RESEARCH PAPER

Antifungal Activity and Flexural Properties of Biosynthesized and Chemically Synthesized Silver Nanoparticle-Loaded Acrylic Denture Bases versus Conventional Acrylic Denture Base: An In-Vitro Study

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ABSTRACT

Background:

Candida albicans adherence to polymethyl methacrylate (PMMA) denture bases is a major cause of denture stomatitis. Modifying it with silver nanoparticles (AgNP) offers a promising antifungal strategy. The present study aimed to evaluate the antifungal effect and flexural properties of denture bases loaded with biosynthesized and chemically synthesized silver nanoparticle liquid suspensions compared to conventional denture base material.

Materials and Methods:

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the biosynthesized (BNS) and chemically synthesized (CNS) AgNP suspensions were determined against *Candida albicans* using the tube dilution method. A total of 63 specimens for the different tests were fabricated and allocated into three groups according to the type of incorporated silver suspensions: Group I (PMMA modified with biologically synthesized silver nanoparticles suspension), Group II (PMMA modified with chemically synthesized silver nanoparticles suspension), and Group III (unmodified conventional PMMA, control group). For antifungal evaluation, 36 specimens were assessed using direct contact test. Using a universal testing machine, three-point bending tests were carried out on 27 specimens to evaluate flexural strength and modulus. A one-way ANOVA was then employed for statistical analysis, followed by Bonferroni's post hoc. Statistical significance was set at $p \leq 0.05$.

Results:

The MIC values for BNS and CNS were 26.75 and 53.5 µg/mL, respectively. The corresponding MFC values were 53.5 and 107 µg/mL. The highest antifungal activity against *Candida albicans* was recorded for Group I, followed by Group II, and lowest for Group III ($p<0.001$). No statistically significant difference in flexural properties were found among the three groups ($p=0.874$).

Conclusions:

Biosynthesized nanosilver suspension demonstrated superior antifungal efficacy against *Candida albicans*, achieving greater inhibition at approximately 50% lower concentrations compared to chemically synthesized nanosilver suspension. In comparison, both biosynthesized and chemically synthesized silver nanoparticle-modified PMMA showed significantly enhanced antifungal activity compared to the unmodified control without compromising flexural strength.

Keywords: Acrylic Denture base, Antifungal, *Candida albicans*, Flexural strength, Green synthesis, Silver nanoparticles.

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Introduction

The growing use of removable dentures in aging populations highlights the need for durable, biocompatible materials. Polymethyl methacrylate (PMMA) has long been established as the standard denture base material, a status attributed to its favorable properties and low cost. However, a critical disadvantage is its susceptibility to microbial adhesion, primarily by *Candida albicans*, which leads to denture stomatitis, a common inflammatory condition. While mechanical reinforcements can improve PMMA's strength, the problem of infection persists. [1,2]

Current preventive strategies, such as topical antifungals and mechanical cleaning, are often ineffective due to poor drug retention, patient compliance issues, and risk of resistance.This has driven research into developing inherently antimicrobial PMMA by incorporating additives like silver nanoparticles (AgNPs). [1]

However, two significant challenges remain: first, conventional chemical synthesis of AgNPs uses toxic reagents. Second, previous methods of blending AgNP powders into PMMA have compromised mechanical properties due to poor dispersion. [3] This highlights a key conflict in the field: balancing potent antimicrobial action with the preservation of material strength.

Biosynthesis of AgNPs using plant extracts offers a safer, "green" alternative, while incorporating AgNPs as a liquid suspension may promote better distribution within the resin. Therefore, this study aimed to evaluate whether PMMA modified with biosynthesized or chemically synthesized AgNP suspensions could improve antifungal activity against *C. albicans* without reducing flexural strength.

The study's null hypotheses were formulated as follows: the incorporation of biologically or chemically synthesized silver nanoparticles into PMMA denture base resin would not result in significant differences in antifungal activity, flexural strength, nor flexural modulus compared with conventional PMMA. Furthermore, no significant differences would exist between biologically and chemically synthesized silver nanoparticle-modified PMMA regarding these properties.

Materials and Methods

Study design: Invitro Experimental Study

- **Preparation of Silver Nanoparticles Liquid Suspensions:**

Biosynthesized silver nanoparticles (BNS) were prepared using green tea extract as reducing agent following the procedures described by **Widatalla HA et al. (2022).** [4] This preparation yielded a final silver nanoparticle concentration of 1 mM**.** While, Chemically synthesized silver nanoparticles (CNS) were prepared following the protocol of **Malina et al. (2012) [5]**, with adjustments in reagent volumes for achieving the final nanoparticle concentration equivalent to that of the biosynthesized nanoparticles.

Morphological analysis:

A JEOL JEM-2100 high resolution transmission electron microscope, operated at 200 kV, was utilized for the TEM observations.

MIC (minimum inhibitory concentration) and MFC (minimum fungicidal concentration) Determination.

MIC and MFC of biologically synthesized silver nanoparticles suspension (BNS) and chemically synthesized silver nanoparticles suspension (CNS) against *Candida albicans* were evaluated by means of broth tube dilution method.[6] Fungal strain (ATCC10231) was obtained from the Microbiology Laboratory, Faculty of Medicine, Cairo University and subcultured on Sabouraud dextrose broth. The inoculum was standardized to a turbidity of 0.5 McFarland corresponding to 1.5 x 10^8 CFU/mL.

Stock solutions from each nanoparticle suspensions were prepared at a starting concentration of 107 µg/mL (0.0107%). Two-fold serial dilutions were subsequently prepared using cation-adjusted Mueller-Hinton broth to obtain concentrations of 107, 53.5, 26.75, 13.375 and 6.6875 µg/mL. Following incubation at 37°C for 24 h, the MIC was determined as the lowest nanoparticle concentration showing no visible fungal growth (turbidity). MIC assessment was performed independently by two blinded examiners. Aliquots from tubes exhibiting no visible growth were plated onto Sabouraud dextrose agar and incubated at 37°C for 24 h, for MFC evaluation. The MFC was defined as the lowest nanoparticle concentration resulting in either complete absence of fungal growth or the presence of fewer than three colonies (≥99% fungicidal activity). In this study, the MFC was selected as the incorporation concentration used to modify the PMMA denture base materials, to ensure fungicidal efficacy and to allow direct comparison between the biological and chemical nanoparticles' synthesis approaches.

- **Specimens Grouping**

Group I: Acrylic denture base specimens loaded with biosynthesized silver nanoparticles (BNS/PMMA).**Group II:** Acrylic denture base specimens loaded with chemically synthesized silver nanoparticles (CNS/PMMA). **Group III:** Conventional Acrylic denture base specimens

(unmodified PMMA, control)

- **Specimens Preparation**

Specimens in Groups I and II were prepared following a standardized protocol, developed through a pilot study and a protocol outlined by **Sun et al. (2021)** to ensure uniform mixing and homogeneous nanoparticle dispersion throughout the PMMA resin. [3]

a) Preparation of acrylic denture base specimens loaded with biologically synthesized silver nanoparticles suspension (BNS/PMMA group)

BNS was first blended with acrylic acid as a coupling agent at a volumetric ratio of 1:3. The modified suspension was then incorporated into methyl methacrylate (MMA) monomer at a ratio of 1:3 to obtain a homogeneous nanoparticle-containing liquid phase. Subsequently, PMMA polymer powder was added to this liquid phase at a volumetric ratio of 2:1 following the manufacturer's instructions, and the mixture was stirred until a uniform dough consistency was reached prior to specimen fabrication. [3]

b) Preparation of acrylic denture base specimens loaded with chemically synthesized silver nanoparticles suspension (CNS/PMMA group)

Specimens in the CNS/PMMA group (Gp II) were prepared following the same protocol described for the BNS/PMMA group, with CNS replacing BNS. [3]

c) Preparation of Conventional Acrylic denture base specimens (PMMA group)

Regarding the control group (PMMA, Gp III), specimens were prepared by directly mixing PMMA powder and MMA following the volumetric powder-to-liquid ratio (2:1) recommended by the manufacturer. [7]

d) Specimens curing

Standardized disc-shaped (10 mm × 2 mm) and rectangular (65 × 10 × 2.5 mm) specimens were fabricated for antifungal and flexural testing, respectively. Specimens corresponding to each experimental group were processed using flasking and compression molding technique. Polymerization was carried out using a heat-curing cycle of 70°C for 90 min followed by 100°C for 30 min. After bench cooling, specimens were deflasked, finished, and polished before testing.

- **Surface mapping and elemental analysis**

Six representative specimens from both, the BNS/PMMA and CNS/PMMA groups were examined using a *A Quattro S (Thermo Scientific, USA)* field-emission scanning electron microscope *(FESEM)* was used to evaluate the the distribution of silver nanoparticles within the acrylic resin matrix. Elemental mapping and compositional analysis were subsequently performed using an energy-dispersive X-ray spectroscopy (EDS/EDAX) system coupled with the FESEM to confirm the presence and localization of silver within the specimens. [8]

- **Antifungal Activity Evaluation**

The antifungal activity of the three experimental groups were evaluated against *Candida albicans* using direct contact method.

a) Preparation of Fungal Suspension:*Candida albicans* suspension was prepared in brain-heart infusion (BHI) broth at a concentration equivalent to 0.5 McFarland standard. Suspension was subsequently diluted to 1 x $10^{\wedge 5}$ fungal cells/mL (final concentration).

b) Antifungal Activity Testing: Sample size for antifungal activity was calculated using G*Power v.3.1.9.7 for one-way ANOVA (effect size f=0.544 from [1], α=0.05, power=80%), requiring 36 specimens (n=12/group). Disc-shaped heat-cured acrylic resin specimens were prepared as previously described and sterilized by autoclaving. Each specimen was inoculated with 10 µL of a standardized *Candida albicans* suspension and incubated at 37°C for 2 h to allow evaporation of the liquid phase and microbial adhesion. Subsequently, specimens were individually transferred to sterile Eppendorf tubes containing 0.5 mL of BHI broth and incubated at 37°C for 12 h. Following incubation, 10 µL aliquots were aseptically collected from each tube and spread onto Sabouraud Dextrose Agar (SDA) plates using sterile glass spreaders. The inoculated plates were incubated at 37°C for 48 h, after which the antifungal activity was evaluated by manually counting the number of colony-forming units (CFU). [1]

- **Flexural Strength and Modulus determination**

Sample size for flexural strength was calculated using G*Power v.3.1.9.7 for one-way ANOVA (effect size f=0.677 from [7], α=0.05, power=80%), requiring 27 specimens (n=9/group). A three-point bending test was employed to determine flexural strength and modulus in accordance with ANSI/ADA specifications.[9] Rectangular specimens were fabricated and positioned horizontally on a custom-made fixture with a span length of 50 mm. Testing was conducted using a computer-controlled Instron universal machine (USA) fitted with a 5 kN load cell, at a crosshead speed of 1 mm/min. Central loading continued until fracture, and the data captured via Instron® Bluehill Lite Software. [10] The Flexural strength (FS) was calculated using the following equation [10]: $\mathbf{FS = 3F\ (L)/2wh^2}$ Where FS: the flexural strength (MPa),F: maximum load at the point of fracture (N), L: distance between the two supports (mm),w:

specimen width (mm), and h: specimen thickness (mm).

Then, the following equation was employed to determine the flexural [11]:

Ef = (L^3 *F) / (4* w *h^3* d) Where, Ef: flexural modulus (Gpa), L: length between two support points (mm), F: force applied at the center of the beam (N), w: width of the beam (mm), h: thickness of the beam (mm), and d: deflection caused by force (mm).

- **Statistical analysis**

IBM SPSS v.20 for windows was used for data analysis. Normality was confirmed by Kolmogorov-Smirnov, and Shapiro Wilk tests. Data are presented as mean ± SD. One-way ANOVA with Bonferroni post hoc correction was used for comparisons among the three groups. Statistical significance was set at $p \leq 0.05$.

Results:

- **Morphological analysis:**

Transmission electron micrographs confirm the formation of nanoparticles for both biologically and chemically synthesized AgNPs,

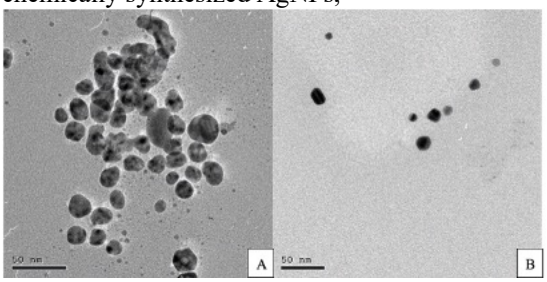


Figure 1. Both biologically and chemically synthesized AgNPs are spherical in shape and monodisperse.

Figure (1): Transmission electron microscopy (TEM) micrographs of (A) biologically synthesized and (B) chemically synthesized silver nanoparticles (AgNPs).

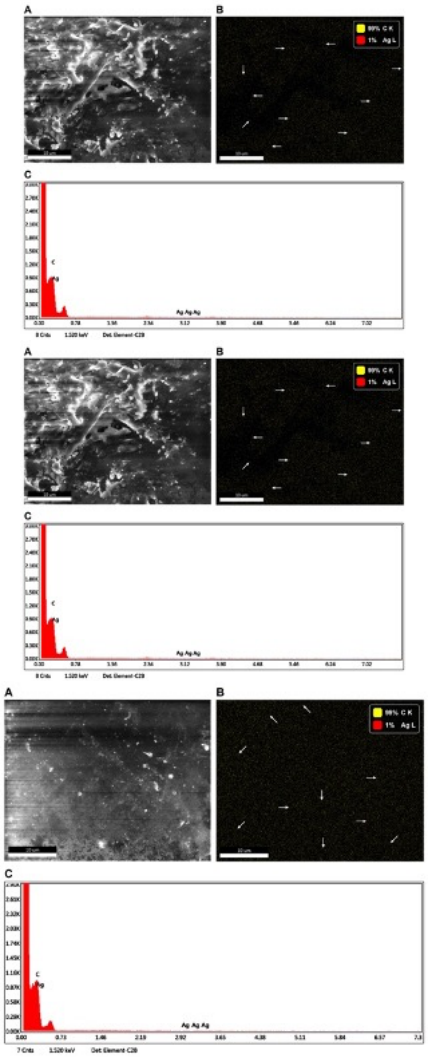
- **MIC and MFC:**

MIC values of BNS and CNS against *Candida albicans* were 26.75 µg/mL and 53.5 µg/mL, respectively. The corresponding MFC values were 53.5 µg/mL for BNS and 107 µg/mL for CNS. Based on these findings, the MFC of each nanoparticle suspension was selected as incorporation concentration for the PMMA denture base resin.

- **Surface mapping and elemental analysis of acrylic denture base specimens**

Scanning electron micrographs revealed a uniform and homogeneous distribution of silver nanoparticles across the surface of the acrylic resin specimens for both biosynthesized and chemically synthesized loaded denture base specimens. Moreover, elemental analysis confirmed the detection of silver, carbon and oxygen, **Figures 2,3** and **Table 1**.

Figure (2): Field emission scanning electron microscopy (FESEM) characterization of acrylic denture base resin loaded with biologically synthesized AgNPs. (A) Surface elemental mapping distribution. (B) Homogeneous distribution of biologically synthesized AgNPs (indicated by white arrows) along the resin surface. (C) Energy dispersive X-ray (EDX) spectrum confirming the presence of elemental silver (Ag) in the modified resin sample



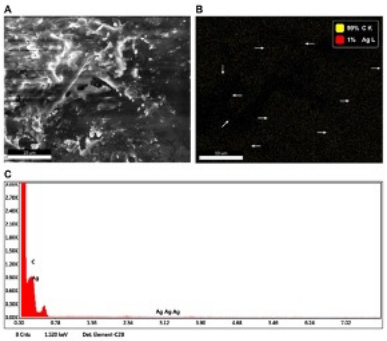


Figure (3): Field emission scanning electron microscopy (FESEM) characterization of acrylic denture base resin loaded with chemically synthesized AgNPs. (A) Surface elemental mapping distribution. (B) Homogeneous distribution of biologically synthesized AgNPs (indicated by white arrows) along the resin surface. (C) Energy dispersive X-ray (EDX) spectrum confirming the presence of elemental silver (Ag) in the modified resin sample.

Table (1): Elemental analysis of acrylic denture base samples loaded with biologically and chemically synthesized AgNPs.

	Smart Quant Results of dentures loaded with biologically synthesized AgNPs			**Smart Quant Results of dentures loaded with chemically synthesized AgNPs**		
Element	Weight %	Atomic %	Error %	Weight %	Atomic %	Error %
C K	71.27	72.25	4.23	57.78	67.73	6.32
O K	27.21	27.58	11.78	36.72	21	10.78
Ag L	1.52	0.17	47.48	1.56	0.18	37.23

- **Antifungal Activity**

The mean CFU values for the investigated groups are presented in **Table 2.** A statistically significant difference was detected among the experimental groups ($p < 0.001$). The BNS/PMMA group exhibited the lowest fungal growth, followed by the CNS/PMMA group, whereas the unmodified PMMA group demonstrated the highest CFU counts.

Table (2) Descriptive statistics and comparison of colony forming unit (CFU) between groups that was done using ANOVA test

Group	**Mean**	**Std. Dev**	**95% Confidence Interval for Mean**		**Min-Max**
			Lower Bound	**Upper Bound**	
I (BNS/PMMA)	48.84 ^{c}	12.80	30.75	66.08	15.0-95.00
II (CNS/PMMA)	61.00 ^{b}	7.27	56.38	65.62	50.0-72.00
III (PMMA)	342.50 ^{a}	49.31	311.20	373.80	250.0-420.00
F value	304.90				
p-value	0.000*				

Significance level $p \leq 0.05$, *significant.
Means with different superscript letters are significantly different.

- **Flexural strength and flexural modulus**

Table 3 summarizes the flexural strength and modulus values expressed as mean ±SD. Among the groups, no statistically significant differences were detected neither for flexural strength (p = 0.874) nor modulus (p = 0.870).

Table (3): Mean and standard deviation values of flexural strength (MPa) and flexural modulus (Gpa) of differently investigated groups:

Group	Flexural Strength (Mpa)		Flexural Modulus (GPa)	
	Mean	Std. Dev	Mean	Std. Dev
I (BNS/PMMA)	81.51[a]	22.72	2.67[b]	0.74
II(CNS/PMMA)	83.43[a]	14.91	2.73[b]	0.49
III (PMMA)	85.77[a]	12.92	2.81[b]	0.42
P-value	0.874 ns		0.870 ns	
F-value	0.136		0.140	

Significance level p≤0.05, ns=non-significant. Means with the same superscript letter are not significantly different.

Discussion:

Over the years, many materials have been used to construct removable complete or partial denture bases. Yet, polymethyl methacrylate (PMMA) is still one of the materials of choice since 1939. This is due to its ease of manipulation, aesthetic appeal, accurate fit, stability in the oral environment, and cost-effectiveness. Inspite of its advantages, PMMA exhibits ease of microbial adherence to its surface, frequent fractures and low thermal conductivity.[2]

Denture stomatitis remains a critical concern for PMMA, due to persistant microbial adhesion. While various reinforcements including metallic fillers, fibers, and styrene rubber grafting have been explored to overcome strength deficiencies in denture base materials, these modifications do not fully resolve the biofilm issue. Prevalence rates of *Candida* species in denture wearers range from 60% to 100%, positioning these organisms as primary etiological agents of oral fungal infections. Within this genus, *C. albicans* remains the predominant pathogen implicated in such conditions. [2]

Generally, maintaining good oral hygiene and denture cleanliness is essential for preventing denture stomatitis. Current management approaches include mechanical cleaning, use of commercial disinfectants, and immersion in antifungal solutions. However, these strategies are often limited by loss of drug rapidly in saliva, inhomogeneous distribution of the drug and the development of antifungal drug resistance. In addition denture hygiene may be compromised in geriatric or hospitalized patients due to reduced motor skills, cognitive impairment, and memory loss. Therefore, alternative strategies such as incorporating antimicrobial agents into conventional PMMA formulations have been proposed to inhibit *Candida* adherence.[3]

Silver nanoparticles were chosen due to the significant attention that was gained by its leading role as an antimicrobial nanomaterial with broad-spectrum effect, high-efficiency and relatively high biocompatibility in comparison to other nanoparticles.[13]

Chemical synthesis remains the most commonly used approach for producing silver nano-particles (AgNPs), which mainly rely on chemical reducing agents as sodium borohydride, sodium citrate, along with stabilizing agents like thiols, amines, and polymers. However, these methods pose environmental concerns due to the generation of toxic chemicals and hazardous by-products. Meanwhile, the whole world is moving toward green technology and prioritizing eco-friendly solutions and sustainability. Accordingly, It's essential to adopt environmentally conscious alternatives as green synthesis of silver nanoparticles.[12]

Plant-mediated synthesis was selected in the present study because it represents a simple, environmentally friendly, and cost-effective approach for silver nanoparticle production.This is consistent with the findings of **Wahab et al. (2021)** who demonstrated the potential of plant-derived silver nanoparticles in combating multidrug-resistant microorganisms. In addition to their antimicrobial efficacy, biosynthesized silver nanoparticles have been reported to exhibit enhanced biocompatibility and reduced environmental impact compared with conventional chemical synthesis methods, highlighting their potential applications in biomedical and dental materials.[14]

Previous approaches relied on mechanical blending of nanosilver powder particles with PMMA powder using ball milling technique to produce AgNP-PMMA denture base materials. While this approach imparted antimicrobial activity to the resulting denture base resin, it was often associated with

nanoparticle agglomeration and non-uniform distribution within the polymer matrix, which adversely affected esthetic appearance and, in some cases, compromised mechanical performance. Therefore, in the current study nanosilver suspensions were used rather than nanoparticle powders in order to provide more homogenous dispersion of nanoparticles throughout the PMMA matrix. This approach was intended to enhance nanoparticle distribution while preserving the esthetic and mechanical properties of the denture base material, while maintaining effective antimicrobial activity.[3]

Nanoparticle toxicity remains a critical concern as a threshold exists between concentrations toxic to microorganisms and those harmful to human cells. Previous studies have shown that low concentrations less than 10 μM are relatively safe when tested on spontaneously immortalized human keratinocyte cell line (HaCaT cells).[15] Accordingly, MIC and MFC were determined in the present study to identify the lowest effective antifungal concentration of silver nanoparticles. MIC was assessed using the tube dilution method, a quantitative and highly reproducible gold standard technique that minimizes variability associated with diffusion-based assays.[16]

Results of the current study revealed that BNS suspension exhibited a potent fungicidal effect at a significantly lower concentration than that of CNS suspension. Accordingly, BNS was incorporated into PMMA at 50% of its stock concentration (53.5 μg/mL), whereas CNS was used at its full concentration (107 μg/mL). This enhanced antifungal efficacy of BNS may be due to the synergysitic interaction between the silver nanoparticle core and the biologically derived capping agents. These capping agents, including phenols, flavonoids and methylxanthins, not only stabilize the nanoparticles but also possess inherent antifungal activities, thereby, enhancing their biological activity through complementary mechanisms.[17]

The antifungal activity of the nanosilver core is primarily mediated through the release of silver ions, that disrupt the fungal cell membrane, interferes with intracellular enzymes and generates reactive oxygen species (ROS). Secondary, the bioactive capping layer may further enhance antifungal activity by weakening the fungal cell wall, inhibiting essential fungal enzymes and disrupt signalling pathways. For instance, epigallocatechin-3-gallate (EGCG) has been reported to inhibit ergosterol and induce fungal cell apoptosis.[14] In addition, polysaccharides and proteins within the capping layer such as rhamnogalacturonan-I (RG-I), arabinogalactans and glucans, may enhance nanoparticles adhesion to the fungal cell wall, facilitating more efficient silver ion delivery. This synergistic mechanisms may explain the superior antifungal performance observed for the BNS group.[18]

For antifungal activity evaluation for the different experimental groups, the direct contact method was used as it closely simulates the clinical interaction between denture-based materials and microbial biofilms. This method enables quantitative assessment of microbial viability through colony-forming unit (CFU) counting and provides greater reproducibility compared with the zone of inhibition assay, which depends on antimicrobial diffusion and is influenced by variables such as agar thickness, nutrient composition, and incubation conditions.[19]

The significantly higher antifungal activity exhibited by the BNS/PMMA group compared with the CNS/PMMA could be due to the synergism between the silver nanoparticle core and the biologically derived capping agents, which provide in a dual antifungal mechanism. These findings are consistent with **Jamdagni et al., (2018)** who reported superior antifungal activity of green-synthesized silver nanoparticles compared with chemically synthesized counterparts against multiple fungal species.[20] Conversely, **Chandrakar et al. (2025)** found comparable antifungal efficacy between biologically and chemically synthesized silver nanoparticles, however, biologically synthesized nanoparticles demonstrated enhanced biofilm inhibition, which was attributed to the presence of bioactive capping agents that improved stability and activity.[21]

In addition, the use of nanosilver suspensions in the present study facilitated improved nanoparticle dispersion within the PMMA matrix, increasing the available surface area for interaction with fungal cells and thereby enhancing antifungal performance. This observation aligns with **Sun et al. (2021)**, who demonstrated superior dispersion and antimicrobial efficacy of nanosilver suspensions compared with nanopowder incorporation.[3]

Interestingly, FE-SEM/EDS mapping demonstrated a homogeneous distribution of silver nanoparticles in both modified groups, suggesting that the observed differences in antifungal activity were primarily related to nanoparticle characteristics and surface chemistry rather than differences in dispersion within the PMMA matrix. Furthermore, uniform distribution is advantageous for maximizing the effective surface area of the nanoparticles and ensuring consistent antimicrobial activity throughout the resin matrix. These observations corroborate the findings of **Sun et al. (2021)**, who highlighted the benefits of incorporating silver nanoparticles as

suspensions rather than powders to achieve homogeneous dispersion and improved antimicrobial performance.[3]

Regarding mechanical properties, flexural strength and modulus are critical determinants of denture base durability, as reduced flexural properties increase the risk of intra and extra oral fracture. In the present study incorporation of nanosilver suspension, whether biosynthesized or chemically synthesized, did not adversely affect the flexural strength or modulus of PMMA, with no differences in the statistical significance observed among groups. This finding is likely attributed to the use of nanosilver in suspension form, which promotes uniform nanoparticle dispersion within the polymer matrix. This was confirmed by FE-SEM/EDS elemental mapping, which demonstrated homogenous distribution of nanoparticles with absence of agglomerations. Such uniformity prevents the formation of stress concentration sites that typically initiate crack propagation and mechanical failure.[22]

In contrast, incorporation of nanosilver in powder form has been frequently associated with reduced flexural strength due to nanoparticle agglomeration caused by strong inter-particle forces. These agglomerates act as stress raisers, facilitating crack initiation under load. This interpretation is supported by **Rashad (2018)**, who reported improved flexural strength when nanosilver was incorporated in liquid form rather than as a powder at similar concentrations.[23] While, **Gupta (2018)** and **Rajesh and Shardul (2019)** demonstrated reduced flexural strength due to nanoparticle agglomeration and poor interfacial bonding, suggesting that inadequately dispersed silver nanoparticles act as defects within the PMMA matrix. .[24,25]

Nanoparticle concentration also is critically important for mechanical performance. At higher concentrations, silver nanoparticles may form nano-sized oxides and interfere with polymerization, thereby disrupting the PMMA network and compromising its mechanical properties.[25, 26] Consistent with this concept, **Pai et al., (2023)** and **El-Hussein (2024)** reported concentration-dependent reductions in flexural strength following nanosilver incorporation.[27, 28] In contrast, **Hycynth (2018)** reported an improvement in flexural strength at higher nanoparticle concentrations, which was attributed to enhanced stress transfer and favorable interactions between silver nanoparticles and PMMA chains.[26] These conflicting findings suggest that the flexural strength is influenced by multiple factors as concentration, nanoparticle size, surface characteristics, dispersion quality, and the method of incorporation into the polymer matrix. Accordingly, the minimum fungicidal concentration was selected in the present study to achieve effective antifungal activity while preserving the mechanical integrity of the denture base resin.

Furthermore, the biologically synthesized nanoparticles may have benefited from the presence of polyphenols, flavonoids, and EGCG within the capping layer. These compounds have been reported to exhibit antioxidant activity and may contribute to stabilization of the polymer matrix. In addition, hydroxyl groups present in these phytochemicals may interact with the carbonyl groups of PMMA through hydrogen bonding, potentially enhancing interfacial adhesion and mechanical stability.[29, 30] The use of acrylic acid as a coupling agent may have further improved nanoparticle–matrix integration through interactions with both silver nanoparticles and the PMMA network, promoting efficient stress transfer and minimizing the likelihood of nanoparticles acting as structural defects, that ultimately preserving flexural properties.[3]

Based on the current study results, the first null hypothesis was partially rejected, as silver nanoparticle modification significantly improved antifungal activity compared with conventional PMMA without affecting flexural strength or flexural modulus. The second null hypothesis was rejected, as biologically synthesized silver nanoparticles exhibited significantly greater antifungal efficacy than chemically synthesized silver nanoparticles, despite no significant differences in flexural properties between the two modified groups.

Limitations of this study include the short-term evaluation period and the need for clinical validation, as findings may not fully translate to the complex oral environment. To ensure clinical viability, future research should assess the long-term stability by evaluating sustained antifungal efficacy and flexural strength degradation following hydrothermal aging. In-vivo animal studies and clinical trials to validate the antimicrobial efficacy, biocompatibility and mechanical performance of silver nanoparticle-loaded denture bases.

Conclusions:

Modification of PMMA with either biosynthesized or chemically synthesized silver nanoparticle suspensions significantly improved its antifungal properties against *Candida albicans* compared with unmodified PMMA, while preserving flexural properties. Notably, Biosynthesized AgNPs exhibited superior antifungal efficacy by achievivg higher antifungal effects at approximately half the concentration required for chemically synthesized silver nanoparticles. Within the limitations of this in-

vitro study, biosynthesized silver nanoparticles appear to be a promising antifungal modification for PMMA denture base materials.

Authors' contribution:

Conceptualisation: G.A.R and S.I.H. Data acquisition: S.K.R and R.A.H. Data analysis and interpretation: S.K.R and R.A.H. Design: G.A.R and S.I.H. Investigation: S.K.R and R.A.H. Supervision: G.A.R and S.I.H. Validation: G.A.R and S.I.H. Visualisation: G.A.R and S.I.H. Writing original draft: S.K.R. Writing review and editing: : S.K.R, G.A.R and S.I.H.

Conflict of interest:

The authors declare no conflict of interest.

Ethics:

The study was approved by Research ethics committee of Faculty of dentistry, Cairo University (approval number: 5-11-22).

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Data Availability Statement:

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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