Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 13s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Phyto-Fabrication Of Palladium Nanoparticles Using Caralluma Fimbriata And Its Potential Antibiofilm Activity Against Dental Pathogens (In Vitro)

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Cite this paper as: M. Vignesh, C. Ragavendran, Dr. Delphine Priscilla Antony S, (2025) Phyto-Fabrication Of Palladium Nanoparticles Using Caralluma Fimbriata And Its Potential Antibiofilm Activity Against Dental Pathogens (In Vitro). *Journal of Neonatal Surgery*, 14 (13s), 157-163.

ABSTRACT

Aim: Phyto-fabrication of palladium nanoparticles using Caralluma fimbriata and its mic and antibiofilm activity against dental pathogens (in vitro). In recent decades, there has been a significant focus on synthesizing metal nanoparticles, such as gold, silver, zinc, platinum, and palladium, within material and biological sciences. Palladium (Pd) has gained particular attention due to its diverse applications as a catalyst in various organic transformations, encompassing carbon-carbon cross-coupling, oxidation, and reduction reactions.

Materials and methods: Preparing an aqueous broth from plants for bio-nanoparticle synthesis and biofabrication. Cf-capped pdnp, characterization techniques, antibacterial potential, synthesis of palladium nanoparticle, SEM, XDR analysis

Results: The results indicated that Pd NPs possessed antibacterial and antibiofilm activities, which were supported by MIC data, SEM images, and X-ray diffraction patterns. The results suggest that Pd NPs have the potential to serve as effective agents against the tested bacteria and warrant further investigation for their biomedical applications.

Conclusion: The comprehensive investigation into the phytofabrication of palladium nanoparticles (Pd NPs) using Caralluma fimbriata and its subsequent evaluation for minimum inhibitory concentrations (MIC) and antibiofilm activity against dental pathogens has provided valuable insights. The in vitro assays conducted in this research enhance the field of green synthesis of nanoparticles and their possible applications in dentistry.

Keywords: *Phyto-fabrication. Palladium nanoparticles (Pd NPs), Caralluma fimbriata. MIC (Minimum Inhibitory Concentrations), Antibiofilm activity*

1. INTRODUCTION

Over the past decades, the synthesis of metal nanoparticles such as gold, silver, zinc, platinum, and palladium has attracted considerable attention in material and biological sciences. (1). For a variety of applications, palladium (PD) as a catalyst for various organic conversions, including carbon cruises, oxidation, and reduction reactions, has attracted special attention (2). Traditional methods involve the synthesis of PD nanoparticles either physically or chemically, with toxic and dangerous reduction and stabilization. However, there has been a significant change to a bio-winning approach for synthesizing metal nanoparticles in pursuit of environmentally friendly practices. (3).

These biological technologies' biocompatibility and environmentally friendly nature provide a promising alternative to traditional physical and wet chemical methods. In the field of biological synthesis of palladium nanoparticles (PD-NPs), various sources of plant extracts, microorganisms, marine organisms, and more serve as green reducing agents (4). Among these approaches, utilizing bio-resources, especially plant extracts, for NP synthesis appears promising due to their easy accessibility, quick processing, cost-effectiveness, and suitability for large-scale biosynthesis (5).

As a result, plant-mediated synthesis of PD-NPs has recently attracted considerable attention, with plant-derived antibacterial information indicating a broad and almost unexplored source of genetic mechanisms for resistance. (6). Ongoing studies aim to harness this resource for medicinal purposes and further investigate plants for the development of new drugs, whether synthetic or natural. The ultimate objective of plant antimicrobials is to provide a suitable and efficient origin with significant therapeutic potential. These compounds hold promise for developing human antimicrobial drugs to address infections, especially those caused by microorganisms(7). There have been many bacteria, fungi, and viruses over the past 20 years, causing severe diseases, which is why we are testing natural substances in tropical and subtropical countries. The variable extracts derived from traditional medicinal plants have been tested and revealed the effectiveness of microorganisms against microorganisms, and the effectiveness of microorganisms revealed that microorganisms occurred against microorganisms (8).

This highlights the crucial role of plants in the further development of modern medicine with new principles. The growing interest in traditional ethnomedicine promises to discover innovative therapeutically active ingredients(9). Medical plants are increasingly integrated into pharmaceuticals, neutraceuticals, cosmetics, and dietary supplements. In this context, the plant provided approximately 7,000 important pharmaceutical connections with the Western pharmacopeia(10). Natural products await hospitalization as new antibiotics, but there is an urgent need to identify new substances that are active against highly resistant pathogens(11).

Biomolecules originating from plants emerge as potential alternatives for controlling antibiotic-resistant human pathogens. In this context, we aim to exploit palladium nanoparticles using caralluma fimbriata and its mic and antibiofilm activity against dental pathogens. This leaf extract is known for several medical uses traditionally validated by ethnic drug research. The extract contains phytochemical components such as polyphenols, flavonoids and hydroxy acids that function as active ingredients for organic reduction, As far as we know(12).

In addition to PD-NP synthesis, the versatility of these NPs is also investigated as multifunctional catalysts for the reduction reaction of Cr(III) with water in aryl halides, alcohol oxidation, and Cr(III) reduction reaction of Cr(III) with water. Furthermore, we evaluate the antibacterial activity of PD-NP against a novel multi-resistant clinical bacterial isolate, Cronobacter sakazakii AMD04(13).This reported novelty in PD-NP synthesis lies in the proper selection of organic resources. This not only serves synthesis, but also plays an important role in communicating several catalytic and antibacterial properties. It is important that the multifunctional catalytic aspects of synthesized PDNPs, including C-C prerequisites, oxidation and reduction reactions, represent additional assets.(14).In our study, we aim to prepare palladium nanoparticles using caralluma fimbriata (CF) through phytofabrication and assessing the MIC and antibiofilm activity against dental pathogens

2. MATERIALS AND METHODS

Preparing a plant aqueous broth for bio-nanosynthesis

Caralluma fimbriata (cf.) Fresh leaves weighing 100 g were crushed finely and gently added to 500 ml of actionized water while shaking frequently. The aqueous solution was stirred continuously for 60 min and 60 min and then cooled to ambient temperature (26 ± 2.00 °C). According to filters of aqueous extracts of leaves with Whatman No. 1 (Grade 1:11µm-Por size) filter paper, the aqueous solution for further bio-synthesis was 4-C.

Bio-fabrication of CF-capped PdNPs

Characterization techniques

PDNPs were successfully recorded in traditional stew production processes. To produce Dinatriumtetrachloropalladat(II)(Na2PDCL4), 5 mL of Bengaluru Sigma Aldrich (product code: 20 rings) was purchased in combination with 100 mL of sterile Milli-Q water. The solution was stirred aggressively at 350 rpm for 2 hours. Perfect inventory status was obtained throughout bioactive synthesis to avoid light-suppressed changes in magnetic stir fry CF biomass isolates when held at 60°C. The darkness was maintained by PDNPs during synthesis by covering the cup with a 0.03 mm thick aluminum foil sheet. The CF extract has been changed from pale yellow to dark brown, indicating the effective formation of biological PDNPs. The darker CF PDNPS solution was centrifuged at 12,000 rpm at 12,000 rpm to complete the reaction, and the pellet was separated from the supernatant. Therefore, the remaining CF-PDNPs were generated by storing the pellet, and the homogenate was swirled for 30 min. After at least three sterile Milli-Q washes, the pellet was frozen. Dried powders without CF-Kacken-PDNP were stored in clean brown bottles until the physical and chemical properties and evaluation of photocatalytic activity were evaluated.

Antibacterial potential

Biosynthetic CF-PD-NPs were tested against gram (-ve and +ve) bacteria, and their antibacterial properties were performed by Liang et al. (2022) A light fix. *S. mutans* (ATCC 25923) and C. albicans (ATCC 25922) were evaluated using two different concentrations by CF-PDNPS-biosynthesis using agar diffusion methods. Next, 20 mL of sterile Muller Hinton Agar (MHA) was added to the Petriplates (Hi-Media, Mumbai). In the cured medium, 0.1 mL of bacterial culture was exchanged and dried for 10 min [29]. A 5 mm fountain then struck the medium. -PDNP was administered overnight to bacterial strains at 50 and 100 ¼g/ml. Chloramphenicol (3î¼g/well) (positive control), negative control was 10% dimethyl sulfoxide (DMSO) (50î 1⁄4 g/well). The inhibition zone was evaluated by incubating plates at 36 ± 1.00 °C for 24 h in a microbiological incubator followed by three experiments.

Statistical analysis

Data are mean (n = 3) ± SE. Columns with different letters denote statistically significant differences (ANOVA followed by Tukey's HSD test, P < 0.05).

3. RESULTS

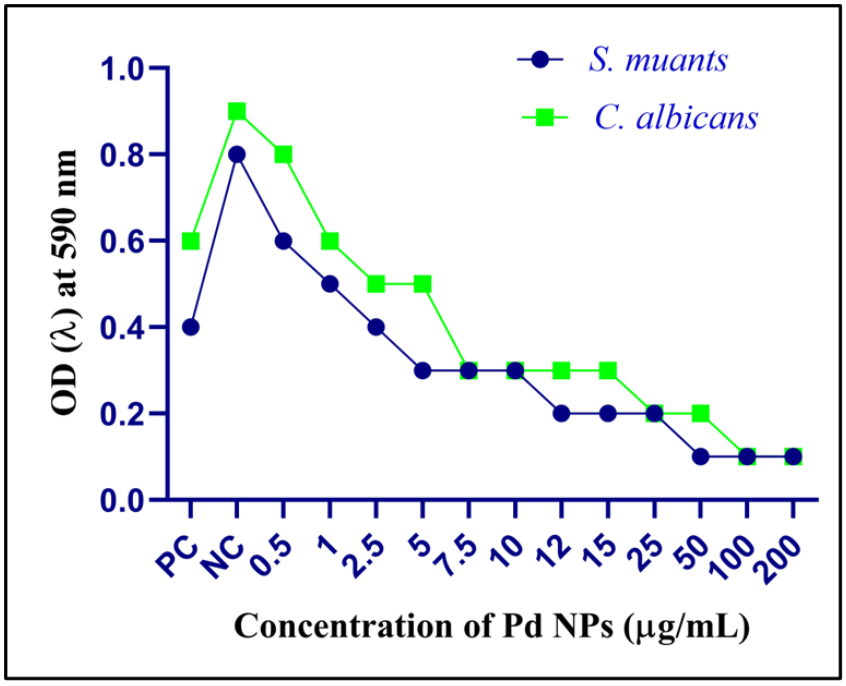

Fig. 1. Minimum inhibitory concentrations of Pd NPs and standard antibiotics against the three bacteria that were tested.

To perform this assay, NB was used to manufacture PDNPs at different concentrations. After 24 h of treatment, MIC values were determined by the initial significant inhibitory concentration of PDNP. + pu, positive control, bacterial population only (no treatment); -ve, negative control NB (no treatment). From figure 1, Minimum inhibitory concentrations (MICs) of palladium nanoparticles (PD-NPs) were assessed against streptococcus-mutans and candida albicans by measuring optical density (OD) after 24 h exposure. The results indicate a concentration-dependent inhibition of microbial growth for both organisms.For *S. mutans*, the OD values initially increased at 0.5 µg/mL but showed a gradual decline as the Pd NP concentration increased. Complete inhibition was observed at concentrations above 25 µg/mL, with OD values approaching 0.1, indicating significant antibacterial activity.

Similarly, *C. albicans* showed an increase in OD at lower concentrations (0.5–2.5 µg/mL), followed by a steady decline. The MIC for *C. albicans* appeared higher than for *S. mutans*, with noticeable inhibition occurring around 10–15 µg/mL and complete suppression observed at 100–200 µg/mL. The negative control (NC) exhibited no OD values, confirming the sterility of the medium, whereas the positive control (PC) displayed high OD readings, indicating normal microbial growth without Pd NP treatment. These findings suggest that in a concentration-dependent manner, PD-NPs have both bacterial and fungal pathogenicity for strong antibacterial properties against bacterial and antifungal properties

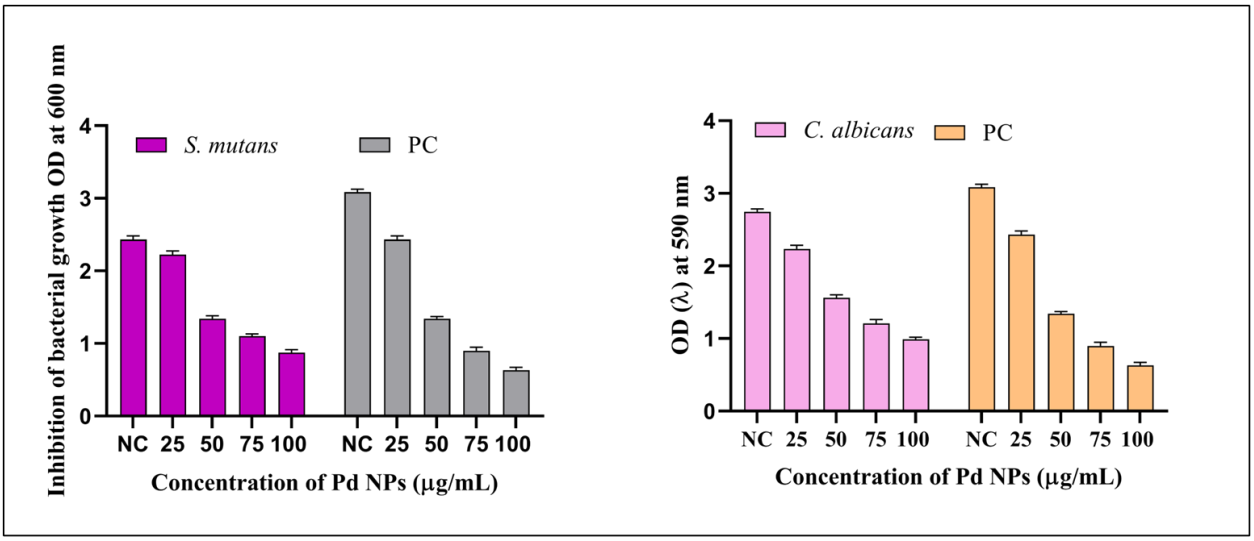

Figure. 2. Antibiofilm activity of Pd NPs against S. mutans and C. albicans.

From Figure 2, The antibiofilm potential of palladium nanoparticles (Pd NPs) against *Streptococcus mutans* and *Candida albicans* was evaluated at different concentrations (25, 50, 75, and 100 µg/mL). The assessment was conducted by measuring the optical density (OD) at 600 nm for *S. mutans* and 590 nm for *C. albicans*. The biofilm inhibition assay for *S. mutans* (Figure 2, left panel) demonstrated a dose-dependent reduction in bacterial biofilm formation. The negative control (NC) exhibited the highest OD (~3.0), indicating robust biofilm growth. However, treatment with Pd NPs resulted in a significant decrease in OD values, the highest level of inhibition was recorded at a concentration of 100 µg/mL.. The positive control (PC) also showed notable biofilm formation, similar to the NC. For *C. albicans* (Figure 2, right panel), a similar trend was observed. The NC displayed high OD values (~3.5), indicating strong biofilm formation, while increasing concentrations of Pd NPs progressively reduced OD values. The most significant antibiofilm activity was observed at the highest concentration (100 µg/mL).The PC, like in the *S. mutans* experiment, maintained high OD values, confirming biofilm growth in untreated conditions.

These results highlight the potent antibiofilm efficacy of Pd NPs against both *S. mutans* and *C. albicans* in a concentration-dependent manner.

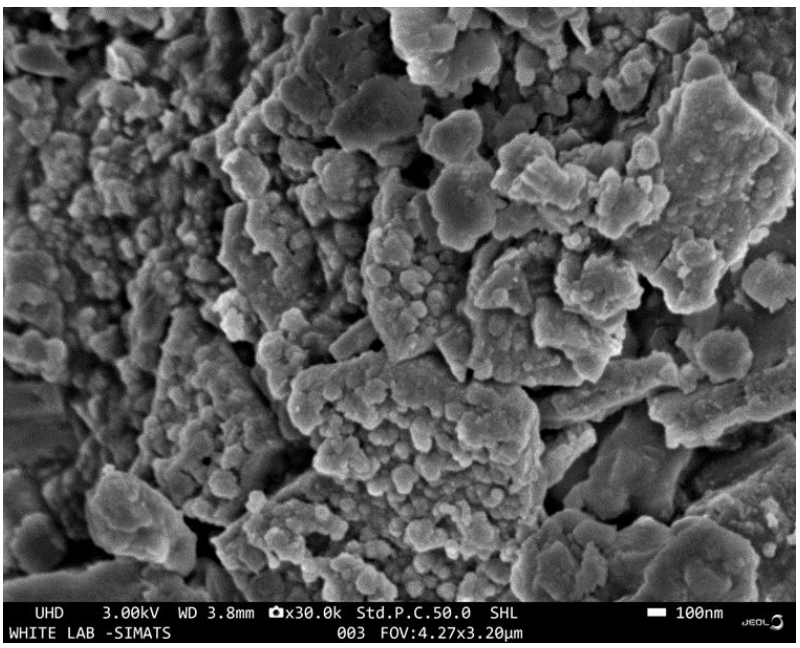

Fig. 3. Sem images of biosynthesized palladium NPs

From Figure 3, the morphology and structural characteristics of the biosynthesized palladium nanoparticles. Scanning electron microscopy (SEM) was used to examine the palladium nanoparticles (Pd NPs). The SEM micrograph (Figure X) demonstrates that the Pd NPs exhibit a highly aggregated structure, with irregularly shaped nanoclusters forming rough, dense surfaces. The observed morphology suggests the presence of polydisperse nanoparticles with a tendency to form agglomerates, which could be attributed to the inherent properties of biologically synthesized nanoparticles and possible interactions between biomolecules and metal ions during the synthesis process.At a magnification of 30,000×, the Pd NPs appear to have a granular texture with varying particle sizes. The scale bar (100 nm) indicates that the nanoparticles are within the expected nanometer range, confirming the successful biosynthesis of Pd NPs. The presence of smaller nanostructures decorating larger particles suggests a hierarchical assembly, which could enhance their surface area and reactivity. These findings support the successful green synthesis of Pd NPs with SEM analysis confirming their nanoscale dimensions and unique surface morphology, which may contribute to their potential applications in antimicrobial, catalytic, and biomedical fields.

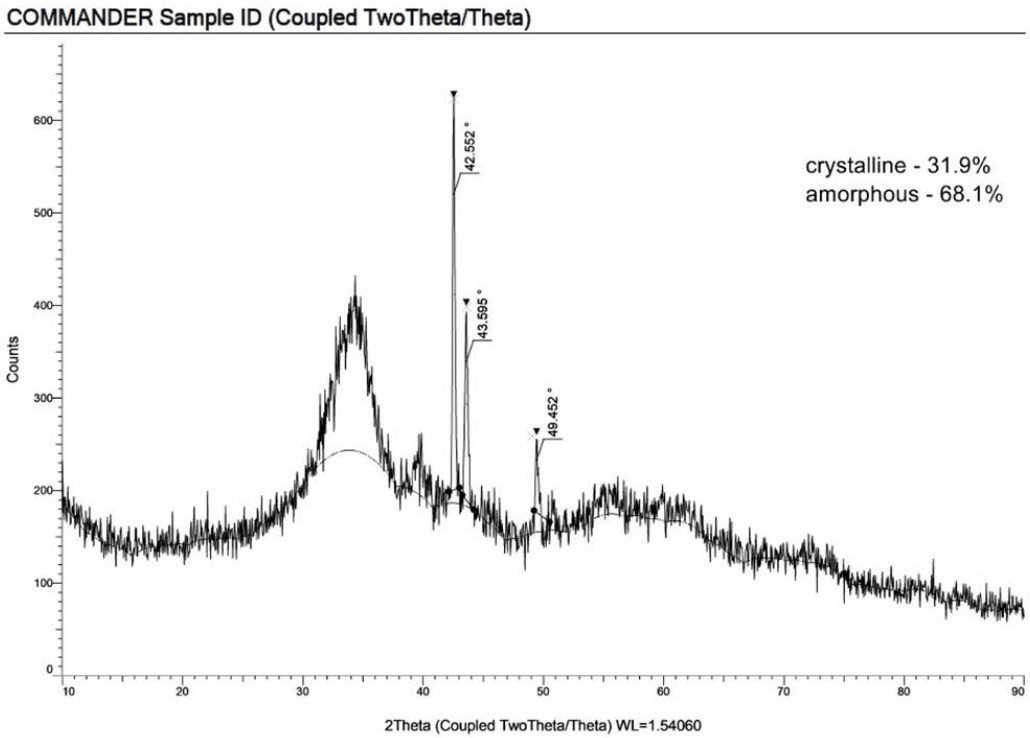

Figure 4. XDR results, The crystalline structure and phase composition of X-ray diffraction (XRD) analysis was performed to characterize the biosynthesized palladium nanoparticles (Pd NPs). The XRD spectrum (Figure X) reveals both crystalline and amorphous characteristics, with an estimated composition of 31.9% crystalline and 68.1% amorphous phases.The diffraction pattern exhibits distinct peaks at 12.552°, 43.855°, and 48.452° in the 2θ range, suggesting the presence of crystalline domains within the sample. The broad nature of the peaks, particularly around 40–50°, indicates nanoscale crystallites with possible strain or lattice distortions. The predominant amorphous region suggests the presence of non-crystalline components, which may result from biomolecule-mediated synthesis, surface functionalization, or partial oxidation. The presence of both crystalline and amorphous phases highlights the unique structural features of the biosynthesized Pd NPs, which could influence their catalytic, electronic, and antimicrobial properties. The high amorphous content may contribute to enhanced surface reactivity, while the crystalline regions provide structural stability.

4. DISCUSSION

Minimum Inhibitory Concentrations (MIC) of Palladium Nanoparticles (Pd NPs) and standard antibiotics against three tested bacteria. MIC is a crucial parameter indicating the minimum concentration of an antimicrobial agent required to prevent visible bacterial growth.The assays were conducted by preparing different concentrations of Pd NPs using Nutrient Broth (NB). After 24 hours of MIC values were identified according to the lowest concentration at which treatment inhibited growth.at which a significant inhibition of bacterial growth occurred.

The inclusion of positive and negative controls enhances the validity of the results. The positive control, denoted as +Ve, represents the bacterial population without any treatment, allowing for a baseline assessment of bacterial growth. The negative control, denoted as -Ve, consists of only NB without bacteria and treatment, providing a control for any intrinsic effects of the nutrient Broth. The data from this figure provides valuable insights into the antimicrobial potential of Pd NPs compared to standard antibiotics. The identification of MIC values it is essential for evaluating the efficacy of Pd NPs in preventing bacterial growth. Any observed variations in MIC values between different bacterial strains may suggest varying susceptibility to the treatment, highlighting the specificity of Pd NPs against the tested pathogens. The antibiofilm activity depicted in Figure 2 showcases the effectiveness of Pd NPs against the development of biofilms by *S. mutans* and *C. albicans*. Biofilm formation is a critical aspect of microbial pathogenicity, and disrupting these structures is essential for combating infections.

The results presented in this figure offer insights into the ability of Pd NPs to inhibit biofilm formation, a key factor in preventing the establishment and persistence of dental pathogens. Figure 3 provides SEM images of the biosynthesized palladium nanoparticles were captured using scanning electron microscopy.SEM is a powerful technique that allows for the visualization of nanoscale structures, providing information about the size, shape, and surface morphology of the synthesized NPs.

The images offer a direct observation of the characteristics of the Pd NPs, further validating the success of the phytofabrication process using Caralluma fimbriata. The diffraction pattern obtained from X-ray analysis presented in Figure 4 offers insights into the crystallographic structure of the Caralluma fimbriata-synthesized palladium nanoparticles (CF PdNP). XRD analysis is crucial for identifying the crystalline nature of nanoparticles. The pattern, by comparing various peaks related to the topic, aids in confirming the successful synthesis and crystallinity of the Pd NPs. The findings presented in this study align with current literature on phytofabrication of metal nanoparticles (15). Similar investigations involving plant extracts for nanoparticle synthesis have demonstrated antimicrobial efficacy against various pathogens (16). The MIC and antibiofilm activities of Pd NPs reported in this study adds to the expanding evidence highlighting the potential of plant-mediated synthesis for biomedical applications(17).

5. CONCLUSION

Biosynthesized Pd NPs exhibited strong antimicrobial and antibiofilm efficacy against *S. mutans* and *C. albicans* On a concentration-dependent manner. MIC assays confirmed microbial inhibition, with *S. mutans* suppressed above 25 µg/mL and *C. albicans* requiring 100–200 µg/mL. The highest concentration (100 µg/mL) significantly reduced biofilm formation. SEM and XRD analyses revealed their polydisperse, aggregated structure, and mixed-phase composition (31.9% crystalline, 68.1% amorphous), contributing to their reactivity and stability. These findings suggest Pd NPs as promising candidates for antimicrobial applications, warranting further investigation into their mechanism, cytotoxicity, and clinical potential**.** Further research on this study endeavors can propel the translational potential of C. fimbriata-synthesized Pd NPs in the realm of dental health, offering sustainable and Efficient approaches for addressing dental pathogens.

FUTURE SCOPE

Further research on this study endeavors can propel the translational potential of Caralluma fimbriata-synthesized Pd NPs in the realm of dental health, offering sustainable and effective solutions for combating dental pathogens.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

ACKNOWLEDGMENT

The authors would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing research laboratory facilities and the required equipment to carry out the study successfully.

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