

Rice vinegar removes *Candida albicans* from denture acrylic resins

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ABSTRACT

Introduction

Denture stomatitis, mainly caused by *Candida albicans*, often affect denture wearers. To manage these patients, denture hygiene is of utmost importance. There is a need for low cost, easily accessible denture disinfectants.

Aims

To investigate the efficacy of rice vinegar and other disinfecting solutions in removing *C. albicans* from acrylic resins.

Materials and methods

Hundred and eighty acrylic resin plates were contaminated with *C. albicans* strains and divided into five groups. These were immersed in apple cider vinegar (ACV), white wine vinegar (WWV), rice vinegar (RV), chlorhexidine (CHX), and sterile distilled H₂O (control). The plates were incubated at room temperature for 30 minutes, 1 hour and 8 hours. *Candida* removing ability of the disinfecting solutions was evaluated, and data was analyzed using two-way ANOVA with Tukey post-test. Significance level of $p < 0.05$ was used.

Results

RV, ACV, WWV and CHX showed the highest efficacy

(100%) in removing both *C. albicans* strains at 8 hours ($p > 0.05$). CHX was the most effective disinfectant in removing both *C. albicans* strains at 30 minutes, 1 hour, and 8 hours (99%-100%).

Conclusion

RV was as effective as ACV, WWV and CHX in removing *C. albicans* from acrylic plates at 8 hours.

Keywords

Rice vinegar. Denture acrylic resin. *Candida albicans*. Disinfection.

INTRODUCTION

According to the World Health Organization, globally approximately 30% of people aged between 65-74 years are edentulous,¹ with many replacing lost teeth with dentures. Denture hygiene remains the mainstay preventive measure in the management of denture-wearing patients.²

Denture stomatitis, a mucous membrane inflammation, occurs on the denture-bearing mucosa of denture wearers. It is a common finding amongst denture wearers (15-70%), with women commonly affected.^{3,4} Several factors, including patient age, age of denture, oral hygiene, diet, smoking, denture trauma, continuous denture wearing, salivary flow, denture base material, cellular immunity, pH of the denture plaque, and oral microorganisms contribute to the onset of the disease.⁴⁻⁸ *C. albicans* is reported as the primary causative agent of the disease.^{9,10}

Denture base acrylic resin is a significant contributing factor to the oral colonization of the denture by *C. albicans*. Dentures have rough fitting surfaces, which are conducive for adherence of *Candida* species and formation of the biofilm.¹¹ In order to control this colonization and prevent *Candida* infections, the removal of these organisms from dentures is imperative. Antifungal agents are used for treatment of *Candida* associated denture stomatitis. However, if the dentures are not properly cleaned and disinfected, denture stomatitis recurs when treatment is ceased.¹² Whilst more people have access to dental care, thus retaining their natural teeth for longer, most elderly people continue to lose their teeth, rendering them prone to denture stomatitis.¹³

Chlorhexidine gluconate (2%) antiseptic mouthwash has been used as a soaking solution to suppress adhesion of *Candida* to acrylic dentures.¹⁴ However, the use of chlor-

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hexidine leads to a brown discoloration of the denture, and has a bitter taste, which may reduce compliance.¹⁵

Although previous studies have tested the removal of *C. albicans* from a denture base using acetic acid/vinegar,¹⁶⁻²⁰ no South African study has tested and compared the effect of different types of vinegars on the removal of *C. albicans* from acrylic resin bases over different time periods. In addition, there are no documented studies on the use of rice vinegar (RV) as a denture cleanser.

Several denture cleansers are readily available on the market; however, the high cost leads to less than 60% of denture wearers using commercially available cleansers.¹⁶ Therefore there is a need for low cost, easily accessible denture disinfectants. Vinegar is cheap and readily available in South Africa. The objective of the study was to investigate the efficacy of RV and other disinfecting solutions in the removal of *C. albicans* from denture acrylic resin.

MATERIALS AND METHODS

Denture acrylic resin plates and disinfecting solutions

One hundred and ninety-two denture acrylic resin plates measuring 10×10×3mm were prepared according to the manufacturer's instructions (Confi-Dent agencies, Johannesburg, South Africa). The plates were kept in a flask containing sterile normal saline, and sterilized in an autoclave at 121°C/15 min.

The vinegars tested were 6% white wine vinegar (WWV), 5.5% RV, and 5% apple cider vinegar (ACV), all obtained from Instant Trading Co. (Pty) Ltd, Durban, South Africa. Chlorhexidine (CHX) (0.12%; Sunstar Americas, Inc, Ontario, Canada) was included as a positive control and sterile distilled water as a negative control.

C. albicans biofilm formation on denture acrylic resin plates

This study was conducted in the Department of Oral Biological Sciences, University of the Witwatersrand. *C. albicans* strains used in the study were the ATCC 90028 (National Health Laboratory Services (Johannesburg, South Africa), and a clinical *C. albicans* isolate. This isolate was obtained from an HIV positive patient (hereafter referred as clinical *C. albicans* strain) from a previous study and was stored in a -80°C freezer in the Department of Oral Biological Sciences. Ethical clearance to conduct the study was obtained from the Human Research Ethics Committee (Certificate No: W-CJ-150205-3).

For biofilm formation, a method described by Jafari, et al.¹⁷ (2012) was followed, with slight modifications. The two *Candida* strains were grown on Sabouraud's Dextrose Agar (SDA) plates at 37°C for 48 hours. *Candida* suspensions were prepared by dissolving colonies in 20ml of sterile normal saline and turbidity adjusted to 0.5 McFarland standard (Difco Laboratories, Detroit, MI, USA), equivalent to 1.5 × 10⁸ *Candida* cells per milliliter. Ninety sterile denture acrylic resin plates were contaminated with the *C. albicans* ATCC 90028 strain, while 90 were con-

taminated with a clinical *C. albicans* strain. This was done by immersing the plates in 3ml sterile Sabouraud's Broth in test tubes and adding 200µl of *Candida* suspension to each tube. The tubes were incubated at 37°C in a shaking incubator (100rpm) for 48 hours.

Disinfection of denture acrylic resin plates

The 90 denture acrylic resin plates contaminated with the *Candida* ATCC 90028 strain and 90 contaminated with a clinical *C. albicans* strain were randomly divided into five groups of 18 each. Group 1 plates were immersed in 20ml of 5% ACV, group 2 in 20ml of 6% WWV, group 3 in 20ml of 5.5% RV, group 4 in 20ml of 0.12% CHX and group 5 in 20ml of distilled water (H₂O; control).

The plates from each group were all incubated at room temperature and after 30 minutes, 6 plates were removed from each group and processed to determine viable *Candida* cells (colony forming units; CFUs). The same procedure was repeated after 1 hour and after 8 hours. Six acrylic plates contaminated with *Candida* ATCC 90028 strain and six contaminated with a clinical strain, but not exposed to any of the disinfecting solutions were included as controls.

Cell viability

To determine *Candida* cell viability, the denture acrylic resin plates were aseptically suspended in 20ml of sterile saline and vortexed for microbial cell detachment. Serial dilutions were prepared from this suspension by mixing 0.1ml of each suspension with 0.9ml of sterile phosphate buffered saline (1:10), which was further diluted by adding 0.1ml of this suspension into another 0.9ml of PBS (1:100). One hundred microliters of these dilutions were spread in duplicate on SDA plates and incubated for 48 hours at 37°C.

These tests were repeated three times for each of the two *C. albicans* strains for reproducibility of results. The *Candida* removing ability of ACV, WWV, RV, CHX, and H₂O was calculated by using the formula: $(CFU_b - CFU_a) \div CFU_b \times 100$, where CFU_b = Number of CFUs before disinfection and CFU_a = Number of CFUs after disinfection.

Statistical analysis

The data was analyzed using GraphPad Prism (Graph Pad Software Inc., La Jolla, CA). Two-way-ANOVA with Tukey's post-test was used for comparison of the *Candida* removing ability of ACV, RV, WWV, CHX, and H₂O at different times. A significance level of less than 0.05 (p<0.05) was used.

RESULTS

The removing ability of different disinfecting solutions at different time intervals are illustrated in **Table 1** (ATCC strain) and **Table 2** (Clinical strain). At 8 hours, ACV, WWV, RV, and CHX showed the highest efficacy (100%) in removing ATCC 90028 and clinical *C. albicans* strains from acrylic resins. CHX was the most effective disinfectant in removing both strains of *C. albicans* at 30 minutes, 1 hour, and 8 hours (99% - 100%).

For ATCC at 30 minutes and 1 hr, WWV was the next most effective after CHX (92 and 97% respectively). For clinical strain, at 30 minutes and 1 hr, WWV was the next most effective after CHX (82 and 73% respectively).

Sterile water could not completely remove either of the *C. albicans* strains at 30 minutes, 1 hour and 8 hours.

For the ATCC 90028 strain, there was no statistically significant difference between ACV, WWC, RV and CHX at 30 minutes, 1 hour and 8 hours ($p > 0.1$) in removing this strain from denture acrylic resin plates. There was statistically significant difference between ACV, WWV, RV, CHX when compared to water at 8 hours ($p = 0.0002$).

There was no statistically significant difference between ACV, WWC, RV and CHX at 30 minutes, 1 hour and 8 hours in removing the clinical strain from denture acrylic resin plates ($p > 0.3$). However, there was statistically significant difference between ACV, WWV, RV, CHX, and water at 8 hours ($p < 0.05$).

DISCUSSION

Denture stomatitis is caused mainly by the adherence and colonization of *C. albicans* on the fitting surface of the denture. In order to control this infection, it is important to remove this organism by disinfecting dentures,

and there is a need for low cost and easily accessible denture disinfectants.

Vinegar is cheap, has low toxicity²¹ and is readily available in South Africa. It is a sour liquid comprised mainly of acetic acid, which is prepared by the fermentation of alcoholic beverages, mainly white and red wines.²² *C. albicans* is the main causative agent of denture stomatitis, and thus using this solution in the disinfection of dentures might prove beneficial both therapeutically and as a preventive measure.²³

This study aimed to investigate the efficacy of RV and other disinfecting solutions in the removal of *C. albicans* from denture acrylic resin. In this study, RV was found to be as effective as CHX, ACV and WWV in removing *C. albicans* from acrylic denture resin at 8 hours.

Previous studies have tested the removal of *C. albicans* from a denture base using acetic acid/vinegar.^{16,17,20,23,24} However, there are no documented studies on the use of RV as a denture cleanser. In this study, RV (5.5%), WWV (6%) and ACV (5%) removed 100% of *C. albicans* ATCC 90028 and clinical *C. albicans* strains from denture acrylic resin plates at 8 hours but did not completely remove these strains at 30 minutes and 1 hour. This shows that longer soaking periods are required to remove *C. albicans* from acrylic dentures using these vinegars.

Table 1. Efficacy of different disinfecting solutions in the removal of *C. albicans* ATCC strain from denture acrylic resin plates.

	30 minutes				1 hour				8 hours			
	CFUs				CFUs				CFUs			
	Before Disinfection	After Disinfection	Removing Ability	P value	Before Disinfection	After Disinfection	Removing Ability	P value	Before Disinfection	After Disinfection	Removing Ability	P value
Apple cider vinegar		3483	89%	>0.1		1750	94%	>0.1		0	100%	>0.9999
White wine vinegar		2533	92%			883.33	97%			0	100%	
Rice vinegar		10100	83%			4783.33	91%			0	100%	
Chlorhexidine	48666	0	100%		48666	0	100%		48666	0	100%	
Water		4583.33	86%			5516.67	75%			11416.67	74%	0.0002
	30 minutes				1 hour				8 hours			
	CFUs				CFUs				CFUs			

ACV - Apple cider vinegar; WWV - White wine vinegar; RV - Rice vinegar; CHX - Chlorhexidine; H₂O; CFUs - Colony forming units

Table 2. Efficacy of different disinfecting solutions in the removal of *C. albicans* clinical strain from denture acrylic resin plates.

	30 minutes				1 hour				8 hours			
	CFUs				CFUs				CFUs			
	Before Disinfection	After Disinfection	Removing Ability	P value	Before Disinfection	After Disinfection	Removing Ability	P value	Before Disinfection	After Disinfection	Removing Ability	P value
Apple cider vinegar		10350	60%	>0.1		8633.33	66%	>0.3		0	100%	<0.05
White wine vinegar		4616.67	82%			7400	73%			0	100%	
Rice vinegar		5266.67	80%			8900	65%			0	100%	
Chlorhexidine	26000	116.67	99%		26000	233.33	99%		26000	0	100%	
Water		6100	77%			7583.33	71%			5650	78%	<0.05

ACV - Apple cider vinegar; WWV - White wine vinegar; RV - Rice vinegar; CHX - Chlorhexidine; H₂O - Water; CFUs - Colony forming units

There are no documented studies where the efficacy of RV against *C. albicans* on denture acrylic resin plates was tested. To the best of our knowledge, this is the first documented study where RV was tested and found to be effective as a denture cleanser. However, Choi, et al.²⁵ (2015) reported that dark RV has strong antioxidant and antimicrobial activity, and was more effective than carbenicillin (50 µg/ml) and tetracycline (50 µg/ml) in inhibiting growth of bacterial species.

Mota, et al.²⁰ (2014) reported 4% ACV to be fungicidal between 30-180 minutes, in contrast to the current study. They also found that ACV prevented *C. albicans* from adhering to the acrylic resin, and suggested that ACV could be used as an alternative therapeutic agent in denture stomatitis patients.

There are very few documented studies on the effectiveness of ACV in removing *C. albicans* from denture materials. However, using gel diffusion methods, ACV was effective against *C. albicans* after 24 hours' exposure.²⁶⁻²⁸

A study by Jafari, et al.¹⁷ (2012) showed that 5% and 10% white vinegar removed 99% and 100% of the *C. albicans* attached to acrylic plates respectively after 8 hours, confirming the efficacy of white vinegar, in agreement with the current study. Another study found that using a higher concentration (125ml/200ml) of vinegar was effective in removing *C. albicans* from acrylic plates in a shorter time (1 hour), while a lower concentration (75ml/200ml) took longer to remove *C. albicans* (8 hours).²⁹ Their results suggest that increasing vinegar concentration would shorten the efficacy time.

In contrast to our results, Pinto, et al.¹⁶ (2008) reported that 10% vinegar could not eliminate *C. albicans* from dentures and saliva of denture wearers after 45 days of overnight exposure, but reduced the amount (cfu/mL) of *Candida* species in the saliva.

Vinegar was ineffective in reducing *C. albicans* colonization on denture materials after 10 minutes' exposure,^{30,31} whilst 50% vinegar diluted in water was less effective than alkaline peroxide in removing *C. albicans* biofilms after 30 minutes.¹⁸ Kumar, et al.³² (2012) reported 4% and 50% vinegar to be less effective in removing *C. albicans* from acrylic resin after 8 hours exposure compared to commercial solutions, which is also in contrast to our study findings. Higher concentrations of vinegar (100%) with shorter exposure times were effective in removing *C. albicans* from dentures.^{23,24,33,34}

The results of the current study show that 5.5% RV, 6% WWV, and 5% ACV are effective (100% efficacy) and can be used as denture cleansers, allowing denture wearers to soak their dentures overnight, ready for use the next day. This will be beneficial for denture wearers, as all these vinegars are cheap and are available in every household around the world.

In the current study, 0.12% CHX was the most effective disinfectant in removing both tested *C. albicans* strains (99 - 100%) at 30 minutes, 1 hour, and 8 hours. This is not surprising as CHX is considered the gold standard agent for controlling dental plaque effectively.³⁵

Water could not completely remove either *C. albicans* strains at 30 minutes, 1 hour, and 8 hours. These results are in agreement with previous studies, where water was less effective in removing *C. albicans* from dentures³⁶⁻³⁸ confirming that water alone is not suitable for disinfection of dentures. The ability of water to reduce *C. albicans* from acrylic resin can be explained by that water is hypotonic as compared to the cellular contents of microorganisms, and this lead to osmotic pressure, causing the flow of water into the cells and disrupting the microorganisms.³⁹

The limitation of this study is that it was an *in vitro* study. Further studies are needed to confirm the efficacy of RV *in vivo*. In addition, the long term effects of RV on the denture acrylic requires further study to assess its effect on the mechanical strength of denture acrylic over time, amongst others. Further studies are needed to determine the duration of time or the number of times the same RV solution could be used, before it loses its effectiveness. Such a study will further confirm the cost-effectiveness of vinegar as a disinfectant.

CONCLUSION

Within the confines of this study, 5.5% RV was as effective as 5% ACV, 6% WWV and 0.12% CHX in removing *C. albicans* from dentures after overnight soaking. This is the first study where RV was tested and found effective as a denture cleanser.

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