



ANTIBACTERIAL AND ANTIBIOFILM ACTIVITY OF DENTAL PATHOGENS USING LEAF EXTRACT DERIVED PALLADIUM NANOPARTICLES FROM ANDROGRAPHIS

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ABSTRACT

OBJECTIVE: Dental diseases such as caries, periodontal disorders, and infections around dental implants are increasingly common and present a significant challenge to global public health. Palladium, a noble metal, has inherent antimicrobial properties that become more pronounced when reduced to the nanoscale, enhancing its ability to inhibit bacterial growth and biofilm formation. This study holds considerable potential in the context of dental medicine, where the need for effective treatments against drug-resistant infections is urgent. If successful, the application of palladium nanoparticles synthesized from *Andrographis* could provide a novel, safer, and more natural approach to combating dental pathogens. **MATERIALS AND METHODS:** Palladium nanoparticles (PdNPs) were synthesized using *Andrographis paniculata*. The antibacterial properties of PdNPs were assessed through the agar well diffusion and broth microdilution assays against *Streptococcus mutans* and *Candida albicans*. The antibiofilm potential was evaluated using the crystal violet staining technique, where PdNPs at different concentrations were tested for their ability to inhibit biofilm formation and disrupt existing biofilms. Biofilm biomass was quantified by measuring absorbance at 570 nm. **RESULTS AND DISCUSSION:** The palladium nanoparticles synthesized using andrographis showed inhibition of 15mm and 13mm at 100µg/ml and 50 µg/ml at concentrations respectively against *S. mutans* and inhibition of 16mm and 5mm at 100µg/ml and 50 µg/ml at concentrations respectively against *C. albicans*. Zone of inhibition is tabulated into bar graphs showing the route at each quantity of nanoparticles. The plate was incubated at 36°C for 48 h, followed by crystal violet staining and spectrophotometric absorbance measurements (OD600 & 530). The absorbance was used to calculate the “biofilm formation” on the y axis. x axis represents Pd-NPs from different concentrations (50 to 200µg/mL) where the synthesized PdNPs exhibited a prominent 570 nm absorption peak. **CONCLUSION:** In summary, PdNPs were synthesized using *Andrographis* leaf extract as a reducing agent. When tested against clinical pathogens, PdNPs demonstrated the most significant antibacterial activity, reflecting inhibition of 15mm and 13mm at 100µg/ml and 50 µg/ml at concentrations, respectively against *S. mutans* and inhibition of 16mm and 5mm at 100µg/ml and 50 µg/ml at concentrations respectively against *C. albicans*. This approach is cost-effective and environmentally friendly. Furthermore, the synthesized PdNPs exhibited a prominent 570 nm absorption peak. These PdNPs showed promising effectiveness against the bacterial strains under investigation. These results suggest that green-synthesized PdNPs could serve as efficient alternative antibacterial agents, inhibiting the growth of infections. In conclusion, we recommend PdNPs as versatile antimicrobial agents with broad-spectrum activity.

KEYWORDS: *Andrographis*, Palladium nanoparticles, Antibacterial activity, Antibiofilm activity



INTRODUCTION

Dental diseases such as caries, periodontal disorders, and infections around dental implants are increasingly common and present a significant challenge to global public health. These conditions are often linked to the formation of bacterial biofilms on the surfaces of teeth and oral tissues(1). Bacterial biofilms are clusters of microorganisms embedded in a self-produced extracellular matrix, making them highly resistant to Dental diseases such as caries, periodontal disorders, and infections around dental implants are increasingly common and present a significant challenge to global public health. traditional antimicrobial treatments. This biofilm-associated resistance is a primary reason for the persistence of infections and the difficulty in eradicating them(2). With the growing issue of antimicrobial resistance (AMR), which limits the effectiveness of conventional antibiotics, there is a pressing need to identify new treatment strategies, especially in the context of dental medicine, to address the challenges posed by biofilms and the pathogens that produce them.(3)

Nanotechnology has emerged as a potential solution to overcome the limitations of traditional antibiotics. Nanoparticles (NPs), due to their small size and large surface area, have unique antimicrobial properties that enable them to effectively interact with bacterial cells at the molecular level(4,5). Palladium (Pd) nanoparticles, in particular, have shown promising antibacterial effects against a range of pathogens, including those commonly found in the oral cavity. Palladium, a noble metal, has inherent antimicrobial properties that become more pronounced when reduced to the nanoscale, enhancing its ability to inhibit bacterial growth and biofilm formation(6). However, traditional methods of synthesizing palladium nanoparticles often involve the use of harmful chemicals, which may pose environmental and health risks. As a result, there is growing interest in alternative, greener approaches to produce these nanoparticles.

One such sustainable method is the green synthesis of palladium nanoparticles using plant extracts, which provides an eco-friendly and cost-effective alternative to conventional chemical methods(4). *Andrographis paniculata*, a medicinal plant known for its antibacterial, anti-inflammatory, and antioxidant properties, has been identified as a potential source for the green synthesis of palladium nanoparticles(7). The plant contains bioactive compounds like andrographolide, which contribute to its antimicrobial effects. This research seeks to explore the potential of *Andrographis paniculata* leaf extract for the synthesis of palladium nanoparticles, testing their antibacterial and antibiofilm properties against common dental pathogens such as *Streptococcus mutans*, *Porphyromonas gingivalis*, and *Aggregatibacter actinomycetemcomitans*(7,8). We hypothesize that the



synergistic combination of *Andrographis* extract and palladium nanoparticles will enhance antimicrobial activity, offering a promising alternative to conventional antibiotics(9). Furthermore, this study aims to evaluate the ability of these nanoparticles to disrupt biofilms, which are notoriously difficult to treat with standard antibiotic therapies. The outcomes of this research could provide innovative approaches for addressing dental infections and help reduce reliance on antibiotics, contributing to the global fight against antimicrobial resistance.(10,11)

This study holds considerable potential in the context of dental medicine, where the need for effective treatments against drug-resistant infections is urgent. If successful, the application of palladium nanoparticles synthesized from *Andrographis* could provide a novel, safer, and more natural approach to combating dental pathogens. Beyond its dental applications, this research could also influence other areas of medicine, where biofilm-associated infections pose similar challenges. By demonstrating the potential of plant-derived palladium nanoparticles in managing dental infections, this research aims to contribute to the advancement of nanomedicine and provide new solutions for the treatment of resistant infections, both in oral health and broader healthcare fields. The aim of this study is to find the antibacterial and antibiofilm activities of *S.mutans* and *C.albicans* against palladium nanoparticles derived from the leaf extract of *Andrographis*

MATERIALS AND METHODS

1. Collection and Preparation of Plant Material

The first step in the synthesis of palladium nanoparticles (PdNPs) involves collecting fresh leaves of *Andrographis*. The leaves are carefully harvested from a healthy plant to ensure they are free from disease or contamination. Once collected, the leaves are thoroughly washed with distilled water to remove any surface dust or impurities. After washing, the leaves are air-dried at room temperature or dried in an oven at a low temperature (typically around 40°C) to prevent the degradation of bioactive compounds. Once completely dry, the leaves are ground into a fine powder using a mortar and pestle or a mechanical grinder.(12)

2. Preparation of Leaf Extract

A specific quantity of the dried leaf powder (e.g., 10 g) is taken and mixed with a suitable solvent, typically distilled water or ethanol, in a ratio of 1:10 (leaf powder to solvent). The mixture is then heated for about 30–60 minutes under mild boiling conditions or left to macerate for several hours at room temperature to extract the bioactive compounds from



the leaf powder. After the extraction process, the solution is filtered using Whatman filter paper or a fine mesh to remove the solid leaf debris, leaving behind a clear plant extract that contains various phytochemicals, including polyphenols, flavonoids, and terpenoids, which can act as reducing agents in the nanoparticle synthesis process.(12,13)

3. Synthesis of Palladium Nanoparticles

Once the leaf extract is prepared, the synthesis of palladium nanoparticles is initiated by adding a palladium salt, typically palladium chloride (PdCl₂), to the extract. A common ratio is 1 mM palladium chloride solution to the plant extract, but this may vary depending on the desired nanoparticle concentration. The reaction mixture is stirred at room temperature or mildly heated (typically around 50–70°C) for several hours, during which the leaf extract's reducing agents reduce the palladium ions (Pd²⁺) to form palladium nanoparticles (PdNPs). During this process, the phytochemicals in the leaf extract of *Andrographis* not only serve as reducing agents but may also act as stabilizers, preventing the aggregation of the nanoparticles and ensuring that they remain well-dispersed in the solution. The color change of the reaction mixture from pale yellow to dark brown or black indicates the formation of palladium nanoparticles, a typical sign of successful nanoparticle synthesis.(6)

4. Antibacterial Activity of PdNPs

The antibacterial activity of the synthesized palladium nanoparticles (PdNPs) was evaluated using two distinct methods. First, the Agar Well Diffusion Method was employed, where bacterial cultures of *S. mutans* and *Candida albicans* were grown overnight in BHI broth at 37°C. The bacterial suspension, adjusted to 0.1 OD at 600 nm, was spread on the surface of nutrient agar plates. Wells (6 mm in diameter) were created on the agar, into which different concentrations of PdNPs (50, and 100 µg/mL) were added. The plates were incubated at 37°C for 24 hours, and the zones of inhibition around each well were measured to assess the antibacterial activity. Additionally, the Broth Microdilution Method was used to determine the minimum inhibitory concentration (MIC) of PdNPs. In this method, various concentrations of PdNPs (1–100 µg/mL) were prepared in BHI broth, and 100 µL of bacterial suspension was added to each well in a 96-well microtiter plate. After incubation at 37°C for 24 hours, bacterial growth was assessed by measuring the optical density at 600 nm, and the MIC was identified as the lowest concentration of PdNPs that completely inhibited bacterial growth.(14)(15)

5. Antibiofilm Activity of PdNPs



The antibiofilm activity of palladium nanoparticles (PdNPs) was assessed using the crystal violet staining method in 96-well microtiter plates. For biofilm formation, a standardized bacterial suspension (0.1 OD at 600 nm) was added to each well and incubated at 37°C for 24 hours to allow the biofilm to develop. After incubation, the wells were washed with sterile PBS to remove non-adherent bacteria, and the biofilm was stained with a 0.1% crystal violet solution for 15 minutes. The excess stain was washed off, and the bound dye was solubilized using 95% ethanol. The absorbance at 570 nm was measured to quantify the biofilm biomass. To evaluate the effect of PdNPs on biofilm formation, PdNPs at varying concentrations (50, 100, 150, 200 µg/mL) were added to the bacterial culture during inoculation, and the biofilm formation was measured using the crystal violet assay. Additionally, the ability of PdNPs to disrupt pre-formed biofilms was examined by treating the biofilms with PdNPs (50, 100, 150, 200 µg/mL) for 24 hours, followed by quantification of the biofilm biomass. The percentage reduction in biofilm biomass was calculated in comparison to the untreated control. (16)(17)

6. Data Analysis

The statistical examination was performed using Graphpad Prism software, and One-way ANOVA was used to determine the significance level. Every test was carried out three times, and Graph Pad Prism ver.8.3 (Graph Pad Software, La Jolla, CA) was used to draw the graph.

RESULTS

The palladium nanoparticles synthesized using andrographis showed inhibition of 15mm and 13mm at 100 µg/ml and 50 µg/ml at concentrations respectively against *S. mutans* and inhibition of 16mm and 5mm at 100 µg/ml and 50 µg/ml at concentrations respectively against *C. albicans*. Zone of inhibition is tabulated into bar graphs showing the route at each quantity of nanoparticles. The plate was incubated at 36°C for 48 h, followed by crystal violet staining and spectrophotometric absorbance measurements (OD₆₀₀ & 530). The absorbance was used to calculate the “biofilm formation” on the y axis. x axis represents Pd-NPs from different concentrations (50 to 200 µg/mL) where the synthesized PdNPs exhibited a prominent 570 nm absorption peak.

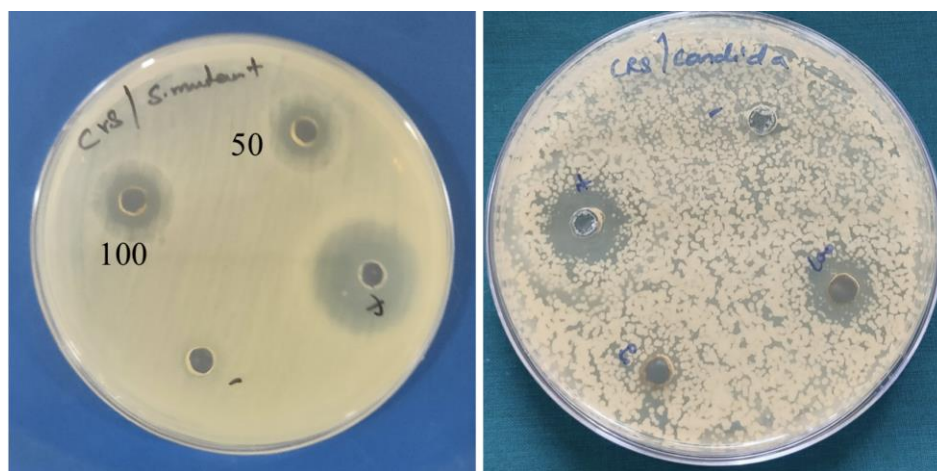


Fig. 1. Antibacterial activity of Pd NPs against dental caries pathogens

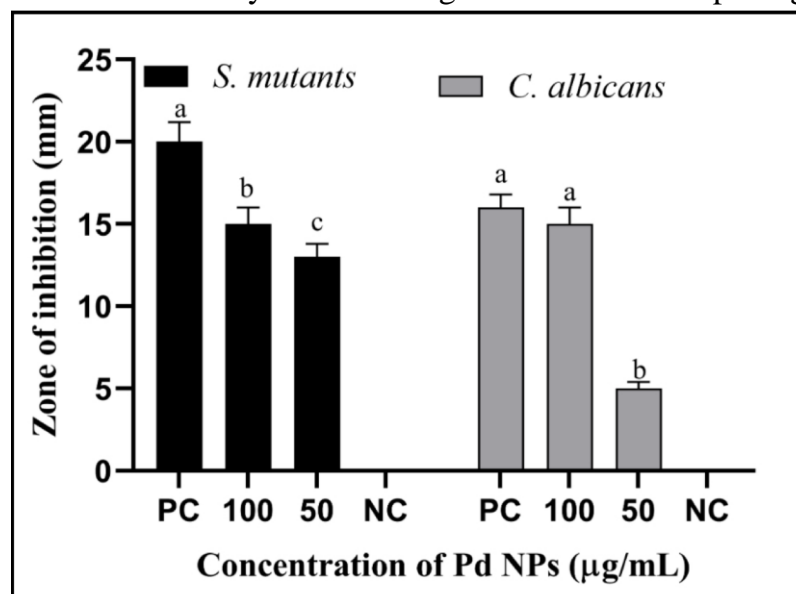


Fig. 2. Antibacterial potential of Pd NPs against *S. mutans* and *C. albicans*.

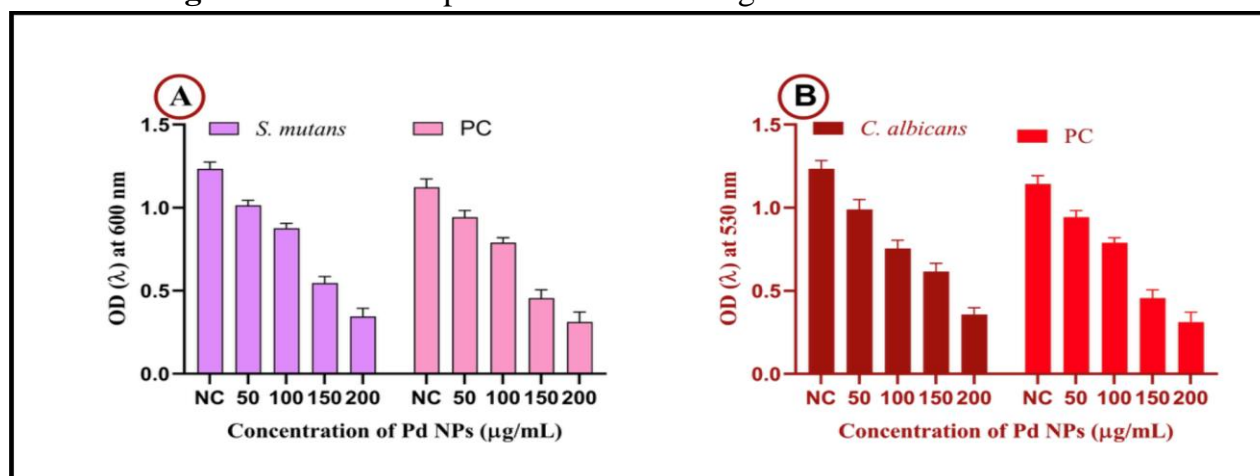




Fig. 3. Antibiofilm activity of Pd-NPs against A). *S. mutants* and B). *C. albicans*.

DISCUSSION

Our findings indicate that PdNPs effectively inhibited the growth of *S. mutans*. This antimicrobial effect could be due to the interaction of the nanoparticles with the bacterial cell wall and membrane. Previous studies suggest that metal nanoparticles, including PdNPs, can disrupt the bacterial membrane structure, increasing its permeability and causing leakage of intracellular material. Moreover, PdNPs may catalytically produce ROS, amplifying oxidative damage to vital cellular components. In addition to direct damage, PdNPs have been shown to impact *C. albicans* biofilms.

Palladium nanoparticles (PdNPs) have shown significant potential in disrupting biofilm formation by *Streptococcus mutans* and *Candida albicans*, both of which are known to create robust biofilms that contribute to persistent infections and resistance to standard therapies. In the case of *S. mutans*, PdNPs interfere with the early stages of biofilm development by hindering bacterial adhesion to surfaces and compromising the integrity of the extracellular matrix that supports the biofilm(18,19). This weakening of the biofilm structure increases its vulnerability to antimicrobial treatments. For *C. albicans*, PdNPs target the fungal biofilm by interacting with the cell membrane and wall, leading to structural damage and increased membrane permeability, causing leakage of intracellular materials.

This study examined the antibacterial and antibiofilm properties of palladium nanoparticles (PdNPs) synthesized using *Andrographis* leaf extract against key dental pathogens, *Streptococcus mutans* and *Candida albicans*. Previous research has shown that PdNPs have strong antimicrobial effects due to their small size, large surface area, and distinctive physical and chemical characteristics, which enable them to penetrate microbial cell membranes. For example, Seku et al. (2022) found that PdNPs were effective against *Escherichia coli* and *Staphylococcus aureus*, where they disrupted cell wall integrity and induced oxidative stress via the production of reactive oxygen species (ROS)(20) In a similar manner, our results demonstrate that PdNPs derived from *Andrographis paniculata* significantly inhibit the growth of *S. mutans*, a major cause of tooth decay(21). The plant-based approach to nanoparticle synthesis may have further enhanced these antimicrobial effects through the additional bioactive compounds in the leaf extract that may work in synergy with the PdNPs(21,22)

The ability of PdNPs to disrupt biofilm formation by *S. mutans* and *C. albicans* is another crucial finding, as biofilms are a key factor in the persistence and resistance of dental infections(23). Biofilms act as protective barriers, making pathogens more difficult



to eliminate with traditional treatments. Previous studies, such as the one by Paluch et al. (2022), reported that PdNPs derived from plant sources like *Cucurbita pepo* could inhibit biofilm development in both *S. mutans* and *C. albicans*(23,24). The disruption of biofilms by PdNPs in this study can likely be attributed to the nanoparticles' ability to interact with and destabilize the biofilm matrix, causing the release of cellular contents and weakening the structure(25). Our findings align with these observations, as PdNPs from *Andrographis paniculata* not only hindered the formation of new biofilms but also helped break down pre-existing biofilms, highlighting their potential as a treatment for biofilm-related oral infections.(25,26)

Compared to chemically synthesized PdNPs, those derived from *Andrographis paniculata* offer several advantages, including improved safety and biocompatibility. The plant's natural compounds might enhance the stability and activity of PdNPs, providing additional antimicrobial benefits(27)(28) Research by Utami et al. (2024) has shown that *Andrographis* Contains bioactive substances like flavonoids, alkaloids, and diterpenes, which have their own antimicrobial properties(29). These compounds may work synergistically with PdNPs, amplifying their effect against dental pathogens. Together, the findings suggest that PdNPs synthesized from *Andrographis* leaf extract could represent an effective, eco-friendly alternative to conventional antimicrobial treatments, especially for combating biofilm-related dental infections.(27,30,31)

CONCLUSION

In summary, PdNPs were synthesized using *Andrographis* leaf extract as a reducing agent. When tested against clinical pathogens, PdNPs demonstrated the most significant antibacterial activity reflecting inhibition of 15mm and 13mm at 100µg/ml and 50 µg/ml at concentrations respectively against *S. mutans* and inhibition of 16mm and 5mm at 100µg/ml and 50 µg/ml at concentrations respectively against *C.albicans*. This approach is cost-effective and environmentally friendly. Furthermore, the synthesized PdNPs exhibited a prominent 570 nm absorption peak. These PdNPs showed promising effectiveness against the bacterial strains under investigation. These results suggest that green-synthesized PdNPs could serve as efficient alternative antibacterial agents, inhibiting the growth of infections. In conclusion, we recommend PdNPs as versatile antimicrobial agents with broad-spectrum activity.

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