Comparative evaluation of color stability of formulated silver nanoparticle incorporated herbal denture cleanser and commercial denture cleanser on acrylic tooth - an in vitro study.

Running title: Assesing the effectiveness of various denture cleansers

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INTRODUCTION:

Edentulism, the complete loss of natural teeth, is a significant oral health condition that affects both function and quality of life. It can result from a variety of causes, including advanced dental caries, periodontal disease, trauma, and systemic conditions, and is more prevalent among older adults. The absence of teeth compromises mastication, speech, and facial aesthetics, often leading to nutritional deficiencies and psychosocial impacts such as reduced self-esteem and social interaction. While removable and fixed prosthetic options, such as complete dentures, implant-supported prostheses, and overdentures, can restore some lost functions, prevention through good oral hygiene, regular dental care, and public health measures remains the most effective approach to reducing the incidence of edentulism. According to the World Health Organization's Study on Global Ageing and Adult Health, the prevalence of edentulism among Indian adults aged 50 and above is 15.3%[1].A broader estimate from the Global Burden of Disease (GBD) 2016 model indicates that about 38.9 million Indians between ages 15–79 live with severe tooth loss or edentulism.[2]

Color stability is a crucial property of artificial teeth in removable partial dentures (RPDs), as it directly impacts the esthetic longevity of the prosthesis. Artificial teeth are typically made from acrylic resin, polymers or porcelain, each with varying resistance to discoloration. Acrylic resin teeth, though popular due to ease of adjustment, chemical bonding to denture bases, and cost-effectiveness, are more prone to color changes over time due to their higher water sorption and surface porosity, which allow absorption of stains from dietary chromogens tobacco, and mouth rinses. Porcelain teeth are the most color-stable because their glazed surface resists stain penetration; however, they are heavier, more brittle, and require mechanical retention in RPDs.

Poor denture cleanliness refers to the inadequate removal of plaque, food debris, and stains from the surface of complete or partial dentures, leading to a range of oral and systemic problems. Denture plaque, similar to dental plaque, is a biofilm composed of bacteria, fungi (particularly *Candida albicans*), and salivary proteins that adheres strongly to the acrylic surface. Inadequate cleaning allows this biofilm to accumulate, causing denture stomatitis, unpleasant odor (halitosis), altered taste sensation, and increased staining. Over time, poor hygiene may also contribute to inflammatory

lesions, angular cheilitis, and an elevated risk of aspiration pneumonia, particularly in elderly or immunocompromised individuals.

On the positive side, regular use of effervescent or enzymatic cleansers reduces microbial load, helps prevent denture stomatitis, minimizes calculus buildup, and maintains a more esthetic appearance. However, prolonged or improper use can have drawbacks.Alkaline peroxide-based cleansers may cause gradual surface roughness in acrylic resin if used excessively, making dentures more susceptible to staining and plaque accumulation[3]. Sodium hypochlorite solutions, though highly effective against fungi and bacteria, can bleach acrylic resin and corrode metal components in removable partial dentures[4,5]. Acid-based cleansers help dissolve mineral deposits but may soften the resin if exposure is too long.

Color stability of artificial teeth is a critical factor in esthetic dentistry, as it directly influences the natural appearance, patient satisfaction,and long-term success of dental prostheses[6,7]. Discoloration, whether due to dietary pigments, smoking, chemical exposure from denture cleansers, or material degradation, can lead to an aged or unaesthetic appearance, potentially diminishing a patient's confidence[8]. Since artificial teeth are prone to color changes over time potentially influenced by denture cleansers. The study tested the null hypothesis that denture cleansers do not cause significant color alteration in acrylic resin teeth. The objective was to evaluate and compare the antimicrobial and color stability of commercially available denture cleansers and formulated nano herbal denture cleanser on acrylic resin teeth.

MATERIALS AND METHODS:

***Study Design and Sample Size Calculation*:**

This study was a comparative in vitro study aimed to evaluate the antimicrobial and color stability of formulated herbal denture cleanser and to further compare its efficiency with commercial denture cleanser.The study was done with the standardized protocol. In order to calculate G-Power, OpenEpi, Version 3 was used and sample size was decided as 45 with 95% confidence interval, 80% power and alpha error of 0.05 ensuring to identify significant difference between groups.

Institutional Ethics Committee Approval

Approval for the study was obtained from Institutional Ethical Committee with the NO. 303/2024/IEC/TMDCH.

Study Duration

Study was conducted over a period of one year starting from March 2024 till April 2025 starting from preparation of samples, nanoformulation and data analysis.

Study Groups

The study included one group, being subdivided into Control, Standard and Test containing 15 sample size each making a total of 45.

Herbal Nanoparticle Synthesis

The synthesis of herbal-incorporated silver nanoparticles (AgNPs) follows a systematic process, including extract preparation, synthesis, purification, and characterization[9]. Fresh Annona muricata(Graviola) and Psidium guajava(Guava) leaves are washed, shade-dried, and ground into powder. Aqueous extraction is performed by boiling 10 g of powder in 100 mL deionized water at 60–70°C for 30 minutes. The extract is filtered and stored at 4°C. A 1mM silver nitrate solution is mixed with the extract (1:3 ratio) at pH 7–9, with a color change indicating AgNP formation. The nanoparticles are purified via centrifugation, dried at 50°C.

Formulation of denture cleanser

The synthesized herbal-incorporated silver nanoparticles (AgNPs) are carefully integrated into a specially formulated denture cleanser to enhance its antimicrobial and antifungal properties. This formulation is designed to effectively cleanse dentures while minimizing microbial biofilm formation. Key components of the formulation include surfactants, which facilitate the removal of debris and microbial contaminants by reducing surface tension and allowing efficient penetration of the cleansing solution. Effervescents, chelating, lubricating, stabilizing agents are also incorporated to aid in breaking down and eliminating biofilms, ensuring thorough cleaning of denture surfaces and reducing microbial adherence. Additionally, flavoring agents are added to improve palatability and user compliance.

Preparation of denture cleanser solution

To evaluate the antimicrobial efficacy of different denture cleansers on acrylic tooth, three groups were prepared such as Standard (Clinsodent Denture Cleanser), Test (Formulated Herbal AgNP Denture Cleanser), and Control (Plain Water). For the

standard group, 2 grams of commercially available Clinsodent powder was accurately weighed and dissolved in 20mL of distilled water, ensuring a homogeneous solution. Similarly, for the test group, 2 grams of the formulated herbals incorporated AgNP denture cleanser was mixed with 20 mL of distilled water and stirred thoroughly to ensure uniform dispersion of active ingredients. Meanwhile, the control group consisted of plain distilled water without any active denture-cleaning agents, serving as a baseline for comparison. All solutions were prepared fresh, stored in sterile containers, and used immediately for antimicrobial testing using the agar well diffusion method to assess their effectiveness against Streptococcus mutans and Candida albicans by measuring the zone of inhibition around the wells.

Preparation of candidal broth

To prepare the Candida broth, isolated colonies of Candida albicans were first grown on Rose Bengal Agar. The well-formed colonies were carefully isolated and inoculated into Mueller-Hinton Broth, followed by incubation at 37°C for 24-48 hours to allow optimal fungal growth[10]. The sterile acrylic tooth specimens were immersed in candidal broth. The specimens was then further incubated for 16 hours at 37°C in an incubator to ensure sufficient proliferation. Once the incubation period was completed, the inoculated specimens were washed thoroughly under running tap water to remove any loosely adhered cells. The washed specimens were then immersed in three different experimental groups, for a duration of 8 hours which mimick the protocol overnight soaking of dentures, following which candidal cell count was calculated.

Statistical Analysis:

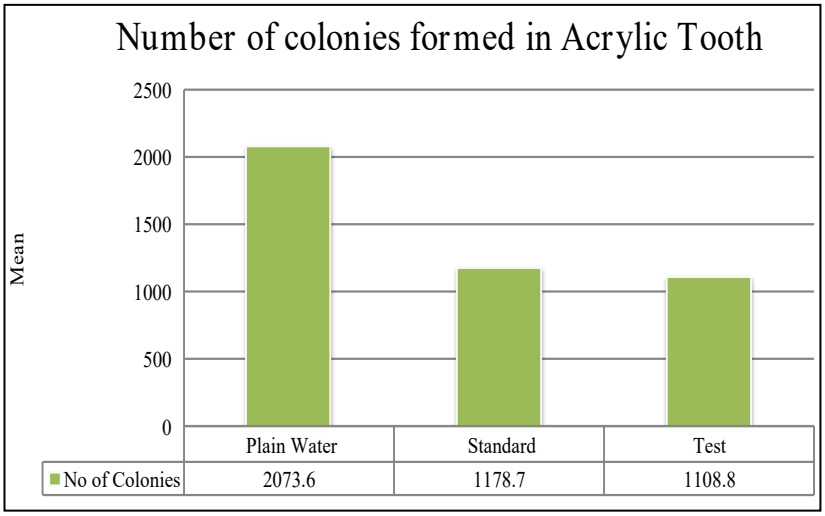
The data obtained was subjected to statistical analysis. The data recorded were transferred and tabulated to the computer - Windows Microsoft Excel (2007) - for the purpose of the data analysis. Statistical Package of Social Science (SPSS; IBM Chicago Inc., USA) was used for statistical analysis.The significance level was fixed to be $p \leq 0.05$ for the analysis. All continuous data were subjected to Kolmogorov Smirnov test for normality. It was found that the data was normally distributed and hence parametric tests of significance were used. Data of the colonies formed among the acrylic tooth were tested using the various types of cleansers using ANOVA. For all comparisons, p value of < 0.05 was considered to be statistically significant.

RESULTS:

Table 1-Evaluation of colonies formed among Acrylic Teeth

Variables	N	Mean	Std. Deviation	Std. Error of Mean	Median	Minimum	Maximum	Range
Control	15	2073.6	0.25314	0.13095	2072	2062.00	2082.00	20.00
Standard	15	1178.7	0.58675	0.17233	1100	1008.00	1424.00	416.00
Test	15	1108.8	0.32781	0.60520	1115	1056.00	1150.00	94.00

Control-Plain Water; Standard-********* ; Test-**********



Graph 1- Number of colonies in acrylic tooth

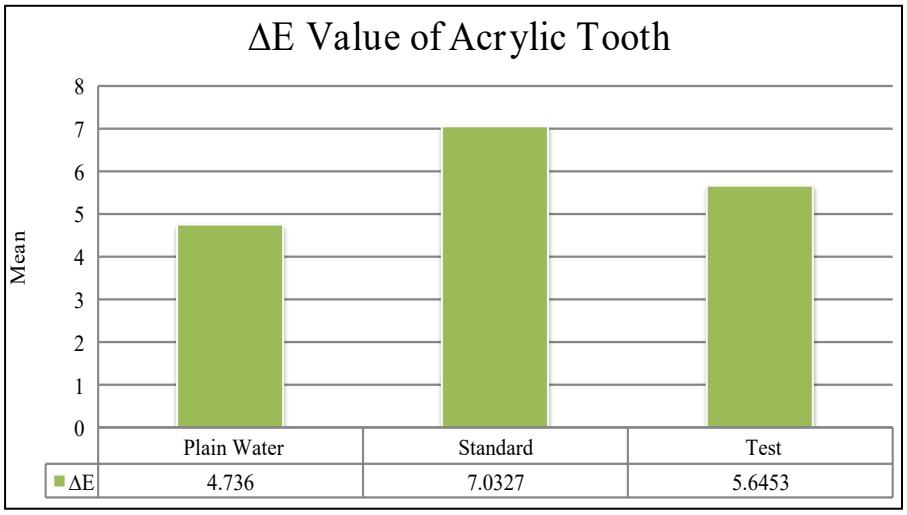
Table 1 and Graph 1 depicts the descriptive statistical analysis of the number of microbial colonies formed in the acrylic tooth while using the control, Standard and the test cleansing solution. It was found that the mean colonies formed was highest in Control (2073.6) followed by Standard(1178.7) and Test (1108.8) cleanser.

Table 2-Evaluation of colour among acrylic teeth

Variables	N	Mean (Delta E)	Std. Deviation	Std. Error of Mean	Median	Minimum	Maximum	Range
Control	15	4.736	1.14088	0.29457	5.27	3.31	5.98	2.67
Standard	15	7.0327	1.81339	0.46821	7.37	3.62	8.38	4.76
Test	15	5.6453	0.50876	0.13136	5.79	4.98	6.22	1.24

Control-Plain Water; Standard-********* ; Test-**********

Table 2 and **Graph 2** depicts the descriptive statistical analysis of the property of colour among the acrylic tooth using the control, Standard and the test cleansing solution by evaluation of the Delta E . It was found that the mean value of ΔE was highest in Standard(7.0327, Significant difference, colour appear distinctly different) followed by Test (5.6453, Significant difference, colour appear distinctly different) and Control (4.736, Moderate difference and easily noticeable)cleanser.



Graph 2- ΔE Value of Acrylic Tooth

DISCUSSION:

The interaction of denture cleansers with acrylic teeth plays a crucial role in controlling microbial colonization and maintaining prosthesis hygiene[11,12]. Acrylic resin, due to its porous nature, provides a favourable surface for bacterial and fungal adhesion, making it prone to plaque accumulation and biofilm formation.[13] When exposed only to plain water, as seen in the control group, the acrylic surface does not undergo any chemical or antimicrobial action, resulting in heavy microbial growth. In contrast, chemical cleansers such as Clinsodent release oxygen and active cleaning agents that interact with the acrylic surface by loosening debris, disrupting microbial cell walls, and reducing colony formation without significantly altering the denture material[14]. The formulated nano-herbal denture cleanser interacts differently, combining the physical advantage of nanoparticles, which penetrate surface irregularities and enhance antimicrobial contact, with the bioactive compounds of herbal extracts that exhibit bactericidal and fungicidal effects. This dual mechanism not only reduces microbial adhesion more effectively but also offers a biocompatible

approach that is less likely to damage the acrylic structure compared to harsh chemical cleansers[15,16]. Thus, the interaction of denture cleansers with acrylic teeth determines the extent of microbial reduction, with the nano-herbal formulation showing promising results as a safe and efficient alternative.

The color stability of acrylic teeth, expressed as ΔE values, varied according to the cleansing medium, reflecting the interaction between the material surface and the agents used. Immersion in distilled water produced the lowest color change (ΔE ≈ 4.74), which can be attributed to water sorption and minor leaching of residual monomers from the acrylic resin; although noticeable, the absence of strong chemical action limited further discoloration[17]. Acrylic resin (PMMA) is hydrophilic enough to absorb water[18]. Prolonged immersion causes water sorption and plasticization, mild leaching of residual monomer/plasticizers, and subtle surface softening that reduces gloss. These processes alter refractive index and light scattering, producing a noticeable but comparatively lower color shift. No oxidizers or detergents are present, so chemical degradation is limited, hence ΔE is the lowest of the three, though still above common perceptibility thresholds. In contrast, Clinsodent denture cleanser showed the highest ΔE value (≈ 7.03), as its alkaline-peroxide system releases oxygen and creates an effervescent, oxidizing environment that roughens the acrylic surface, enhances water uptake, and alters the resin matrix, leading to greater optical changes and pigment leaching, thereby compromising esthetics despite its cleansing efficacy.

Clinsodent which acts as a control showed ΔE ≈ 7.03. This may be due to various chemical constituents present in the commercial denture cleanser. Alkaline-peroxide, sodium perborate release oxygen and create an alkaline, effervescent environment. They increase surface roughness/microporosity via oxidative attack and saponification of surface contaminants. Enhance water uptake into the now roughened surface, leaching of pigments and matrix components occurs altering the translucency[19,20].It leave the surface more receptive to adsorption of extrinsic chromogens from storage media.Together these effects amplify optical change, explaining the highest ΔE among groups and indicating a trade-off between antimicrobial potency and color stability

The formulated nano-herbal denture cleanser resulted in a moderate ΔE (≈ 5.65), lower than Clinsodent but higher than water,indicating that its milder chemistry and nanoparticle activity preserved surface integrity better while still exerting antimicrobial effects. However, slight discoloration may also arise from herbal pigments adhering to the surface.The nano-herbal formulation likely operates with milder pH and less aggressive oxidation, relying on phytochemicals (e.g., polyphenols, terpenoids) and nanoparticles for antimicrobial action. Two opposing mechanisms influence color such as nanoparticles can occlude micro-irregularities and reduce biofilm retention,gentler chemistry limits matrix degradation, so less roughness and water sorption than with peroxide cleansers[21,22].The tinting potential of herbals carry intrinsic chromophores that may impart a slight hue or adsorb to the surface.The net result is a moderate Δ, but greater than water (because any active chemistry tends to change optics) but lower than Clinsodent, reflecting a better balance between hygiene and esthetics.

CONCLUSION:

Within the limitations of this in vitro study, it can be concluded that both commercial (Clinsodent) and formulated nano-herbal denture cleansers were effective in reducing microbial colonization on acrylic resin teeth.The formulated nano-herbal denture cleanser offers an effective and biocompatible alternative to conventional chemical cleansers, maintaining antimicrobial efficacy while minimizing color alteration of acrylic teeth, thereby enhancing long-term esthetics and patient satisfaction.Further research should focus on long-term in vivo studies to evaluate the clinical performance, patient compliance, and safety profile of nano-herbal denture cleansers. Comparative trials with larger sample sizes and diverse patient groups can help validate their effectiveness and establish them as a viable alternative to conventional chemical cleansers in routine prosthodontic care.

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