Research Article

Water Sorption and Antimicrobial Performance of Alkali-Treated *Moringa oleifera* Reinforced Heat-Cured Denture Base Resin: An *In-vitro* Experimental Study

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Abstract: Background: Heat-cured polymethyl methacrylate (PMMA) is susceptible to water sorption and microbial colonization leading to dimensional instability and denture stomatitis. Natural lignocellulose based fillers like *Moringa oleifera* , may improve the functional and antibacterial properties of PMMA.

Objective: To evaluate water sorption and antimicrobial activity of heat-cured Polymethyl Methacrylate resin reinforced with alkali-treated *Moringa oleifera* powder.

Materials and Methods: This *in-vitro* experimental study was performed at Azra Naheed Dental College and University of Lahore, from 30th March 2025 to 31st December 2025 after obtaining approval from of Ethical Review Board (Ref: ANDC/RAC/2025/66) dated: 29th March 2025. Heat-cured PMMA specimens were prepared as control (0% moringa) and moringa-reinforced groups (5%, 10%, 15% w/w). Powder was washed, oven-dried, milled, sieved, and alkali-treated (5% NaOH), then neutralized and re-dried. Water sorption (%) was measured by mass change after immersion in distilled water for one month. Antimicrobial activity was evaluated using an agar disc diffusion method by measuring zones of inhibition. One-way ANOVA and Tukey HSD were used, followed by Pearson correlation. p-values ≤0.05 were taken as significant.

Result: Water sorption decreased with higher moringa content (control 1.82±0.12 to 15% 1.62±0.08; p=0.008). The Antimicrobial zones increased with the increase in concentration (control 0.00±0.00 to 15% 12.17±0.75; p<0.001). In moringa groups, the correlations were very small and non-significant.

Conclusion: Alkali-treated moringa reinforcement reduced PMMA water sorption and produced measurable antimicrobial inhibition in a concentration-dependent manner.

Keywords: Candida, Denture stomatitis, *Moringa oleifera* , PMMA denture base, Water sorption.

INTRODUCTION

Polymethyl methacrylate (PMMA) is used for denture bases because it is economical, and esthetically acceptable [1]. The oral cavity is a moist and biologically active where dentures are exposed to saliva, beverages, cleansing agents, mechanical and thermal variation [2]. These exposures and physical or mechanical changes affect the polymer structure and surface properties. Moreover, PMMA can absorb water and saliva during service, and this can reduce dimensional stability and hygiene and may cause this denture to become a harboring agent for bacteria and fungi [3].

Water sorption is a transport phenomenon in denture polymers where water molecules diffuse through the polymer network and occupy free space within the chains in the matrix, as well as micro-voids formed during mixing and polymerization [4]. This absorbed water can plasticize the polymer chains and may reduce stiffness and can lead to deformation [5]. Water uptake can also contribute to swelling and these mechanisms are controlled by diffusion kinetics, matrix polarity, porosity, and the quality of the filler to matrix interface [6].

Microbial colonization is another major concern for removable dentures as they provide a surface that supports microbial biofilms and often lead to denture stomatitis, a common inflammatory condition in denture wearers [7]. Previous studies have reported a frequent, multifactorial infection associated with Candida containing biofilms and hygiene related issues ranging from 20% to 67% patients [8-10].

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Candida in dentures cause mucosal inflammation because they adhere to acrylic surfaces, grow and cause pathogenicity and later form structured biofilms [8]. Conventional hygiene practices reduce microbial load but often fail to achieve effective suppression, especially on older immunocompromised patient or in patients with limited dexterity or overnight wear [9]. Previous studies have evaluated antimicrobial modifications of PMMA by incorporating bioactive agents or fillers including organic, inorganic, and phytochemical approaches, with Candida commonly used as the target [11, 12].

Polymethyl methacrylate modifications that targets water sorption and microbial growth is clinically important where a dispersed particulate phase can change diffusion pathways by increasing tortuosity and reducing accessible free volume, which may reduce water uptake if porosity is controlled and interfacial bonding is effective [4]. On the other hand water sorption may increase if the filler is hydrophilic and swell by absorbing water [6]. At the same time, a bioactive fillers may provide antifungal and antibacterial effects through surface contact or release of active molecules. The challenge was to deliver antimicrobial activity without affecting the hydrophilicity, or porosity [11, 13].

Moringa oleifera is a plant-based filler in dental biomaterials because it contains multiple antimicrobial phytochemical components, including phenolics, flavonoids, alkaloids, tannins, saponins, and terpenoids [14]. Previous studies have reported antifungal activity of moringa leaf preparations against Candida albicans, supporting its relevance to denture-associated candidiasis [15, 16]. Moreover, alkali treatment of lignocellulose fillers is a proven approach to reduce unwanted surface impurities and improve filler to matrix compatibility and interaction [17].

This study assessed heat cured PMMA reinforced with alkali-treated moringa powder at 5%, 10%, and 15% (w/w), and it effect on water sorption and antimicrobial activity.

MATERIALS AND METHODS

This *in-vitro* experimental study was performed after the approval of Ethical Review Board of Azra Naheed Dental College (Ref: ANDC/RAC/2025/66) dated: 29th March 2025. The study duration from 30th March 2025 to 31st December 2025. Sample fabrication was done at Azra Naheed Dental College, whereas testing was performed at University College of Medicine and Dentistry, University of Lahore. Four groups were prepared i.e., Group A: Control PMMA (0% moringa), Group B: PMMA+5% moringa (w/w), Group C: PMMA+10% moringa (w/w), and Group D: PMMA+15% moringa (w/w).

Moringa oleifera leaves were washed, oven-dried, chopped, milled, and sieved at 100 µm. Powder was alkali-treated with 5% NaOH, then washed with distilled water till neutral pH (~7) was achieved. The powder was re-dried at 50°C for 12 h. Using 90% power, 95% confidence level, and a 5% level of significance, using $Z_{1-\beta}=1.28$, $Z_{1-\alpha/2}=1.96$, $\sigma_1=5.8$, $\sigma_2=6.9$, $\mu_1=115.1$, and $\mu_2=128$, a sample of 6 per group was calculated. The bars were randomly allocated to each group.

A stainless-steel die (80mm×10mm×4mm) was used to form heat-cured PMMA specimens. Die was invested in plaster using dental flask. The dies were removed and a separator was applied before adding PMMA in dough stage. The alkali treated moringa powder was added in the PMMA powder in the concentration 5%, 10% and 15%, and mixed till homogenous. The powder and monomer were mixed at a 3:1 ratio. After packing the flasks were closed using compressing moulding technique and a long curing cycle was applied i.e., 74°C for 8 to 9 hours, followed by 100°C for 1 hour. Specimens were trimmed, finished and polished and stored in distilled water before testing (Fig. **1**).

Fig. (1). Fabrication of Specimens (**A**) Stainless Steel Die (**B**) Alkali Treated Moringa Powder (**C**) Plaster Mould (**D**) Acylic Bars for Testing (**E**) Prepared Samples Sealed in Pouches.

Each specimen was dried and weighed to assess initial weight. Specimens were immersed in distilled water, following ISO 62:2008, for one month, then removed, dried, and re-weighed to obtain final weight. Water sorption (%) was calculated by the formula: Percent water sorption= (Final weight−Initial weight)/ Initial weight×100.

Antimicrobial activity was evaluated using a disc diffusion assay. PMMA specimens were placed on agar surfaces seeded with Candida species and other microbial species. Plates were incubated at 37°C for 24 hour. Zones of inhibition were measured in diameters around each sample.

All microbiological procedures were performed using institutional biosafety policies consistent with Biosafety Level-2 (BSL-2) practices. Laboratory personnel used appropriate personal protective equipment and performed aerosol generating steps using biosafety cabinet when required. Work surfaces were disinfected before and after procedures using an appropriate glutaraldehyde disinfectant, and all contaminated disposables like plates, swabs, etc., were collected as biohazard waste and decontaminated prior to disposal according to institutional protocols.

STATISTICAL EVALUATION

Data was analyzed using SPSS version 24. The descriptive statistics was used to present frequencies and means with standard deviations. The normality was assessed using Shapiro Wilk test. The data was normally distributed. One-way ANOVA was used to compare groups, followed by Tukey HSD for pairwise comparisons. p-values ≤0.05 was taken as significant.

RESULT

Water sorption was evaluated by immersing the samples in water and measuring the change in weight. The mean water sorption and antimicrobial activity and their comparison is shown in Table **1**. Water sorption decreased with increasing moringa concentration, with a significantly difference (p=0.008). The maximum water sorption was observed in Group A and minimum was seen in Group D. The control group showed no inhibition, whereas, moringa groups showed concentration-dependent inhibition with a significant difference between groups (p<0.001). The maximum inhibition was shown by 15% (Fig. **2**).

Table 1. Water Sorption and Antimicrobial Activity and their Comparison among Control and Moringa-Reinforced PMMA Groups.

Characterization	Group	Mean ± SD	F	p
Water Sorption	Group A: (Control)	1.82±0.12	5.22	0.008
	Group B: (5% Moringa)	1.77±0.12		
	Group C: (10% Moringa)	1.63±0.10		
	Group D: (15% Moringa)	1.62±0.08		
Antimicrobial Activity	Group A: (Control)	0.00 ± 0.00	145.74	0.001
	Group B: (5% Moringa)	8.67 ± 0.82		
	Group C: (10% Moringa)	11.33 ± 1.37		
	Group D: (15% Moringa)	12.17 ± 1.60		

p-values were determined using one way ANOVA.

Post-hoc testing showed significant reductions in water sorption for Group A vs Group C (p=0.033) and Group A vs Group D (p=0.018) (Table **2**). All pairwise comparisons between control and moringa groups regarding antimicrobial activity were significant, and inhibition increased significantly between 5% vs 10% and 5% vs 15% (Table **3**).

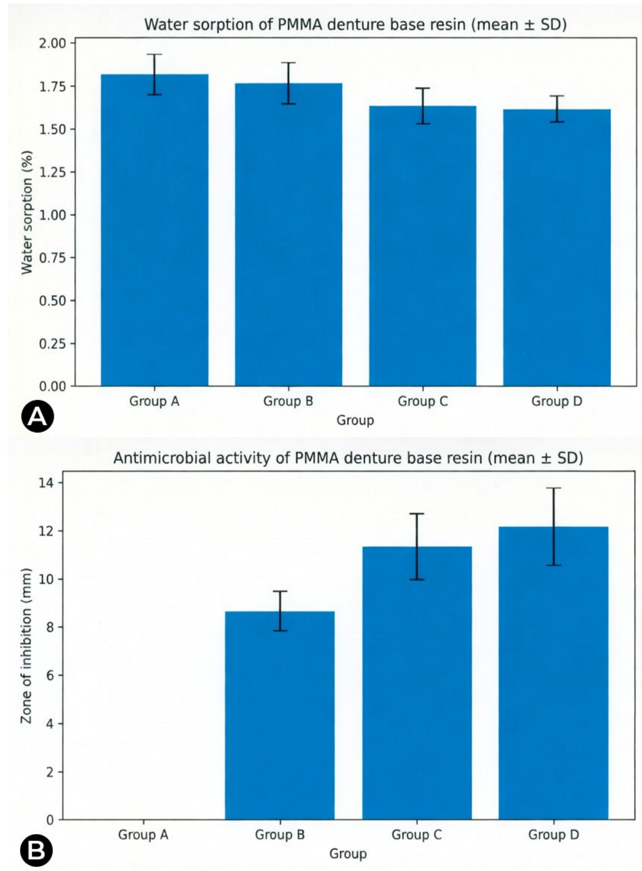

Fig. (2). (**A**) Water sorption and (**B**) Antimicrobial Activity in Control and Experimental Groups.

Table 2. Post-Hoc Tukey HSD Test Results for Water Sorption.

Group Comparison	Mean Difference	p
Group A vs Group B	0.05	0.845
Group A vs Group C	0.18	0.033
Group A vs Group D	0.20	0.018
Group B vs Group C	0.13	0.161
Group B vs Group D	0.15	0.098
Group C vs Group D	0.02	0.993

p-values were determined using post hoc Tukey test.

Table 3. Post-Hoc Tukey HSD Test Results for Antimicrobial Activity.

Group Comparison	Mean Difference	p
Group A vs Group B	-8.67	<0.001
Group A vs Group C	-11.33	<0.001
Group A vs Group D	-12.17	<0.001
Group B vs Group C	-2.67	0.003
Group B vs Group D	-3.50	<0.001
Group C vs Group D	-0.83	0.587

p-values were determined using post hic Tukey test.

In moringa groups, the correlations between water sorption and antimicrobial activity were non-significant. Within-group Pearson correlation analysis between water sorption and antimicrobial activity was not significant for the 5%, 10%, or 15% moringa groups (Table **4**). Water sorption decreased as moringa content increased indicating that higher moringa loading is associated with lower sorption and higher antimicrobial activity.

Table 4. Pearson Correlation between Water Sorption and Antimicrobial Activity for Experimental Groups.

Group	r	p	Interpretation
5% moringa	0.067	0.899	Negligible, not significant
10% moringa	0.047	0.929	Negligible, not significant
15% moringa	0.304	0.558	Weak positive, not significant

p-values were determined using Pearson correlation.

DISCUSSION

There was a gradual reduction in water sorption from 1.82% in control to 1.62% in 15% moringa, with statistically significant decreases at 10% and 15%. This finding was consistent with previous literature as a 2024 study added hydroxyapatite nanoparticles to heat cured PMMA resin and reported decreased water sorption and justified it by indicating that water sorption is affected by porosity, increased crosslinking, and lower residual monomer [4, 18]. This could be due to reduced water diffusion through increased tortuosity due to fillers as they hinder the diffusion water path and fewer microvoids [6].

Alkali treatment can further enhance this by improving filler to matrix interaction and lesser weak interfacial zones acting as water reservoir [19]. Recent PMMA composite studies emphasizes that water aging is highly dependent on filler chemistry and dispersion [4, 18]. Previous studies on PMMA reinforced with nanoparticles and fibers reported that absorbed water in oral or conditions like oral cavity can induce plasticizing effect and ultimately durability [4, 12, 18]. The decreased sorption in the current study indicates that moringa did not introduce excess porosity and acted as a diffusion barrier.

Antimicrobial activity in this study reported no inhibition for control and significant inhibition for all moringa experimental groups, increasing from 8.67 mm to 12.17 mm with increasing concentration. This concentration dependent pattern is consistent with the idea that higher moringa filler concentration increases the availability of antimicrobial phytochemicals at the interface leading to more availability of the particles for interaction [14, 20]. The findings align with literature reporting that these plants based herbal agents can suppress Candida and other oral pathogens [15, 16, 20]. A study on moringa leaf extract reported antifungal activity against C albicans with a significant minimum inhibitory concentration [16]. Lignocellulose based fillers act by membrane disruption and biofilm interference, and recent studies have reported various plant-derived oils, extracts and powders as viable antifungal agents to add in denture base polymers and liners [13, 21, 22].

Moreover, these lignocellulose based fillers are cheap, renewable and effective alternatives to expensive fillers made in industries from non-renewable sources like metals, ceramics and polymers [23]. This study also aligns with the sustainable developmental goals and agenda 2030 by using cost effective renewable plat based addition in dentures improving antimicrobial activity while keeping water sorption within acceptable limits, as well as the dimensional stability. Moreover, it may indirectly reduce biofilm retention [24, 25].

However, this study is *in-vitro* and used a diffusion-based assay rather than a biofilm model, therefore, future studies should consider biofilm models and assess Candida biofilm, surface roughness changes after water aging, and sustained antimicrobial performance after prolonged immersion. Moreover, *in-vitro* conditions do not fully replicate the conditions inside oral cavity and require in-vivo testing in future.

CONCLUSION

Alkali-treated *Moringa oleifera* reinforcement of heat-cured PMMA reported reduced water sorption at 10% to 15% loading and a concentration dependent antimicrobial inhibition in disc diffusion testing. These results support moringa as a dual-function filler for denture base resins by keeping water sorption and dimensional stability in control and producing antimicrobial effect.

AUTHORS' CONTRIBUTION

Rabiya Saif and **Hammad Hassan**: Conceptualization, Study Design, Methodology, Data analysis and interpretation, Writing draft.

Mehvish Sajjad: Study Design, Methodology, Data analysis and interpretation, Writing draft, Critical review and revision the manuscript.

Naila Umer: Conceptualization, Methodology, Data analysis and interpretation, Critical review and revision the manuscript, Final approval, final proof to be published.

Hira Asghar: Study Design, Methodology, Data analysis and interpretation, Writing draft.

Usman Ashraf: Methodology, Data analysis and interpretation, Writing draft.

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Declared none.

ETHICAL DECLARATIONS

Data Availability Statement

Data are available upon reasonable request. The data used to support the findings of this study are available from the corresponding author upon request.

Ethical Approval

The study was approved by Ethical Review Board of Azra Naheed Dental College (Ref: ANDC/RAC/2025/66) dated: 29th March 2025. The duration of the study from 30th March 2025 to 31st December 2025.

Consent to Participate

Informed consented.

Consent for Publication

All of the authors give consent for publication of this manuscript.

Conflict of Interest

Declared none.

Competing Interest/Funding

Declared none.

Use of AI-Assisted Technologies

The authors declare that NO generative artificial intelligence (AI) or AI-assisted technologies were utilized in the writing of this manuscript, in the creation of images/graphics/tables/captions, or in any other aspect of its preparation.

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