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Research Article

Effectiveness of Reagent-Grade and Food-Grade Citric Acid as Denture Cleanser in Reducing *Candida albicans* Colonies

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Abstract

Candida albicans is an opportunistic pathogenic fungus commonly found in the oral cavity, with increased prevalence among denture wearers. Citric acid is known for its antimicrobial properties. Reagent-grade citric acid has been previously studied, while food-grade citric acid has not been investigated as a denture cleanser. This study, thus, aims to evaluate the effectiveness of reagent-grade and food-grade citric acid as denture cleansers in reducing *Candida albicans* colonies. The samples were heat-cured acrylic resin plates (n=28) contaminated with *Candida albicans* ATCC 10231 suspension, then soaked for 30 minutes in different solutions: reagent-grade and food-grade citric acid at concentration of 4%, 5%, 7%, and distilled water as negative control. *Candida* colonies on the plates were cultured, incubated, and counted using the Total Plate Count (TPC) method. Data were analyzed using Welch's ANOVA followed by post-hoc T-test. As a result, the average number of *Candida albicans* colonies in the reagent-grade citric acid groups (4%, 5%, 7%) were (2.15, 1.75, 0.29) $\times 10^4$ CFU/ml. In the food-grade citric acid groups (4%, 5%, 7%), the averages were (6.55, 4.27, and 3.17) $\times$ 10^4 CFU/ml. The distilled water group had the highest number of colonies (13×10^4 CFU/ml). The lowest reduction in colony count was in the 4% food-grade citric acid group (49.6%), while the highest reduction was in the 7% reagent-grade citric acid group (97.7%). Welch's ANOVA indicated a significant difference ($p<0.05$) among all treatment groups. Reagent-grade and food-grade citric acid as acrylic denture cleansers are effective in reducing *Candida albicans* colonies.

Keywords: *Candida albicans*; denture cleanser; food-grade citric acid; reagent-grade citric acid

INTRODUCTION

Polymethyl Methacrylate (PMMA) is widely utilized as a denture base material. PMMA has good aesthetics, low toxicity, low specific gravity (lightweight), non-irritation, affordability, and ease of manipulation and repair.[1,2] The denture base is in direct contact with saliva and food debris in the oral cavity, thus becoming a place of attachment for microorganisms.[3] Attachment of microorganisms to oral tissues and dentures contributes to the colony formation and the development of infections.[4] *Candida albicans* is the most frequent microorganism that causes infections in denture wearers.[5–7*]

Candida albicans is an opportunistic fungal pathogen and is commonly found as a commensal microorganism in the oral cavity of approximately 45-65% of healthy individuals. In denture wearers, its prevalence increases to 60-100%. According to Manikandan et al.,[8] *Candida*-associated denture stomatitis (CADS)

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affects approximately 65-70% of individuals who wear dentures. Denture stomatitis refers to inflammation of the oral mucosa that is in contact with dentures. Etiological factors contributing to denture stomatitis include trauma, allergies, poor oral hygiene, salivary pH, age, gender, smoking, immune system deficiency, and infection by *Candida* species.[9]

Denture cleaning methods are generally classified into mechanical and chemical approaches.[10] Mechanical methods include the use of toothbrushes and ultrasonic devices, whereas chemical methods involve soaking the denture in cleaning solutions.[11] Commercially available denture cleansers are often difficult to obtain and less affordable for the general population, especially those living in remote areas with low income.[12–14] Alternative materials that have been studied and demonstrated strong antimicrobial effects against *Candida albicans* and *S. aureus* include citric acid.[15–19] These findings support the potential of citric acid as an alternative denture cleanser.

Citric acid exhibits both bactericidal and bacteriostatic effects, and solutions containing it can be used as sterilizing agents.[20,21] Faot et al.[10] reported that citric acid effective in reducing the viability of *C. albicans*. Citric acid has also demonstrated effective disinfectant properties, with a 70% success rate in eliminating *Candida*.[22] Food-grade citric acid is a safe-to-consume type of citric acid that is more affordable and easy to find on the market.[23,24]

Studies on the use of reagent-grade citric acid as a denture cleaner against *Candida albicans* remain limited, and no study has been conducted on using food-grade citric acid as an acrylic denture cleanser. Therefore, this study aims to evaluate the effectiveness of reagent-grade and food-grade citric acid as a denture cleanser in reducing *Candida albicans* colonies.

MATERIALS AND METHODS

This study was conducted at the Dentistry Laboratory and Central Laboratory of Padjadjaran University from January to May 2024. A total of 28 acrylic resin (PMMA) plates were used as samples, with the number determined based on Federer's formula.

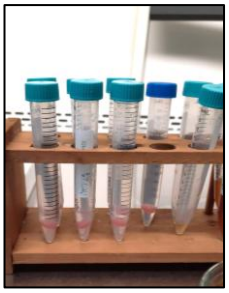

Figure 1. A total of 28 samples were divided into seven groups for treatment.

There were seven groups of samples, with each group containing four samples. The samples were soaked in 4%, 5%, and 7% reagent-grade citric acid (Sigma-Aldrich, Merck KGaA, Germany); 4%, 5%, and 7% food-grade citric acid (Cap Gajah, Golden Sinar Sakti, Indonesia); and distilled water as the control group. Shokri[25] reported that citric acid has an inhibitory effect on pathogenic fungi at concentrations ranging from 2.5-10%. The immersion duration for all samples was 30 minutes.

Heat-cured acrylic resin plate samples (ADM, England) were fabricated using circular wax patterns (10 mm in diameter, 2 mm in thickness).[10] The wax patterns were placed on top of the plaster-cast mixture in a dental flask. The dental flask was soaked in boiling water to eliminate the wax, creating a mold. The mold was smeared with a separation medium (cold mold seal), and acrylic resin material in the dough phase was packed into the dental flask.[22] The flask containing the

packed acrylic resin material underwent a curing process by immersion it in boiling water.[10,26] The acrylic plates were then sterilized using an autoclave and stored in a sterile environment until use.[16,27]

Candida albicans ATCC 10231 inoculum was subcultured onto fresh Sabouraud's Dextrose Agar (SDA) media and incubated under facultative anaerobic conditions using a desiccator at 37°C for 18-24 hours.[27,28] The culture was then refreshed in Sabouraud's Dextrose Broth (SDB) media for 18-24 hours at 37°C, yielding a suspension with turbidity adjusted to the McFarland standard.[27] *Candida albicans* colonies grown on SDB media were confirmed by Lactophenol Cotton Blue (LCB) staining, and their morphology, which appeared round to oval with the presence of germ tubes, was observed using light microscopy.

The contamination process of the acrylic plates with *Candida albicans* was carried out by immersing the plates in a petri dish containing a suspensions of *Candida albicans* ATCC 10231. The Petri dish was then incubated in a desiccator (37°C, 24 h).[27]

Solutions of reagent-grade and food-grade citric acid at concentrations of 4%, 5%, and 7% were prepared by dissolving 0.4 g, 0.5 g, and 0.7 g of citric acid in 10 ml of distilled water, respectively.

Acrylic resin samples contaminated with *Candida albicans* ATCC 10231 were immersed in the treatment solutions for 30 minutes. Afterwards, the samples were transferred into 10 ml of physiological NaCl and homogenized for 30-60 seconds. The physiological NaCl solution containing the samples was serially diluted up to a 10^{-3} dilution, and 1 ml of the final dilution was then inoculated onto SDA media using the Pour Plate method.

The colony count of *Candida albicans* grown on SDA media was measured using a colony counter (HiMedia LA 660, HiMedia Laboratories Pvt. Ltd., India) and expressed in Colony Forming Units (CFU/ml). *Candida albicans* colonies were observed to appear creamy white in color with a round or oval shape.[29] The colony counts were then analyzed to evaluate the effectiveness of each soaking solution.

Analysis of the data included the Shapiro-Wilk test for normality and Levene's test for homogeneity. Hypothesis testing was performed using Welch's ANOVA, followed by post hoc analysis with the T-test. Statistical analysis was conducted using SPSS Statistics version 29.0.2.0 (IBM, USA, 2023) with a significance level of 0.05. This study hypothesized that both reagent-grade and food-grade citric acid are effective in reducing the colony count of *Candida albicans* on acrylic plates.

RESULTS

Table 1 presents the colony counts of *Candida albicans* ATCC 10231 across all treatment groups.

Table 1. Colony count of *C. albicans* ATCC 10231

Immersion Groups	Mean ± St. Dev ($\times 10^4$ CFU/ml)	Colonies Reduction
RCA-4	2.15 ± 0.58	83.20 %
RCA-5	1.75 ± 0.51	86.20 %
RCA-7	0.29 ± 0.02	97.70 %
FCA-4	6.55 ± 1.62	49.60 %
FCA-5	4,27 ± 0.35	67.15 %
FCA-7	3.17 ± 0.22	75.57 %
C (-)	13.00 ± 1.68	0

RCA-4 : Reagent-grade citric acid 4%
RCA-5 : Reagent-grade citric acid 5%
RCA-7 : Reagent-grade citric acid 7%
FCA-4 : Food-grade citric acid 4%
FCA-5 : Food-grade citric acid 5%
FCA-7 : Food-grade citric acid 7%
C (-) : Negative control (distilled water)

Table 1 shows that the sample group soaked in reagent-grade citric acid had a lower mean colony count of *Candida albicans* compared to the food-grade citric acid group. The lowest reduction was

observed in the FCA-4 group (49.6%), while the highest reduction occurred in the RCA-7 group (97.7%). The normality test indicated that the data were normally distributed ($p>0.05$), and the homogeneity test demonstrated that the data were heterogeneous ($p<0.05$). Statistical tests were then continued using Welch's ANOVA due to the heterogeneity of variance.

Welch's ANOVA revealed significant differences in the mean *Candida albicans* colony counts across the seven treatment groups, supporting the effectiveness of both reagent-grade and food-grade citric acid as acrylic denture cleansers in reducing *C. albicans* ATCC 10231.

To determine the significance between treatment groups, the T-test results were analyzed and are presented in Table 2. A statistically significant difference ($p<0.05$) was observed between the distilled water group and both the reagent-grade and food-grade citric acid groups. Additionally, a significant difference was observed between the reagent-grade and food-grade citric acid group. In contrast, non-significant differences ($p>0.05$) were observed between the RCA-4 and RCA-5 groups, as well as between the FCA-4 and FCA-5 groups. These findings indicate that statistically significant difference reflect variations in the effectiveness of treatment groups in reducing *Candida albicans* ATCC 10231 colony counts, whereas non-significant difference suggest comparable effectiveness among the groups.

Table 2. The results of the T-Test

	RCA-4	**RCA-5**	**RCA-7**	**FCA-4**	**FCA-5**	**FCA-7**	**C (-)**
RCA-4							
RCA-5	0.344						
RCA-7	0.008	0.011					
FCA-4	0.008	0.007	0.005				
FCA-5	0.002	0.000364	0.000187	0.064			
FCA-7	0.032	0.007	0.000102	0.024	0.003		
C (-)	0.000404	0.000420	0.000632	0.002	0.001	0.001	

DISCUSSION

This study uncovered a significant difference in the *Candida albicans* colony count between the reagent-grade citric acid group and the food-grade citric acid group, with the reagent-grade group exhibiting a lower mean colony count. This difference may be attributed to the variations in chemical composition between the two types of citric acid. Reagent-grade citric acid has a very high purity level ($\geq 99.50\%$) and minimal impurities ($\leq 0.02\%$), whereas food-grade citric acid may contain additional substances compliant with food standards, which could explain the difference in effectiveness between reagent-grade and food-grade citric acid.[30,31]

The effectiveness of citric acid against *C. albicans* reported by Eliuz[15] differs from the findings of the present study. Eliuz reported that 3% citric acid exhibited 46.03% effectiveness, whereas in this study, 4% reagent-grade citric acid demonstrated an effectiveness of 83.2%. These differences may be attributed to variations in citric acid concentration and in the measurement methods employed. Eliuz[15] used an inhibition zone test, while the present study employed a colony count test method. Despite the methodological differences, Eliuz[15] concluded that citric acid was effective against *C. albicans*. Similarly, Sharma et al.[19] also reported that citric acid had a higher inhibitory activity

against *C. albicans* compared to other tested organic acids.

The results of this study demonstrated a reduction in *C. albicans* colony counts across all groups soaked in citric acid. These findings are consistent with those of Faot et al.,[10] who reported a decrease in *C. albicans* on acrylic plates soaked in a citric acid-based denture cleaner. Similarly, research by A. Mahmood et al.[16] showed that citric acid denture cleansers could significantly reduce the number of *C. albicans* colonies on heat-cured acrylic plates. Furthermore, a study by Nittla et al.[22] reported that citric acid at a concentration of 20% achieved a 70% success rate in eliminating *Candida.*

Several factors can influence antimicrobial activity, including pH, concentration, exposure time, and temperature.[32] This theory supports the findings of the present study, which showed that the citric acid becomes more effective at higher concentrations. Citric acid is one of the organic acids that disrupt microbial metabolism through undissociated citric acid molecules penetrating the cell membrane and release hydrogen ions.[33]

Moreover, the findings of this study indicate that both reagent-grade and food-grade citric acid are effective in reducing the colony counts of *C. albicans* on acrylic dentures, with effectiveness ranging from 49.6% to 97.7%. These results are supported by Izumi et al.[33] who reported a 65% reduction in *C. albicans* colony count when samples were treated with a 3% citric acid solution compared to untreated controls. Similarly, a study by Rajendran A. et al.[34] demonstrated a 67.6% reduction in colonies following the combined use of a citric acid-based denture cleanser and toothbrushing, concluding that this combination was effective in reducing *Candida* on dentures.[34]

In this study, reagent-grade and food-grade citric acid solutions at concentrations of 4%, 5%, and 7% demonstrated effectiveness values above 60%, except for the 4% food-grade citric acid solution, which showed the lowest effectiveness at 49.6%. The disinfection process is considered adequate if it eliminates 60-90% of microorganisms, reduces their number at room temperature, and is non-toxic.[35] Findings from Izumi et al.[33] and Rajendran et al.[34] further support this statement, reporting effectiveness values above 65%. The present study indicates that food-grade citric acid exhibited better effectiveness at concentrations of 5% and 7%.

A study by Izumi et al.[33] concluded that organic acid solutions with citric acid as the main component are not harmful and only slightly affect the surface roughness and color stability of denture base. Research by A. Mahmood et al.[16] also found that acrylic resin materials can be safely soaked in a 4% citric acid-based denture cleanser and other organic acids for 10 minutes without causing damage. In addition, research by Cakan et al.[36] stated that soaking acrylic plates in a citric acid-containing denture cleanser for 8 hours daily over 140 days resulted in lower surface roughness compared to three types of sodium perborate-based effervescent cleansers. These findings provide valuable insight into the effects of citric acid solutions on the mechanical properties of dentures.

Furthermore, both reagent-grade and food-grade citric acid have been proven effective in reducing the colony counts of *C. albicans*, making them potential candidates for use as acrylic denture cleansers. The limitation of this study is that the long-term effects of using reagent-grade and food-grade citric acid on acrylic resin materials remain unknown; therefore,

further research is necessary to explore this aspect. Additionally, this study only used heat-cured acrylic resin samples, so further research could be carried out to investigate the effects of reagent-grade and food-grade citric acid on other denture base materials, such as soft acrylic or metal frameworks.

CONCLUSION

Reagent-grade and food-grade citric acid solutions used as acrylic denture cleansers are effective in reducing the colony count of *Candida albicans*. Reagent-grade citric acid solutions exhibit greater effectiveness than food-grade citric acid solutions. This study demonstrates that higher citric acid concentrations enhance its effectiveness against *Candida albicans.*

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