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ORIGINAL ARTICLE

EVALUATION OF MECHANICAL AND ANTIMICROBIAL PROPERTIES OF ALKALI TREATED MORINGA POWDER REINFORCED POLYMETHYLMETHACRYLATE ACRYLIC RESIN: AN IN-VITRO EXPERIMENTAL LABORATORY STUDY

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Abstract

Background: poly methyl methacrylate (PMMA) denture base resin is widely used in Prosthodontics but has limitations such as low fracture resistance and absence of antimicrobial activity. Natural fillers like *Moringa oleifera* powder are being explored to improve its mechanical strength and antimicrobial properties.

Objective: To evaluate the effect of incorporating different concentrations of Moringa powder (5%, 10%, and 15%) as a reinforcement in PMMA (polymethyl methacrylate) denture base resin on mechanical and antimicrobial properties..

Material and Methods: This in-vitro experimental laboratory-based study was conducted after the approval of the IRB of Azra Naheed Dental College, from Mar to Dec 25on PMMA resin reinforced with Moringa powder at 5%, 10%, and 15% by weight. The samples were prepared in accordance with ISO 178 and ISO 179-2 standards for mechanical testing. Flexural strength was evaluated using a universal testing machine (three-point bending test). Impact strength was tested using the Izod method. Antimicrobial activity was assessed using the disc diffusion method, and zones of inhibition were measured and averaged across all tested microorganisms. Data was analyzed using one-way ANOVA and post-hoc Tukey tests. SPSS v24 was used for analysis. p-value less than or equal to 0.05 was taken as significant.

Results: There were significant improvements in impact strength (p=0.002), flexural strength (p=0.044) and antimicrobial properties (p=0.001) with the incorporation of moringa powder. The 10% moringa group reported the highest flexural strength (89.83±3.54 MPa) and impact strength (10.13±0.68 J). The 15% Moringa group exhibited the largest antimicrobial activity, with the highest zone of inhibition (12.17±1.60 mm).

Conclusion: Incorporating moringa oleifera powder into PMMA denture base resin significantly improved both mechanical and antimicrobial properties, with the 10% concentration providing the best outcomes. Moringa powder shows potential as a natural reinforcement for improving the performance of PMMA dental materials.

Keywords: Antimicrobial activity, Denture base resin, Flexural strength, Impact strength, Moringa oleifera.

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Introduction:

Polymethylmethacrylate (PMMA) is a widely used denture base polymer because of its esthetics, easy processing, biocompatibility, and low cost. However, conventional heat-polymerized PMMA exhibits certain limitations like moderate flexural and impact resistance and microbial adhesion and biofilm formation.[1] These limitations lead to fractures during use (e.g., accidental dropping, cyclic mastication stresses) and infections like denture stomatitis.[2] Recent studies have reported that Candida species are commonly associated with denture stomatitis, with denture base surfaces acting as biofilm reservoirs that maintain microbial load and cause recurrent inflammation/infection.[2,3]

A. Authorship Contribution: [1,6]Substantial contributions to the conception or design of the work; or the acquisition, Data analysis, Literature review, [2]Drafting the work or revising it critically for important intellectual content, [3,4,6]Final approval of the version to be published, Topic Selection & Supervision

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To improve clinical durability and strength, recent studies have focused on modifying PMMA through various reinforcements like fibers/fillers/nanoparticles, and antimicrobial functionalization.[4, 5]Recent systematic and narrative reviews report that modifications in PMMA can improve toughness and flexural strength, but it strongly depends on filler type, particle shape, concentration of fillers, interfacial bond, and dispersion.[6, 7] An important challenge is the excessive filler loading which can agglomerate, causes voids, and stress concentration which can lead to compromise in strength, therefore, identifying an "optimal" filler loading is essential for stable improvements in strength.[3]

A newer innovation following the UN Agenda 2030 for Sustainable Goals, is the use of natural fillers that may provide both mechanical strength and antimicrobial activity.[8] Moringa oleifera contains multiple bioactive elements like lectins and polyphenolic compounds which reportedly exhibit antimicrobial and antifungal activity, especially against Candida species.[9] In dentistry, moringa seed extracts have been studied against oral Candida species from denture wearers, supporting its potential role in denture stomatitis prevention.[10] Antimicrobial fillers in acrylic denture base material can reduce microbial growth, but not at the expense of mechanical strength, therefore, it is important to evaluate both mechanical and antimicrobial performance simultaneously.[11]The use of alkali-treated moringa represents a potentially novel and more functionally effective approach for simultaneously improving the mechanical and antimicrobial properties of denture base PMMA.

The present in-vitro experimental study investigated the effect of incorporation of moringa powder into heat-cured PMMA at different concentrations (5%, 10%, 15%) compared with unmodified PMMA control.

Material and Method:

Study design: This in-vitro laboratory-based experimental study.

Setting and duration: study was conducted from March 2025 to December 2025, after the approval of the Institutional Review Board of Azra Naheed Dental College, Superior University (Ref: FRB/BMS/05/09/2025), dated March 20, 2025. All laboratory procedures were performed in accordance with relevant in-vitro experimental laboratory standards, and mechanical testing was carried out following ISO 178 and ISO 179-2 specifications for flexural and impact strength assessment. The samples were made using PMMA heat cure acrylic resin reinforced with moringa particles in the concentration 5%, 10% and 15%.

Sample size: A sample size of 6 per group was calculated using 90% power of study, 5% level of significance using the formula $n=[(Z_{1-\alpha/2}+Z_{1-\beta})^2(\sigma_1^2+\sigma_2^2)]/(\mu_1-\mu_2)^2$, and primary outcome variable of flexural strength, where$Z_{1-\beta}=1.28$, $Z_{1-\alpha/2}=1.96$, $\sigma_1=5.8$, $\sigma_2=6.9$, $\mu_1=115.1$, and $\mu_2=128.12$ A total of 24 heat-cured PMMA acrylic bars were produced and divided into four groups with concentration of moringa powder as follows (n=6/group) i.e., Control (0%), 5%, 10%, and 15% moringa).

Figure 1: (a) Moringa Powder and Heat Cure Acrylic Powder Mixture (b) Mould Fabrication (c) Un-trimmed and Unfinished samples (d) Final Cured Samples.

The moringa powder was sourced from a local vendor, washed, dried and refined by ball milling to obtain a fine, homogeneous powder, which was later passed through a sieve of 100-µm mesh, and only particles ≤100 µm were extracted utilized. To improve PMMA matrix-to-filler adhesion, the alkali surface treatment was done by immersion in 6% sodium hydroxide ($NaOH$) solution to remove surface waxes, oils, lignin/hemicellulose fractions. Later, it was washed with distilled water till neutral pH was achieved and dried in laboratory oven to obtain alkali treated moringa powder ready for inclusion.

The moringa powder was dry mixed manually in the PMMA acrylic resin heat cured powder for 5 minutes using stainless steel spatula after careful weighing in the concentrations 5%, 10% and 15% respectively, to obtain uniform distribution. This was followed by additional blending in a sealed container using mechanical shaking for 10 minutes to enhance homogeneity and minimize particle agglomeration. The PMMA-moringa powder was then mixed with liquid in the ratio 3:1 (w/v), in a clean glass jar, till dough stage was achieved.

Samples were madeby packing the dough in the moulds, made using standardized dies following dimensions 80mm×10mm×4mm (ISO 178 and 179).[13,14]The packed flasks were bench-cured for 30 minutes and then polymerized using a conventional water-bath curing cycle at 74°C for 90 minutes, followed by terminal boiling at 100°C for 30 minutes. After curing completion, the flasks were allowed to cool gradually to room temperature before deflasking, finishing, and polishing.For antimicrobial testing, PMMA discs were fabricated (10mm diameter and 2mm thickness). After polymerization, finished and polishing samples were subjected to flexural and impact strength testing.

Flexural strength was measured using a universal testing machine under three-point bending. The Impact strength was tested using an Izod impact testing machine. Antimicrobial activity was assessed via disc diffusion (Kirby–Bauer method).[15] Microbial susceptibility was accessed by measuring the diameter of the growth inhibition zone (mm) around specimens. Antimicrobial activity was recorded for Candida albicans, Streptococcus mutans, and Candida tropicalis.

Inclusion criteria: Standardized heat-cured PMMA specimens with 0%, 5%, 10%, and 15% alkali-treated moringa powder reinforcement and acceptable dimensions were included.

Exclusion criteria: Specimens with cracks, porosity, air bubbles, dimensional defects, or incomplete polymerization were excluded.

Data collection procedure: Fabricated specimens were tested for flexural strength, impact strength, and antimicrobial activity against *Candida albicans*, *Streptococcus mutans*, and *Candida tropicalis*. All results were recorded on a structured data collection sheet for analysis.

Data analysis: The data was analyzed using SPSS version 24 (IBM, USA). Normality of the data was assessed using Shapiro–Wilk. Group comparisons were evaluated using one-way ANOVA followed by post-hoc Tukey testing for pair wise comparisons. Significance was p-values less than or equal to 0.05.

Results:

A total of 6 samples per group were analyzed for flexural, impact and antimicrobial testing. There was a significant difference between the flexural (p=0.044), impact strength (p=0.002), and antimicrobial activity (p=0.001) among the study groups. The 10% moringa group demonstrated the highest mean flexural strength and impact strength. The greatest overall antimicrobial inhibition was recorded in the 15%, indicating a concentration-dependent antimicrobial effect (Table 1).Antimicrobial activity values represent the combined mean zone of inhibition averaged across Candida albicans, Streptococcus mutans, and Candida tropicalis. The post-hoc Tukey pairwise comparisons for flexural strength, impact strength and antimicrobial testing is exhibited in Table 2.

Table 1: Comparison of Mechanical Properties and Antimicrobial Activity of PMMA Reinforced with Moringa Powder

Characterization	Group	N	Mean±SD	F	p
Flexural Strength	Control (No Moringa)	6	83.67±4.03	3.23	0.044
	5% Moringa	6	85.17±2.56		
	10% Moringa	6	89.83±3.54		
	15% Moringa	6	85.83±4.02		
Impact Strength	Control (No Moringa)	6	8.55±0.71	7.48	0.002
	5% Moringa	6	9.62±0.57		
	10% Moringa	6	10.13±0.68		
	15% Moringa	6	9.13±0.43		
Antimicrobial Activity	Control (No Moringa)	6	0.00±0.00	145.74	0.001
	5% Moringa	6	8.67±0.82		
	10% Moringa	6	11.33±1.37		
	15% Moringa	6	12.17±1.60		

P-values were obtained using one way ANOVA

Table 2: Post-hoc Tukey HSD test pair wise comparison for flexural strength, impact strength, and antimicrobial activity

Characterizations	Group Comparison	Mean Difference	p
Flexural Strength	Control vs. 5% Moringa	-1.50	0.887
	Control vs. 10% Moringa	-6.17	0.035*
	Control vs. 15% Moringa	-2.17	0.725
	5% Moringa vs. 10% Moringa	-4.67	0.144
	5% Moringa vs. 15% Moringa	-0.67	0.988
	10% Moringa vs. 15% Moringa	4.00	0.248
Impact Strength	Control vs. 5% Moringa	-1.07	0.030*
	Control vs. 10% Moringa	-1.58	0.001*
	Control vs. 15% Moringa	-0.58	0.365
	5% Moringa vs. 10% Moringa	-0.52	0.468
	5% Moringa vs. 15% Moringa	0.48	0.524
	10% Moringa vs. 15% Moringa	1.00	0.044*
Antimicrobial	Control vs. 5% Moringa	-8.67	<0.001*
	Control vs. 10% Moringa	-11.33	<0.001*
	Control vs. 15%	-12.17	<0.001

	Moringa		*
	5% Moringa vs. 10% Moringa	-2.67	0.003
	5% Moringa vs. 15% Moringa	-3.50	<0.001 *
	10% Moringa vs. 15% Moringa	-0.83	0.587

p-values were obtained using post-hoc Tukey HSD test.

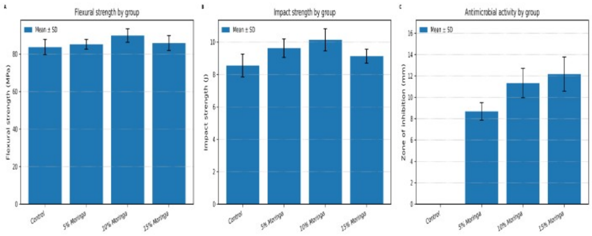

Figure 2(a) Flexural Strength (b) Impact Strength (c) Antimicrobial Activity.

Discussion:

In the current study, adding alkali-treated moringa oleifera powder produced a statistically significant difference in flexural strength across groups (p=0.044), with the 10% moringa group showing the highest mean value, showing that the "optimum filler loading" is consistent with recent studies about PMMA reinforced composites indicating that low-to-moderate filler loading can increase flexural strength if particles are evenly dispersed and transfer stress evenly at the interface, however, performance may decline when filler loading increases to certain extent, leading to agglomeration and void formation or disruption in polymer matrix.[6, 16, 17]

Similar concentration-dependent trendis reported for other natural reinforcements in heat-cured PMMA i.e., curcumin-modified PMMA, bamboo-reinforced PMMA and other lignocelluloses based fillers reported highest flexural strength at an intermediate concentrations and reduced strength at higher concentrations.[18, 19] The flexural improvement at 10% moringa can be due to better particle–matrix interlocking after alkali treatment, cleaner rougher surfaces improve wettability and mechanical anchorage and effective crack deflection and stress distribution within the PMMA matrix.[20] At 15% loading, the slight drop relative to 10% while still near control could be due toincreased clustering, stress concentration at particle interfaces, indicating lower flexural strength at higher filler levels.[5]

Impact strength showed a clear and significant difference (p=0.002), both 5% and 10% moringa groups were significantly higher than control which indicates that moderate moringa loading improved the ability to absorb impact, but excessive loading reduced that property, and this finding aligns with the recent literature about the PMMA reinforced composite, where toughness improves when filler particles promotes stress transfer, fracture reduction and crack propagation; but reduces when excessive filler becomes an initiation site for brittle fracture due to clustering and weak interfacial areas.[6, 21]The same trend has been observed in other PMMA composites containing organic additives like curcumin, almond shells, rice husk, bamboo husk incorporation, and produced significant concentration-related differences in impact strength at various concentrations.[18, 22, 23]the 10% moringa having higher impact strength i.e., improved fracture toughness due to better stress redistribution at the particle–PMMA interface. The reduction at 15% likely reflects particle clusters and microvoids which acts as critical flaws under impact, lowering the energy required for crack initiation and propagation.[20, 24]

Antimicrobial activity showed the strongest effect size where the control group had no inhibition, while antimicrobial activity increased progressively with moringa loading with highest activity in 15% group. These findings are consistent with the literature showing that incorporating antimicrobials into PMMA inhibit microbial growth, although it depends on processing,drug release, chemistry, and biocompatibility.[9, 10]Recent literature shows that moringa has antifungal potential.[9, 10, 25] Moringa oleifera leaf has demonstrated in vitro antifungal activity against Candida albicans with reported MIC level and measurable cellular effects.[10]

In this study, the reason for rising antimicrobial activity with higher moringa loading is increased availability of moringa particles on surface into the agar medium, improving suppression of microbial growth.[25] At the same time, the literature reports that antimicrobial additions can alter PMMA polymer network, therefore, long-term aging, leach ability, color stability, and cytotoxicity should be evaluated before clinical introduction.[12]

Overall, the results suggest that 10% moringa produced the most favorable combination for mechanical strength (both flexural and impact), while 15% moringa provided the highest antimicrobial activity but with a small mechanical drop relative to 10%. The 10% group is most optimized when it comes to strength as well as antimicrobial activity.

This study has limitations like in-vitro design which may not fully replicate the complex oral environment, secondly, the sample size was relatively small, and thirdly, aging procedures were not performed. Antimicrobial activity was assessed using the disc diffusion method, which provides preliminary information on microbial inhibition but does not fully simulate biofilm formation on denture surfaces. Therefore, future studies should include larger sample sizes, aging, surface roughness assessment, color stability, cytotoxicity, and biofilm-based antimicrobial analysis before clinical application.

Conclusion:

Moringa oleifera powder incorporation into heat-cured PMMA improved mechanical as well as antimicrobial properties. 15% moringa reduced mechanical strength but showed highest antimicrobial activity. However, 10% moringa reportedly was the most favorable, with highest mechanical strength and optimal antimicrobial activity, suggesting that future optimization should target improved dispersion as well as improved interfacial bonding at higher loadings or hybrid fillers that maintain strength and toughness while improving antimicrobial activity. Clinically, this modification may contribute to more durable denture base materials with reduced microbial colonization, potentially lowering the risk of denture fracture and denture-associated stomatitis.

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

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