

COMPARISON OF MICROBIAL AND CYTOTOXIC EFFECT OF HERBAL INCORPORATED SILVER NANOPARTICLE AND COMMERCIAL DENTURE CLEANSER ON DENTURE MATERIALS: *IN VITRO* STUDY

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ABSTRACT

Objectives: Effective denture cleaning is vital to prevent oral and systemic infections among the geriatric group. Adding nanoparticles to denture cleansers enhances antimicrobial action by reaching microscopic denture surfaces, reducing infection risk. In this project, we aimed to evaluate the antimicrobial, antifungal, and cytotoxicity effects of a formulated denture cleanser prepared using silver nanoparticles (AgNPs) and herbals on *Streptococcus mutans* and *Candida albicans*, and further compare the efficiency of the formulation against a commercial denture cleanser.

Methods: Herbal-incorporated AgNPs preparation was done by green synthesis and evaluated for antimicrobial efficacy using Time Kill Curve Assay. Denture cleanser was formulated using herbals such as *Psidium guajava*, *Annona muricata*, AgNPs, emulsifying agents, and chelating agents. This formulated denture cleanser was checked for antimicrobial activity and cytotoxicity using the Zebrafish embryonic test. Two types of polymethylmethacrylate (PMMA) resins (Dentsply, Ivocap) with a total sample size of 90 were divided into two groups. These PMMA specimens were inoculated into the candidal broth for 10 h in an incubator. These specimens were washed, and each group with a sample size of 45 was further immersed in a denture cleanser (two groups) – Clinsodent and formulated denture cleanser, and distilled water as a control group for 8 h at room temperature. After 8 h, specimens were washed, and candidal cells adhering to the specimens were counted using a microscope.

Results: Intergroup comparison of the antimicrobial efficiency of Dentsply PMMA and Ivocap denture surface based on the number of colonies among control, standard, and test groups was done using *post hoc* Tukey HSD analysis. The most significant reduction was seen in the herbal denture cleanser group, with mean colony counts of 1692.8 for Dentsply PMMA and 1713.6 for Ivocap, compared to the commercial cleanser at 1980.8 and 2015.2, respectively, and the control at 2628 and 2512.

Conclusion: These results indicate that both cleansers were effective in reducing bacterial colonies, with the herbal denture cleanser demonstrating the greatest antibacterial efficacy for both denture materials.

Keywords: Antimicrobial activity, Cytotoxicity, Denture cleanser, Herbals, Nanoparticles.

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INTRODUCTION

The oral cavity is a dynamic environment constantly exposed to microbial colonization. Dentures, serving as replacements for lost teeth, provide a surface for bacterial and fungal accumulation, leading to infections such as denture stomatitis [1]. Proper denture hygiene is essential to prevent the negative impact on oral tissues [2]. However, many edentulous patients struggle with maintaining denture cleanliness due to improper cleaning, leading to microbial biofilm formation. Over time, biofilms, particularly those containing *Candida albicans*, increase infection risks [3,4].

With advent of Ethnobotanic medicine, defined as the use of plants by humans for medicinal purposes, has gained increasing importance and is rapidly evolving in recent times [5]. Among the higher plant species on this planet, 6% have been studied for biological activity, and a reported 15% have been evaluated phytochemically [6]. This leads to a window for newer searches among plant families for alternatives to ancient therapies. *Annona muricata* (Sour sop) and *Psidium Guajava* (Guava) are attaining global importance for being miracle trees in the field of cancer research and can provide a way for research in many medical fields, including dentistry, claiming their potential against pathogens. *Psidium*

guajava, a popular fruit of Bangladesh, is said to be an evergreen tree found throughout Asia, and it is spread widely in India, Bangladesh, and Malaysia [7,8]. Extracts of many medicinal plants are used as they have significant reducing and capping agents to convert metallic ions to form nanoparticles. Among them, the extracts of *P. guajava* and *A. muricata* are most commonly used. The flavonoids and tannins present in *P. guajava*, along with the acetogenins in *A. muricata*, can act as both reducing and capping agents during the green synthesis of silver nanoparticles (AgNPs). Furthermore, these bioactive compounds are also known to possess inherent antimicrobial properties, thereby contributing synergistically to the overall antimicrobial efficacy of the synthesized nanoparticles.

Bose and Chatterjee [9] prepared AgNPs using *P. guajava* leaf extract. They investigated their antibacterial activity against Gram-negative bacterial strain whereas in another study by Sharmila *et al.*, they demonstrated results for both Gram-negative and Gram-positive bacteria with a zone of inhibition <10 mm [10,11].

Nanotechnology has introduced AgNPs as promising antimicrobial agents. Herbal-mediated AgNP synthesis enhances biocompatibility while reducing cytotoxicity [12,13]. These nanoparticles disrupt

microbial cell membranes, effectively inhibiting growth. Traditional denture cleansers may not fully eliminate microbial biofilms, but AgNPs can penetrate and disrupt them, preventing recolonization. Studies show AgNP-modified dental materials exhibit significant antifungal activity against *C. albicans* [14,15].

Despite advancements, limited research exists on incorporating herbal-based AgNPs into denture cleansers, whereas AgNPs in denture materials have been studied before; the specific combination of these two herbs in a denture cleanser formulation makes this research unique. The objective of this study is to formulate an eco-friendly denture cleanser using *P. guajava* and *A. muricata* leaves and to further investigate their antimicrobial and cytotoxic effects for clinical safety. The hypothesis states no significant difference in the antimicrobial efficacy of cleansers with and without herbal-incorporated AgNPs. The formulated herbal denture cleanser is further compared with a commercial denture cleanser to arrive at an archetypal report for this study.

METHODS

A study was conducted in Chennai over a period of 1 year, starting from March 2024 to April 2025, which involved the preparation of samples, nano formulation, and data analysis. Approval for the study was obtained from the Institutional Ethical Committee with the No. 303/2024/IEC/TMDCH. The study was done with the standardized protocol. The sample size was calculated using OpenEpi, Version 3 Software, which showed a confidence interval of 95% and power of 80%, resulting in a minimum of 15 samples per subgroup. The study protocol was divided into herbal AgNPs synthesis, preparation of acrylic specimens, formulation of AgNPs incorporated herbal denture cleanser, and antimicrobial analysis.

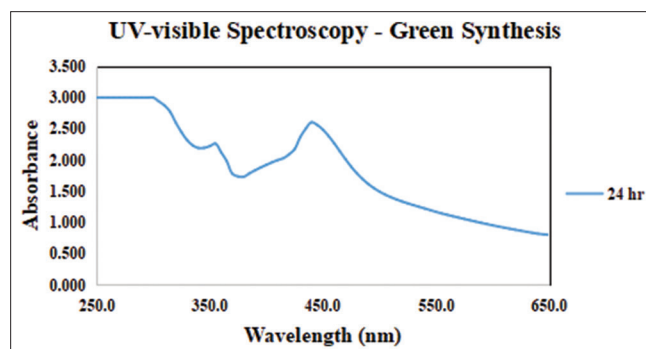
This experimental *in vitro* study was done using two types of polymethylmethacrylate (PMMA) resins (Dentsply, Ivocap) with a total sample size of 90, which was divided into two groups, each further subdivided into control, standard, and test containing 15 samples each, namely Group I – Dentsply (n=45) and Group II – Ivocap (n=45).

Fresh *A. muricata* and *P. guajava* leaves were obtained from the botanical garden in Red hills located in Chennai. These leaves were washed, shade-dried, and ground into powder, which measured 500 g. The obtained material was checked for its authenticity by the botanists and pharmacognosists. Aqueous extraction is performed by boiling 10 g of powder in 100 mL of deionized water at 60–70°C for 30 min [16]. The extract is filtered using Whatman filter paper and stored at 4°C. Adjustment of the reagent silver nitrate is done using 0.1M sodium hydroxide (NaOH) and a calibrated pH instrument to achieve a silver nitrate solution between 7 and 9, which improves the reduction of silver ions. A 1 mM silver nitrate solution was mixed with the extract in 1:3 ratio. The green synthesis of AgNPs from herbals (*A. muricata* and *P. guajava*) extract was evaluated, and the preliminary examination involving the identification of color changes have been noted, confirming the synthesis of AgNPs. In the current study, a color change from dark green to reddish brown was observed, determining the formation of AgNPs. The nanoparticles are purified via centrifugation and dried at 50°C. In this study, ultraviolet-visible spectroscopy was used to evaluate the absorbances within the range of 300–700 nm (Graph 1). The absorbance of AgNPs has maximum peaks at 435 nm, suggesting the existence of AgNPs synthesized by *A. muricata* and *P. guajava*. The morphological characteristics of the synthesized nanoparticles were determined using the standard error of mean analysis. The size was represented by a nanometer in the range of 10–100, 38.14 nm, and it was confirmed by the histogram (Graph 2).

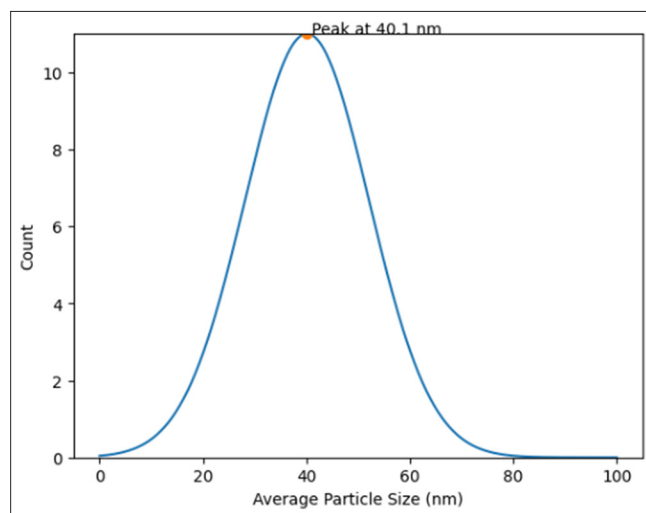
With respect to the methodological procedures followed, concentration-dependent interactions between prepared herbal-incorporated AgNPs and the net growth rate of *Streptococcus mutans* and *C. albicans* over regular time intervals were calculated using the time kill curve assay [17]. The microbial strains used in this study were *S. mutans* and

C. albicans, obtained from the Department of Microbiology, Saveetha Dental College and Hospital, Chennai, Tamil Nadu. The strains were cultured in Mueller-Hinton Broth (MHB) supplemented with varying concentrations of herbal-incorporated Ag nanoparticles (25 µg/mL, 50 µg/mL, and 100 µg). A fresh overnight culture was prepared by inoculating a single colony into the broth and incubating at 37°C for 18–20 h until it reached the logarithmic phase. Amoxrite and fluconazole served as the standard, and the control was cultured broth. Before the experiment, growth curves were established following a 4-h pre-incubation period in a drug-free medium to ensure that all pathogens had reached a stable early-to-mid log phase. Subsequently, at regular time intervals, the percentage of dead cells was quantified by measuring absorbance at 600 nm.

Simultaneously, the synthesized herbal-incorporated AgNPs were carefully integrated into a specially formulated denture cleanser to enhance its antimicrobial and antifungal properties. This formulation was designed to effectively cleanse dentures while minimizing microbial biofilm formation. Key components (Table 1) 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. Effervescent, chelating, lubricating, and stabilizing agents are also incorporated to aid in breaking down and eliminating biofilms, ensuring thorough cleaning of the denture surface. In addition, flavoring agents are added to improve palatability and user compliance. Once formulated, the denture cleanser undergoes rigorous testing to evaluate its antimicrobial and antifungal efficacy against common oral pathogens such as *C. albicans* and *S. mutans*. Furthermore, cytotoxicity assessments are performed to ensure the safety and biocompatibility of the product, confirming that it does not pose any harm to oral tissues.



Graph 1: Ultraviolet visible spectra of green-synthesized silver nanoparticles representing the absorbance



Graph 2: Histogram of silver nanoparticles

Table 1: Composition of formulated denture cleanser

Serial number	Name of the ingredient	Quantity (%)	Uses
1	Sodium bicarbonate	40	Effervescent
2	Citric acid	16	Effervescent
3	Sodium lauryl sulfoacetate	4	Surfactant
4	Sodium hexametaphosphate	6	Sequestering/chelating agent
5	EDTA	10	Sequestering/chelating agent
6	Tartaric acid	20	Effervescent
7	Tea Tree oil	0.4	Lubricating/stabilizing agent, Flavoring agent
8	Peppermint oil	0.3	Lubricating/stabilizing agent, Flavoring agent
9	Polyethylene glycol	5	Emulsifier/dispersant
10	Polyvinylpyrrolidone	1.6	Film-forming agent/stabilizing/binding
11	Nanoparticle	1	Antimicrobial, anti-inflammatory

Herbal-incorporated silver nanoparticles (AgNPs) were synthesized from *Psidium guajava* and *Annona muricata* leaf extracts. EDTA: Ethylenediaminetetraacetic acid

Cytotoxicity of denture cleanser was evaluated using the zebra embryonic cytotoxicity test [18]. A single female fish was mated with two male fish to obtain fertilized embryos. Positive control, such as 0.1 mg/L of potassium dichromate, was used to validate the sensitivity of the embryos, whereas E3 medium served as a negative control. The viable eggs were collected and rinsed using an E3 medium, composed of 5 mmol/L sodium chloride, 0.17 mmol/L potassium chloride, 0.33 mmol/L calcium chloride, and 0.33 mmol/L magnesium sulfate, maintaining a pH of 7.2 without the addition of methylene blue. The experimental design included five test groups exposed to varying concentrations of herbal nanoparticle incorporated denture cleanser (5, 10, 20, 40, and 80 µL) and one control group. Each well contained five fertilized embryos and the E3 medium for incubation.

The fertilized embryos were exposed to the different concentrations of denture cleanser for 96 h, covering the normal zebrafish embryonic hatching period of 48–72 h post-fertilization (hpf). The hatching rate and embryo viability were assessed and recorded at 24-h intervals. Any dead embryos or larvae were immediately recorded and removed. Observations were read using a stereo microscope.

The antimicrobial activity of green-synthesized AgNPs was evaluated using the agar well diffusion technique [19]. Mueller-Hinton Agar was prepared according to standard protocols and sterilized using an autoclave. After sterilization, the molten agar medium was allowed to cool to approximately 45–50°C and poured into sterile Petri plates. Fresh bacterial and fungal suspensions of *S. mutans* and *C. albicans*, respectively, were prepared. Using a sterile cotton swab, both microbial suspensions were uniformly spread onto separate agar plates to ensure even distribution of the test organisms. Wells of 9 mm diameter were then created in the agar using a sterile polystyrene tip, maintaining an adequate distance between them to prevent overlapping of inhibition zones. The wells were filled with different concentrations of formulated denture cleanser (25 µg, 50 µg, and 100 µg), while a commercial denture cleanser, Clinsodent, served as the standard control. The plates were left at room temperature for 15–20 min to allow diffusion of the test compounds before incubation. The bacterial plates were incubated at 37°C for 24 h, whereas the fungal plates were incubated at the same temperature for 48 h. Following incubation, the antimicrobial efficacy of AgNPs was determined by measuring the diameter of the zone of inhibition surrounding each well using a ruler or Vernier caliper, with results recorded in millimeters (mm). The effectiveness of the AgNPs at different concentrations was then analyzed by comparing the inhibition zones with those of the standard control.

The study involved the use of two types of heat-cure PMMA specimens, namely, Ivocap and Dentsply. Circular-shaped specimens, each with a dimension of 1 cm in diameter and 3 mm in thickness, were fabricated. The heat-cure PMMA specimens were fabricated using a standard molding and polymerization process. A wax pattern of 1 cm in diameter and 3 mm in thickness was prepared and invested in a dental flask using

dental stone. After wax elimination, the mold was packed with heat-cure Dentsply PMMA resin following the manufacturer's instructions. The polymerization process was carried out, and the specimens were retrieved, trimmed, polished, and sterilized before antimicrobial testing. The injection molding technique was used to fabricate Ivocap heat-cure PMMA specimens. Wax patterns were created and invested in Type III dental stone within a metallic injection flask. Polymerization was carried out, the PMMA specimens were removed, and the finished specimens were washed, sterilized, and stored in a sterile container for further experimental use.

The antimicrobial efficacy of three groups, such as standard (Clinsodent denture cleanser), test (formulated herbal AgNP denture cleanser), and control (plain water), was evaluated. For the standard group, 2 g of commercially available Clinsodent powder was accurately weighed and dissolved in 20 mL of distilled water, ensuring a homogeneous solution. Similarly, for the test group, 2 g 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 *S. mutans* and *C. albicans* by measuring the zone of inhibition around the wells.

To prepare the Candida broth, isolated colonies of *C. albicans* were first grown on Rose Bengal Agar [20]. The well-formed colonies were carefully isolated and inoculated into MHB, followed by incubation at 37°C for 24–48 h to allow optimal fungal growth. The sterile acrylic specimens were immersed in candidal broth. The specimens were then further incubated for 16 h 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 h which mimics the protocol overnight soaking of dentures, following which swabbing technique is followed where specimen surfaces are swabbed with a sterile cotton, which is then immersed in sterile saline and vortexed to release the collected cells after which candidal cell count was calculated under light microscope (COSLAB HL-10A).

Statistical Package of Social Science (SPSS) (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 the Kolmogorov-Smirnov test for normality. It was found that the data were normally distributed ($p > 0.05$) and hence parametric tests of significance were used. Means and standard deviations were established. The number of colonies among the experimental groups was analyzed using One-way analysis of variance, following which intergroup comparison was made using *post hoc* Tukey HSD analysis.

RESULTS

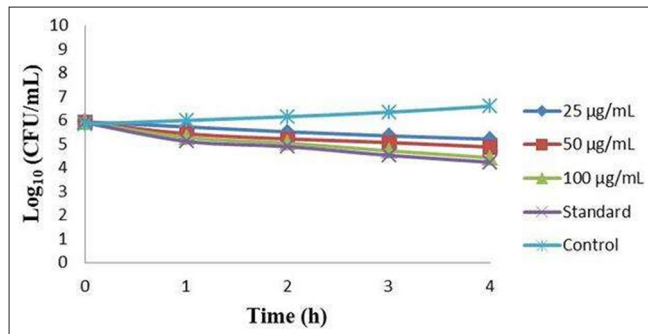
Graphs 3 and 4 represent the bacterial population reduction over time when formulated herbal-incorporated AgNPs are exposed to antimicrobial agents. Tables 2 and 3 reveal that at 100 µg/mL, the studied *S. mutans* and *C. albicans* exhibited reduced colony-forming units with maximum efficiency on *C. albicans*, almost mimicking the standards.

The results from Graphs 5 and 6, comparing the hatching rate (%) and viability rate (%) at different concentrations of AgNP incorporated herbal denture cleanser, demonstrate a dose-dependent cytotoxic effect. The control group, which was not exposed to AgNPs, shows

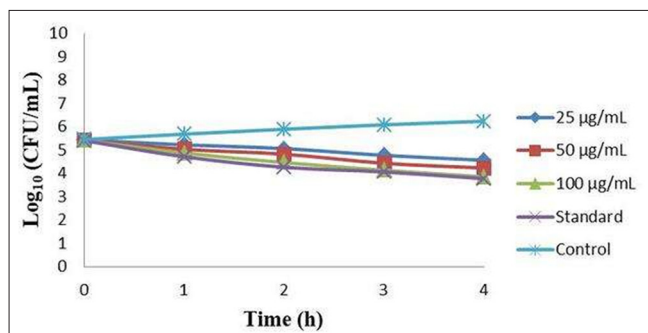
the highest hatching and viability rates, indicating normal biological conditions. At lower concentrations (5–10 µg/mL), both the hatching and viability rates remain relatively high, suggesting minimal toxicity. However, as the concentration increases to 20–40 µg/mL, a noticeable decline is observed in both parameters, with the hatching rate decreasing more significantly than the viability rate, implying that embryonic development is more sensitive to AgNP exposure. At the highest concentration (80 µg/mL), both rates drop sharply, with the hatching rate falling to approximately 50–55% and the viability rate showing a similar reduction, confirming strong cytotoxic effects.

Graph 7 illustrates the antimicrobial activity of a denture cleanser combined with AgNPs against *C. albicans* and *S. mutans* at different concentrations, with a commercial denture cleanser (Clinsodent – 20 µg) as the standard control. The results in Figs. 1 and 2 indicate that herbal infused AgNPs denture cleanser shows enhanced antimicrobial effectiveness, with the highest inhibition seen at 100 µg/mL (Table 4) when compared to the standard.

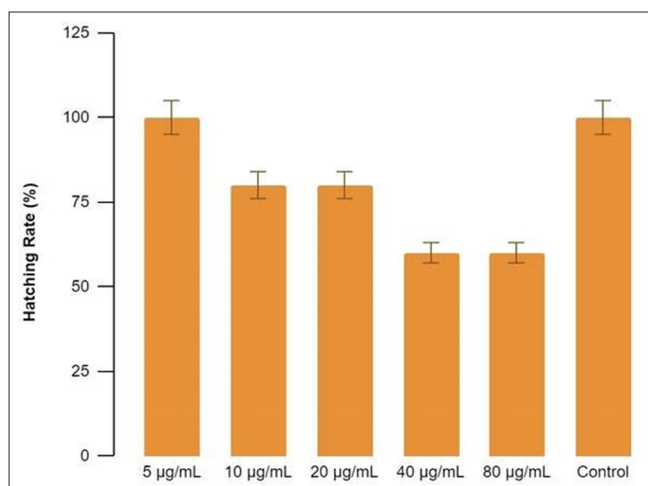
Descriptive statistics were made, and data of antimicrobial efficiency of the Dentsply and Ivocap PMMA specimens were compared based on the number of colonies among the experimental groups using one-way analysis of variance. For all comparisons, $p < 0.05$ was considered to be statistically significant. Similarly, intergroup comparison (Table 5)



Graph 3: Represents the bacterial population (*Streptococcus mutans*) reduction over time when exposed to antimicrobial agents



Graph 4: Represents the bacterial population (*Candida albicans*) reduction over time when exposed to antimicrobial agents



Graph 5: Hatching rate of zebrafish embryos after 96 h of exposure to various concentrations of the formulated herbal silver nanoparticles denture cleanser

Table 2: Antibacterial activity of herbal-incorporated AgNPs against *Streptococcus mutans* expressed as Log₁₀ colony-forming units/mL over time

<i>Streptococcus mutans</i>	Time in hours				
	0	1	2	3	4
25 µg/mL	5.93	5.72	5.51	5.34	5.2
50 µg/mL	5.92	5.43	5.21	5.06	4.87
100 µg/mL	5.91	5.27	5.03	4.71	4.42
Standard (amoxrite)	5.9	5.11	4.89	4.52	4.23
Control (broth)	5.84	5.99	6.15	6.34	6.59

Values represent the mean Log₁₀ colony-forming units per mL (CFU/mL).

Standard: Amoxrite (for *S. mutans*) Control: Mueller-Hinton broth, AgNPs: Silver nanoparticles

Table 3: Antifungal activity of herbal-incorporated AgNPs against *Candida albicans* expressed as Log₁₀ colony-forming units/mL over time

<i>C. albicans</i>	Time in hours				
	0	1	2	3	4
25 µg/mL	5.43	5.22	5.06	4.77	4.56
50 µg/mL	5.42	5.03	4.82	4.43	4.23
100 µg/mL	5.41	4.87	4.46	4.13	3.85
Standard (fluconazole)	5.4	4.71	4.26	4.06	3.77
Control (broth)	5.45	5.68	5.89	6.08	6.23

Values represent the mean Log₁₀ colony-forming units per mL (CFU/mL).

Standard: Fluconazole (for *C. albicans*), Control: Mueller-Hinton broth.

CFU: Colony-forming units, AgNPs: Silver nanoparticles

Table 4: Zone of inhibition (mm) of formulated denture cleanser against *C. albicans* and *S. mutans*

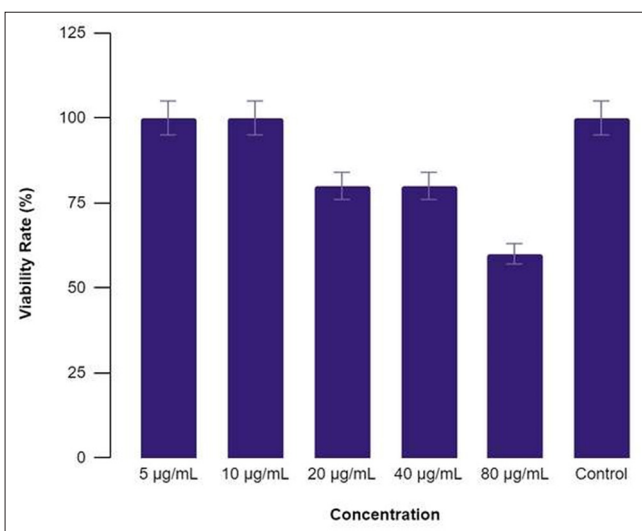
Organisms	Formulated denture cleanser			Standard (Clinsodent) 20 µg/mL
	25 µg/mL	50 µg/mL	100 µg/mL	
<i>C. albicans</i>	18	21	25	15
<i>S. mutans</i>	10	15	17	9

Values are the mean diameter of the inhibition zone (mm), including the well diameter of 9 mm. Standard: Commercial denture cleanser (Clinsodent, 20 mg/mL). *S. mutans*: *Streptococcus mutans*, *C. albicans*: *Candida albicans*

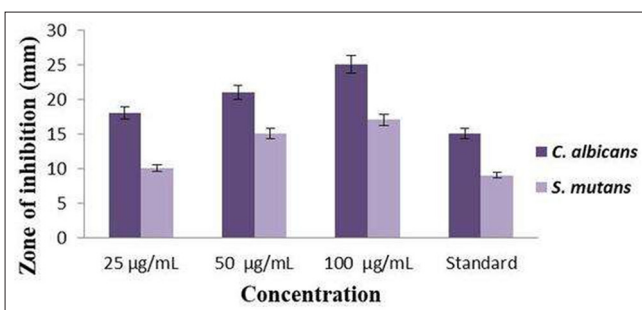
Table 5: Descriptive and comparative evaluation of antimicrobial efficiency of Dentsply polymethylmethacrylate and Ivocap denture base material

Variables	n	Mean	SD	SEM	p-value	Intergroup comparison		p-value
Dentsply PMMA								
Control	15	2628	399.1734	103.0661	<0.001***	Control	Standard	<0.001***
Standard	15	1980.8	501.8075	129.5661		Standard	Test	<0.001***
Test	15	1692.8	237.8965	61.42461		Test	Control	0.125
							Standard	<0.001***
Ivocap								
Control	15	2512	293.1562	75.69261	<0.001***	Control	Standard	<0.001***
Standard	15	2015.2	85.23178	22.00675		Standard	Test	<0.001***
Test	15	1713.6	269.5596	69.6		Test	Control	0.003**
							Standard	<0.001***

***p<0.001, **p<0.01. *Very highly significant, **Highly significant. Control: Distilled water; Standard: Commercial denture cleanser (Clinsodent), Test: Formulated herbal AgNP denture cleanser. Data presented as mean±SD. As determined by one-way ANOVA followed by *post hoc* Tukey HSD test. SD: Standard deviation, PMMA: Polymethylmethacrylate, SEM: Standard error of mean



Graph 6: Viability rate of zebrafish embryos/larvae after 96 h of exposure to various concentrations of the formulated herbal silver nanoparticles denture cleanser



Graph 7: Illustrates the antimicrobial activity of a denture cleanser combined with silver nanoparticles against *Candida albicans* (fungus) and *Streptococcus mutans*

was done using *post hoc* Tukey HSD analysis, which revealed statistical significance with p<0.001.

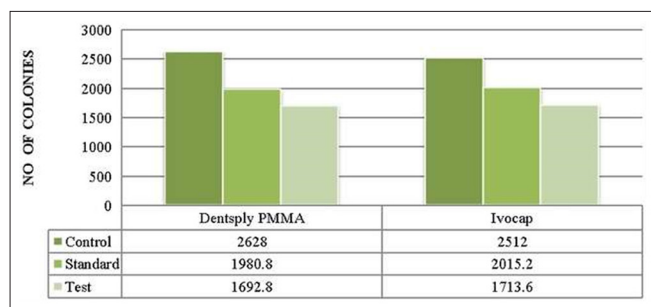
Graph 8 presents a comparison of the number of bacterial colonies found on two different denture materials between three different experimental groups. In the control group, where no cleanser was used, the highest bacterial colony counts were observed, with Dentsply



Fig. 1: Representative images of agar well diffusion plates showing zones of inhibition against *Candida albicans*. Wall labels: S-standard (Clinsodent); t-test (Formulated herbal silver nanoparticles denture cleanser) at concentrations of 25, 50, and 100 µg/mL



Fig. 2: Representative images of agar well diffusion plates showing zones of inhibition against *Streptococcus mutans*. Wall labels: S-standard (Clinsodent); t-test (Formulated herbal silver nanoparticles denture cleanser) at concentrations of 25, 50, and 100 µg/mL



Graph 8: Comparison of the number of bacterial colonies found on two different denture materials between three different experimental groups

showing 2628 colonies and Ivocap showing 2512 colonies. When the standard cleanser was applied, there was a noticeable reduction in bacterial colonies, with Dentsply decreasing to 1980.8 and Ivocap to 2015.2. The most significant reduction was seen in the test cleanser group, where the colony counts were the lowest, at 1692.8 for Dentsply PMMA and 1713.6 for Ivocap.

Based on the antimicrobial assay, the test cleanser demonstrated the greatest antibacterial efficacy for both denture materials. This suggests that AgNPs could be a promising antimicrobial agent, particularly for combating fungal infections in denture users. Overall, the cytotoxicity findings indicate that lower concentrations of the formulated denture cleanser appear safe while higher concentrations can have significant toxic effects, potentially limiting its safe applications.

DISCUSSION

According to a report by the World Health Organization, around 80% of the population in this evolving world shows interest in traditional plant extracts as antioxidants, antibacterial, antifungal, and antiviral agents. *P. guajava* (Guava) can be considered an eco-friendly plant suitable for low-carbon and green growth [21,22]. Guava fruits and leaves are value-added plants known for their cosmetics results and food materials. Guava leaves have been reported to contain secondary metabolites such as flavonoids, sesquiterpenes, triterpenoids, coumarins, alkaloids, and tannins [23]. Many studies on Guava leaves reported various phytochemical activities of concentrated *P. guajava* leaf extract, such as a hyperglycemia inhibitory effect, an antioxidant effect, an antibacterial effect [24-26], and tyrosinase inhibitory activity. Among the constituents, sesquiterpene is reported to have antioxidant activity, antibacterial activity, tyrosinase and collagenase inhibition activity [27-29].

The qualitative studies of *A. muricata* leaf extracts revealed the presence of various Phytochemicals such as phenolic compounds, which are considered important for human health, reducing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macro-molecular oxidation [30]. These compounds also revealed antioxidant potential activity with the presence of alkaloids and flavonoids (anthocyanins and leuco-anthocyanins) and their ability to induce many biological activities because flavonoids are a group of phenolics compounds involved in many biological effects [31-33] such as anti-inflammatory activity, antioxidant, and hepatoprotective activities [34]. Biologically, *A. muricata* is potent against microorganisms due to the presence of acetogenins. These are bioactive compounds found in the Annonacea family. Acetogenins are known to have tumoricidal, anti-malarial, anti-helminthic, antiviral, and antimicrobial effects, suggesting its potentiality in multiple applications. Among the annonaceous-acetogenins, bullatacin, an acetogenin, is a powerful tumoricidal and antibacterial agent. Researchers concentrate on green route synthesis to avoid hazardous chemicals and physical abrasion-based incidents involved in chemical and physical methods involved in the fabrication of metallic nanoparticles. Green synthesis of metallic

nanoparticles includes the reduction of metal ion source compounds by the extract of any plant and capping of newly synthesized nanoparticles by phytochemicals present in the extract. Several researchers have attempted the synthesis of metallic nanoparticles using mainly aqueous extract of *P. guajava* leaves.

Recent studies have explored the incorporation of these green-synthesized AgNPs into dental materials to combat microbial colonization [35,36]. For instance, a study done by Garcia *et al.* in 2024 investigated the antifungal properties of tissue conditioners modified with chitosan and green-synthesized AgNPs, indicating that even minimal concentrations of these nanoparticles effectively inhibited the growth of *C. albicans*, suggesting their potential in preventing denture stomatitis [15,37]. Adam and Khan evaluated the antimicrobial activity of heat-cure denture base resins integrated with varying concentrations of AgNPs, and findings demonstrated significant antimicrobial effects against *C. albicans* and *S. mutans*, highlighting the promise of AgNPs in enhancing the antimicrobial properties of denture materials [38-40].

In the current study, antimicrobial activity of the formulated denture cleanser combined with AgNPs demonstrates a clear dose-dependent effect, as evidenced by the increasing zone of inhibition with higher AgNP concentrations. The largest inhibition zone is observed at 100 µg/mL, indicating that the antimicrobial efficacy of AgNPs intensifies with higher concentrations. This suggests that AgNPs exhibit a strong inhibitory effect on microbial growth, reinforcing their potential as effective antimicrobial agents.

The mechanism involves AgNPs disrupting microbial cell membranes and interfering with cellular metabolism, leading to cell death [41]. In addition, the results highlight a greater susceptibility of *C. albicans* compared to *S. mutans* across all tested concentrations. The inhibition zones for *C. albicans* are consistently larger than those for *S. mutans*, indicating that the denture cleanser combined with AgNPs is more effective against fungal strains than bacterial ones. This trend suggests that *C. albicans* is more sensitive to AgNPs, potentially due to differences in cell wall composition and structural susceptibility. Furthermore, when comparing the test samples to the standard denture cleanser (Clinsodent), it is evident that the denture cleanser alone has a lower antimicrobial effect compared to all AgNP-treated samples, particularly against *C. albicans*. This finding suggests that while the denture cleanser possesses some inherent antimicrobial properties, the addition of AgNPs significantly enhances its effectiveness, making it a promising approach for improving antimicrobial formulations, especially for denture hygiene. This comprehensive approach ensures that the herbal-based AgNP-infused denture cleanser is both effective and safe for long-term use, offering an innovative solution for maintaining optimal denture hygiene.

Based on a material perspective, the significant difference in antimicrobial activity may be due to differences in material composition, polymerization technique, surface properties, and interaction with cleansers. In addition, variations in hydrophobicity and wettability could influence bacterial attachment, with Dentsply potentially being more hydrophilic, thereby limiting bacterial retention. The chemical structure of Dentsply PMMA may also interact more effectively with the active ingredients in cleansers, enhancing antimicrobial action. Due to super-hydrophilic surfaces with a "water sheathing" effect created by AgNPs, microbial attachments are hindered [42]. Furthermore, a higher degree of polymerization and cross-linking in Dentsply could result in a more stable and less permeable material, preventing bacterial penetration into microvoids and making cleansing more efficient.

With the rising resistance of microorganisms to conventional drugs, it is essential to explore new strategies to prevent the emergence of multidrug-resistant strains [43]. Thus, in our current study, the denture cleanser with a combined formulation of *P. guajava* and *A. muricata* with AgNPs demonstrated an antimicrobial effect almost similar to commercial

denture cleansers. With the incorporation of such natural derivatives, the deleterious effects of chemicals on oral tissues can be avoided. This combination at the lowest concentration (10 µg/mL) exhibited maximum action against *C. albicans* and *S. mutans*. Interestingly, our literature does not uncover any prior studies evaluating such a denture cleanser formulation. This highlights the novelty of our findings and the need for further *in vivo* studies in this area of research. However, the key technological challenge in nanoparticle development lies in scaling up the synthesis process while maintaining consistent optimization and reliably predicting their performance [44].

As a limitation, this study being *in vitro* does not simulate the clinically relevant oral conditions, such as saliva, microbial biofilms, long-term effects of denture cleanser use on oral tissues. Further *in vivo* experiments at the molecular level are necessary to validate these research findings. Clinical trials are needed to assess the effectiveness of this denture cleanser formulation in a human setup.

CONCLUSION

Formulated denture cleanser with *P. guajava* and *A. muricata* leaf extract with AgNPs incorporation was efficient on the studied organisms. The present study demonstrated the *in vitro* efficiency of formulated denture cleanser, almost similar to that of commercial denture cleanser, with the highest antimicrobial property against *C. albicans* followed by *S. mutans*. Thus, nature serves as a cure against many harmful oral pathogens, best use of such resources would be a permanent solution for many dental problems.

AUTHOR CONTRIBUTION

KS: Conceptualization, methodology, data collection, analysis, and original draft preparation. US: Supervision and project administration. MA: Manuscript revision. AK: Critical review and validation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to this manuscript.

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ETHICS AND CONSENT TO PARTICIPATE

Not applicable.

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