

Comparison Of Antimicrobial Efficacy Of Herbal Denture Cleansers - An In Vitro Study

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ABSTRACT

AIM: To efficacy of herbal extracts as potential denture cleansing agents at varying concentrations as denture cleansers.

MATERIALS AND METHODS: Plant samples were collected, processed, dried, and ground into powder. Ethanolic extracts were then prepared at varying concentrations. These extracts formed the test groups—Group A, B, and C for the respective plants, while Group X served as the control, using a commercial denture cleanser (Clinsodent) containing sodium perborate. Heat-cured acrylic denture specimens measuring 4 cm × 2 cm × 2 mm were fabricated. Three standard microbial strains were selected for testing. Based on the antimicrobial susceptibility results, each plant extract was further tested on denture specimens. The denture blocks were exposed to 0.5 McFarland standard bacterial and fungal suspensions for 24 hours. Following contamination, the specimens were immersed in the respective plant extracts at different concentrations to assess microbial adhesion. All data were subjected to statistical analysis.

RESULTS: The tested herbal formulations demonstrated effectiveness against *S. aureus*, *S. mutants*, and *C. albicans*. *Justicia adhatoda* exhibited inhibitory activity at a concentration of 50 mg/dl, while *Punica granatum* and *Glycyrrhiza glabra* were effective at 100 mg/dl. The combined herbal denture cleanser formulation could be used at a concentration of 100 mg/dl.

CONCLUSION: Natural (herbal) products used as denture cleansers are effective as commercial denture cleansing tablets. Natural denture cleansers are cost effective, easily available and highly effective against oral pathogens.

Keywords: *Denture blocks, Denture cleanser, Denture hygiene, Glycyrrhiza glabra, Justicia adhatoda, Punica granatum.*

How to Cite: Gajapathi Balaraman, Bharanija. K, MDS, Nishanthini, S. Prasanna Karthik, Murali Karthik. R, MDS, M. Kiran Kanna., (2025) Comparison Of Antimicrobial Efficacy Of Herbal Denture Cleansers - An In Vitro Study, *Journal of Carcinogenesis*, Vol.24, No.6s, 104-109.

1. INTRODUCTION

Nature has provided a complete store house of remedies to cure all ailments of mankind. Herbal products such as Azadirachta indica (neem), Aloe barbadensis (Aloevera), Citrus limon (lemon), Triphalachurna (amla, baheda, harada), Justicia adhatoda (adhatoda), Glycyrrhiza glabra (athimathuram), Punica granatum (pomegranate) are known to have high antimicrobial potential.^[1,2,3,4] Antimicrobial property of herbal products inhibits the growth of various microbes. Microbial flora is also seen in partially edentulous and completely edentulous denture wearers. Dentures enhance accumulation of food, debris and plaque formation on denture surfaces which trigger a condition known as denture stomatitis & halitosis. According to the National Oral Health survey, the prevalence of subjects wearing complete denture in both dental arches around the age group 65-74 yrs (10.2%-11.2%) removable partial denture around the age group 25-45 yrs (6.5%-8%).^[3] Commonly, on the palatal mucosa Candida associated denture stomatitis affects 60%-65% of denture wearers. There are many specialized products available in the market for denture cleansing, but the majority population using dentures have decreased access to continuous supply of such materials hence there is a need to introduce a few natural products to clean dentures, which are easily & economically available. Plants like Adhatoda (Justicia adhatoda), pomegranate (Punica granatum), Athimathuram (Glycyrrhiza glabra) are known for their medicinal properties have been traditionally used in India as therapeutic and antimicrobial aids in various ailments & are potent antifungal products effective against.^[5] Methanolic leaf extract of Justicia adhatoda shows inhibitory action against Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, Candida albicans, Cryptococcus neoformans.^[6] Methanolic extract of pomegranate shows inhibitory action against Staphylococcus aureus, Candida albicans.^[7] The requirements of ideal denture cleansers are that they should have antibiofilm activity, should be affordable, should exhibit bactericidal and fungicidal effects; should be non-toxic, compatible with denture materials, short acting, easy to use; should have an acceptable taste.^[8] Chemical denture cleansers containing active ingredients such as chlorhexidine, sodium perborate, sodium hypochlorite, alkaline peroxides, enzymes, and diluted acids have shown bacteriostatic effect on cleaning the plaque adhering on acrylic resin.^[9] Numerous herbal extracts have demonstrated potent antimicrobial activity without any side effects, even when ingested by elderly individuals.^[3,5,6] This study aimed to assess the effectiveness of herbal extracts, at different concentrations, as potential denture cleansing agents in comparison to a commercially available denture cleanser.

2. MATERIALS AND METHODS:

Plant material collection: Group A- Adhatoda (Justicia adhatoda, leaves), Group B - Pomegranate (Punica granatum, fruit) and Group C- Athimathuram (Glycyrrhiza glabra, root & bark). Plant extract preparation was conducted according to the protocol described in a previous study.^[5]

3. PREPARATION OF HEAT CURE ACRYLIC RESIN BLOCKS:

A metal die was fabricated in dimensions about 4cm in length 2cm in width and 2mm in thickness according to ISO specification.^[10] Metal die was invested in dental flask using dental plaster and dental stone as investing medium. Then deflasking was done to pack heat cure polymerized denture base resin. Specimens of about 4cm x 2cm x 2mm were fabricated. (Figure 1)



Figure 1: Heat cured acrylic resin blocks

4. CONTROL:

Commercial denture cleanser (clinsodent) manufactured by ICPA health products manufacturer limited which contains sodium perborate monohydrate was used as control group.

5. METHODOLOGY:

The study included three test groups (Group A, Group B, and Group C) and one control group (Group X), with 5 denture blocks assigned per group. In total, 80 denture blocks were utilized.

Group X: Control group –immersed in commercial denture cleanser

Group A1: Test group-immersed in 6 mg/dl of ADHATODA

Group A2: Test group-immersed in 12 mg/dl of ADHATODA

Group A3: Test group-immersed in 25 mg/dl of ADHATODA

Group A4: Test group-immersed in 50 mg/dl of ADHATODA

Group A5: Test group-immersed in 100 mg/dl of ADHATODA

Group B1: Test group-immersed in 6 mg/dl of POMEGRANATE

Group B2: Test group-immersed in 12 mg/ dl of POMEGRANATE

Group B3: Test group-immersed in 25 mg/ dl of POMEGRANATE

Group B4: Test group-immersed in 50 mg/ dl of POMEGRANATE

Group B5: Test group-immersed in 100 mg/ dl of POMEGRANATE

Group C1: Test group-immersed in 6 mg/dl of ATHIMATHURAM

Group C2: Test group-immersed in 12 mg/dl of ATHIMATHURAM

Group C3: Test group-immersed in 25 mg/dl of ATHIMATHURAM

Group C4: Test group-immersed in 50 mg/dl of ATHIMATHURAM

Group C5: Test group-immersed in 100 mg/dl of ATHIMATHURAM

6. PREPARATION OF MICROBIAL COATED DENTURE BLOCK:

Each denture block was sterilized using diethyl ether, followed by a rinse with sterile saline. The blocks were then inoculated with 0.5 McFarland standard bacterial and fungal suspensions for 24 hours. After incubation, the presence of microbial pathogens on the denture blocks was confirmed through microbial isolation and identification (Figure 2). Subsequently, the contaminated denture blocks were rinsed with phosphate buffer at pH 7.0

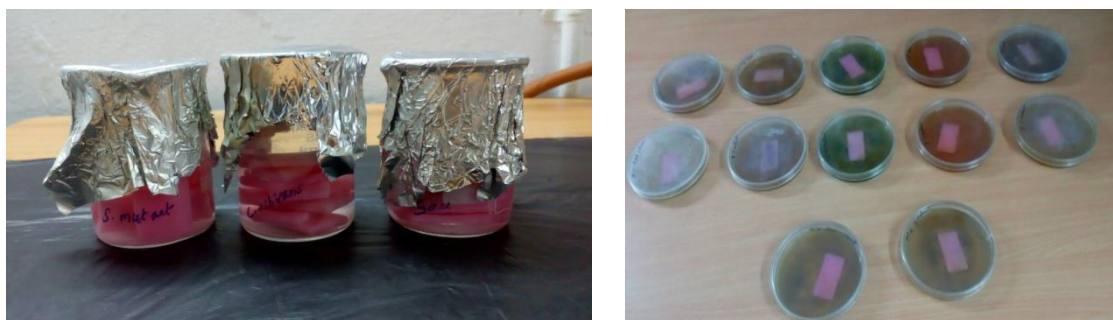


Figure 2: Microbial coated acrylic resin blocks

Plant Extract formulation and Testing

Each plant extract was dissolved in Dimethyl Sulfoxide (DMSO) and diluted with sterile saline. Based on antimicrobial susceptibility testing results,^[5] the extracts were serially diluted to concentrations of 100 mg/dl, 50 mg/dl, 25 mg/dl, 12 mg/dl, and 6 mg/dl.

CLINSODENT TESTING WITH DENTURE BLOCKS

Denture blocks were tested using clinsodent in 100, 200, and 400 mg/ml

Statistical Analysis: One-way ANOVA test and Tukey HSD post-hoc test were used for multiple pairwise comparison within the groups. A P value < 0.05 was considered statistically significant.

Results: The herbal extracts significantly reduced *C. albicans* and *S. aureus* adhesion, with limited efficacy against *S. mutans*, requiring higher concentrations. Adhesion of *C. albicans* and *S. aureus* on denture acrylic was reduced following treatment with *Justicia adhatoda*, *Punica granatum*, and *Glycyrrhiza glabra* extracts at concentrations of 50, 50, and 100 mg/dl, respectively ($P = 0.000$). For *S. mutans*, a reduction in adhesion was observed only at 100 mg/dl with *Punica granatum* and *Glycyrrhiza glabra*, with no activity noted at 50 mg/dl. (Table 1). The denture blocks tested with Clinsodent (control group – Group X) exhibited significant antibacterial or anti-candidal activity at all tested dilutions.

Table 1: Effective plant extract formulations (mg/dl) as Denture Cleansers

S. No.	Microbial pathogens	Justicia adhatoda (Group A)			Punica granatum (Group B)			Glycyrrhiza glabra (Group C)		
		mg/dl	95% CI	P	mg/dl	95% CI	P	mg/dl	95% CI	P
1.	<i>S. aureus</i>	50	7.34 – 8.66	0.000*	50	5.25 - 8.11	0.000*	100	24.06 - 25.94	0.000*
2.	<i>S. mutans</i>	-	-	-	100	5.67 - 8.33	0.000*	100	13.24 - 16.76	0.000*
3.	<i>C. albicans</i>	50	8.85 - 11.15	0.000*	50	6.02 - 7.45	0.000*	100	16.85 - 19.15	0.000*

S. aureus: Staphylococcus aureus, *S. mutans*: Streptococcus mutans, *C. albicans*: Candida albicans, mg: milligram, dl: deciliter. * $P \leq 0.05$ is significant

7. DISCUSSION:

Denture hygiene plays a crucial role in maintaining the oral and general health of edentulous individuals. Complete and partial denture wearers are particularly susceptible to biofilm accumulation on denture surfaces, which serves as a reservoir for pathogenic microorganisms such as *Candida albicans*, *Staphylococcus aureus*, and various streptococcal species. These pathogens can lead to common denture-related conditions, including denture stomatitis, angular cheilitis, halitosis, and even systemic infections in immunocompromised individuals.^[11]

Traditionally, mechanical cleaning using toothbrushes combined with chemical cleansers, such as sodium hypochlorite, chlorhexidine gluconate, or effervescent tablets containing alkaline peroxides, has been the standard protocol for denture hygiene. However, these chemical agents are associated with several drawbacks. Long-term use of sodium hypochlorite, for instance, may result in bleaching of acrylic resin, corrosion of metallic components in partial dentures, and mucosal irritation. Similarly, chlorhexidine, though effective, has been linked to tooth and denture staining and alteration of taste sensation.^[12] This has led to increasing interest in safer, biocompatible, and environmentally friendly alternatives.

In recent years, herbal medicine has garnered attention as a complementary approach in oral healthcare. Herbal extracts are rich in bioactive compounds, such as flavonoids, tannins, alkaloids, and essential oils, which exhibit antimicrobial, antifungal, anti-inflammatory, and antioxidant properties^[5]. Plants like *Azadirachta indica* (neem), *Ocimum sanctum* (holy basil or tulsi), *Adhatoda* (*Justicia adhatoda*), pomegranate (*Punica granatum*), *Athimathuram* (*Glycyrrhiza glabra*), triphala churna have demonstrated inhibitory effects against oral pathogens including *C. albicans* and *S. aureus*, which are commonly found on denture surfaces.^[5,13,14]

Because of their ready availability and many medicinal uses, three medicinal plants (*Justicia adhatoda*, *Punica granatum*, *Glycyrrhiza glabra*) were selected to assess their effectiveness against pathogenic oral microorganisms at varying concentrations, with the aim of evaluating their potential as denture cleansing agents. The process of extraction of the experimental plants employed was according to the methodology adopted in a previous study.^[5]

The results from this present study shows adhesion of **C. albicans** on denture acrylic were reduced with treatment of *Justicia adhatoda*, *Punica granatum*, and *Glycyrrhiza glabra* extracts at 50, 50 and 100 mg/dl respectively in accordance with the literature.^[5,6,15,16] Mehta et al. reported that *Punica granatum* has demonstrated effectiveness as a herbal denture cleanser and shows potential for the development of novel therapeutic options that could enhance patient compliance, which aligns with the findings of the present study.^[15]

Adhesion of **S. aureus** on denture acrylic were reduced with treatment of *Justicia adhatoda*, *Punica granatum* and *Glycyrrhiza glabra* extracts at 50, 50 and 100 mg/dl respectively. Ameer et al. highlighted that *Justicia adhatoda* extract exhibited a significant zone of inhibition against *Staphylococcus aureus* at a concentration of 1000 µg/ml.^[16]

Adhesion of *S. mutans* on denture acrylic were reduced with treatment of *Punica granatum*, and *Glycyrrhiza glabra* extracts at 100 and 100 mg/dl respectively and no activity at 50mg/dl.

These natural agents not only offer a safer alternative to chemical cleansers but also present the added advantage of being cost-effective and culturally acceptable in many parts of the world. Moreover, herbal extracts are generally biodegradable and pose less risk of developing microbial resistance. Therefore, exploring the use of herbal formulations as denture cleansers holds significant promise for improving denture hygiene while minimizing adverse effects on the user and the environment. The tested herbal extracts exhibit superior antimicrobial properties on heat-cured denture base materials. Due to its accessibility in rural areas, affordability, and numerous benefits, these herbal denture cleansers stand out as one of the most promising alternatives to currently used commercial denture cleansers.

8. CONCLUSION:

The tested extracts of all three plants were found to be effective against pathogenic microorganisms present in the oral cavity, further purification and toxicological studies of these plants and in vivo trials should be carried out. Also, enhancement of antimicrobial studies can be done if the phytoconstituents of these plant extracts are purified using various solvents like ethanol, methanol, acetone, etc. With the emerging development of biotechnology and tools for validation of bioactive compounds, it will be a known truth that medicine is not to be searched out; it is to be searched within.

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