Open Access

Original Article

Anti-Candida Efficacy of the Natural Products as a Denture Cleanser on Heat-polymerized Acrylic Resin – an in-Vitro Study

Osama Yasin, Saman Hakeem, Saba Farooq*, M.Iqbal Choudhary**

Department of Prosthodontic, Bahria University Medical & Dental College Karachi Pakistan, *Department of Virology, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi Pakistan, **Department of Organic Chemistry, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi Pakistan

ABSTRACT

Objective: To compare the anti-candidal activity of aloe vera and neem extracts with a sodium perborate-based commercial cleaning tablet on heat-polymerized acrylic resin.

Study Design: In-vitro experimental study

Place and Duration of Study: International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Pakistan from Dec 2024 to Jun 2025.

Methodology: An in-vitro experimental design was used with heat-polymerized acrylic resin (PMMA) discs treated with aloe vera, neem extracts, and a sodium perborate-based commercial denture cleanser as the positive control. Candida albicans biofilm formed on the discs, and the growth was measured by using optical density (OD) readings at a wavelength of 530 nm.

Results: There were significant differences were observed in anti-candidal activity between the positive control and neem extract (p value< 0.001), as well as between the positive control and Aloe vera (pvalue = 0.002). Moreover, a statistically significant difference among all tested Groups (*p* value< 0.001) was noted, with Candida inhibition percentage values following the order: positive control > Aloe vera > neem. However, no significant time-dependent differences in inhibition effectiveness were observed across the 30-minute, 2-hour, 4-hour, and 6-hour intervals (*p* value= 0.247).

Conclusion: Under laboratory conditions, aloe vera and neem extracts exhibited anti-candidal potential at the concentration of 0.1 mg/ml, and could serve as affordable, non-toxic alternatives to traditional denture cleansers.

Keywords: Acrylic resins, Candida albicans, Denture cleansers, Plant extracts, Polymethylmethacrylate,

How to Cite This Article: *Yasin O, Hakeem S, Farooq S, Choudhary MI. Anti-Candida Efficacy of the Natural Products as a Denture cleanser on heat-polymerized acrylic resin – An in-vitro Study. Pak Armed Forces Med J 2026; 76(Suppl-8): S1368-S1372.*
DOI: https://doi.org/10.51253/pafmj.v76i2.14041

INTRODUCTION

Candida-associated denture stomatitis (CADS), often referred to simply as denture stomatitis, is a widespread condition affecting an estimated 65%–70% of denture wearers worldwide, with a higher prevalence among geriatric individuals.[1,2] In Pakistan, denture stomatitis affects 14.9% of complete denture wearers, making it the second most common denture-related oral mucosal lesion, following traumatic ulcers (19.3%).[3]

Dentures can serve as reservoirs for infection, with surface irregularities potentially enhancing the retention of microorganisms even after cleaning.[1,2] To mitigate this, commercially available denture cleansers utilize either chemical or abrasive methods to effectively reduce microbial presence.[2] Formulations often contain sodium perborate or percarbonate, along with detergents such as trisodium phosphate, which release oxygen bubbles that help mechanically lift loosely attached debris.[3] Immersion cleansers should be used for at least 20 minutes, and ideally left to soak overnight when possible.[2,4]

Traditional products for cleaning dentures are expensive and have adverse effects, including staining and erosion of denture base materials.[4] Moreover, growing awareness of the diverse applications of natural products has positioned them as a popular alternative to synthetic denture cleansers.[5] They are safe, low in toxicity, and effective against candida biofilms.[6,7,8] Studies show that neem, aloe vera, triphala, and cashew leaves possess anti-candida properties with minimal toxicity.[7,8] A study comparing the synergistic effect of neem extract and tea tree oil with their individual treatments found that the combined treatment of neem extract and tea tree oil resulted in the lowest colony-forming units (CFU) of Candida albicans (0.40), followed by tea tree oil alone (2.30) and neem extract alone (30.33), demonstrating the most effective reduction in fungal growth.[9] In their study, Salim RH et al. found that aloe vera extract effectively inhibited Candida albicans and

Correspondence: Dr Osama Yasin, Department of Prosthodontic, Bahria University Medical & Dental College Karachi Pakistan
Received: 19 Nov 2025; revision received: 23 Jan 2026; accepted: 26 Jan 2026

Staphylococcus aureus when acrylic resin specimens were soaked for three minutes.[10] Although neem and aloe vera are well-documented for their antifungal and anti-inflammatory properties, but there is a notable gap in the literature regarding comparative studies that simultaneously evaluate their anti-candidal efficacy as denture cleansers. Furthermore, existing research lacks comprehensive evaluations of their antimicrobial effects over varying time intervals, using standardized methodologies. The comparison of these botanical extracts with commercially available denture cleaners has not been thoroughly examined.[10] Therefore, the rationale of this study was to provide evidence-based insights for denture wearers and healthcare professionals about using these readily available natural products as alternative denture cleaners. In this context, the investigation aimed to assess the comparative anti-candida activity of aloe vera and neem extracts with sodium perborate-based cleaning tablet on heat-polymerized acrylic resin.

METHODOLOGY

This laboratory-based study was conducted at the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi. A non-probability consecutive sampling technique was employed for sample selection. Ethical approval was obtained from the Institutional Ethics Committee of Bahria University of Health Sciences, Karachi, Pakistan under approval number BUHS-IRB#047/24. Data collection for the experiment was carried out between December 2024 and June 2025. Sample size estimation was done using G*Power version 3.1.9.2. F-test was applied for ANCOVA with these parameters: effect size was based on differences in Candida albicans adhesion (colony-forming units) = 0.4,9 α = 0.05, and power = 95%. The following formula to find the sample size:

$$n = \frac{\left(Z_{\frac{\alpha}{2}} + Z_{\beta}\right)^2 \cdot (\sigma^2)}{\Delta^2}$$

The estimated sample size was 162. This was divided equally into three Groups: Group A = positive control, Group B = Neem, and Group C = Aloe vera.

Inclusion Criteria: Heat cure polymethyl methacrylate (PMMA) resin discs with dimensions 15 mm × 15 mm and 1.5 mm thickness were included.

Exclusion Criteria: Acrylic discs with porosities, voids or processing errors were excluded.

A total of 162 heat-cured polymethyl methacrylate (PMMA) resin discs were made using a metal mold to replicate conventional acrylic dentures, using Pyrax Heat Cure Denture Base Acrylic Resin. Petroleum jelly was applied to the mold and glass slabs. Next, molten wax was poured into the cavity and allowed to solidify. Using traditional methods, the set wax was turned into heat-cured acrylic resin through flasking, wax removal, packing, and curing. The initial curing was done at 74 °C for 2 hours, followed by 100 °C for 1 hour. The discs were finished, and their dimensions were checked. Thirty defective discs were discarded, leaving 132 valid discs. These were then randomly divided into 3 Groups: 44 discs in each Group (positive control, neem, and aloe vera).

Yeast extract peptone dextrose (YEPD) medium was prepared in 1000 ml of distilled water following the manufacturer's protocol, and the pH was adjusted to 6.8. The medium was sterilized by autoclaving at 121 °C for 15 minutes. For culture preparation, the biofilm-forming strain of Candida albicans (ATCC 36082) was inoculated into sterile vials or Falcon tubes containing YEPD broth and incubated at 37 °C for 48 hours to obtain an active culture for experimentation.

Sterile acrylic resin samples were positioned in the wells of a 6-well tissue culture plate and coated with artificial human serum by dispensing 500 µl of protein solution into each well. The samples were incubated at 37 °C for 1 hour, after which the serum was aspirated and replaced with 50 µl of yeast suspension. To enable yeast adherence and colonization on the acrylic surfaces, the plates were incubated for an additional 2 hours at 37 °C. Finally, 2 ml of YPD medium was added to each well, and the samples were incubated for 144 hours (6 days).

After the 6-day incubation, the samples were prepared for yeast removal tests. Sterile acrylic resin samples were placed in six-well tissue culture plates, and 2 mL of cell culture medium was added to each well. The plates were incubated at 37°C for 6 days to allow the formation of mature candida albicans biofilms on the specimen surfaces. Following incubation, 100 µl of suspension from each well was transferred in triplicate to 96-well microplates. The optical density (OD) at 530 nm was measured using a Multiskan microplate spectrophotometer to standardize the cell concentration to approximately 1 × 10^6 cells/ml (0.5 McFarland standard).

For the positive control, one Fittydent® denture cleaning tablet was dissolved in 200 ml of distilled

water as per the manufacturer's instructions. Each herbal extract was prepared at a concentration of 0.2 mg/ml by dissolving 2 mg of the powdered sample in 10 ml of distilled water. For the experimental assay, 2 ml of the positive control solution, natural extract solution, and Candida albicans cell suspension (1×10^6 cells/mL) were mixed in a 1:1 ratio in the wells. The final concentration of the extract was half (0.1 mg/ml) of the prepared stock.

The antifungal effect was assessed by measuring OD at 530 nm at 30-minute, 2-hour, 4-hour, and 6-hour intervals post-treatment. All treatments and controls were carried out under identical conditions with 12 biological replicates per Group. The percentage inhibition of the cell suspension at each interval was calculated using the formula: Inhibition % = 100 - (OD sample / OD control) × 100. A higher inhibition percentage indicates a stronger antifungal effect.

Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software (version 23.0). The Shapiro-Wilk test showed a *p*-value greater than 0.05, confirming that the anti- candidal inhibition% values of positive control, neem and aloe vera followed a pattern of normal distribution. Continuous variables are expressed as mean ± standard deviation. To compare the inhibition efficiency between the positive control and the intervention Groups (aloe vera and neem), independent samples t-tests were used. A one-way ANOVA followed by Tukey's post-hoc test ranked the efficacy of all Groups and examined time-dependent inhibition efficiency. The *p*-value of ≤ 0.05 was considered significant.

RESULTS

Of the 162 heat-cured polymethyl methacrylate (PMMA) discs fabricated, 132 were included in the final analysis after exclusion of defective specimens. These specimens were evenly distributed into three Groups (n = 44 per Group): positive control, neem extract, and aloe vera extract. Regarding anti-candidal efficacy of experimental Groups, there was statistically significant difference noted between the positive control (Mean= 42.93 ± 8.01) and the neem extract infusion (Mean = 32.01 ± 3.93; *p* value = 0.001). Similarly, the anti-candidal activity of aloe vera extract (Mean = 37.82 ± 2.20) was significantly lower than that of the positive control (Mean 43.99 ± 5.47). The decreasing order of candida inhibition (%) was: positive control > aloe vera > neem (Table-I). When the samples were ranked according to their anti-candidal efficacy, a statistically significant difference was observed among all study Groups (Table-II).Differences were considered statistically significant at $p < 0.05$.

Table-I: Comparison of Anti-candidal Activity Among Experimental Groups (n=132)

Parameters	Positive control Mean + SD (n=44)	Neem Extracts Mean + SD (n=44)	Aloe vera Extracts Mean + SD (n=44)	*p*-value
Mean candida inhibition (%)	43.99 ± 5.47	32.01 ± 3.93	37.82 ± 2.20	$p <$ 0.001

Table-II: Pairwise Comparison of Anti-candidal Activity Among Experimental Group(n=44)

	Positive Control vs Neem	Positive Control vs Aloe vera	Neem vs Aloe vera
Mean candida inhibition (%)	$p < 0.001$	$p = 0.002$	$p = 0.004$

Although, there is maximum anti- candidal inhibitory effect observed after 2 hours. (Table-III), but there was no significant difference observed in the anti-candidal efficacy among all tested samples at the time intervals of 30 minutes, 2 hours, 4 hours, and 6 hours (*p*-value=0.247)(Table-IV).Differences were considered statistically significant at $p < 0.05$.

Table-III: Comparison of Time-Dependent Anti-candidal Activity Across Multiple Time Intervals (n=44 per Group)

Parameters	After 30 mins	After 2 hours	After 4 hours	After 6 hours	*p*-value
anti-candidal inhibition(%)	37.28+2.01	41.2+1.34	35.16+1.98	38.12+1.72	0.247

Table-IV: Pairwise Comparison of Time dependent Anti-candidal Activity

Pairwise time intervals	*p* -value
30mins Vs 2 hours	0.550
30mins Vs 4 hours	0.887
30mins Vs 6 hours	0.992
2 hours Vs 4 hours	0.190
2 hours Vs 6 hours	0.723
4 hours Vs 6 hours	0.747

DISCUSSION

The sodium perborate, a positive control, was most effective, followed by aloe vera and neem. These results partially agree with Ojah et al., who reported the greatest candida reduction with a commercial denture cleanser, followed by neem, triphala, and aloe vera.[12] Notably, both the commercial one and neem demonstrated significantly greater reductions compared to triphala and aloe vera ($p < 0.001$)[12]. Similarly, another study comparing neem tablets with commercial denture cleansers (Clinsodent) identified

as having the highest anti-candidal activity, followed by neem tablets.[13]Although all treatment Groups significantly reduced colony counts, but none of them eradicated biofilm completely, underscoring the need to pair chemical agents with mechanical cleaning for optimal denture hygiene[13] Sinha et al also revealed that sodium hypochlorite (positive control) and Propolis showed the highest antifungal effect.[14] Tea tree oil and turmeric had moderate efficacy, while neem showed limited activity, followed by saline (negative control).[14] This may be due to the variations in the experimental design, exposure times or in measuring techniques.

In contrast to the findings of the present study, some studies report superior efficacy of natural extracts over commercial denture cleansers. For instance, Hamid et al. found that incorporating neem powder into the denture base polymer during its fabrication led to a significant reduction in candida albicans count as compared to the negative control.[15] Similarly, Volety et al revealed that origanum oil(natural extract) exhibits the greatest antifungal activity (mean OD 0.072 ± 0.014), outperforming Fluconazole (0.094 ± 0.155), and suggested it as a viable, side-effect-free alternative to commercial denture cleansers.16 Moreover, the lytic activity of the denture cleansers increased over time, indicating a time-dependent effect on yeast lytic capacity, which contrasts with the findings of the present study, as there was no time-dependent anti-candidal activity, although there was noticed a peak effect at 2-hour interval.16 This difference may be attributed to the longer exposure time of 8 hours used in their study.[16] Additionally, variations in measurement techniques, such as using optical density at 595 nm, could also account for the discrepancy in antifungal efficacy results.

The findings of the present study are partially consistent with those of Memon et al. (2024), who evaluated the comparative efficacy of chitosan and aloe vera on soft-lining samples.[17] In their study, aloe vera significantly reduced candida adherence compared to the control Group, with aloe vera (41.15 CFU) outperforming chitosan (16.05 CFU).[17] The observed differences in results could be attributed to the variations in candida measurement techniques, extract concentrations, and preparation methods. Berteina *et al.,* and Wylie *et al.,* also documented that neem (rich in compounds such as azadirachtin) exhibits significant antimicrobial and anti-inflammatory effects, while aloe vera is widely recognized for soothing properties alongside antifungal activity.[18,19]

In line with the studies mentioned, Mahajan *et al*. broadened the scope of their investigation to assess the anti-candidal efficacy of Homeopathic (Sepia 200CH/30CH), Ayurvedic (Neem extract), commercial denture cleanser (Densive), and antifungal drug (Fluconazole) on acrylic resin. They found that all tested agents significantly reduced Candida albicans biofilm formation against negative control. However, antifungal drug(Fluconazole) exhibited the highest level of anti- candidal efficacy among all the tested agents.[20] Although the highest mean candidal inhibition appeared at the 2-hour interval, the differences in anti-candidal efficacy among the 30-minute, 2-hour, 4-hour, and 6-hour time points were not statistically significant. Mahajan *et al.,* reported that the lytic effect of denture cleansers accelerated with longer exposure, indicating that time plays a significant role in enhancing their yeast-lytic activity.[20] Polat *et al* also observed that there is a variability in the time-dependent antifungal activity with Corega(commercial denture cleanser) and 15% vinegar showing rapid action against fungal adherence within minutes, whereas other commercial denture cleansers, such as Listerine, Protefix, and natural product(tea tree oil), showed a longer time duration for action, particularly on polyamide.[21]

The strengths of the study include the time-dependent anti-candidal evaluation that provides insights into the treatment duration, while robust statistical analysis further strengthens these results. Additionally, the use of optimal density in Candida albicans biofilm quantification ensures accurate and consistent assessment, minimizing variability and enabling reliable comparisons.

Thus, findings of the undertaken study show that aloe vera and neem do counter candida growth, but their effectiveness is lower than that of the positive control under laboratory conditions. Some studies agree with this, while others say these natural extracts work better. It seems how well antifungal performance depends heavily on factors such as concentration, exposure time, and measurement methods. Because of this, we need to figure out the best way to optimize these parameters and make sure they're consistent before we can say they are the better replacements for commercial denture cleaners.

LIMITATION OF THE STUDY

One limitation of the study is its in-vitro design, which may not accurately reflect real-world clinical conditions and the focus on Candida albicans, omitting other potentially relevant microorganisms. Additionally, the use of a single concentration of the natural extracts limits the scope of the findings.

CONCLUSION

Under laboratory conditions, aloe vera and neem extracts exhibited anti-candidal potential at the concentration of 0.1 mg/ml. There was revealed significant differences in anti-candidal efficacy among all Groups (p=0.000), with the positive control exhibiting the highest inhibitory activity, followed by aloe vera and neem.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

OY & SH: Data acquisition, data analysis, critical review, approval of the final version to be published.

SF & MIC: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Manikandan S, Vinesh E, Selvi DT, Kannan RK, Jayakumar A, Dinakaran J. Prevalence of Candida among denture wearers and nondenture wearers. J Pharm Bioall Sci 2022; 14(1): S702-705. https://doi.org/ 10.4103/jpbs.jpbs_781_21
2. Mudgade DK, Motghare P, Priyadarshani Rustum Sarkate DP, Mudgade DK. A comparative evaluation of antimicrobial efficacy of commercially available three types of denture cleanser. Int J LifeSci Biotechnol Pharma Res.2025; 14(2): 1124-1126. https://doi.org/ 10.69605/ijlbpr_14.2.2025.202
3. Saeed S, Ali H, Akhtar I. Frequency and association of various risk factors of oral mucosal lesions among complete denture wearers. J Gandhara Med Dent Sci 2026; 13(1): 78-82. http://doi.org/10.37762/jgmds.13-1.779
4. Salem SAL, Al-Khafaji AMA. The effect of prepared denture cleansers on some physical properties of stained acrylic resin denture base material cured by two different techniques. J Baghdad Coll Dent 2006; 18(2): 1-8.
5. Khurana P, Singhal R, Agarwal SK, Kalpana K. Comparative evaluation of the effect of two plant extract and denture cleanser on the staining and anti-fungal efficacy of denture base resin: An in vitro study. J Dent Oral Biol 2022; 7(3): 1-7.
6. Bhaiyana S, Puri A, Nangia R, Bhat N. Antibacterial effect of aloe vera gel against plaque and caries bacteria: an in-vitro study. Int J Health Sci Res 2019; 2: 21-25.
7. Shetty PJ, Hegde V, Gomes L. Anticandidal efficacy of denture cleansing tablet, Triphala, Aloe vera, and Cashewleaf on complete dentures of institutionalized elderly. J Ayurveda Integr Med 2014 ; 5(1): 11-14. https://doi.org/10.4103/0975-9476.128847
8. Barua DR, Basavanna JM, Varghese RK. Efficacy of neem extract and three antimicrobial agents incorporated into tissue conditioner in inhibiting the growth of C. albicans and S. mutans. J Clin Diagn Res 2017; 11(5): ZC97. https://doi.org/10.7860/JCDR/2017/23784.9950
9. Singhania A, Sathe S, Ranka R, Godbole S, Ranka RK, Dubey SA. Individual and Synergistic Effects of Tea Tree Oil and Neem Extract on Candida albicans Adhesion to Denture Soft Liner. Cureus 2022; 14(8): e27869. https://doi.org/10.7759/cureus.27869
10. Salim RH, Salloum AM, Alsalameh SA, Khazem MR, Hajeer MY, Salloum AM, et al. Antimicrobial properties of aloe vera ethanol extract as a denture disinfectant: An in vitro study. Cureus. 2024; 16(5): 59916. https://doi.org/ 10.7759/cureus.59916
11. Tauseef S, Tauseef F, Siddiqui K. Antifungal Potential of Leaves and Barks of Azadirachta indica: Antifungal Potential of Neem Tree. Pak J Sci Indust Res 2024; 67(1): 8-14. https://doi.org/10.52763/PJSIR.BIOL.SCI.67.1.2024.8.14
12. Ojah P, Luniyal C, Nair C, Astekar M, Pal A, Chopra M. Anti candidal efficacy of commercially available triphala, neem, denture cleanser and natural aloevera leaf on heat polymerized acrylic resin. J Indian Prosthodont Soc 2021; 21(2): 167-172. https://doi.org/ 10.4103/jips.jips_599_20
13. Sharma C, Makhija A, Yadav B, Singh A, Shetty O. A Comparative Evaluation of Anti-candidal Efficacy of Commercially Available Neem Tablets and Denture Cleansers on Complete Dentures: An In Vivo Study. World J Dent 2024; 15(1): 13-18. https://doi.org 10.5005/jp-journals-10015-2355
14. Sinha DJ, Vasudeva A, Gowhar O, Garg P, Sinha A, Prakash P. Comparison of antimicrobial efficacy of propolis, Azadirachta indica (Neem), Melaleuca alternifolia (Tea tree oil), Curcuma longa (Turmeric) and 5% sodium hypochlorite on Candida albicans biofilm formed on tooth substrate: an in-vitro study. J Pharm Biomed Sci. 2015; 5(6): 469-474.
15. Hamid SK, Al-Dubayan AH, Al-Awami H, Khan SQ, Gad MM. In vitro assessment of the antifungal effects of neem powder added to polymethyl methacrylate denture base material. J Clin Exp Dent 2019; 11: e170–e178. http://doi.org 10.4317/jced.55458
16. Volety S, Shetty PP, Shetty G. Antifungal effects of herbal extracts and fluconazole on heat-polymerized acrylic denture base resin as denture cleanser: An in vitro study. J Contemp Dent Pract 2021; 22(2): 162-165. https://doi.org 10.5005/jp-journals-10024-3042
17. Memon MR, Memon H, Shoro M, Bhurgri H, Issrani R, Iqbal A, et al. Effectiveness of Chitosan versus Natural Aloe Vera on Candida Adherence in Denture Soft Lining Material. Scientifica 2024; 2024(1): 9918914. https://doi.org 10.1155/2024/9918914
18. Berteina-Raboin S. Comprehensive overview of antibacterial drugs and natural antibacterial compounds found in food plants. Antibiotics 2025; 14(2): 185. https://doi.org.3390/antibiotics14020185
19. Wylie MR, Merrell DS. The antimicrobial potential of the neem tree Azadirachta indica. Front Pharmacol 2022; 13: 891535. https://doi.org.10.3389/fphar.2022.891535
20. Mahajan S, Indrakumar HS, Nirwan AS, Jain S, Kaushal P, Jain S. A Comparative evaluation of the efficacy of Homeopathic (Sepia 200 CH/Sepia 30CH), Ayurvedic (Neem extract), Commercial denture cleanser (Densive) and Antifungal drugs (Fluconazole) against Candida Albicans biofilm on heat cure acrylic resin-an in vitro study. J Prosthodont 2024; 19(1): 18-33.
21. Polat Sagsoz N, Orhan F, Baris O, Sagsoz O. In Vitro Evaluation of Plant Antimicrobials Against Candida albicans Biofilm on Denture Base Materials: A Comparison with Chemical Denture Cleansers. Polymers 2025; 17(21): 2869. https://doi.org 10.3390/polym17212869