

# Comparative Effectiveness of Microwave, Chemical, and Herbal Disinfectants on Surface Microhardness of Heat Cured Acrylic Resin- An *in vitro* Study

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## ABSTRACT

**Background and Aims:** Disinfection of acrylic dentures is essential to prevent pathogen transmission; however, its effect on the surface microhardness of denture base resins remains unclear. This study aimed to evaluate and compare the effects of chemical, microwave, and herbal disinfection methods on the microhardness of heat-cured acrylic denture base resin.

**Methods:** In this *in vitro* study, 200 rectangular specimens were fabricated and divided into four groups (n=50): control (Group X), chemical (Group C), microwave (Group M), and herbal (Group H). Each experimental group was further split into two subgroups based on disinfection duration—one cycle (7 days) and three (21 days) cycles. Surface microhardness was measured using Vickers hardness testing (50 g force for 10 seconds). Data were analyzed using Kruskal-Wallis, Dunn post hoc, and Wilcoxon signed-rank tests (p<0.05).

**Results:** After 7 days, the microwave group showed the highest microhardness, followed by the chemical, control, and herbal groups. At 21 days, chemical disinfection resulted in the highest microhardness, followed by microwave, control, and herbal groups. Statistically significant differences were observed between groups for both time points.

**Conclusion:** Microwave and chemical disinfection had the most favorable effects on the surface microhardness of heat-cured acrylic resins at 7 and 21 days, respectively, while herbal disinfection consistently showed the lowest values.

**Keywords:** Denture cleansers, denture cleansing methods, denture-based resins, microhardness

## INTRODUCTION

PMMA (Polymethyl methacrylate) resin remains a material of choice for denture bases, owing to its proven clinical

performance, aesthetic appeal, desirable physical characteristics, cost efficiency, and ease of manipulation.<sup>[1,2]</sup> PMMA prosthetics face significant drawbacks, including

dimensional inaccuracies, susceptibility to microbial adhesion, inadequate mechanical strength, and potential allergic reactions, which can compromise their clinical effectiveness.<sup>[3]</sup> Surface roughness and pitting in acrylic denture bases create favorable biofilm formation and microbial colonization conditions.<sup>[4]</sup> Prostheses contaminated with pathogenic microorganisms can act as vectors for cross-contamination. Poor denture hygiene can lead to bad breath, staining, and calculus build-up, which may further contribute to denture stomatitis, angular cheilitis, and opportunistic infections.<sup>[5]</sup>

Denture sterilization is essential for preventing cross-contamination and protecting the oral mucosa in patients with removable dental prostheses. An ideal denture cleanser should be biocompatible and non-toxic, effectively removing deposits without damaging the denture material and being easy for patients to use.<sup>[4]</sup> Generally, denture cleaning methods are divided into three categories: mechanical (e.g., brushing), chemical (e.g., creams, liquids, or tablets), and physical (microwave irradiation).<sup>[6]</sup> Additionally, plant oils and their extracts, which have long been used in food preservation for their antioxidant and antimicrobial properties, have been studied and shown to be effective as denture cleansers.<sup>[7]</sup>

While dental cleansers are essential for denture sterilization, studies have evaluated their effects on denture resins' physical and mechanical properties.<sup>[8,9]</sup> Prolonged use of alkaline peroxides in chemical cleansers can bleach acrylic resin.<sup>[10]</sup> Alkaline hypochlorites can tarnish and discolour metal components, such as cobalt-chromium alloys in partial dentures, and also bleach acrylic resin. Chlorhexidine gluconate solutions cause notable discoloration with routine soaking, making them unsuitable for daily use.<sup>[11,12]</sup> Furthermore, microwave disinfection can affect mechanical properties like flexural strength, impact strength, color stability, and surface roughness.<sup>[13]</sup>

Surface properties are crucial to the clinical success of prostheses, and one key property is surface hardness. Denture surface hardness is measured by its resistance to indentation, which reflects its ability to withstand scratching.<sup>[14]</sup> Measuring the surface hardness of a denture base resin reveals how well it can withstand the forces exerted during chewing. Therefore, if the hardness significantly decreases with repeated disinfection cycles, the wear on the denture base material increases, which can result in the fracture of the denture.

Although studies have investigated the effects of dental cleansers on surface properties like color stability, solubility, and surface hardness, there is a paucity of evidence on the comparative efficacy of three different dental cleansers on the microhardness of dental resins. Therefore, this study aimed to assess and compare the effects of chemical, microwave, and herbal disinfectants on the surface microhardness of heat-polymerized acrylic denture base resin.

## **MATERIALS & METHODS**

**Study setting and location:** The present study was conducted in the dental clinic, and the testing procedure was carried out in the technical laboratory of Cuttack, India, using a surface hardness tester called the Vickers hardness test. Sample size calculation was performed using a significance level ( $\alpha$ ) of 0.05 and a power of 0.95. A sample size of 194 was obtained, which was rounded to 200 with 50 samples per group. Ethical approval was not required because no human or animal subjects were involved.

### **Study procedure:**

The test specimens were fabricated using heat-cured polymethyl methacrylate (PMMA) resin (DPI Heat Cure PINK™, Dental Products of India Ltd., India; 2135). These samples underwent evaluation using a Vickers microhardness tester machine (Micro Vickers VH-1MDX, Economet, Chennai Metco).

### Sample preparation:

**I. Fabrication of metal dies:** A stainless-steel die was created to produce two batches of 100 bar specimens each (**Figure 1**), by ISO/FIDs 1567 standards, each measuring 65mm x 10mm x 3.3mm. The metal dies

featured a central threaded hole to facilitate easy removal from the stone mold. Initially, a cold mold seal (Major 2, Dental Products of India, batch no- RRL 051) was applied to the surface of the metal die and then left to dry at room temperature.

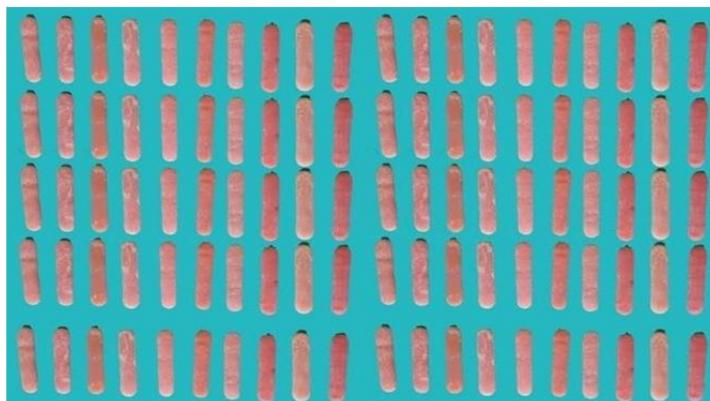


Figure 1: Rectangular specimens prepared with heat-cured acrylic resin

### II. Preparation of PMMA resin samples:

Conventional heat-polymerized denture base resin (DPI Heat Cure) was employed in this study. Following the manufacturer's instructions, 200 samples were fabricated by mixing 3 ml of monomer with 7.5 g of polymer powder in a porcelain container. During the dough stage, the resin was packed into the die and cured in an acrylic bath at 74°C for eight hours, followed by a final boil at 100°C for one hour. The specimens were then bench-cooled overnight. After polymerization, the resin samples were removed from the mold, trimmed of excess material, and subsequently finished and polished. The 200 rectangular specimens were then equally divided into four groups of 50 specimens each. Among these four groups, 1 group served as a control, and the other three were divided into two subgroups.

**Group X:** Control group

**Group C:** Chemical disinfectant (Clinsodent powder) group. Further divided into two subgroups namely, C1 (following 1 cycle) and C3 (following 3 cycles)

**Group M:** Microwave disinfectant group. Further divided into two subgroups, namely,

M1 (following 1 cycle) and M3 (following 3 cycles)

**Group H:** Herbal disinfectant (Thyme essential oil) group. Further divided into two subgroups, namely, H1 (following 1 cycle) and H3 (following 3 cycles)

### Chemical disinfection of the samples

**(Group C):** The chemical disinfectant solution was prepared by dissolving one tablespoon of Clinsodent powder (ICPA Health Care Pvt. Ltd.) in 150 ml of water in a clean container. After initial storage in distilled water, 50 test specimens were immersed in the prepared solution at room temperature for 10 minutes. Following disinfection, the samples were rinsed under tap water for 30 seconds and then stored in distilled water at room temperature for seven days. (C1 group)

### Microwave disinfection of the samples

**(Group M):** Another set of 50 specimens was irradiated using a domestic microwave unit (LG, Model 7880 PSR, India) at 650 W for 3 minutes. Following irradiation, the samples were stored in distilled water at room temperature for seven days. (M1 group).

### Herbal disinfection of the samples

**(Group H):** The herbal disinfectant solution was formulated by combining 5 ml of thyme essential oil (Nature Man, 100% Pure & Natural Essential Oil), 5 ml of 0.5% surfactant (Purenso), 80 ml of ethanol, and 910 ml of distilled water. From this mixture, 150 ml was used to immerse the Group H specimens for 15 minutes. Following immersion, the 50 samples were rinsed under tap water for 30 seconds and then stored in distilled water at room temperature for seven days. (H1 group).

For the C3, M3, and H3 groups (21-cycle groups), the disinfection procedure was repeated three times, with a seven-day interval between each cycle. Following the final disinfection cycle, the specimens were stored in distilled water at room temperature for seven days.

**III. Testing of samples:** To mimic intraoral conditions, all specimens were pre-conditioned by immersion in distilled water at 37°C for 48 hours in an incubator before mechanical testing.

**Surface hardness test:** Microhardness was tested at Cuttak, India. The first testing was done after seven days (One Cycle), and the second was done after 21 days (three cycles). The hardness of the samples was assessed using the Vickers hardness test (VHT), a commonly employed method for evaluating surface resistance to indentation. This test involves pressing a diamond indenter, shaped as a right pyramid with a square base and an included angle of 136° between opposing faces, into the material's surface using a force of 50 grams (0.5 N) applied for 10 seconds. Three marks were made on the surface of each specimen, one in the center and one at each end. The average of the three indentations was considered the specimen hardness and expressed as VHN.

### STATISTICAL ANALYSIS

Data sets for each group were evaluated for normality, and mean and median values were computed accordingly. Due to the violation of normality assumptions identified by the Shapiro–Wilk test, non-parametric methods were utilized for statistical analysis. The mean values of the four subgroups were compared using a Kruskal-Wallis test and Dunn-Bonferroni *post hoc* analysis. The Wilcoxon Signed-Rank Test was done for pairwise comparisons. All statistical analyses were conducted using SPSS Version 24.0 (IBM Corp., Armonk, NY, USA). A *P-value* of  $\leq 0.05$  is considered statistically significant.

### RESULT

**Table 1** shows the microhardness values of the control, microwave (M1), Clinsodent (D1), and herbal (T1) disinfection after seven days. The microhardness was highest in the microwave group ( $16.34 \pm 0.13$ ), followed by the chemical ( $16.10 \pm 0.18$ ) and control group ( $15.59 \pm 0.17$ ). The lowest microhardness was found in the herbal disinfection group ( $13.09 \pm 0.34$ ). A statistically significant variation in microhardness across the groups was observed using the Kruskal–Wallis test ( $P < 0.001$ ). Dunn-Bonferroni *post hoc* analysis (Table 2) examined the differences between the groups. It was found that there was a statistically significant difference among all three groups, with the greatest mean difference observed between the Microwave and herbal groups (3.250).

**Table 1: Comparison of microhardness of study groups after seven days between the groups using the Kruskal-Wallis test**

Groups	Mean $\pm$ SD	<i>P-value</i>	<i>H value</i>
Clinsodent group	16.10 $\pm$ 0.18	0.000*	85.64
Microwave group	16.34 $\pm$ 0.13		
Herbal group	13.09 $\pm$ 0.34		
Control group	15.59 $\pm$ 0.17		

\**P-value* < 0.001

**Table 2: Dunn (post-hoc) test showing difference between the microhardness values of different groups (seven days)**

Treatment pair	Mean diff	P-value	T value	Confidence Interval (CI)
Clinsodent vs control	.505	0.000*	-14.42	-0.574 to -0.435
Microwave vs control	.745	0.000*	-24.61	-0.805 to -0.685
Herbal vs control	2.50	0.000*	46.59	2.398 to 2.612
Clinsodent vs microwave	.240	0.000*	7.64	0.178 to 0.302
Clinsodent vs herbal	3.010	0.000*	-55.32	-3.119 to -2.902
Microwave vs herbal	3.250	0.000*	-63.13	-3.352 to -3.148

\*P-value<0.001

**Table 3** shows the microhardness values of the control, microwave (M3), Clinsodent (D3), and herbal (H3) disinfection after 21 days. The microhardness was highest in the chemical group (15.8±0.47), followed by the microwave (15.64±0.15) and control group (15.60±0.10). The lowest microhardness was found in the herbal disinfection group (12.83±0.34). Kruskal-Wallis test showed a statistically significant

difference ( $P<0.001$ ) in microhardness among the groups. Dunn-Bonferroni *post hoc analysis* (Table 4) examined the differences between the groups. It was found that there was a statistically significant difference among all three groups, with the greatest mean difference observed between the Clinsodent and herbal groups (3.256).

**Table 3: Comparison of microhardness after 21 days between the groups using the Kruskal-Wallis test**

Groups	Mean±SD	P-value
Clinsodent group	15.8±0.47	0.000*
Microwave group	15.64±0.15	
Herbal group	12.83±0.34	
Control group	15.60±0.10	

\*P-value<0.001

**Table 4: Dunn (post-hoc) test showing difference between the microhardness values of different groups (21 days)**

Treatment pair	Mean diff	P-value	T value	Confidence Interval (CI)
Clinsodent vs control	0.20	0.004*	-2.94	-0.335 to -0.065
Microwave vs control	0.04	0.119**	-1.56	-0.090 to 0.010
Herbal vs control	2.77	0.000*	55.26	2.6705 to 2.8695
Clinsodent vs microwave	0.16	0.008*	-2.29	-0.298 to -0.021
Clinsodent vs herbal	2.97	0.000*	-36.20	-3.139 to -2.807
Microwave vs herbal	2.81	0.000*	-53.46	-2.914 to -2.706

\*P-value<0.05, \*\*P-value>0.05

**Table 5** shows intragroup comparisons of the study groups' microhardness at 7 and 21 days using the Wilcoxon Signed-Rank Test.

The results showed a significant difference between all the groups on the 7<sup>th</sup> and 21<sup>st</sup> days, except for the control group.

**Table 5: Comparison of microhardness of study groups at seven days and 21 days using the Wilcoxon Signed-Rank Test**

Groups	Seven days (mean±SD)	21 days (mean±SD)	P-value	Confidence Interval (CI)
Clinsodent group	16.10±0.18	15.8±0.47	0.000*	-0.441 to -0.159
Microwave group	16.34±0.13	15.64±0.15	0.000*	-0.756 to -0.644
Herbal group	13.09±0.34	12.83±0.34	0.010*	-0.395 to -0.125
Control group	15.59±0.17	15.60±0.10	0.720	-0.0454 to 0.0654

\*P-value<0.05

## DISCUSSION

Maintaining denture hygiene and implementing proper disinfection protocols are crucial for preventing cross-contamination and ensuring oral mucosal health. The surface properties of denture bases are crucial for the longevity of dentures during use. In vitro techniques have been widely used to assess surface roughness and hardness, offering valuable information about the mechanical characteristics of tested materials. The present in vitro study investigated how chemical, microwave, and herbal disinfection methods affect the surface microhardness of heat-cured denture base acrylic resin.

Acrylic resins are commonly used in dental prostheses owing to their advantageous characteristics. Nevertheless, they remain vulnerable to abrasive forces. Because surface hardness indicates a material's ability to resist scratching or abrasion, prostheses with lower hardness values may be more susceptible to degradation during mechanical, chemical, or microwave cleaning. As such, hardness measurements are frequently utilized to predict the wear behavior of dental materials.<sup>[2]</sup> The Vickers microhardness test provides a standardized approach to evaluating the hardness of rigid polymers by quantifying their resistance to penetration under a predetermined load.<sup>[15]</sup> This test provides an efficient means of assessing the degree of monomer-to-polymer conversion in resin systems composed of a powder phase (polymethyl methacrylate with benzoyl peroxide) and a liquid phase (methyl methacrylate stabilized with hydroquinone).<sup>[16]</sup>

The Kruskal-Wallis test revealed statistically significant differences in microhardness between all three disinfection methods for one cycle (seven days) and three cycles (21 days). When inter-group differences were analysed using the Dunn test, all the groups showed a significant difference in microhardness at seven days. Similar observations were made at 21 days between the groups, except between the

Clinsodent and microwave groups, which showed a non-significant difference. It can be hypothesized that these methods had varying effects on the surface hardness due to manufacturing characteristics, such as pressing and cross-linking agents.

After one (seven days) and 3 (21 days) disinfection cycles, the cured acrylic resin samples fabricated on stainless steel dies showed the highest microhardness in the microwave and Clinsodent groups, respectively. In contrast, the thyme essential oil group had the lowest microhardness in both cycles. These results align with a previous study by Konchanda *et al.*<sup>[17]</sup> On the contrary, Silva *et al.*<sup>[18]</sup> reported that neither disinfection method had a significant impact on the hardness of heat-cured resin, whether it was immersion in sodium perborate (50°C for ten minutes) or microwave irradiation (650 watts for six minutes), regardless of the number of microwave cycles.

When intra-group comparisons were made, all three groups showed a significant reduction in microhardness at 21 days compared to seven days for all three methods. Several factors may explain this result. Water absorption during the disinfection process can serve as a plasticizing agent, facilitating the release of residual stresses formed during polymer processing, which in turn reduces surface hardness.<sup>[19]</sup> Repeated exposure of dentures to disinfectant solutions has been reported to affect their physical properties. Certain chemical constituents in these solutions may contribute to softening and degradation of the resin surface. Additionally, disinfectants that release oxygen can promote hydrolysis and breakdown of the polymerized resin matrix.<sup>[20]</sup> A study by Gandhi *et al.*<sup>[21]</sup> demonstrated a reduction in the microhardness of various artificial tooth types following three cycles of microwave disinfection. However, no significant change in microhardness was observed when the teeth were treated with chemical disinfectants, specifically 2%

glutaraldehyde and 1% sodium hypochlorite.

A significant finding was that three cycles of microwave disinfection led to a markedly greater reduction in surface microhardness ( $p = 0.000$ ). This suggests that the microwave procedure led to more significant softening of the teeth compared to chemical methods. This reduction in surface hardness could be attributed to water absorption at the surface, which is accelerated by the increased temperature during microwave disinfection. The plasticizing effect caused by this process reduces the surface hardness of the denture base. However, earlier research found no hardness changes in most acrylic resin denture teeth after two microwave cycles, provided the specimens were pre-immersed in water for 90 days.<sup>[22]</sup> The results showed a significant difference between microwave disinfection and control at both 7 and 21 days, which is in line with the study by Gandhi *et al*<sup>[21]</sup> who showed a decrease in microhardness of different types of acrylic teeth after three cycles in microwave disinfection. In contrast, studies by Ribeiro *et al*<sup>[23]</sup> and Konchanda *et al*<sup>[17]</sup> reported no significant differences in the mean surface hardness values between control specimens and those exposed to microwave disinfection, even at durations of up to five minutes

Similar observations were made for the Clinsodent disinfecting agent, which showed a significant reduction in microhardness ( $p=0.003$ ) from seven to 21 days. Such changes may be due to continuous polymerization processes, the liberation of unreacted monomers, and their subsequent reaction with oxygen-generated free radicals. This was in line with Kurt *et al*.<sup>[24]</sup> who investigated the effects of cleaning solutions (alkaline peroxide, 1% sodium hypochlorite, and 0.1% polymeric-guanidine solution) on heat-polymerized PMMA and found a decrease in Vickers hardness values. Similar findings have been reported in other studies<sup>[25,26]</sup> where the hardness of heat-polymerized PMMA was

reduced. Dayan *et al*<sup>[27]</sup> also reported that exposure to sodium hypochlorite (5%) led to a reduction in the surface hardness of heat-polymerized polymethyl methacrylate (PMMA). However, Gandhi *et al*<sup>[21]</sup> found no significant change in microhardness when the denture teeth were immersed in chemical disinfectants, specifically 2% glutaraldehyde and 1% sodium hypochlorite. Machando *et al*<sup>[28]</sup> also showed that neither immersion in sodium perborate solution nor microwave irradiation significantly affected the surface hardness of the denture base.

The study also revealed a significant difference in the microhardness of denture material from seven days to 21 days when herbal thyme oil was used as a disinfectant. The decrease in microhardness of the thyme essential oil group both after seven days and 21 days was statistically significant compared with the control group. According to Namala *et al*.<sup>[29]</sup> denture cleansers containing thyme essential oil demonstrated superior efficacy in maintaining the surface integrity of denture base resins when compared with conventional commercial products.

The study's limitations include using specimens with only a specific shape and size, excluding complex forms such as complete and removable dentures. Also, the long-term effect on hardness was not considered. To validate the findings, further research incorporating these complex shapes is required. Additionally, the study utilized only one type of denture resin, suggesting that future studies should explore various denture resin types.

## CONCLUSION

Considering the limitations of this in vitro study and the statistical analysis of the results, the following conclusions are warranted:

1. A statistically significant difference in surface micro-hardness was observed between the three disinfection methods for both one cycle (seven days) and three cycles (21 days).

2. After one cycle (seven days) and three cycles (21 days), surface microhardness was highest for the microwave group and Clinsodent group, respectively, and lowest for the herbal group.

#### **Declaration by Authors**

**Ethical Approval:** Not required because no human or animal subjects were involved.

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**Conflict of Interest:** The authors declare no conflict of interest.

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