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Incorporation of Striga Orobanchioides Extract in Denture Adhesives & Its Antifungal Effect on Acrylic Resins: An Invitro Study

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KEYWORDS

Denture Stomatitis, Candida Albicans, Denture Adhesive, Striga Orobanchioides, Antifungal Activity, Biofilm Inhibition, Acrylic Denture Base, Color stability, Spectrophotometer.

ABSTRACT:

Introduction: Denture stomatitis is a common condition affecting removable denture wearers, primarily caused by Candida albicans colonization and biofilm formation on the denture base. The prolonged use of denture adhesives, while beneficial for retention and stability, may alter oral microflora and increase susceptibility to fungal infections.

Aim: This study evaluates the antifungal efficacy of Striga orobanchioides extract-based denture adhesive in preventing C. albicans adhesion and biofilm formation on acrylic denture bases.

Material and methodology: Ethanolic extracts of Striga orobanchioides were incorporated into a denture adhesive formulation. The antifungal activity was assessed using microbiological assays to determine the inhibition of C. albicans growth and biofilm formation. The color stability was also evaluated to ensure clinical applicability.

Results: Findings suggest that Striga orobanchioides extract exhibits significant antifungal activity against C. albicans, reducing its adhesion to the denture surface.

Conclusion: The incorporation of Striga orobanchioides extract into denture adhesives demonstrates promising antifungal potential, offering a novel approach to managing denture stomatitis. Further clinical studies are required to confirm its long-term efficacy and safety for routine use.

INTRODUCTION

With the prevalence of tooth loss & increase in the aging population, there is a growing need for oral rehabilitation, mostly commonly done by removable complete dentures. Success and acceptance of complete dentures by the patient depends upon various factors, one of which is retention. A well-retentive denture prevents displacement during speech and eating, minimizing discomfort and psychological distress. Effective retention enhances patient satisfaction and acceptance of the prosthesis [1]. Denture adhesives are commonly used for improving the retention of removable complete dentures [2].

Factors related to use of dentures include poor fitting, which exacerbate oral mucosal trauma and irritation, long term use of dentures, lack of appropriate denture care and hygiene, the presence of pathogenic microbial infection (primarily *C. albicans*), and continual wearing of dentures [3].

C. albicans has been identified as the major causative microbial agent in denture stomatitis, and plays a major role in geriatric patients with poor oral hygiene & patients with physical or mental disabilities [4]. An essential event in the initiation of denture stomatitis is the adhesion of *C. albicans* to the denture base. Adhesion of *C. albicans* facilitates the formation of a mature biofilm on denture surfaces, which can lead to denture stomatitis under favorable conditions. Controlling *C. albicans* adhesion and biofilm formation on the denture base is essential for preventing denture stomatitis [5].

Denture adhesives play a valuable role in patient care by improving denture stability, enhancing functionality, and boosting patient confidence [6]. The long-term use of adhesives alters the oral microflora or predisposes the individual to oral candidiasis. Effective management of denture stomatitis requires treating the oral tissues and the denture itself. Incorporating anti-Candida agents into denture adhesives may help prevent *C. albicans*

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colonization on removable prostheses, thereby reducing inflammation and the severity of stomatitis [5].

Medicinal plants have proven to be effective as a source of compounds in the treatment of many diseases and disorders. They have been used extensively as pure compounds or as crude material.

Striga genus comprises approximately 30 species of parasitic herbs. These plants are typically scabrous in texture and tend to become discoloured or black upon drying. Among them, *Striga orobanchioides Benth.* is known for its medicinal properties, particularly its use in diabetes management. Petroleum ether extracts and ethanolic extracts of *S. densiflora Benth* and *S. orobanchioides benth* have been approved for their pharmacological activities against pathogenic and nonpathogenic bacteria [7]. All these might effectively approve the intense traditional uses of the genus Striga as pharmaceutical agents for many diseases [8].

Therefore, the aim of the study was to evaluate the antifungal efficacy and staining ability *of striga orobanchioides* extract-based denture adhesive on the surface of acrylic denture base.

MATERIALS AND METHODS

Study Design – An Experimental in vitro **s**tudy was conducted in the Department of Prosthodontics, Crown & Bridge, and Implantology at Rural Dental College, Department of Microbiology, RMC and Pravara Rural College of Pharmacy, Loni. The study was approved by the Institutional Ethical Committee of Pravara Institute of Medical Sciences. (IEC PIMS DU)

PREPARATION OF STRIGA OROBANCHIOIDES EXTRACT:

The extract was prepared in the Pravara Rural College of Pharmacy, Loni in which the *S. orobanchioides* plant was dried for 3 days in a shed and then powdered and collected in round bottom flask. Absolute alcohol was then added to the dried powder in concentration of 300 grams /300ml (weight by volume). Reflux extraction technique was used for 3 hours and after filtration extract was collected (Figure 1).

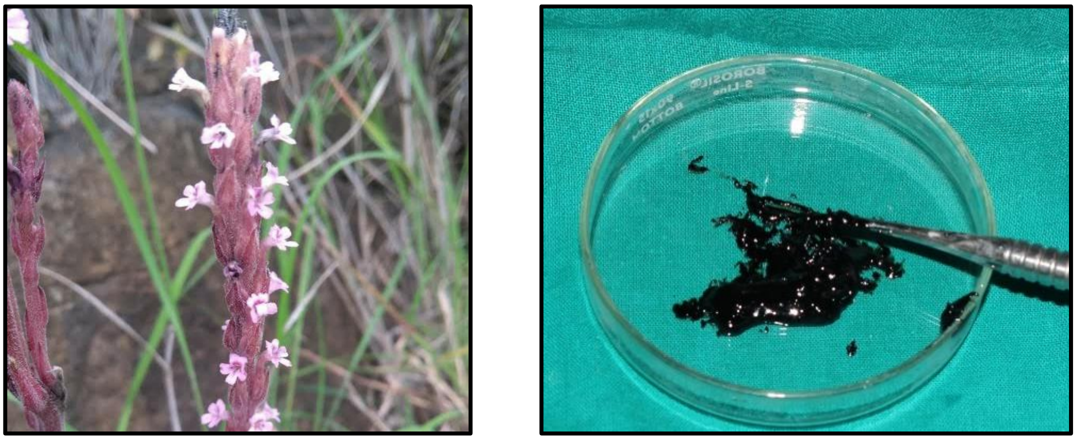

Figure 1: a) Striga orobanchioides plant **b) Striga orobanchioides extract**

RANDOMIZATION OF THE SAMPLES:

A total sample size of 30 was selected with 97% confidential level equally divided into 3 groups namely A, B, C (Flowchart 1).

Sample size n = $[DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1) + p*(1-p)]$

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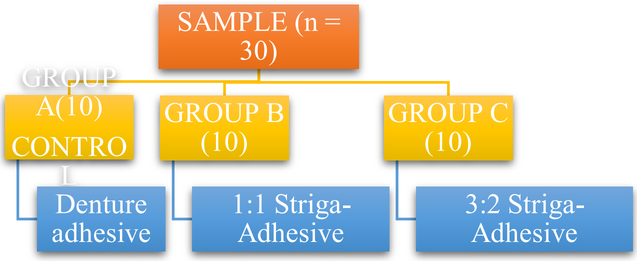
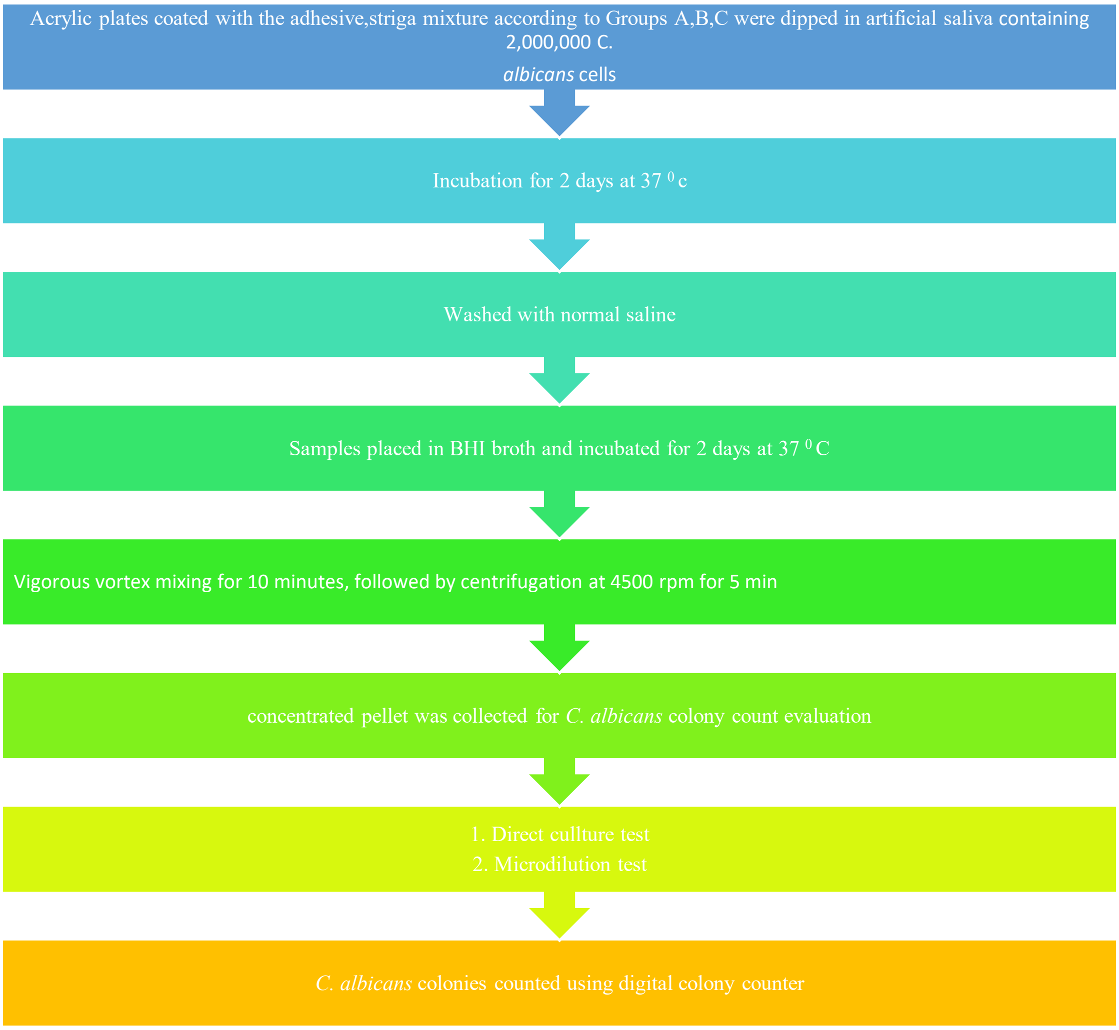


Flowchart 1: Description of study sample distribution

A stent measuring 10 mm × 2 mm was fabricated as a mould for specimen preparation, and a putty impression index was made to standardize the mould dimensions. The index was flasked using dental plaster in a conventional denture flask, with base flasking followed by counter flasking after applying a separating medium. Heat-cure acrylic resin was packed into the mould and the flask was closed under pressure to remove excess material. Curing was carried out in a water bath at 73°C–100°C for 1 hour, followed by 30 minutes of bench cooling. The acrylic plate was then carefully deflasked and sectioned into uniform 10 × 10 × 2 mm specimens using a precision cutting machine.

1.ADHESION ASSAY OF C. ALBICANS TO PMMA



Flowchart 2: ADHESION ASSAY OF C. ALBICANS TO PMMA

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C. albicans adhesion was assessed using the direct culture method (Figure 2). A 100 µl aliquot of the centrifuged pellet was inoculated onto SDA plates and incubated at 37°C for 24 hours. Colony-forming units (CFUs) were counted using a digital colony counter.

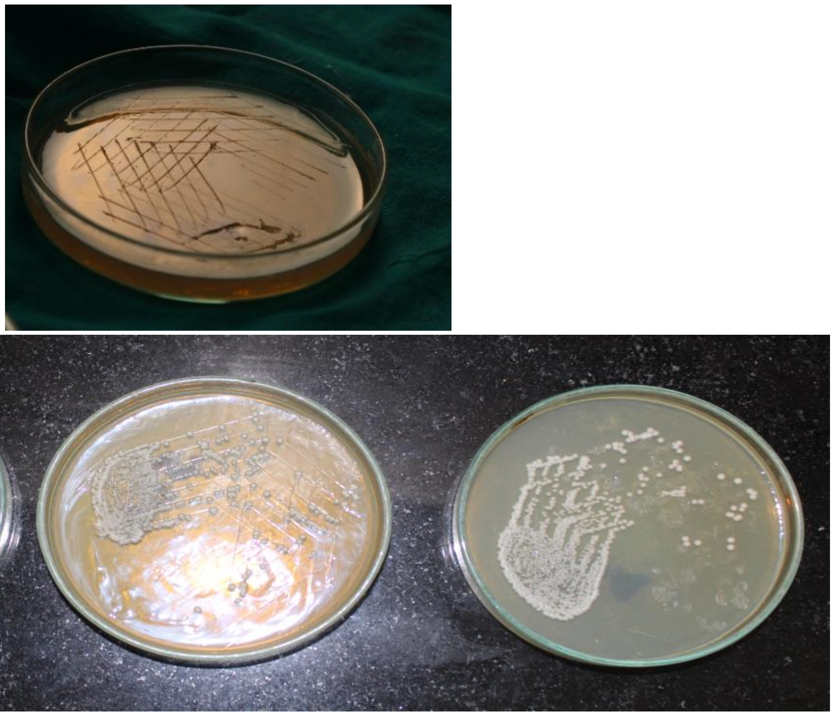

Figure 2: Direct culture test

For the microdilution test, five tubes with 900 µl saline were prepared. A 100 µl aliquot of the pellet was added to the first tube (1/10 dilution) and serially diluted up to 1/100,000 (Figure No.3). From each dilution, 10 µl was plated on SDA and incubated at 37°C for 24 hours. Colonies were then counted digitally (Flowchart 2).

FIGURE 3: Microdilution

To assess the antimicrobial properties of the test specimens, both positive and negative culture controls were included with each set of samples. The microbiological evaluation followed a standardized protocol:

Preparation of Microbial Inoculum

- *C. albicans* was cultured on SDA and incubated at 37°C for 24–48 hours.
- A standardized microbial suspension was prepared using **McFarland standard (0.5)** to ensure a uniform concentration of microorganisms for testing.
- **Positive Control:**
  - A sample without any antimicrobial treatment was included to confirm normal microbial growth.
  - This ensured that the microorganism was viable and the culture conditions were suitable for growth.

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- **Negative Control:**

o A sample without microbial inoculation was used to confirm sterility and rule out contamination from external sources.

o This ensured that any observed growth in the test group was due to the inoculated microorganism and not from an external contaminant.

- **FOR COLOR STABILITY OF SPECIMEN**

Color stability was assessed using the CIELab color system (L*, a*, b*) with a digital spectrophotometer, Vita Easyshade V (VITA Zahnfabrik, Germany), at baseline before immersion.

Table 1: Study Sample distribution for assessing color stability

Groups	**Sample size (Total 15)**
Group A- Control group	05
Group B - 1:1 Striga-Adhesive	05
Group C - 3:2 Striga-Adhesive	05

A total of 15 samples were divided into three groups (Table 1). Initially, spectrophotometer readings were recorded for all samples as pre-reading values [E, L, a, b]. Subsequently, adhesive was applied to Group A samples, striga: adhesive 1:1 mix to Group B samples, and striga: adhesive 3:2 mix to Group C samples using an applicator tip. The samples were then placed in artificial saliva & left for 4 hours, the samples were removed, washed with saline, and subjected to spectrophotometer analysis again to record post-reading values [E, L, a, b]. According to **I**SO/TR-28642:2016, a perceptibility threshold of $\Delta E \leq 1$ and an acceptability threshold of ΔE between 1.2 and 2.7 were considered clinically acceptables [9].

STATISTICAL ANALYSES

All statistical analyses were performed using SPSS version 25, which facilitates a range of statistical tests, including normality assessments, chi-square tests, and non-parametric comparisons such as Kruskal-Wallis. Colony growth across different dilution levels (10^1 to 10^5) was analyzed using pairwise comparisons with appropriate statistical tests, such as the Mann-Whitney U Test or other non-parametric methods.

RESULTS

A Chi-Square test revealed a statistically significant difference in Candida colony presence among the groups ($p < 0.05$). Group A showed 100% growth, Group B had 40%, and Group C showed the least at 20%. This indicates maximum inhibition in Group C and highest growth in Group A (Table 2).

Table 2: Comparison of *C. albicans* Colony by Direct Culture Test

Group	**Present Colonies (%)**	**Absent Colonies (%)**	**Chi-Square Statistic**	**p-value**
A	100%	0%	13.93	0.00095
B	40%	60%		
C	20%	80%		

Dilution Test Results

The dilution test data show bacterial growth across different dilution levels (10^1 to 10^5) (Table 3).

Table 3: Comparison of *C. albicans* Colony Count Across Different Dilution levels (10^1 to 10^5)

Group	**10^1**	**10^2**	**10^3**	**10^4**	**10^5**
A	Ø	Ø	>100	50.4	8.7

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B	Ø	63.25	41.5	4.5	0.75
C	11	7.5	4.5	0.5	0

(Ø - overgrowth)

Table 4: Within Groups Comparison of *C. albicans* Colony Count Across Serial Dilution Levels

Group	Comparison	p-value	Interpretation
A	10^3 vs 10^4	0.001953	Significant Difference
A	10^4 vs 10^5	0.001953	Significant Difference
B	10^2vs 10^3	0.125	No Significant Difference
B	10^3vs 10^4	0.125	No Significant Difference
B	10^4 vs 10^5	0.125	No Significant Difference
C	10^2 vs 10^3	0.5	No Significant Difference
C	10^3vs 10^4	0.5	No Significant Difference
C	10^4vs 10^5	0.5	No Significant Difference

Group A exhibited **overgrowth at 10^1 and 10^2**, with a **high presence of *Candida albicans* colonies** at **10^3 and lower dilutions**. This suggests Group A had the highest fungal load, requiring greater dilution for a significant reduction in colony counts.

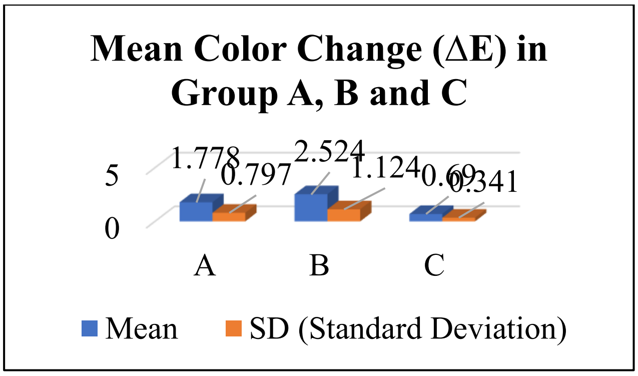
Group B displayed **moderate *Candida albicans* colony counts**, with a **progressive decline** as dilution increased**. It exhibited moderate *Candida* growth**, with a **gradual but statistically insignificant decline** across dilutions indicating **partial but insufficient inhibitory effects**.

Group C demonstrated **the lowest fungal colony counts**, showing **early suppression of fungal proliferation**. The colony count was **near zero at 10^4 and completely inhibited at 10^5**, indicating **most potent antifungal effect**, with **near-total inhibition** observed at higher dilutions **(Table 4).**

Mean Color Change (ΔE) per group

The color stability of each group was assessed using the average ΔE values, standard deviation (SD), standard error (SE), and 95% confidence intervals (CI).

Group B exhibited the highest color change (**ΔE = 2.524**), followed by Group A (**ΔE = 1.778**), while Group C showed the least color change (**ΔE = 0.690**)



Graph 1: Mean Distribution of Degree of colour change (ΔE) in groups

Group A vs. Group B: Group B showed a higher mean ΔE (2.524) than Group A (1.778), indicating lower color stability. Both groups had significant p-values, with Group B exhibiting greater discoloration.

Group A vs. Group C: Group C had a lower mean ΔE (0.690) than Group A, showing better color stability. Its p-value (0.089) was not significant, suggesting superior resistance to discoloration (Graph 1).

DISCUSSION

Denture adhesives play a key role in enhancing denture retention; but their prolonged use can promote microbial growth, especially *Candida albicans*, a key cause of denture stomatitis [3]. Conventional adhesives lack antifungal properties, enabling biofilm formation on acrylic resins and leading to infections and mucosal irritation. Sampaio-Maia et al suggested that C. albicans colony morphology is linked to virulence factors such as its ability to adhere, invade tissues, cause damage, evade the immune system, and develop resistance to antimicrobial treatments [10]. Natural antifungal agents have gained attention as biocompatible additives in denture adhesives to mitigate fungal proliferation.

S. orobanchioides Benth (family: Scrophulariaceae) is a parasitic plant found on roots of species like *Lepidagathis* and *Dysophylla*, typically in red, gravelly soils up to 6000 ft elevation [11]. Traditionally used in Ayurveda for its antidiabetic effects, it also exhibits antimicrobial and antifungal properties [7,12]. Incorporating its extract into denture adhesives could enhance retention while inhibiting *Candida albicans* adhesion and biofilm formation.

Study evaluated the antifungal efficacy of the ethanolic extract of *S. orobanchioides* in denture adhesives at varying concentrations and its effect on the colour stability of acrylic resins. The addition of *the* extract to denture adhesives has demonstrated significant antifungal activity against *C. albicans* growth on acrylic resins.

In **Group A (Control group– with no extract in adhesive)** exhibited highest *C. albicans* colonization at lower dilutions (10^1–10^3), with significant fungal growth persisting at 10^3. The absence of antifungal agents led to increase *C. albicans* adhesion and biofilm formation. Pereira-Cenci et al. concluded that denture adhesives without antimicrobial properties led to fungal growth due to their moisture retention and prolonged contact with oral tissues [13].

In **Group B (*Striga orobanchioides : adhesives* concentration; 1:1)** showed partial inhibition but statistically insignificant inhibition of *Candida* growth with increasing dilutions. This suggests the extract had antifungal effects but at insufficient concentration to suppress fungal growth. Chanda & Rakholiya noted plant antifungals like flavonoids, alkaloids, and tannins act dose-dependently, needing optimal concentrations for effective inhibition [14].

Group C (*Striga orobanchioides: adhesives* Concentration; 3:2) showed the most potent antifungal effect, significantly reducing *C. albicans* growth at 10^3 and nearly eliminating it at 10^5. Mahmoud et al. supported plant-based antifungals like *S. orobanchioides* as effective natural alternatives. Bioactive compounds in *S. orobanchioides* disrupt the fungal membranes, inhibiting biofilms, or block ergosterol synthesis in *Candida* cell walls [15]. Color stability is essential for assessing the long-term esthetic performance of denture materials. The results showed that Group B exhibited the highest color change followed by Group A while Group C had the least color change.

Goiato et al. studied that denture material composition affects color stability and staining.[6] The greater discoloration seen in Group B may be due to higher stain absorption, while moderate discoloration in Group A suggests materials without color-protective agents are more prone to change. Polyzois et al. noted that disinfectant effects on denture color depend on material composition, disinfectant type, exposure time, and chemical reactions, absorption properties [16].

Group C showed superior color stability with a significantly lower ΔE value ($p = 0.089$), suggesting enhanced stain resistance. This could be due to modifications in polymer structure or the incorporation of antifungal and hydrophobic agents. Pereira et al. found that bioactive compounds can reduce water absorption and stain adherence, thus maintaining color integrity for longer periods [17].

This underscores the importance of material selection in maintaining the color stability of denture adhesives. The significant discoloration in Groups A and B highlights the potential for esthetic deterioration over time, whereas Group C showed promising results in resisting color change. The combination of striga and adhesive plays a role, as concentration of *striga* is increasing stability is enhanced.

The study supports *S. orobanchioides* as a promising natural antifungal in denture adhesives, with higher concentrations significantly reducing *C. albicans* growth. Flavoring agents like honey, stevia, licorice, citrus, vanilla, or artificial sweeteners (e.g., aspartame, sucralose) can mask bitterness and improve taste and compliance [18,19].

Further research is needed to assess the long-term effects of these bioactive compounds on oral tissues, denture material integrity, and microbiome balance. Plant-based

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antifungal denture adhesives may offer a novel approach for preventing denture stomatitis and enhancing oral health outcomes in denture wearers.

CONCLUSION

S. orobanchioides-based denture adhesive demonstrated significant antifungal activity, inhibiting *C. albicans* growth more effectively than commercially available adhesives.

- Commercially available denture adhesive exhibited antifungal properties but was comparatively less effective in reducing fungal growth.
- The S. orobanchioides extract-based adhesive caused minimal discoloration of acrylic resins, supporting its suitability for long-term clinical use without affecting denture aesthetics.

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