <0.05

DISCUSSION

The addition of cassava starch to the soft-lining material in this study resulted in no significant color difference (Figure 1), indicating that cassava starch incorporation does not affect the aesthetic quality of the soft-lining material. …. Ergun *et al,*[19] explained that the addition of zirconium dioxide to denture liners resulted in a color change, although this was not statistically significant. In addition, Ataol *et al,*[20] concluded in their study that the addition of carbon nanoparticle to soft denture liner resulted in clinically unacceptable color changes at all different ratios tested. Soft denture liners are applied to the fitting surface of acrylic dentures to improve patient comfort. However, their use still poses a problem: *Candida albicans* adhere more readily to dentures coated with soft denture liners.

This research proves that the average absorbance of *Candida albicans* in the group of acrylic plates coated with soft denture liner is the highest and significantly higher than that in the group of acrylic plates without coatings (Table 1 and 2). The results of this study are supported by Tasopoulos *et al*,[21] who explained that long-term soft denture liners accumulate a significant amount of C. albicans. The soft denture liner's ability to retain moisture creates a favorable environment for fungal growth. In addition, the release of plasticizers during use can alter the surface structure, making it rougher and increasing the risk of *Candida albicans* colonization.

The low-dimensional stability of the soft denture liner material can also create microcracks that serve as sites for microbial colonization.[22] Burket[23] reported in his research that, under normal conditions, *Candida albicans* does not cause disease, but it can become pathogenic when the microbiota is imbalanced, leading to overgrowth. This imbalance is more common in denture wearers because the oral cavity becomes more moist and closed, favoring the growth of Candida. The results of this study also confirmed those of Morikava et al,[23] and Singhania et al,[25] who reported that *C. albicans* colonization is widespread on soft denture liner.

The potential for increased colonization by *Candida albicans* is what triggers denture stomatitis. Denture stomatitis is a chronic inflammation of the oral mucosa that generally develops in areas covered by dentures, especially when oral hygiene is poor or dentures do not fit properly.[26] The pathogenesis begins with the initial adhesion of *Candida albicans* to the mucosal surface and to denture materials, aided by adhesion proteins such as Hwp1 and Als3, which facilitate strong adhesion even to inert surfaces such as acrylic and soft denture liner.[24] Once adhesion occurs, the fungus undergoes a morphological transition from blastospores to hyphae, a more invasive form that then penetrates the mucosal epithelium and triggers a local immune response.[27] Modifications to soft denture liners are being developed to reduce the potential for *Candida albicans* adhesion;

one such modification was tested in this study by adding herbal materials to the soft denture liner.

This study aimed to test the potential of cassava starch (*Manihot esculenta Crantz)* as an antifungal agent in soft denture liner. The results showed a significant difference ($p < 0.05$) in three research groups, namely the acrylic group without the use of a soft denture liner (negative control group), the acrylic group with the use of a soft denture liner (positive control group), and the acrylic group with the use of a soft denture liner that had been added with 15% cassava starch (treatment group). The LSD test results showed the absorbance values for *Candida albicans* in the treatment group was significantly lower than the positive control group (Table 2). The results of this study are in line with research conducted by Memon et al, who added other materials such as chitosan and aloe vera to soft denture liners.

Cassava starch has potential as an antifungal agent due to its saponins, flavonoids, and tannins, which inhibit fungal growth and attachment.[15,28] Flavonoids are known to inhibit the proliferation of *Candida albicans* cells by disrupting membrane and cell wall integrity and by inhibiting cell metabolism and transport. This compound can form protein complexes that interact directly with fungal cell walls. This interaction can disrupt cell function, inhibiting replication and transcription in these microorganisms.[29,30]

In addition, saponins reduce fungal virulence by inhibiting the yeast-to-hyphae transition, a process essential for invasiveness and biofilm formation, through the downregulation of virulence genes and key signaling pathways such as cAMP–PKA and MAPK.[31] Supporting this mechanism, Lu Li et al,[32] reported that saponins inhibited early biofilm formation by approximately 77.6% at a concentration of 0.16 mg/mL and were also effective against mature biofilms by suppressing cell aggregation, inducing morphological changes, and blocking mycelial transformation.

In addition to flavonoids and saponins, tannins have been widely reported to exhibit antifungal and antibiofilm activities against *Candida albicans***.** Polymeric proanthocyanidin tannins were shown to inhibit both planktonic growth and biofilm formation by reducing fungal adhesion and disrupting biofilm structure.[33] Similarly, Widodo et al,[34] demonstrated that tannins significantly inhibited mono-species *C. albicans* biofilm formation by damaging the extracellular polymeric substance matrix. More recent studies have shown that tannic acid reduces fungal burden and tissue invasion in models of invasive candidiasis and enhances the antifungal efficacy of fluconazole, including against resistant strains.[35] Collectively, these findings indicate that the antifungal potential of cassava starch is mediated through multiple mechanisms that suppress fungal growth, biofilm formation, and virulence of *Candida albicans***.**

This study has certain limitations, as only a single concentration (15%) of cassava starch was incorporated into the soft-lining material. Future studies should investigate a range of concentrations to comprehensively evaluate the antifungal efficacy of cassava starch against *Candida albicans*. Moreover, mechanical and physical properties were not assessed following starch incorporation; therefore, further evaluation is necessary to determine its effects on the adhesion of the soft-lining material to the fitting surface and on its elastic behavior.

CONCLUSION

This study shows that cassava starch (*Manihot esculenta Crantz)* 15% inhibits *Candida albicans* adhesion to the soft denture liner. The implication of this study is that cassava starch has the potential to serve as an alternative material for soft denture liners. However, this study still has limitations, so further research is needed to determine the physical or mechanical changes in soft denture liners supplemented with other materials.

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