Antimicrobial and Mechanical Effects of Aloe vera Extract Incorporation into Heat-Polymerized Acrylic Denture Base Resin: An In Vitro Study

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ABSTRACT

Background: Heat-polymerized polymethyl methacrylate (PMMA) is widely used for denture bases, but microbial colonisation and fracture remain important limitations. Aloe vera contains phytochemicals with potential antimicrobial activity; however, incorporation of plant-derived material may alter resin mechanics. This study evaluated antimicrobial activity, flexural strength and impact fracture energy of PMMA modified with different concentrations of Aloe vera extract.

Methods: This in-vitro study used 100 independent specimens allocated to four separate tests: Candida albicans (n=25), Staphylococcus aureus (n=25), flexural strength (n=25) and impact testing (n=25). Each test included five specimens at each concentration (0%, 3%, 5%, 7% and 10% w/w). Aloe vera inner-leaf material was extracted with absolute ethanol for approximately 24 hours, filtered and oven-dried at 40°C. The extract was incorporated into Pyrax heat-polymerized PMMA using a 2:1 polymer-to-monomer ratio (g:mL). Specimens were cured at 73–74°C for 1 hour 30 minutes, followed by 100°C for 30 minutes. Antimicrobial activity was assessed by exposure to organism-inoculated broth, serial dilution and viable colony enumeration. Flexural strength and impact fracture energy were also assessed.

Results: Alkaloids, tannins, saponins, flavonoids and anthraquinones were detected, while reducing sugars were not detected. Mean C. albicans counts decreased from 30.4 million CFU/mL in the control group to 68,000 CFU/mL at 10% Aloe vera. Mean S. aureus counts decreased from 40.4 million CFU/mL in the control group to 566,000 CFU/mL at 7%, although the 3% group had a higher count than the control. Overall differences in $\log_{10}$-transformed microbial counts were significant for C. albicans (Welch's $F(4, 9.43)=6216.54$, $p<0.001$) and S. aureus (Welch's $F(4, 9.58)=9203.34$, $p<0.001$). Flexural strength decreased from 67.56 MPa in the control group to 55.40 MPa at 10%, with a significant difference among concentrations ($H=23.03$, $df=4$, $p<0.001$). Mean impact fracture energy also decreased from 0.282 J in the control group to 0.180 J at 10% ($H(4)=22.62$, $p<0.001$).

Conclusion: Aloe vera incorporation at 5–10% was associated with substantial reductions in recoverable microbial counts, alongside reductions in flexural strength and impact fracture energy. No concentration produced the best outcome across all measures. Further standardised testing, material characterisation and biocompatibility assessment are needed before clinical application.

Keywords: Aloe vera; polymethyl methacrylate; denture base resin; antifungal activity; antibacterial activity; flexural strength; impact fracture energy.

INTRODUCTION

Heat-polymerized polymethyl methacrylate (PMMA) remains widely used for removable denture bases because of its favourable handling characteristics, acceptable aesthetics, relatively low cost and established clinical use [1,2,10]. Despite these advantages, conventional PMMA has limitations, including susceptibility to fracture under functional or accidental loading and the capacity to support microbial attachment and colonisation [2,3].

Candida albicans is an important microorganism in denture-associated stomatitis, while Staphylococcus aureus is also relevant to investigations of microbial contamination and colonisation of oral and biomaterial surfaces [2,3]. Strategies that modify denture-base materials to reduce microbial burden have therefore attracted research interest.

Approaches to modifying PMMA include incorporation of fibres, fillers, nanoparticles and antimicrobial agents. Although such modifications may improve selected properties, additives can also alter polymer-matrix continuity and mechanical performance [2,3]. Aloe vera contains several classes of phytochemicals associated with biological activity, and previous in-vitro studies have investigated Aloe vera preparations against microorganisms relevant to denture hygiene [4,9]. However, incorporating Aloe vera directly into PMMA may produce effects different from applying the extract as a denture disinfectant.

An antimicrobial modification would be of limited practical value if it substantially compromised the mechanical integrity of the denture base. This study therefore investigated the effects of incorporating 0%, 3%, 5%, 7% and 10% (w/w) Aloe vera extract into heat-polymerized PMMA on recoverable counts of C. albicans and S. aureus, flexural strength and impact fracture energy. The null hypotheses were that Aloe vera concentration would not be associated with differences in microbial counts or mechanical outcomes.

Study design and specimen allocation

This laboratory-based in-vitro experimental study evaluated heat-polymerized PMMA containing 0%, 3%, 5%, 7% and 10% (w/w) Aloe vera extract. A total of 100 independent specimens were prepared and allocated to four separate tests: C. albicans (n=25), S. aureus (n=25), flexural strength (n=25) and impact testing (n=25). Each test included five specimens per concentration. The 0% formulation served as the unmodified PMMA control. Specimens were not reused across tests.

Collection and preparation of Aloe vera extract

Fresh, mature Aloe vera leaves were purchased in Benin City, Edo State, Nigeria, and authenticated at the Herbarium Unit, Department of Plant Biology and Biotechnology, University of Benin. The voucher specimen number was UBH-A528. The leaves were washed; the outer rind and yellow latex were removed; and the inner parenchymatous material was separated, washed and homogenised. The homogenised material was soaked in absolute ethanol (analytical reagent grade; JHD) for approximately 24 hours. The mixture was subsequently filtered, and the resulting filtrate was oven-dried at 40°C to obtain the extract. The dried extract was pulverised into powder and stored in a clean, dry, airtight container until use. The initial mass of homogenised material and final mass of dried extract were not recorded; therefore, extraction yield was not calculated.

Preparation of modified PMMA specimens

Heat-polymerised polymethyl methacrylate (PMMA) denture-base resin (Pyrax, India) was used for specimen fabrication. The polymer powder and monomer liquid were proportioned at a ratio of 2:1, comprising two parts polymer powder by weight (g) to one part monomer liquid by volume (mL), in accordance with the manufacturer's instructions. Aloe vera extract powder was incorporated into the polymer powder at concentrations of 0%, 3%, 5%, 7% and 10% (w/w), calculated relative to the weight of the polymer powder. For every 10 g of polymer powder, 0, 0.3, 0.5, 0.7 and 1.0 g of Aloe vera extract were added, respectively. The corresponding monomer liquid was then added according to the manufacturer's recommended mixing ratio. The mixture was allowed to reach the dough stage, taking care to minimise air incorporation and promote uniform dispersion. The control was prepared in the same manner without extract. The dough was packed into prepared moulds, and the flasks were clamped before polymerisation. A short curing cycle was employed for all concentrations, including the control. The flasks were placed in a water bath at room temperature, which was gradually heated to 73–74°C and maintained for 1 hour 30 minutes. The temperature was subsequently raised to 100°C and maintained for 30 minutes. Following polymerisation, the flasks were allowed to cool inside the water bath before deflasking and retrieval. The specimens were trimmed and polished, and their dimensions were checked using a digital vernier caliper. Visibly defective, porous or cracked specimens, and specimens outside the required dimensions, were excluded and replaced.

Mechanical-test specimens measured 64 × 10 × 3.3 mm.

Specimen conditioning

After fabrication, trimming and polishing, specimens were immersed in distilled water at approximately 37°C for 24 hours. They were then removed, gently dried and transferred aseptically for the relevant tests.

Microorganisms and inoculum preparation

In-house control isolates of C. albicans and S. aureus were identified using the VITEK 2 Compact automated microbial identification system and the corresponding identification cards. Following identification, isolates were preserved at −80°C. Before testing, stored isolates were recovered and screened using conventional microbiological methods. Standardised suspensions were prepared and adjusted to a turbidity equivalent to the 0.5 McFarland standard.

Antimicrobial assay against Staphylococcus aureus

A standardised S. aureus suspension was prepared in sterile nutrient broth and adjusted to 0.5 McFarland turbidity. Specimens containing 0%, 3%, 5%, 7% and 10% Aloe vera were aseptically transferred into sterile test tubes containing the inoculated nutrient broth, immersed and gently agitated. Tubes were incubated at 37°C for 24 hours. Specimens were then removed aseptically and the broth suspensions subjected to ten-fold serial dilution. Six sterile tubes containing 9 mL sterile normal saline each were labelled by dilution. One millilitre of incubated broth was transferred to the first tube to obtain 10^{-1}; 1 mL was then transferred sequentially between tubes to obtain further ten-fold dilutions. An aliquot of 0.1 mL from an appropriate dilution was spread onto Mueller–Hinton agar (MHA) and incubated at 37°C for 24 hours.

Visible colonies were counted and expressed as CFU/mL.

Antifungal assay against Candida albicans

A standardised Candida albicans suspension was prepared in sterile Sabouraud broth and adjusted to a turbidity equivalent to the 0.5 McFarland standard. Specimens containing 0%, 3%, 5%, 7% and 10% Aloe vera were aseptically transferred into sterile test tubes containing the inoculated Sabouraud broth, immersed and gently agitated. The tubes were incubated at 37°C for 24 hours. The specimens were then removed aseptically, and the broth suspensions were subjected to ten-fold serial dilution using six sterile tubes, each containing 9 mL sterile normal saline. One millilitre of incubated broth was transferred into the first tube to obtain a 10^{-1}

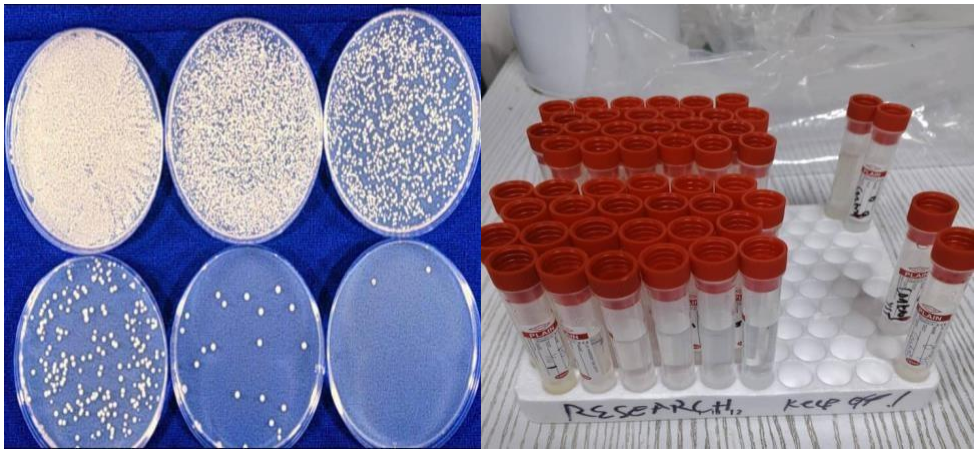
dilution, followed by sequential transfers to obtain further ten-fold dilutions. Appropriate dilutions were cultured on Mueller–Hinton agar supplemented wth 2% dextrose and 0.5 µg/Ml methylene blue. Following incubation, visible fungal colonies were counted and expressed as CFU/mL.





Viable count and percentage reduction

CFU/mL was calculated as: (number of colonies × reciprocal of dilution) / volume plated (mL). For the S. aureus assay, the plated volume was 0.1 mL. Percentage reduction relative to the 0% PMMA control was calculated as [(C − T)/C] × 100, where C is the mean CFU/mL from the 0% control and T is the mean CFU/mL from an Aloe vera-modified group. A negative value indicates an increase relative to control.



Flexural strength testing

Twenty-five rectangular heat-polymerised PMMA specimens were prepared for flexural strength testing. The specimens were divided according to the five Aloe vera extract concentrations: 0%, 3%, 5%, 7% and 10%, with five specimens prepared for each concentration. Each specimen measured 64 mm × 10 mm × 3.3 mm.



The specimens were subjected to static loading using a loading apparatus. The load was progressively increased at two-minute intervals until fracture occurred. The fracture load for each specimen was recorded in kilogram-force (kgf) and converted to Newtons (N) using a gravitational acceleration of 9.81 m/s².

Flexural strength was calculated using the following equation:

$\Sigma f = 3FL/(2bd^2)$, Where:

Σf= flexural strength (MPa) F = fracture force (N)

L = distance between the supports (mm)



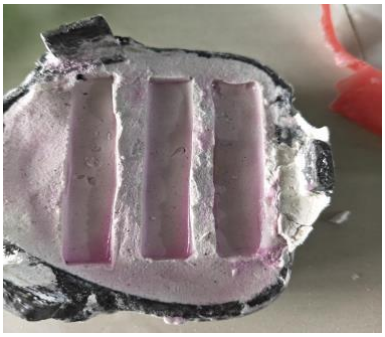


b = width of the specimen (mm)

d = thickness of the specimen (mm)

Impact testing

Impact testing was performed using an AIC–EMIC plastic impact-testing machine equipped with a pendulum of nominal energy 0.5 J, following the Charpy impact-testing principle. The specimens measured 64 × 10 × 3.3 mm (length × width × thickness), with a notch depth of 1.2 mm and a support span of 50 mm. Five specimens were tested at each Aloe vera extract concentration (0%, 3%, 5%, 7% and 10%), giving a total of 25 specimens. The energy absorbed at fracture was read directly from the machine display and recorded in joules (J). The recorded values were used to evaluate the effect of Aloe vera extract incorporation on the impact fracture energy of the heat-polymerized acrylic denture-base material.





Statistical analysis

Microbial counts were converted to colony-forming units per millilitre (CFU/mL) using the recorded dilution factors and plated volumes. The CFU/mL values were $\log_{10}$-transformed before inferential analysis. Welch's one-way analysis of variance (ANOVA) was used to compare $\log_{10}$-transformed microbial counts across the five Aloe vera extract concentrations (0%, 3%, 5%, 7% and 10%), followed by Games–Howell post-hoc pairwise comparisons.

Flexural strength and impact fracture energy were analysed separately using the Kruskal–Wallis test, followed by Dunn's pairwise comparisons with Holm adjustment for multiple comparisons. Descriptive results were presented as mean and standard deviation. All statistical tests were two-sided, and statistical significance was set at $\alpha = 0.05$.

RESULTS

Phytochemical screening

Qualitative screening detected alkaloids, tannins, saponins, flavonoids and anthraquinones; reducing sugars were not detected (Table 1).

Constituent	Result
Alkaloids	Detected (+)
Tannins	Detected (+)
Saponins	Detected (+)
Flavonoids	Detected (+)
Anthraquinones	Detected (+)
Reducing sugars	Not detected (−)

Table 1. Qualitative phytochemical screening of Aloe vera extract. Antifungal activity against Candida albicans

Mean recoverable C. albicans counts declined with increasing Aloe vera concentration. The reduction at 3% was comparatively small, whereas marked reductions occurred at 5%, 7% and 10%. The lowest mean count was observed at 10% (Table 2). Welch's ANOVA on log10-transformed CFU/mL showed an overall difference among concentrations ($F(4, 9.43)=6216.54$, $p<0.001$).

Aloe vera	Mean CFU/mL	SD (CFU/mL)	Reduction vs control
0%	30,400,000	1,673,320	Reference
3%	27,200,000	836,660	10.53%
5%	740,000	54,772	97.57%
7%	312,000	21,679	98.97%
10%	68,000	13,038	99.78%

Table 2. Recoverable C. albicans counts and descriptive percentage reduction.

Antibacterial activity against Staphylococcus aureus

The 3% group had a higher mean S. aureus count than the 0% control, whereas lower counts occurred at 5%, 7% and 10%. The lowest mean count occurred at 7%, with a slightly higher count at 10% (Table 3). Welch's ANOVA on log10-transformed CFU/mL indicated an overall difference among concentrations ($F(4, 9.58)=9203.34$, $p<0.001$

Aloe vera	Mean CFU/mL	SD (CFU/mL)	Reduction/increase vs control
0%	40,400,000	2,701,851	Reference
3%	45,600,000	2,509,980	12.87% increase
5%	6,140,000	487,852	84.80% reduction
7%	566,000	19,494	98.60% reduction

10%	662,000	16,432	98.36% reduction

Table 3. Recoverable S. aureus counts and descriptive percentage reduction/increase.

Flexural strength

The flexural strength of heat-polymerised PMMA decreased with increasing Aloe vera extract concentration. The control group recorded a mean flexural strength of 67.56 ± 0.00 MPa, while the 3%, 5%, 7% and 10% groups recorded 64.18 ± 0.00 MPa, 61.48 ± 1.51 MPa, 57.43 ± 0.00 MPa and 55.40 ± 1.85 MPa, respectively.

Aloe vera concentration	Specimen A (MPa)	Specimen B (MPa)	Specimen C (MPa)	Specimen D (MPa)	Specimen E (MPa)	Mean ± SD (MPa)
0%	67.56	67.56	67.56	67.56	67.56	67.56 ± 0.00
3%	64.18	64.18	64.18	64.18	64.18	64.18 ± 0.00
5%	60.81	60.81	64.18	60.81	60.81	61.48 ± 1.51
7%	57.43	57.43	57.43	57.43	57.43	57.43 ± 0.00
10%	54.05	57.43	54.05	57.43	54.05	55.40 ± 1.85

Table 4. Individual and mean flexural strength values of PMMA specimens containing different Aloe vera concentration

Values are presented as individual specimen measurements and mean ± standard deviation (SD), with five specimens per concentration. Flexural strength was calculated using the three-point bending equation.

$\sigma f = 3FL / 2bd^2$

Impact fracture energy

Mean impact fracture energy declined from 0.282 J in controls to 0.180 J at 10% Aloe vera, a descriptive reduction of approximately 36.2%. The Kruskal–Wallis test indicated an overall difference among groups ($H(4)=22.62$, $p<0.001$).

Aloe vera	A (J)	B (J)	C (J)	D (J)	E (J)	Mean ± SD(J)
0%	0.28	0.28	0.29	0.28	0.28	0.282 ±0.004
3%	0.25	0.27	0.25	0.25	0.25	0.254 ±0.009
5%	0.25	0.24	0.26	0.25	0.25	0.250 ±0.007
7%	0.22	0.23	0.22	0.23	0.23	0.226 ±0.005
10%	0.18	0.19	0.17	0.18	0.18	0.180 ±0.007

Table 5. Individual machine-displayed impact fracture-energy readings and group summaries.

DISCUSSION

This in-vitro study found that Aloe vera incorporation was associated with lower recoverable counts of C. albicans and S. aureus at concentrations of 5–10%, while measured flexural strength and impact fracture energy declined as extract concentration increased. These findings indicate a trade-off between the observed microbiological response and mechanical performance.

The antifungal response showed a small reduction at 3%, followed by marked reductions at 5%, 7% and 10%. The 10% group had a mean C. albicans count 99.78% lower than the control. The S. aureus response was not

strictly concentration-dependent: the 3% group had a higher mean count than control, and the lowest count occurred at 7%, not 10%. The two organisms should therefore be interpreted separately.

Qualitative screening detected several broad phytochemical classes, but did not quantify individual constituents or identify the compounds responsible for the observed response. Extract composition may vary with plant material and processing.

Previous studies have investigated Aloe vera preparations against denture-associated microorganisms [4,9], but differences in extraction, formulation and exposure method limit direct comparisons. Further work should use larger samples, chemically characterised extract, validated mechanical testing and clinically relevant biofilm models.

The assay measured recoverable viable counts in broth after exposure to specimens. It did not determine MIC, MBC, minimum fungicidal concentration or inhibition of mature biofilms. The findings was interpreted as differences in recoverable microbial burden under the stated in-vitro conditions, not as evidence of sterilisation or clinical antimicrobial efficacy.

No concentration provided the best outcome across all measures. The 10% formulation produced the lowest C. albicans count but also the lowest flexural strength and impact fracture energy. The 7% formulation produced the lowest S. aureus count, The 3% formulation showed a slight reduction in flexural strength compared with the control, while providing only limited antifungal activity and an increase in S. aureus count. These findings indicate that no single formulation provided the best balance of antimicrobial activity and mechanical performance. The Kruskal–Wallis test revealed a statistically significant difference in flexural strength among the five Aloe vera concentrations ($H = 23.03$, $df = 4$, $p < 0.001$). Dunn's post-hoc pairwise comparisons with Holm adjustment showed significant differences between the control and 7% groups (adjusted $p = 0.0038$), the control and 10% groups (adjusted $p = 0.0003$), and the 3% and 10% groups (adjusted $p = 0.0236$). These findings indicate that increasing Aloe vera extract concentration was associated with a reduction in the flexural strength of the acrylic denture-base material.

Limitations include the small number of specimens per concentration, qualitative rather than quantitative extract characterisation, and the absence of biofilm, surface roughness, water sorption, solubility, colour stability, residual monomer, ageing, extract-release and biocompatibility assessments. The initial mass of homogenised Aloe vera material and final mass of dried extract were not recorded, so extraction yield could not be calculated. The release of Aloe vera constituents from the PMMA matrix was not investigated; therefore, the mechanism responsible for the observed antimicrobial effects could not be established.

CONCLUSION

Within the limitations of this in-vitro study, incorporation of Aloe vera extract into heat-polymerized PMMA was associated with substantial reductions in recoverable C. albicans and S. aureus counts at concentrations of 5–10%. The response differed between organisms, with an increase in S. aureus counts at 3% and the lowest S. aureus count at 7%. Increasing extract concentrations were also associated with reductions in flexural strength and impact fracture energy. No concentration demonstrated an overall advantage across all outcomes. The findings provide preliminary laboratory evidence and do not establish clinical suitability.

RECOMMENDATIONS

Further studies should increase specimen numbers; standardise and document Aloe vera extraction and chemical composition; assess extract dispersion and release from PMMA; verify mechanical procedures and calculations against applicable standards and instrument manuals; and evaluate surface properties, water sorption, solubility, colour stability, residual monomer, ageing, cytotoxicity and biocompatibility. Antimicrobial testing should include standardised biofilm models and additional clinically relevant organisms before clinical application is considered.

DECLARATIONS

Ethics approval: The thesis reports approval by the University of Benin Teaching Hospital Research Ethics Committee (Approval No. ADM/E22/A/VOL. VII/148312717) and the Federal University of Technology, Owerri (Reg. No. 00010/FUTO/SHEC/P0024).

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the research, authorship, or publication of this manuscript.

Author Contributions

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Etinosa Richard Eriamiatoe : laboratory investigation, microbiological analysis, methodology, validation, or manuscript review.

Clare Uchenna Emeagha: Validation and manuscript review. Ukwueze Regina Chidiebere: Mechanical investigation and validation Oshoke Inaboya: Validation and data Analysis.

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