RESEARCH PAPER

Green Synthesis of Silver Nanoparticles Using Moringa oleifera Flower Extract and Its Antibiofilm Activity Against Oral Pathogen

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ABSTRACT

Silver nanoparticles (AgNPs) have emerged as promising alternatives to conventional antibiotics due to the rising problem of antimicrobial resistance among oral pathogens. This study reports the green synthesis of AgNPs using Moringa oleifera flower extract and evaluates their antibacterial and antibiofilm activity against oral pathogens. The biosynthesis was achieved through an eco-friendly method, with phytochemicals acting as reducing and stabilizing agents, confirmed by a visible color change. The synthesized AgNPs exhibited significant antibacterial activity against Streptococcus mutans and Enterococcus faecalis, with enhanced efficacy compared to the plant extract alone, indicating a synergistic effect. A clear dose-dependent inhibitory pattern was observed. Furthermore, the AgNPs demonstrated strong antibiofilm activity, significantly reducing biofilm formation in both pathogens. Higher concentrations led to substantial disruption of established biofilms, suggesting interference with bacterial adhesion and biofilm maturation. Overall, M. oleifera flower extract–mediated AgNPs show promising antibacterial and antibiofilm potential, highlighting their applicability in managing oral biofilm-associated infections through a sustainable and eco-friendly approach.

Keywords: Silver nanoparticles, Moringa oleifera, antibiofilm, oral pathogens, green synthesis

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INTRODUCTION

Nanotechnology has emerged as one of the most rapidly advancing fields in modern science, with wide-ranging applications in medicine, environmental science, agriculture, and material engineering [1]. Among various nanomaterials, metal nanoparticles particularly silver nanoparticles (AgNPs) have attracted considerable attention due to their unique physicochemical properties, including high surface area-to-volume ratio, enhanced reactivity, and remarkable antimicrobial potential. These properties make AgNPs highly suitable for biomedical applications, especially in combating microbial infections [2, 3].

The increasing prevalence of antibiotic-resistant microorganisms has become a major global health concern. Conventional antibiotics are gradually losing efficacy due to the emergence of multidrug-resistant bacterial strains [4]. This has prompted the search for alternative antimicrobial agents that are both effective and less prone to resistance development. In this context, silver nanoparticles have demonstrated broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, including clinically relevant oral pathogens [2, 5].

Traditional methods for the synthesis of nanoparticles, such as chemical reduction and physical approaches, often involve toxic reagents, high energy requirements, and environmental hazards. To overcome these limitations, green synthesis approaches using biological systems have gained significant interest [6]. Plant-mediated synthesis, in particular, offers a simple, cost-effective, and eco-friendly alternative. Plant extracts contain a diverse array of bioactive compounds such as phenolics, flavonoids, alkaloids, proteins, and polysaccharides, which can act as natural reducing and stabilizing agents during nanoparticle formation [7, 8].

Moringa oleifera (M. oleifera), commonly known as the drumstick tree, is widely distributed in tropical and subtropical regions, including India. It is well known for its rich nutritional and medicinal properties, owing to the presence of various phytochemicals such as vitamins, minerals, amino acids, and antioxidants [9–11]. Although *M. oleifera* has been extensively studied for applications in water purification and as a nutritional supplement, its direct antimicrobial activity is relatively limited. However, its phytochemical composition makes it an excellent candidate for the green synthesis of nanoparticles, potentially enhancing its biological activity when combined with metallic ions such as silver [12, 13].

Oral health is significantly impacted by biofilm-forming bacteria, particularly *Streptococcus mutans (S. mutans)* and *Enterococcus faecalis (E. faecalis)*, which play a crucial role in the development of dental plaque and caries. These microorganisms adhere to tooth surfaces and produce extracellular polymeric substances that facilitate biofilm formation, making them more resistant to antimicrobial agents. Therefore, targeting biofilm formation is a key strategy in preventing and managing oral diseases [14, 15].

In this setting, the development of nanoparticle-based antibiofilm agents represents a promising therapeutic approach. Silver nanoparticles, due to their small size and high reactivity, can penetrate bacterial cell walls, disrupt cellular processes, and inhibit biofilm formation effectively. Therefore, the present study aims to synthesize silver nanoparticles using *M. oleifera* flower extract through a green synthesis approach and to evaluate their antibacterial and antibiofilm activity against key oral pathogens.

MATERIALS AND METHOD

2.1 Chemicals and Reagents

Silver nitrate ($AgNO_3$) of analytical grade was procured from Sigma-Aldrich. All reagents used in the study were of analytical grade, and double-distilled water was used throughout the experiments [16] .

2.2 Preparation of *Moringa oleifera* Flower extract

Fresh flowers of *M. oleifera* were collected, thoroughly washed with distilled water to remove impurities, and air-dried at room temperature. The dried flowers were finely powdered [17]. 10 g of the powdered material was mixed with 100 mL of distilled water and heated at 60°C for 30 minutes. The mixture was then cooled and filtered using Whatman No. 1 filter paper. The obtained filtrate was stored at 4°C and used as the reducing and stabilizing agent for nanoparticle synthesis [18].

2.3 Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized using *Moringa oleifera* flower extract by adding varying volumes (0-10 mL) of the extract to a 0.5 mM aqueous silver nitrate ($AgNO_3$) solution, maintaining a final reaction volume of 100 mL. The reaction conditions were optimized with the pH adjusted to 11.0, temperature maintained at 60°C, and stirring speed set at 300 rpm. The optimized mixture consisted of 10 mL of *M. oleifera* flower extract and 90 mL of 0.5 mM $AgNO_3$ solution, which was incubated for 2 hours under constant stirring. The formation of AgNPs was preliminarily confirmed by a visible color change from pale yellow to reddish-brown [19, 20].

2.4 Purification of Synthesized Nanoparticles

Following synthesis, the reaction mixture was centrifuged at 8000 rpm for 20 minutes. The obtained pellet was washed three times with distilled water to remove unbound phytochemicals and residual ions. The purified nanoparticles were dried at 80°C and stored for further characterization and biological assays [21].

2.5 Antibacterial Activity

The antibacterial activity of the synthesized AgNPs was evaluated against oral pathogens, including *S. mutans* and *E. faecalis*. The bacterial cultures were grown under standard conditions, and antimicrobial activity was assessed using standard methods (e.g., agar well diffusion method). Zones of inhibition were measured to determine antibacterial efficacy [22].

2.6 Antibiofilm Assay

The antibiofilm activity of AgNPs was evaluated against *S. mutans* and *E. faecalis*. Bacterial cultures were incubated with varying concentrations of AgNPs under suitable conditions.

Biofilm formation was quantified using standard staining methods (such as crystal violet assay), and the percentage of biofilm inhibition was calculated in comparison to untreated controls. Fig 1 summarizes the study [23].

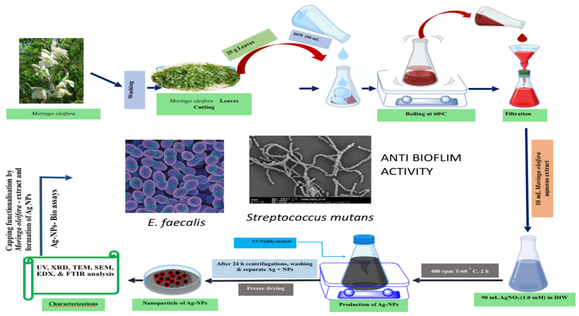

Fig 1: *Schematic representation of the green synthesis of silver nanoparticles (AgNPs) using M. oleifera flower extract and their subsequent characterization and antibiofilm evaluation.* The workflow illustrates plant collection and washing, preparation of aqueous extract, synthesis of AgNPs using silver nitrate under controlled conditions (60°C), followed by purification (centrifugation and freeze-drying). The synthesized nanoparticles were systematically characterized, and their antibiofilm activity was evaluated against oral pathogens, including *S. mutans* and *E. faecalis.*

2.7 Statistical Analysis

All experiments were performed in triplicates, and the data were expressed as mean ± standard deviation. Statistical significance was analyzed using appropriate statistical tests, with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1 Synthesis of Silver Nanoparticles

The successful synthesis of AgNPs using *M. oleifera* flower extract was initially confirmed by a visible color change in the reaction mixture. Upon incubation under optimized conditions (pH 11, 60°C), the solution gradually changed from pale yellow to reddish-brown within 2 hours, indicating the reduction of silver ions (Ag^{+}) to metallic silver (Ag^{0}) and the formation of nanoparticles.

3.2 Antibacterial Activity

The synthesized AgNPs exhibited significant antibacterial activity against oral pathogens, including *S. mutans* and *E. faecalis*. Compared to the plant extract alone, AgNPs showed enhanced antibacterial efficacy, indicating the synergistic effect of silver ions and plant-derived biomolecules. The antibacterial activity increased with increasing concentrations of AgNPs, demonstrating a dose-dependent inhibitory effect.

3.3 Antibiofilm Activity

The antibiofilm activity of AgNPs was evaluated against *S. mutans* and *E. faecalis*, and the results demonstrated a significant reduction in biofilm formation compared to untreated controls. A clear concentration-dependent inhibition was observed, with lower concentrations showing moderate inhibition, while higher concentrations resulted in substantial disruption of established biofilms. These findings indicate that AgNPs effectively interfere with bacterial adhesion and biofilm maturation processes (Fig. 2 and 3).

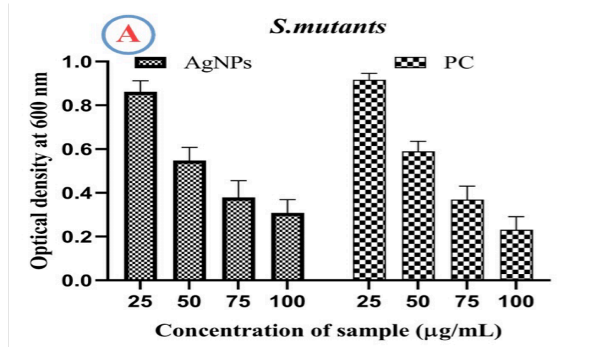

Fig 2: A. Antibiofilm activity of silver nanoparticles against oral pathogens. Graph showing the inhibitory effect of AgNPs on biofilm formation by *S. mutans*. A concentration-dependent reduction in biofilm formation is observed, indicating the effectiveness of AgNPs in disrupting bacterial adhesion and biofilm development.

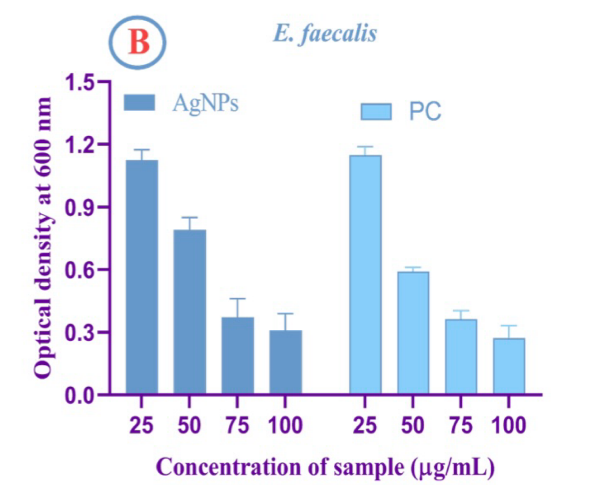

Fig 3: B. Antibiofilm activity of silver nanoparticles against oral pathogens. Graph showing the inhibitory effect of AgNPs on biofilm formation by *E. faecalis*. A concentration-dependent reduction in biofilm formation is observed, indicating the effectiveness of AgNPs in disrupting bacterial adhesion and biofilm development.

DISCUSSION

The present study, a green and efficient method for biosynthesis of silver nanoparticles was developed using

M. oleifera flower extract. The developed method has numerous significant advantages over conventional methods. Specifically, the synthesis of AgNPs using *M. oleifera* flower extract does not produce any toxic substances. The ability of oral *streptococci* and *Enterococci* to establish biofilm growth on the surface of teeth is crucial to the formation of dental plaque and dental cavities. *S. mutans* and *E. faecalis* in particular adheres to teeth and utilizes dietary sucrose to synthesize extracellular insoluble glucans that can serve as scaffolding for other microbes to colonies. Tooth decay ensues when *S. mutans* and *E. faecalis* and other microbes residing within the plaque biofilm produce acids that erode enamel. From the above result we could see that the Ag nanoparticle has a anti biofilm activity which could be used as a preventive measure for dental caries

In the current work, *M. oleifera* seed extract was used to create an environmentally friendly and effective approach for the production of silver nanoparticles. The created approach offers many noteworthy benefits over traditional techniques. To be more precise, no harmful compounds are produced during the manufacture of AgNPs utilizing *M. oleifera* seed extract. Furthermore, due to surface plasmon resonance events, there was an increase in color intensity from yellow to black when the incubation time was increased from 0 to 2 h, after which no additional color changes were noticed [19]. These findings suggest that *M. oleifera* flower extract contains bioactive compounds that not only reduce silver ions to form nanoparticles but also help stabilize the synthesized AgNPs. This highlights the effectiveness of the extract in green nanoparticle synthesis. Moreover, the observed results are consistent with previous studies, including a recent report demonstrating eco-friendly synthesis of silver nanoparticles using *M. oleifera* flowers, with a characteristic absorption peak at 429 nm, confirming successful nanoparticle formation [19].

Additionally, in the present study, the antibacterial efficacy of *M. oleifera* flower extract-mediated AgNPs was evaluated against oral pathogens, including *S. mutans* and *E. faecalis*. The synthesized AgNPs exhibited significant antibacterial activity, showing greater efficacy compared to the plant extract alone, which indicates a synergistic interaction between silver ions and plant-derived bioactive compounds. Furthermore, the antibacterial activity increased with increasing concentrations of AgNPs, demonstrating a clear dose-dependent inhibitory effect against the tested pathogens.

In addition to their antibacterial potential, the synthesized AgNPs also displayed strong antibiofilm activity against *S. mutans* and *E. faecalis*. A significant reduction in biofilm formation was observed when compared to untreated controls. The inhibition was concentration-dependent, with lower concentrations producing moderate effects, while higher concentrations led to substantial disruption of established biofilms. These findings suggest that the AgNPs interfere with bacterial adhesion and biofilm maturation, which are critical steps in the pathogenesis of oral infections. The enhanced antimicrobial activity of the synthesized nanoparticles can be attributed to their small size and high surface area-to-volume ratio, which facilitate effective interaction with bacterial cell membranes. In the present study, the average size of the *M. oleifera* flower extract–AgNPs was found to be approximately 4.0 nm, which likely contributed to their strong bactericidal activity. Moreover, AgNPs are known to penetrate bacterial cells and interact with intracellular components such as proteins and nucleic acids, ultimately leading to cell death.

These findings are consistent with earlier reports demonstrating the potent antibacterial activity of *M. oleifera* mediated silver nanoparticles against a wide spectrum of pathogenic bacteria. For instance, earlier reports demonstrated that *M. oleifera* flower extract AgNPs exhibited strong antibacterial activity against *Pseudomonas aeruginosa* (ATCC 25853), Gram-negative *Escherichia coli* (ATCC 25922), and Gram-positive *Staphylococcus aureus* (ATCC 29213), with comparatively higher activity against Gram-negative bacteria. However, some studies have shown that AgNPs synthesized from *M. oleifera* leaves displayed limited antibacterial activity against *Klebsiella pneumoniae* [24], suggesting that the type of plant material used in nanoparticle synthesis plays a crucial role in determining antimicrobial efficacy. Additionally, AgNPs derived from *M. oleifera* seeds have been reported to exhibit larvicidal activity against *Aedes aegypti*, the dengue vector [25], highlighting their diverse biological applications. Similarly, selenium nanoparticles have demonstrated antimicrobial activity against halophilic bacteria [26]. However, the AgNPs synthesized in the present study exhibited superior antibacterial and antibiofilm efficacy, particularly against oral pathogens, emphasizing their enhanced potential for biomedical applications.

Overall, the results of the present study, in comparison with previous findings, highlight the potent antibacterial and antibiofilm properties of *M. oleifera* flower extract–mediated AgNPs, particularly against oral pathogens, and support their potential application in managing biofilm-associated infections.

CONCLUSION

In conclusion, the present study demonstrates that *M. oleifera* flower extract-mediated AgNPs can be successfully synthesized through a simple and eco-friendly approach, yielding stable nanoparticles with potent biological activity. The synthesized AgNPs exhibited significant antibacterial effects against oral pathogens, particularly *S. mutans* and *E. faecalis*, with enhanced efficacy compared to the plant extract alone, indicating a synergistic action of silver ions and phytochemicals. Additionally, the nanoparticles showed strong antibiofilm activity, effectively inhibiting biofilm formation in a concentration-dependent manner. Overall, these results suggest that *M. oleifera* flower extract–derived AgNPs hold promising potential as effective antimicrobial and antibiofilm agents, particularly for the management of oral infections, and may serve as a basis for the development of novel therapeutic strategies.

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CONFLICT OF INTEREST

Nil.

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