RESEARCH PAPER

Harnessing Solanum nigrum for Palladium Nanoparticles Biosynthesis and Exploring Its Anti-Biofilm Potential

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ABSTRACT

The study explores the biosynthesis of palladium nanoparticles [Pd NPs] using the medicinal plant Solanum nigrum through a green synthesis approach and evaluates their anti-biofilm activity against dental caries–causing pathogens. Dental caries is a chronic infectious disease primarily caused by bacteria such as Streptococcus mutans that metabolize carbohydrates and produce acids leading to tooth demineralization. Green synthesis of nanoparticles is an environmentally friendly, economical, and safe method that utilizes biological sources such as plants, microorganisms, and algae to produce nanomaterials. *Solanum nigrum* bark contains bioactive phytochemicals including alkaloids, flavonoids, and tannins which act as reducing and stabilizing agents in nanoparticle synthesis. In this study, bark extracts were prepared and used to synthesize Pd NPs, which were characterized using analytical techniques including FTIR, XRD, and SEM to determine their structural and morphological properties. The antibiofilm activity of the synthesized nanoparticles was evaluated against Streptococcus mutans and Enterococcus faecalis using a 96-well microtiter plate assay with different nanoparticle concentrations ranging from 25–100 µg/mL. Results showed a color change indicating nanoparticle formation, and characterization confirmed the presence of crystalline Pd NPs. The antibiofilm assay demonstrated significant inhibition of bacterial biofilm formation, particularly at higher concentrations, with notable reduction in optical density values indicating decreased bacterial growth. The findings suggest that phyto-synthesized palladium nanoparticles derived from Solanum nigrum possess promising antimicrobial and antibiofilm properties and may serve as a potential eco-friendly therapeutic agent for controlling dental pathogens and biofilm-associated infections.

Keywords: Anti biofilm activity, *Solanum nigrum,* palladium nanoparticles, Dental caries pathogens.

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INTRODUCTION

Cariogenic bacteria that attach to teeth, mainly Streptococcus mutans, are the cause of dental caries, a common chronic infectious illness that gradually demineralizes tooth structure by metabolizing carbohydrates to create acid [1]. Green synthesis is the use of an environmentally friendly, economical, safe, and clean method to create nanomaterials [2]. The green synthesis of nanomaterials uses microorganisms as substrates, including yeast, fungi, bacteria, algae, and some types of plants. The emergence of bacteria that are resistant to many drugs in healthcare facilities has limited the treatment options available to doctors, leading to the need for more costly remedies [3].

Solanum nigrum bark boasts a diverse array of phytochemicals, including alkaloids, flavonoids, and tannins [4]. These compounds become pivotal in the synthesis of palladium nanoparticles, influencing their properties and potential applications[5]. The use of *Solanum nigrum* bark aligns with green synthesis principles, emphasizing sustainability in nanoparticle fabrication. This eco-friendly approach capitalizes on natural resources, minimizing environmental impact and promoting a greener alternative to conventional synthesis methods [6]. The biosynthesis of nanoparticles from microbes and plant extracts has attracted researchers' attention because their small size, large surface area, orientation, and physical properties make them suitable to be used in medical sciences[7]. Furthermore, they have a low cost and are not harmful to nature.

Alkaloids, flavonoids, and tannins are only a few of the many phytochemicals found in the bark of Solanum nigrum [4]. These substances play a crucial role in the production of palladium nanoparticles, affecting both their characteristics and possible uses [5]. The utilization of Solanum nigrum bark emphasizes sustainability in the production of nanoparticles and is consistent with green synthesis principles. Using natural resources to the fullest extent possible minimizes environmental effect and promotes a more environmentally friendly option to traditional synthesis methods [6]. Because of their tiny size, wide surface area, orientation, and other desirable qualities that make them acceptable for use in the medical sciences, researchers have focused their attention on the production of nanoparticles from microorganisms and plant extracts [7]. They are also inexpensive and do not hurt the environment.

In order to create nanoparticles from silver-doped TiO2, iron nanoparticles [FeNPs], and gold nanoparticles and investigate their potential as antibacterial and anticancer agents, several research have used aqueous extracts of Solanum nigrum as a bioreductant [11]. We expect that the use of phyto-synthesized nanomaterials will expand the research topics related to the fight against dangerous bacteria and biofilms as well as the range of novel applications for metal oxide nanoparticles [12]. This survey may pave the way for additional research in this field. Palladium [Pd] based nanoparticles [NPs] were produced in the current work using a green chemical technique, and the bark extract of Solanum nigrum showed strong antibacterial action against Enterococcus faecalis and Streptococcus mutans.

MATERIALS AND METHODS

Collection of *Solanum nigrum*

The bark of Solanum *nigrum* were collected and thoroughly washed under running water, dried at room temperature, and then cut into small pieces to remove adhering dust particles.

Leaf extract preparation

An aqueous extract of Solanum nigrum was made by mixing 10 g of chopped leaves with 100 mL of double-distilled water in a 500 mL Erlenmeyer flask. The mixture was filtered through Whatman no. 1 filter paper after being left to stand at 60 °C for 20 minutes. Palladium nanoparticles were created using the filtered bark extract. Extra leaf solution was kept at -20 °C until needed again.

Biosynthesis of silver nanoparticles

Ten milliliters of the leaf extract was mixed with 90 mL of a 1.0mM in distilled water solution. Within 24 h, a color change from yellow to brown was observed. This indicated the formation of colloidal Pd NPs. After 15 min of centrifugation at 10,000 rpm, Pd NPss were obtained. Before the pellet was characterized, it was spun in a centrifuge three times, freeze- dried, and ground into a powder. Only then was it put back into deionized water.

Green Synthesis of Pd NPs

The supplier of the palladium nitrate hexahydrate [Pd [NO3]2. 6H2O] was Sigma-Aldrich Chemicals in India. Fresh leaves were washed three times in the presence of distilled water to remove dust, chopped, and added to water [1:10] at 60ºC while being continuously stirred for 30 min. After filtering, the mixture was cooled and kept at 40ºC for additional use [13]. 24h spent shaking the leaf extract with 0.2M palladium nitrate [1: 9]. The colour change of the liquid from brown into a semi-solid creamy colour indicated the formation of Pd NPs. The phytochemicals found in biomaterials [such plant extract] can function as reducing agents, transforming the metal precursors into metal nanoparticles [NPs]. Materials having phytochemicals may act as both reducing and stabilizing agents because they include antioxidants and toxic-free substances.

Anti biofilm susceptibility screening by 96-microtiter well plate method

A 96-microtitre well plate was used to conduct a quantitative investigation on biofilm development. Freshly grown bacteria were added to Brain Heart Infusion [BHI] broth and the mixture was then incubated for 72 h at 37 ºC. The cell suspensions were diluted at a ratio of 1:100 in the freshly made BHI broth medium after 24 h. Bacterial cells that were not exposed to Pd NPs were regarded as the positive control. Pd NPs were also added to the treated bacterial cultures at a concentration ranging from 25, 50, 75 and 100 µg/mL. The sterile BHI broth medium remained empty. Then, 200 µL culture suspensions with and without Pd NPs treatment were added to the sterilised 96-well microplates, which were then incubated for a further 24 h at 37 ºC without shaking. Three replicates of each bacterial suspension were stored. By rotating the plates over, all of the treated and untreated cells in the microtiter wells were discarded. Free-floating cells and undesirable material were

then removed by washing the plates three times in phosphate buffered saline [PBS, pH 7.2].

Minimal inhibitory concentrations

MICs of AgNPs against different bacterial strains were determined using an amended broth macro-dilution method. For the estimation of MIC, the stock solutions of AgNPs [0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15, 25, 50, 100, and 200 µg/mL] were prepared. The sterile Mueller–Hinton broth was transferred into a sugar test tube [13×100 mm] containing 2.0 mL of bacterial inoculum [culture density of 5×10^5 CFU/mL] in two sets for each bacterial strain [14]. Subsequently, each test tube was mixed with 2.0-mL individual concentrations of AgNPs, limiting the final tube volume to 4.0 mL, resulting in a 1:2 dilution followed by incubation for 24 h at 37 °C. The optical density [O.D.] of microbial growth was measured at 600 nm. The lowest dose of AgNPs exhibiting no growth after incubation was considered the MIC endpoint. The turbidity of bacterial growth and MBC values were determined by swabbing the bacterial culture on MHA plates followed by incubation at 37 °C for 24 h. The MBC is considered the concentration of extract at which bacteria are completely killed.

Antibacterial activity

The *E. alba*-mediated silver nanoparticles were tested for antibacterial activity by employing the agar well diffusion method. The clinical pathogenic strains of *S. mutans* and *C. albican*s were acquired from the Department of Cariology, Saveetha Dental College Tamil Nadu, India. The pathogenic cultures were properly sub-cultured and maintained in our laboratory. In the antibacterial assays, AgNPs [50 and 100 µg/mL] were poured into the wells of Mueller–Hinton agar [MHA] plates, respectively, after which they were incubated for 24 h at 37 °C and 25 °C, respectively. The antibiotic chloramphenicol was used as a positive control. The growth inhibition zones were measured by the zone inhibition scale [Hi- Media, India].

Statistical analysis

The Statistical Package for the Social Sciences [SPSS] was used to do statistical analyses after the information that was assessed was graph in a Microsoft Excel spreadsheet [SPSS Statistics; version 23, IBM, SPSS Inc, Chicago, United States of America]. Analysis of the group samples' means and standard deviations was done using descriptive statistical methods. Three sets of samples' means were compared using a one-way analysis of variance [ANOVA] test for numerical data. To identify which of the three groups is in charge of the significant difference, the post hoc Tukey's test was used.

RESULTS

When zinc acetate dihydrate is introduced to Solanum nigrum extracts, physiochemical changes occur in the aqueous solution. One of the most obvious indications that the green synthesis of Pd NPs was successful is the mixture's hue shift. This change is apparent within 50 minutes of the synthesis process. This was thought to be the initial step of the synthesis of NP. In the current investigation, the color changes from yellow to light brown. It is believed that the chemical elements flavonoids and phenolics present in peel extract are responsible for converting zinc ions into palladium nanoparticles. After three hours, the solution's color stopped changing, indicating that the Pd salt had completely bio-reduced into NPs. .

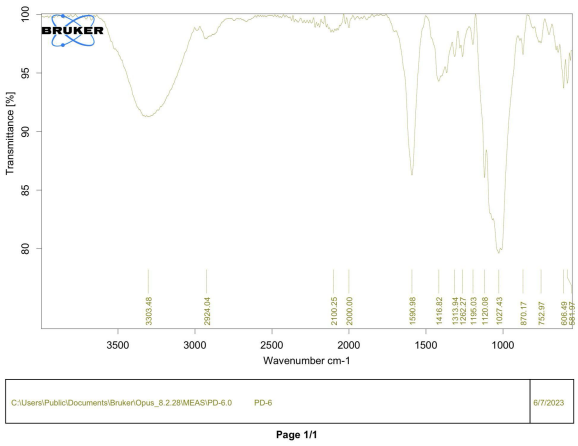

Figure 1 : FDIR analysis of Pd NPs

The FTIR analysis was carried out to identify the functional groups in biomolecules responsible for the bio-reduction of silver ions and stabilization of Pd NPs. Chemical groups absorb IR radiation and convert it into rotational and/or vibrational energy signals. The resultant signals present as a spectrum and represent a molecular fingerprint of sample. Each molecule or chemical structure has a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification. FTIR spectra results showed that CFE of Solanum nigrum MAE 11 exhibited strong and medium absorption bands at different wavenumbers [3303.48 2924.04, 2100.25, 2000.00, 1590.98, 1416.82, 870.17, cm−1]. 3303.48 of O–H stretch of carboxylic acids functional group. 2924.04 corresponds C–H stretch of alkanes group.

2100.25 corresponds -C=C- stretch of alkynes, 2000.00 corresponds C=O stretch of carbonyls [general], 1590.98 corresponds C-C stretch [in-ring] of aromatics, 1416.82 C-H bend of alkanes, 870.17 corresponds C-CI stretch of alkyl halides.

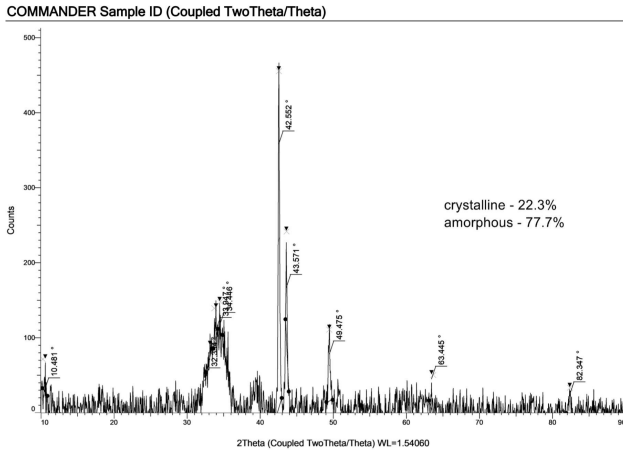

Fig. 2: XRD analysis of synthesized Pd NPs

The Pd NP were subjected to an XRD analysis in order to determine their size, phase identification, and crystalline nature. The results of the XRD pattern demonstrated an unambiguous confirmation of the cubic crystalline lattice of Pd NP in the 2θ values of 20–80°. The XRD spectrum exhibited three distinct diffractive peaks corresponding to the lattice planes of the Bragg's reflection of Pd NP 10.481°,43.571°,49.475°,63.445°,82.347°,67.94° [fig. 2].

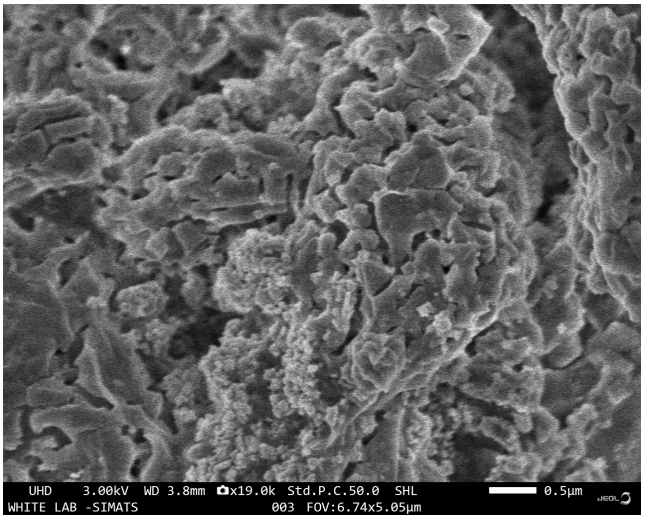

Fig. 3: SEM pictures of synthesized Pd NPs.

SEM micrographs showed that as the amount of MEA increases, the grain sizes steadily decrease. The findings of the UV-visible diffuse reflectance spectroscopy show that the Pd NPs have a large band gap energy and wider absorption bands [fig. 3].

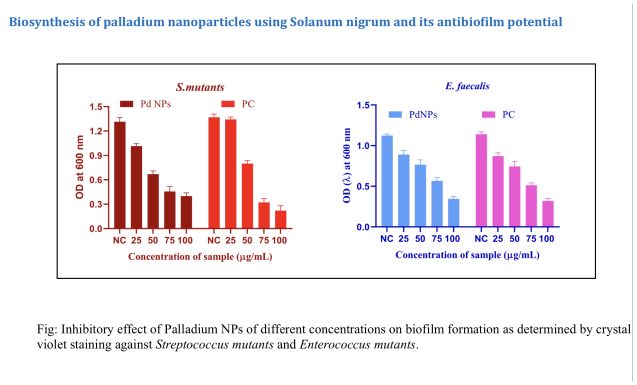

Fig: Inhibitory effect of Palladium NPs of different concentrations on biofilm formation as determined by crystal violet staining against *Streptococcus mutants* and *Enterococcus mutants*.

Fig 4: Microtiter plates demonstrating the antibiofilm activity of Pd NPs. Microtiter plate optical density [OD] 600nm reading [mean± standard error] of biofilm formation with Pd NPs added at concentration of 25 to 100µg/mL. A] *Streptococcus mutans* and B]. *Enterococcus faecalis* [fig. 4].

DISCUSSION

Palladium nanoparticles were produced by applying the Solanum nigrum bark aqueous extract [AN-Pd NPs] method of phytonanotechnology. The true threat to human survival is not antibiotics made by chemicals, but rather pathogenic microorganisms that are becoming resistant to them [15]. However, a significant amount of systemic toxicity is brought on by pharmaceuticals that are produced chemically [16, 17]. By measuring biofilm growth with crystal violet in the presence of different Pd NP concentrations, the antibiofilm activity of Pd NPs was assessed [18]. At 100 µg/mL, Pd NPs effectively inhibited the formation of biofilms [p = 0.005]. Pd NPs demonstrated notable antibiofilm activity against S. mutans in a dose-dependent manner [Fig.]. At an OD of 1.123, the positive control group devoid of Pd NPs demonstrated growth. Significant colony growth inhibition would reduce OD. The addition of 50 µg/mL Pd NPs [OD of 0.349] slightly inhibited bacterial growth, which was further reduced when Pd NP concentrations were increased [OD of 0.3 at 100 µg/mL]. By measuring biofilm growth with crystal violet in the presence of different Pd NP concentrations, the antibiofilm activity of Pd NPs was assessed [19]. At 100 µg/mL, Pd NPs effectively inhibited the formation of biofilms [p =

0.005]. Pd NPs demonstrated notable antibiofilm activity against S. mutans in a dose-dependent manner [19, 21] At an OD of 1.123, the positive control group devoid of Pd NPs demonstrated growth. Significant colony growth inhibition would reduce OD. Bacterial growth was mildly inhibited by the addition of 50 μg/mL Pd NPs [OD of 0.349] and was decreased further with the addition of increasing Pd NPs concentrations [OD of 0.3 at 100 μg/mL][22][19,22]

MRSA and ESBL are two examples of bacteria that form biofilms with a protective layer to withstand antibiotic treatment, as we compared to another study. Higher C. sinensis extract concentrations [14%–68%] in PS11 are linked to better biofilm removal by P. aeruginosa, which produces ESBL. According to research by Abraham et al. [23], methanolic caper extraction dramatically decreased the formation of biofilms and the synthesis of extracellular polymeric substances [EPS] in Proteus mirabilis, P. aeruginosa, Serratia marcescens, and Escherichia coli. [24]. Similarly, at higher concentrations [12%–59%] in SA2, MRSA biofilm reduction performs better. The function of antibiofilm[25] is in line with previous studies on various terrestrial plant species from across the world, as opposed to another study. For example, an Indian study discovered that Vetiveria zizanioides root extract inhibited the production of MRSA biofilms [26]. Similarly, a different Brazilian study found that Piper regnellit's dichloromethane extract prevents the growth of biofilms [27].Despite the fact that bacteria are resistant to antibiotics, biofilm infections are significant from a clinical perspective [28]. To eradicate biofilm producers, high concentrations of antibacterial agents may be required. Although low-concentration combination therapy can be helpful in the treatment of staphylococcal biofilm-related infections [29] such as those caused by MRSA, this may not always be possible in vivo due to toxicity and accompanying side effects. The process of choosing the right antimicrobial agent requires early screening, identification of biofilm producers, and subsequent testing for antimicrobial sensitivity [30].

The comparative analysis (31) revealed that the bark extract of Solanum nigrum was reported to have higher antibiofilm action as compared to other articles. Because of this, using Solanum nigrum as a model to discover more effective drugs or to monitor the development of microbial biofilms is suggested. But bacteria show that they are resistant to antibiotics (32). Biofilm infections are significant clinically. It may be necessary to use high antibiotic concentrations to eradicate biofilm producers (33). MRSA infections and other staphylococcal biofilm-related infections may be successfully treated with low-concentration combination therapy (34]. However, because of toxicity and related side effects, this might not always be feasible in vivo. Early identification and assessment of biofilm producers is essential for selecting an appropriate antimicrobial agent.

CONCLUSION

In conclusion, a reducing agent known as aqueous extract from Solanum nigrum bark was employed in the production of Pd NP. Studies had shown that Pd NP exhibited the strongest antibacterial activity against clinical pathogens. This is affordable and makes use of eco-friendly methods. Based on findings from a number of analytical characterization methods, such as green synthesis of Pd NP, SEM, EDAX, and anti-biofilm activity, Pd NP has been demonstrated to exist. Our findings suggest that the bark of Solanum nigrum could someday be developed into an advanced antimicrobial drug.

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