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Probiotics and *Aloe vera* as eco-conscious fungal barriers in dentures: Original study

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Abstract

Introduction: Edentulism is among the most prevalent oral health issues affecting humans, and dentures remain the most common treatment option. Natural substances and essential oils have shown great potential as therapeutic agents against oral infections. With growing awareness of the benefits of natural products, they are increasingly being considered as alternatives to synthetic materials. Hence, this study was designed to evaluate and compare the antifungal properties of *Aloe vera* and probiotics on heat-cured polymethyl methacrylate (PMMA) resin.

Settings and Design: *In vitro* - experimental study.

Aim: To evaluate and compare the effects of probiotics and *Aloe vera* extract on the adherence of *Candida* species to heat-cured denture base resin made of polymethyl methacrylate.

Materials and Methods: A total of specimens were carefully prepared using modeling wax to form discs measuring 10 mm × 10 mm with a thickness of 2 mm. These were divided into four groups:

- **Group A:** *Aloe vera* solution
- **Group B:** Probiotic solution
- **Group C:** Combination of probiotics and *Aloe vera* gel in equal proportions
- **Group D:** Control group

All specimens were immersed in their respective solutions for 15 minutes at 37 °C.

Results: A significant reduction in *Candida* cell counts on PMMA surfaces was observed following treatment with both probiotic and *Aloe vera* solutions. Heat-cured denture base materials demonstrated a notable decrease in *Candida* adhesion after exposure to these natural agents.

Keywords: *Candida albicans*, denture cleansers, *Aloe vera*, probiotics, denture base resins

1. Introduction

Oral hygiene has long been recognized as a vital component of overall health and well-being. However, among elderly individuals, there is often insufficient awareness about proper oral hygiene practices and their management. One common condition affecting this population is denture-related stomatitis also referred to as *Candida*-associated denture-induced stomatitis which is characterized by mild inflammation and redness of the oral mucosa beneath dentures. The prevalence of *Candida* among denture wearers is estimated to range between 60% and 100%.

Denture cleansers are generally classified into two categories: chemical and natural. While chemical cleansers can be effective, they may also cause undesirable side effects. In contrast, natural cleansers such as *Aloe vera* offer several advantages they are effective, easily available, and cost-efficient. *Aloe vera*, often called the “lily of the desert,” is one of the oldest known medicinal plants and belongs to the Liliaceae family. The word *aloe* originates from the Arabic term “*Alloeh*,” meaning “shining bitter substance,” while “*vera*” is Latin for “true.” *Aloe vera* gel possesses significant antimicrobial properties, making it a promising agent for use as a natural denture cleanser.

Probiotics defined as live microorganisms that, when administered in adequate amounts, confer health benefits to the host also play a crucial role in maintaining oral and gut health. Probiotic bacteria exert competitive, antagonistic, and immunological effects against pathogenic microbes. Oral candidiasis typically arises from dysbiosis, or an imbalance in the

normal microbiota, leading to overgrowth of *Candida* species. Probiotics help restore microbial balance by promoting the growth of beneficial bacteria, which in turn enhance the body's natural defense mechanisms.

Studies have shown that certain probiotic strains, such as *Lactobacillus paracasei* and *Lactobacillus rhamnosus* (isolated from the oral cavities of healthy individuals), inhibit *Candida albicans* and *Streptococcus mutans* through the production of organic acids, hydrogen peroxide, and bacteriocins. Similarly, commercial probiotic formulations containing *Lactobacillus plantarum* and *Lactobacillus reuteri* have demonstrated strong *in vitro* inhibition of *C. albicans*, primarily due to low pH and hydrogen peroxide production. Moreover, *in vivo* studies have revealed that probiotic-enriched foods such as cheese containing *L. rhamnosus* GG, *L. rhamnosus* LC705, and *Propionibacterium freudenreichii* ssp. *shermanii* JS can significantly reduce *Candida* prevalence in elderly individuals.

Given the mounting evidence supporting the protective and therapeutic roles of probiotics on mucosal surfaces, further research is warranted to explore their applications, formulations, and delivery methods for the prevention and management of oral diseases.

Material and method: For this study, 40 samples of heat-cured acrylic resin were fabricated, each measuring 10 mm × 10 mm × 2 mm. The samples were then divided into four groups as follows:

- **Group A:** *Aloe vera* solution
- **Group B:** Probiotic solution
- **Group C:** Combination of probiotics and *Aloe vera* gel in an equal ratio
- **Group D:** Control group

Preparation of PMMA Specimens

Using a modeling wax sheet, test samples were prepared by precisely cutting the wax into pieces measuring 10 mm × 10 mm × 2 mm (Fig. 01). This procedure was repeated until a total of 40 wax patterns were obtained. The wax samples were then flaked, and polymethyl methacrylate (PMMA) heat-cured denture resin (DPI, Mumbai, India) was used for packing the molds (Fig. 02).

Following the manufacturer's instructions, the resin samples were fabricated by mixing the polymer powder and monomer liquid in the recommended proportions. The flasks were then secured in a dental clamp for 30 minutes before undergoing polymerization in an acrylizer unit (Confident Company, India). A long curing cycle at 70 °C for 9 hours was employed to minimize residual monomer content and ensure good transparency of the final material.

After polymerization, the cured resin specimens were finished and polished using standard laboratory techniques in accordance with the manufacturer's guidelines.

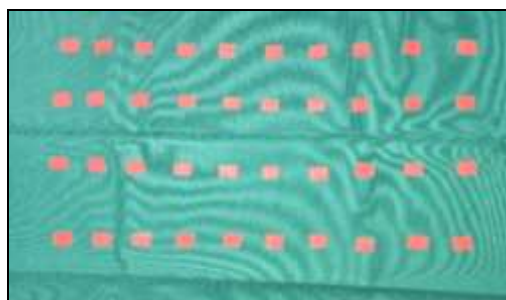


Fig 1: Preparation of wax samples (10 mm × 10 mm × 2 mm) for testing.



Fig 2: Flasking and packing of molds with heat-cured PMMA denture resin.

Preparation of Aloe vera solution

Fresh *Aloe vera* leaves were thoroughly washed with distilled water to eliminate any surface impurities. The leaves were then cut open, and the inner gel (pulp) was carefully extracted, as shown in Figure 03. The collected gel was dried in a hot air oven at 80 °C to obtain it in powdered form.

Subsequently, 25 grams of the dried *Aloe vera* powder was dissolved in 100 mL of distilled water to prepare the extract. The resulting solution was then subjected to rotary evaporation to concentrate the active components, as illustrated in Figure 04.



Fig 3: Extraction of inner gel (pulp) from freshly cut *Aloe vera* leaves.



Fig 4: Concentration of *Aloe vera* extract using rotary evaporation.

Preparation of probiotic solution

A commercially available probiotic powder was used to prepare the test solution. A total of 25 grams of the probiotic powder was accurately weighed and dissolved in 100 mL of distilled water. The mixture was stirred continuously to ensure complete dissolution of the powder. Thorough mixing was performed to obtain a uniform and homogeneous solution, as shown in Figure 05.

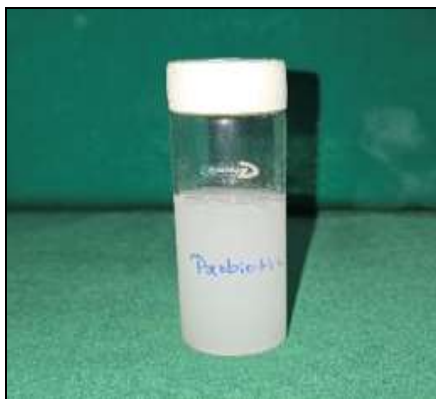


Fig 5: Preparation of probiotic test solution by dissolving and mixing probiotic powder in distilled water.

Preparation of probiotic and Aloe vera mixture solution

Equal quantities of commercially available probiotic powder and freshly prepared *Aloe vera* powder were combined in a 1:1 ratio. The powders were accurately weighed to maintain uniformity and then added to an appropriate volume of distilled water. The mixture was stirred thoroughly to obtain a homogeneous solution, ensuring complete dissolution of both components. (Figure 06)

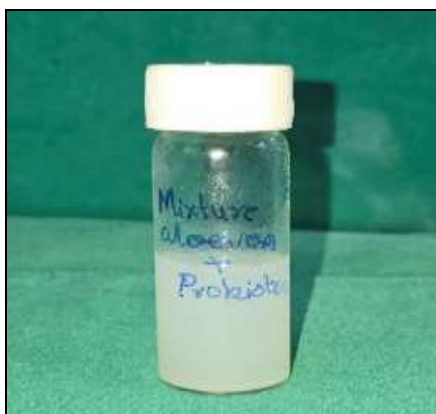


Fig 6: Preparation of *Aloe vera*-probiotic mixture.

Evaluating the adherence of *Candida albicans*

The prepared acrylic discs were incubated with *Candida albicans* strains that had been isolated from patients diagnosed with candidiasis. Each disc was carefully placed into a sterile test tube containing Brain Heart Infusion (BHI) broth inoculated with the *Candida* culture, as shown in Figure 07. The test tubes were then incubated at 37 °C for 24 hours to facilitate microbial interaction and colonization, as illustrated in Figure 08.

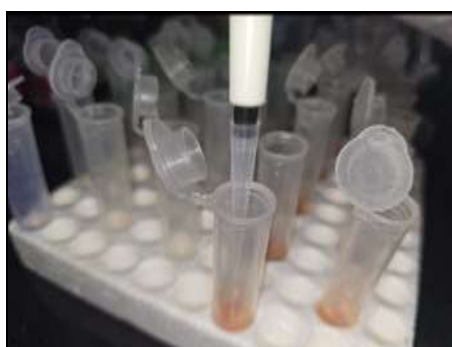


Fig 7: Inoculation of acrylic discs with *Candida albicans*.

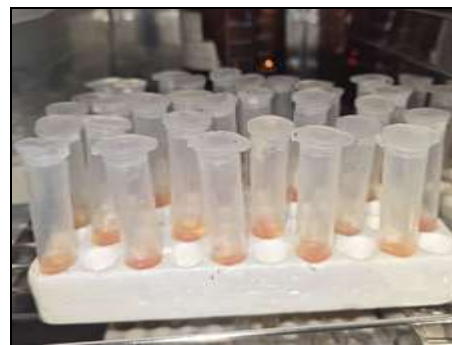


Fig 8: Incubation of inoculated samples at 37 °C for 24 hours.

After 24 hours of incubation, the sample discs were carefully washed and vortexed in distilled saline to remove any loosely adhered *Candida albicans* cells. Following this, ten samples from each group were treated with the respective solutions *Aloe vera* solution (Figure 09), probiotic solution (Figure 10), and the mixture of both (Figure 11) for a period of 15 minutes.

After treatment, the discs were again washed and vortexed in distilled saline to remove any residual solution and non-adherent fungal cells. This step ensured uniform exposure to the test agents and effective removal of surface contaminants prior to further microbiological analysis.



Fig 9: Treatment of samples with *Aloe vera* solution.



Fig 10: Treatment of samples with probiotic solution.



Fig 11: Treatment of samples with *Aloe vera*-probiotic mixture.

After the final vortexing, the resulting solutions were utilized for smear testing under strict sterile conditions (Figure 12). The solutions were aseptically smeared onto sterile Petri dishes (Figure 13), which had been pre-divided into four experimental groups Group A, Group B, Group C, and Group D (Figure 14).

Throughout the procedure, strict aseptic techniques were meticulously maintained to prevent contamination. The inoculated Petri dishes were then incubated at 37 °C for 24

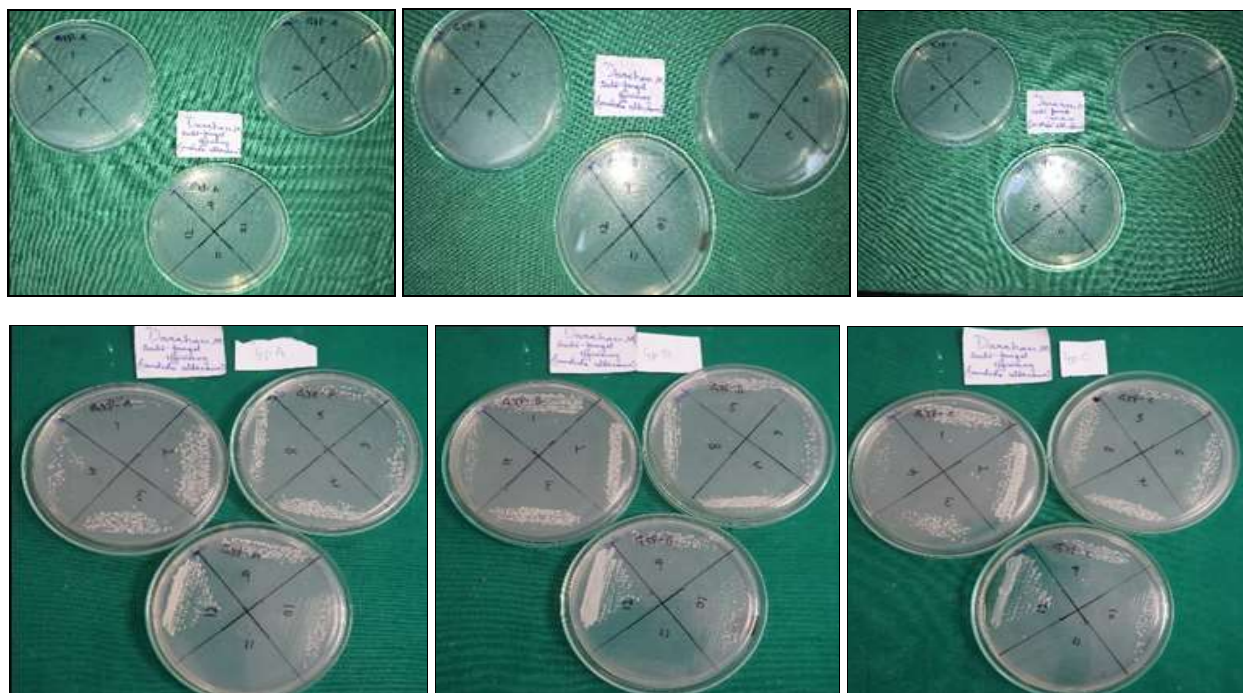
hours to facilitate fungal colony growth. After incubation, the number of *Candida* colonies was counted and recorded for quantitative analysis.



Fig 12: Preparation of samples for smear testing.



Fig 13: Smearing of samples onto sterile Petri dishes.



After 24hr incubation

Fig 14: Division of Petri dishes into experimental groups.

Results

Table 1: Colony Forming Units (CFU) Count for Different Groups

Samples	Group A	Group B	Group C	Group D
01	22	110	118	286
02	93	92	112	254
03	81	64	55	214
04	45	50	39	228
05	44	69	34	-
06	51	31	54	-
07	68	79	86	-
08	56	85	76	-
09	62	47	67	-
10	37	23	39	-
11 (Control Extract)	NG	NG	NG	-
12 (Organism)	210	257	207	-

Descriptive Statistics

Table 2: Descriptive table

Group	N	Mean CFU	Standard Deviation (SD)	Median	Range (Min-Max)
Group A (Aloe Vera)	10	55.9	21.6	53.5	22-93
Group B (Probiotics)	10	65.0	29.2	66.5	23-110
Group C (Mixture)	10	63.9	29.2	60.5	34-118
Group D (Control)	10	245.5	32.7	241.0	214-286

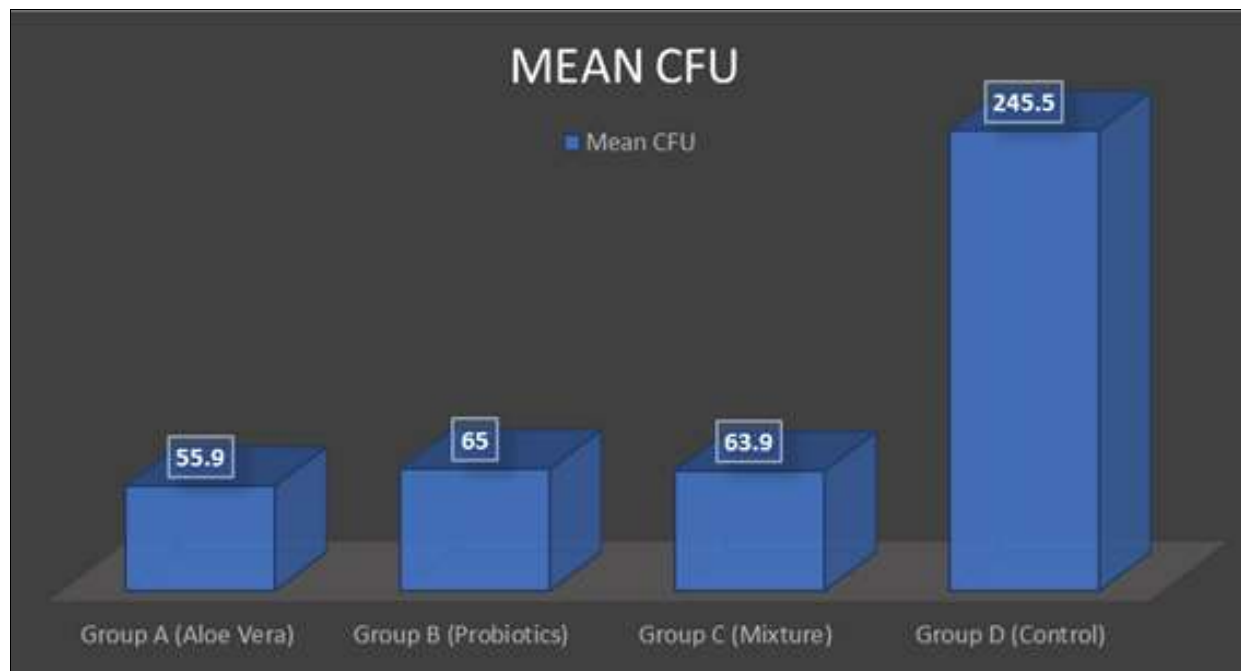


Table 2: One-Way ANOVA Results (Comparison of Mean CFU Across Groups)

Source	Sum of Squares	df	Mean Square	F-value	p-value
Between Groups	156,892.4	3	52,297.5	78.63	<0.001
Within Groups	21,288.6	32	665.3	-	-
Total	178,180.9	35	-	-	-

Table 3: Post-Hoc Tukey HSD Test (Pairwise Comparisons)

Comparison	Mean Difference (CFU)	95% CI	p-value
Group D vs. Group A	189.6	(153.2, 226.0)	<0.001
Group D vs. Group B	180.5	(144.1, 216.9)	<0.001
Group D vs. Group C	181.6	(145.2, 218.0)	<0.001
Group A vs. Group B	-9.1	(-42.5, 24.3)	0.891
Group A vs. Group C	-8.0	(-41.4, 25.4)	0.925
Group B vs. Group C	1.1	(-32.3, 34.5)	0.999

Discussion

The use of natural products as antimicrobial agents offers several advantages over synthetic antibiotics, whether systemic or local. Natural compounds are not only effective as bactericidal and fungicidal agents, but they are also readily available, biocompatible, and cost-effective^[12].

Among these, Aloe vera is well known for its diverse medicinal properties and is regarded as one of the richest natural plants for promoting health^[13]. In this study, Aloe vera (*Aloe barbadensis*) powder was utilized. The powder, obtained through spray-drying the juice extracted from the plant's leaves, was chosen because the whole leaf extract which includes both the gel and the latex contains bioactive antifungal components beneficial for the study.

In critically ill patients, prophylactic management of *Candida* infections is typically achieved using imidazole derivatives or nystatin administered orally. Recent reviews of these treatments have confirmed their effectiveness in reducing invasive *Candida* infections. However, despite their efficacy, both systemic and topical antifungal agents are associated with adverse effects^[14]. Additionally, the emergence of antifungal-resistant yeast strains has highlighted the urgent need for alternative or adjunctive antifungal therapies^[15].

Several studies investigating probiotics against oral *Candida* have been conducted, primarily in asymptomatic or mildly symptomatic individuals. These studies indicate that

probiotics, while not a standalone treatment for *Candida* infections, can serve as a valuable adjuvant when used alongside conventional antifungal therapies, enhancing their overall effectiveness.

Conclusion

The study was conducted with the objective of evaluating the effect of probiotics and *Aloe vera* on the adherence of *Candida* species to heat-cured denture base material. Based on the findings, the following conclusions can be drawn:

- Reduction in *Candida albicans* count:** Treatment of denture base resin (PMMA) with probiotic and *Aloe vera* solutions resulted in a noticeable decrease in *Candida albicans* colony count compared to untreated specimens.
- Comparable efficacy among treatments:** There was no significant difference observed among the three test groups ($p > 0.89$), indicating that probiotic, *Aloe vera*, and their combination exhibited similar antifungal effectiveness.
- Potential as natural alternatives:** Due to their availability, cost-effectiveness, and numerous therapeutic benefits, natural agents such as *Aloe vera* represent promising alternatives to conventional denture cleansing tablets and mouthwashes. It is therefore imperative that

further research on medicinal plant extracts especially those with unexplored antimicrobial properties be prioritized, particularly in developing countries, where access to expensive pharmaceutical products is often limited.

Limitation

- However, this study has certain limitations, primarily due to the small sample size and specific selection criteria, which may limit the generalizability of the findings.
- The experiment was conducted under controlled laboratory conditions, which may not fully replicate the oral environment.
- Multiple external factors, such as temperature variations, salivary composition, and patient hygiene habits, may influence the results in clinical settings.
- The study was performed over a 24-hour observation period, providing only a short-term assessment of antifungal activity.
- The surface roughness of the heat-cured acrylic resin could have influenced the adherence of *Candida albicans*, potentially affecting the accuracy of colony counts.
- Future research should aim to evaluate the long-term efficacy of probiotics and *Aloe vera* under *in vivo* conditions, using larger sample sizes and extended observation periods to validate their potential as natural antifungal agents for denture hygiene.

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