

## Research Article

# The dual role of bio-inspired palladium nanoparticles in antibacterial action and wound healing: An *in vitro* and *in vivo* study

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## ABSTRACT

Nanoparticles have become essential in theragnostic applications due to their multi-functionality. However, conventionally synthesized nanoparticles are often limited by high production costs and moderate efficacy. To address these challenges, this study focuses on bio-inspired palladium nanoparticles (PdNPs), an entirely novel nanomaterial synthesized with the *Plectranthus amboinicus* leaf extract offering an economical, green, biocompatible, and stable substitute. To characterize biosynthesized PdNPs, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), UV-Vis spectroscopy, energy-dispersive X-ray spectroscopy (EDS), field emission scanning electron microscopy (FESEM), high-resolution transmission electron microscopy (TEM), and zeta potential analysis were employed. The nanoparticles, measuring 5–40 nm, displayed diverse shapes (spherical, triangular, and rectangular), with XRD revealing a face-centered cubic (fcc) crystalline structure. The zeta potential value of -12.9 mV indicated high stability due to the surface charge of the PdNPs. Therapeutically, PdNPs exhibited broad-spectrum antibacterial activity, particularly against *E. coli* ( $14 \pm 0.3$  mm inhibition zone), along with potent antioxidants (71.41  $\pm$  0.94%), anti-diabetic (77%), and anti-inflammatory (72%) properties. Remarkably, PdNPs-based ointments in a mouse excision wound model demonstrated a 74.76% wound closure within 10 days in a mouse model, with complete healing achieved by day 14. This study therefore underscores the broad applicability of PdNPs emphasizing its novelty and potential as a competitive alternative to conventional therapies making it ideal for numerous biomedical applications such as wound healing, tissue repair, dentistry, regenerative medicine, and biosensing platforms.

## 1. Introduction

Metal nanoparticles (MNPs), which involves a broad range of examples with at least one dimension between 1 and 100 nm, have become an appealing class of materials in recent years. MNPs have unique properties beneficial in various applications like industrial catalysis (Solomon *et al.*, 2024), food packaging (Joshi *et al.*, 2024), biosensing (Kumalasari *et al.*, 2024), batteries (J. Zheng *et al.*, 2024), superconductor systems (Atchaya and Meena Devi, 2024), medicine (Issaka *et al.*, 2024; Naser *et al.*, 2024; Panda *et al.*, 2021; Puri *et al.*, 2024; Todaria *et al.*, 2024; Q. Zheng *et al.*, 2024), and bacterial disinfection (Paul *et al.*, 2018). Palladium nanoparticles (PdNPs) have diverse applications due to their unique characteristics and catalytic activity (Lin *et al.*, 2023; Losada-Garcia *et al.*, 2022; Seku *et al.*, 2024; Vinnacombe-Willson *et al.*, 2023). PdNPs play a crucial role in the automobile industry specifically in catalytic converters by neutralizing

harmful gases, including carbon monoxide, unburned hydrocarbons, and nitrogen oxides and turning them into less detrimental compounds (Aarzo *et al.*, 2022). They also serve as catalysts in various organic syntheses and electro-catalysts in fuel cells to promote the oxidation and reduction reactions involved in electricity generation (Dhumal *et al.*, 2024; Shukla *et al.*, 2023). In water treatment, PdNPs remove impurities and minimize pollutants, such as chlorinated hydrocarbons and heavy metals (Arsiya *et al.*, 2017; Emam, 2022; Shokouhimehr *et al.*, 2019; Vijwani *et al.*, 2012). They use air purification devices to catch and neutralize pollutants, hazardous gases, and conductive inks for flexible and wearable electronics (Cai *et al.*, 2018; Chen *et al.*, 2011; Palliyarayil *et al.*, 2020). Palladium nanoparticles are functionalized and implemented in cancer therapy (Alinaghi *et al.*, 2024; Li *et al.*, 2024), drug delivery systems (Shanthi *et al.*, 2015), biosensors (Orzari *et al.*, 2024; Phuong *et al.*, 2024), imaging agents (Liu *et al.*, 2020; Nie *et al.*, 2014), and antimicrobial agents (Hamid *et al.*, 2024; Nie *et al.*, 2014).

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Several approaches have been designed modified to synthesize MNPs including the widely adopted top-down and bottom-up techniques (Lin et al., 2023; Vinnacombe-Willson et al., 2023). Broadly divided into two primary categories, chemical and physical, each of these approaches has their pros and cons, and selecting one is influenced by some aspects, such as preferred nanoparticle properties, scalability, and intended applications (El-Khawaga et al., 2023; Gupta et al., 2023; Verma et al., 2016). While these processes provide for the accurate regulation of nanoparticle size and morphology, they are energy-intensive and often generate hazardous by-products (Kumari et al., 2023; Saleh and Fadillah, 2023). To address these limitations, green synthesis methods utilize biological agents such as biomass or organisms as eco-friendly reducing and stabilizing agents (C and T, 2024; Shahid-ul-Islam et al., 2023). These strategies employ various microorganisms (viruses, bacteria, yeast, algae and fungi) and organisms (plants) to synthesize MNPs under mild reaction conditions, minimizing energy consumption and ecological impact (Arteaga-Castrejón et al., 2024; Bokolia et al., 2024; Karunakaran et al., 2023; Verma et al., 2022).

Despite these advanced relatively greener approaches, there is a critical need to explore greener and more sustainable synthesis methods for MNPs, which do not rely on energy-intensive chemical and physical techniques that produce hazardous by-products (Bokolia et al., 2024) (Meena et al., 2024). Bio-inspired methods that utilize plant extracts have become a green alternative to conventional techniques, leveraging phytochemicals, such as alkaloids, flavonoids, polyphenols, and terpenoids serving as naturally occurring stabilizing and reducing agents. These aid in the reduction of metal ions to generate nanoparticles without the need of synthetic reducing agents and act as capping agents, providing stability, preventing aggregation, and improving colloidal stability of the nanoparticles (O. Adeyemi et al., 2022). It is considered environmentally benign because it does not require the use of noxious chemicals and solvents that are frequently used in traditional synthesis processes (Vijayaram et al., 2024). Bio-inspired synthesis frequently occurs under mild conditions, such as reduced temperatures and air pressure. Compared to traditional methods, plant-based synthesis is cost-effective, environmentally benign, and biocompatible. Moreover, the resultant nanoparticles exhibit excellent therapeutic properties, including stability, permeability, and reduced toxicity (Huang et al., 2024). Therefore, researchers continue investigating and developing new methods to make MNPs emergence more sustainable and environmentally friendly.

This study focuses on *Plectranthusamboinicus*, a perennial Solanaceae herb with a wide distribution in tropical and warm regions. It has a variety of cultural uses in traditional medicine. Diverse phytochemicals in the plant extract of *P. amboinicus*, such as carvacrol, thymol, flavonoids, triterpenoids, and rosmarinic acid, contribute to the herb's biological activities, including antimicrobial, anti-inflammatory, antiviral, antiepileptic, antitumorogenic, wound healing, and antioxidant effects ("Health-promoting properties of *Plectranthus amboinicus*: a comprehensive review," 2023; Nizar Ahamed et al., 2023). The phytochemical composition of *P. amboinicus* has shown high therapeutic and nutritional properties, garnering significant interest from the pharmaceutical industries for its potential medicinal applications (Augustus et al., 2024; Gupta et al., 2024; Paramasivam et al., 2020).

The primary objective of this research was therefore the development of a green synthesis technique to synthesize bioinspired PdNPs using alcoholic leaf extracts of *P. amboinicus*. By integrating the unique properties of PdNPs and *P. amboinicus*-derived phytochemicals, this study aimed to develop a multifunctional nanotherapeutic agent. The bio-inspired PdNPs were assessed for their antimicrobial, antioxidant, anti-diabetic, and anti-inflammatory activities, as well as their wound-healing efficacy in a mouse excision wound model. By utilizing the combined advantages of the MNPs and plant extract, this strategy aimed to establish the synthesized PdNPs as a promising candidate for multifaceted therapeutic applications.

## 2. Materials and Methods

### 2.1 Materials and characterization techniques

Before employing glassware for synthesis, it is correctly cleansed with aqua regia (a mixture of HCl and HNO<sub>3</sub> in a 3:1 ratio) followed by

rinsing with double-distilled water. Palladium chloride (PdCl<sub>2</sub>, 99.9%) procured from Sigma-Aldrich was applied without further processing. Analytical-grade chemicals and Milli-Q-grade water were used for all experiments. The leaves of *P. amboinicus* were harvested from the university nursery, University of Rajasthan, Jaipur, India. The UV-visible (UV-Vis) absorption spectra of biosynthesized PdNPs were measured using an Agilent Technology Cary 60 Visible spectrophotometer with wavelength of 200–800 nm. Fourier transform infrared (FTIR) spectra of PdNPs were recorded on a FT-IR Spectrum 2 (Perkin Elmer) in the 400–4000 cm<sup>-1</sup> range. Energy-dispersive X-ray spectroscopy (EDS)-equipped field emission scanning electron microscopy (Apreo 2S Highvac, Thermofisher Scientific) was utilized to investigate the morphology and element mapping of PdNPs. Transmission electron microscopy (Tecnai G2 S-TWIN, 200KV) was used to examine the size. The zeta potential of the re-dispersed nanoparticles was assessed using a Zetasizer Nano ZSP (ZEN 5600). The X-ray diffraction (XRD) pattern of PdNPs was determined using XPERT PRO PANalytical XRD.

### 2.2 Leaf extract preparation

The leaves of *P. amboinicus* were obtained from the university nursery on the university campus. The acquired leaves were cleansed with tap and distilled water to eliminate any impurities. The cleaned leaves were grated into little pieces. In a 500 mL conical flask, 40 g of the grated leaves were placed, and 200 mL of water was added (leaves: water = 1:5 ratio). The conical flask was placed at heating plate and heated at 70–80 °C until the water began to boil. The solution changed to a brown color during heating and was subsequently cooled after boiling. The extract was kept at room temperature for later use after being cooled and filtered through Whatman No.1 filter paper to separate the liquid component (leaf extract) from the solid plant material.

### 2.3 Synthesis of PdNPs

The procedure for synthesizing palladium nanoparticles (PdNPs) is as follows. For a 1 mM PdCl<sub>2</sub> solution (100 mL), dissolve 0.0741 g of PdCl<sub>2</sub> in 90 mL of ultrapure water. While stirring on a hot magnetic stirrer, add 2–3 drops of HCl to the solution to ensure complete dissolution. Next, 10 mL of the leaf extract was added to 90 mL of the 1 mM PdCl<sub>2</sub> solution and stir the mixture at 80°C for 30 mins. The reaction solution was kept undisturbed until the color changed from yellow to black. After the color shift, the solution was left to stabilize the nanoparticles under static conditions for 48 hrs. Finally, the solid nanoparticles were then collected by centrifuging the reaction mixture for 10 mins at 6000 rpm and dispersed again in Milli-Q water. The centrifugation and scattering processes were performed twice to eliminate any remaining PdCl<sub>2</sub> and *P. amboinicus* leaves extract solution from the final product. Following collection, the nanoparticles were dried up in a hot air oven (Fig. 1).

### 2.4 Antibacterial efficacy of PdNPs

The Agar Well Diffusion approach was employed for the *in vitro* antibacterial assay. The individual test materials were diluted with

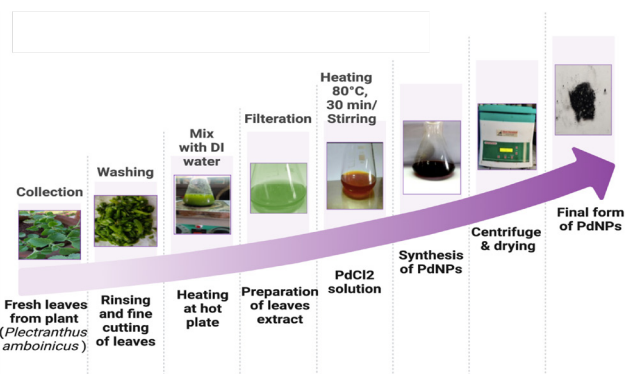


Fig. 1. Schematic diagram of the green synthesis of PdNPs using an aqueous extract of *P. amboinicus*.

0.5% dimethyl sulphoxide (DMSO), and four varying concentrations (conc.) (25 µg/mL, 50 µg/mL, 75 µg/mL, and 100 µg/mL) of palladium chloride, *P. amboinicus* leaf extract, and PdNPs were created. Sterilized petri dishes containing the nutritional agar (NA) medium were employed to inoculate gram-negative and -positive bacterial strains including *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*), and *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), respectively. This inoculum was spread evenly across the plate with a spreader and let to stand for 30 mins. Wells of 6 mm diameter were created in the seeded agar plates. A control well was also constructed at the same distance. All concentrations of palladium chloride, *P. amboinicus* leaf extract, PdNPs, and the standard medication (30 µg/mL) were poured into the pre-organized wells of seeded plates. The plates were incubated for 24 hrs at 37°C. The inhibition zone (IZ) around each well was employed to estimate the antibacterial spectrum of the test material. The sizes of the inhibition zones developed from the test samples and the commercial positive control (Streptomycin) were compared. The studies were carried out in triplicate, with the mean absorbance values reported.

## 2.5 Antioxidant activity of PdNPs

The efficiency of the PdNPs in scavenging DPPH radicals was compared to that of the standard ascorbic acid. For the experiment, 3 mL of a 0.1 mM DPPH solution in methanol was dissolved with variable conc. (20, 40, 60, 80, 100, 150, 200, 250, 300, 500, and 1000 µg/mL) of palladium chloride, leaf extract, and synthesized PdNPs. The Mixture was agitated vigorously to achieve consistency and then kept for 30 mins in dark at an ambient temperature. The optical density was assessed at 517 nm using a UV-Vis spectrophotometer. The DPPH radical inhibition (%) was estimated using the following Eq. (1):

$$\% \text{DPPH radical inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad (1)$$

The control samples's absorbance ( $A_{\text{control}}$ ) and test sample's absorbance ( $A_{\text{sample}}$ ) are key components in the formula for evaluating the DPPH radical inhibition. It's important to substitute the correct values of  $A_{\text{control}}$  and  $A_{\text{sample}}$  into the formula to accurately determine the percentage of DPPH radical inhibition.

## 2.6 Anti-inflammatory activity of PdNPs

Anti-inflammatory activity was assessed employing a altered BSA method established by Williams *et al.* with varying conc. of 100, 250, 500, 1000, and 2000 µg/mL of aspirin and PdNPs (Williams *et al.*, 2002). A 0.4% w/v BSA solution was made by dissolving one Tris-buffered saline tablet in 15 mL deionized water, producing a buffer containing 0.05 M Tris and 0.15 M sodium chloride at pH 7.6 at 25°C. The pH was lowered to 6.4 using glacial acetic acid. PdNPs were dissolved in DMSO to prepare stock solutions at a concentration of 50 µg/mL (0.005% w/v). These PdNPs aliquots were mixed with 1 mL of 0.4% w/v BSA buffer in test tubes. The mixtures were incubated in a water bath at 72°C for 20 mins, followed by cooling for another 20 mins. A spectrophotometer was used to determine turbidity at 660 nm, with air serving as the blank. The studies were carried out in triplicate, with the mean absorbance values reported. The following formulae was used to determine the inhibition of BSA denaturation:

$$\text{Percentage inhibition of BAS Denaturation}(\%) = 100 \times \left( 1 - \frac{A_1}{A_2} \right)$$

where A1 is the reference's absorbance and A2 is the sample's absorbance.

## 2.7 Anti-diabetic assay of PdNPs

The chromogenic DNSA method was employed in order to conduct an inhibition study. The assay solution contains 500 µL of 0.02 M sodium phosphate buffer (pH 6.9, supplemented with 6 mM NaCl), along with 1 mL of salivary amylase, and 400 µL test samples with conc.

varying between 20 to 1000 µg/mL incubated for 10 mins at 37°C. Subsequently, 580 µL of a 1% w/v starch solution was added to each tube, followed by further incubation for 15 mins at 37°C. To terminate the reaction, 1.0 ml of DNSA reagent was added, followed by 5 mins in boiling water, cooling at room temperature, and OD measurement at 540 nm. The control without PdNPs had 100% enzymatic activity. Acarbose was added as a negative control along with the test sample in the reaction mix without any enzyme to remove the absorbance brought on by PdNPs. The percentage of alpha amylase inhibition was computed using the following:

$$\% \text{ Relative enzyme activity} = \frac{\text{Enzyme activity in test sample with PdNPs}}{\text{Enzyme activity in control}} \times 100$$

$$\% \text{ Inhibition in the alpha-amylase activity} = 100 - \% \text{ Relative enzyme activity}$$

## 2.8 Wound healing activity of PdNPs

### 2.8.1 Preparation PdNPs ointment

In order to formulate the ointment with PdNPs, 30 g of absolute Vaseline is heated in a water bath at 60°C using a bain-marie technique. Then, 0.3 g of PdNPs is added (1% w/w). Ultimately, the ointment that had been created undergoes sonication at 60°C for 30 mins in order to achieve a consistent and uniform texture (Zare-Bidaki *et al.*, 2023).

### 2.8.2 Animal care and handling

The mice (male Swiss albino mice) weighing 25–30 g were utilized in the present study. Animals were acquired from the Central Animal Facility (CAF) National Institute of Pharmaceutical Education and Research (NIPER), Mohali, Chandigarh (Reg. No: (108/GO/Re/Rc/Bi/Bt/99/CPCSEA). All animal experiment were approved from the Institutional Animal Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur India letter no. UDZ/IAEC/V/07 dated 16-03-2022. Animals were kept in the animal house facility at the Department of Zoology, University of Rajasthan, Jaipur, during the experimental work. Polyacrylic cages were used to house the animals, ensuring standard conditions of 20–30°C temperature, 50–70% humidity, and a 12:12 light-to-dark cycle. A 7-day acclimation period was observed prior to the experiments, and the animals were fed dry pellets and tap water *ad libitum*. The study design for the wound healing potential of the biosynthesized PdNPs animals was divided into three groups as follows.

Group A: *Control Group*: - No treatment was administered to the animals in this group.

Group B: *Positive Control Group*: - Animals of this group were treated with Nitrofurazone (0.2%w/w) ointment

Group C: *Drug Treated Group*: - Animals of this group were treated with the Vaseline ointment containing PdNPs.

### 2.8.3 Excision wound model

Excisional wounds are widely used as a model for studying wound healing, as they closely mimic acute clinical wounds that heal by second intention, where the skin edges are left unsutured. The experimental procedure involved anesthetizing animals with diethyl ether, followed by shaving the dorsal back to prepare for a wound. Ethanol (70%) served as an antiseptic for the shaved area. A circular excision wound, extending through the full thickness of the skin, was then created on the predetermined shaved region without subsequent dressing. No local or systemic antimicrobial agents were administered. Each mouse was housed individually in a separate cage throughout the study. This methodology aimed to investigate the wound healing efficacy of the PdNPs without the interference of antimicrobial treatments, allowing for an assessment of natural recovery with and without drug treatment. Nitrofurazone (0.2%w/w) ointment was applied as a positive control.

### 2.8.4 Assessment of wound contraction

The experimental animals were divided into three groups following the production of wounds, as previously mentioned. The excision wound margins were traced via a clear plastic sheet, and the surface area of the wound was evaluated planimetrically. The size of wounds was measured in mm<sup>2</sup> by putting the transparent sheet on graph paper every day throughout the monitoring period; photographs of the dorsal surface of the mice were taken on the 1<sup>st</sup>, 6<sup>th</sup>, 12<sup>th</sup>, and 18<sup>th</sup> day. Wound contraction was estimated using the following formula:

$$\text{Percent wound contraction} = \frac{\text{Initial wound area} - \text{unhealed area}}{\text{Initial wound area}} \times 100$$

### 2.9 Statistical analysis

The experiment was carried out in three replicates for each treatment and overall results are presented as mean  $\pm$  standard deviation (SD). Student's t-test and one-way ANOVA were used to analyze the collected data. p-values < 0.05 imply statistical significance.

## 3. Results and Discussion

### 3.1 UV-vis analysis of PdNPs

UV-vis spectroscopy is a common technique employed to characterize metal nanoparticles. The UV-vis absorbance of *P. amboinicus* leaves extract, PdCl<sub>2</sub> solution and bio-inspired synthesized PdNPs was observed within 200–800 nm wavelength. The reaction occurs between *P. amboinicus* leaves extract and PdCl<sub>2</sub>; the reaction mixture's color shifts from yellow to black. The reaction mixture showed a dark brown color due to the stimulation of surface plasmon resonance (SPR) of PdNPs. UV-vis absorption spectra of the PdCl<sub>2</sub> solution showed a distinct absorption peak at 425 nm, revealing Pd (II) ions presence in the solution. After the reaction, the absorption peak at 425 nm of the precursor PdCl<sub>2</sub> had disappeared, indicating that the precursor Pd (II) reduction was completed (Basavegowda et al., 2015; Kuniyil et al., 2019). Due to the surface Plasmon, PdNPs often do not exhibit any noticeable peaks (Fig. 2a).

### 3.2 FT-IR analysis of PdNPs

FT-IR spectroscopy is an incredibly effective analytical technique that reveals important information about chemical structures and functional groups. The FT-IR spectrum of the *P. amboinicus* leaves extract revealed important absorption peaks at 3420, 2925, 2853, 1617, 1430, 1385, 1078 and 617 cm<sup>-1</sup> (Fig. 2(b), blue). *P. amboinicus* leaf extract's FTIR spectra showed absorption peaks at 3420 and 1617 cm<sup>-1</sup>, indicating O–H and >C=C< stretching of flavonoids or polyols. The vibrations at 1078 and 1385 cm<sup>-1</sup> were related to C–O stretching and the C–H bending of flavonoids or polyols. Additionally, the leaf extract displayed bands at 2925 and 2853 cm<sup>-1</sup>, which corresponded to the C–H stretching vibrations mode of aliphatic compounds. In contrast, the FT-IR spectrum of PdNPs showed significant absorption peaks at 3372, 2920, 2820, 1560, 1410, 1342, 1024 and 648 cm<sup>-1</sup>, clearly indicating the occurrence of phytoconstituents that serve as capping agents (Fig. 2(b), red). After the bio reduction of PdCl<sub>2</sub> by the leaf extract, there were noticeable modifications in the positions and intensity of the stretching vibrations, suggesting the involvement of polyphenols or flavonoids. The presence of the O–H group of polyols in the bio reduction process was confirmed by the band at 3372 cm<sup>-1</sup>. Furthermore, the minor peaks at 2920 and 1560 cm<sup>-1</sup> corresponded to C–H and C–O stretching vibrations, while the peaks at 1024 cm<sup>-1</sup> were associated with the C–O stretching vibration in flavonoids or polyols (Dauthal and Mukhopadhyay, 2013; Jayamani et al., 2023; Sarmah et al., 2019).

### 3.3 X-ray diffraction analysis

The XRD diffractograms in Fig. 2(c) display the PdNPs synthesized through plant mediation. These diffractograms exhibit intense diffraction peaks at  $2\theta = 40.0, 46.4, 67.9, 81.8, \text{ and } 86.5^\circ$ , corresponding to the

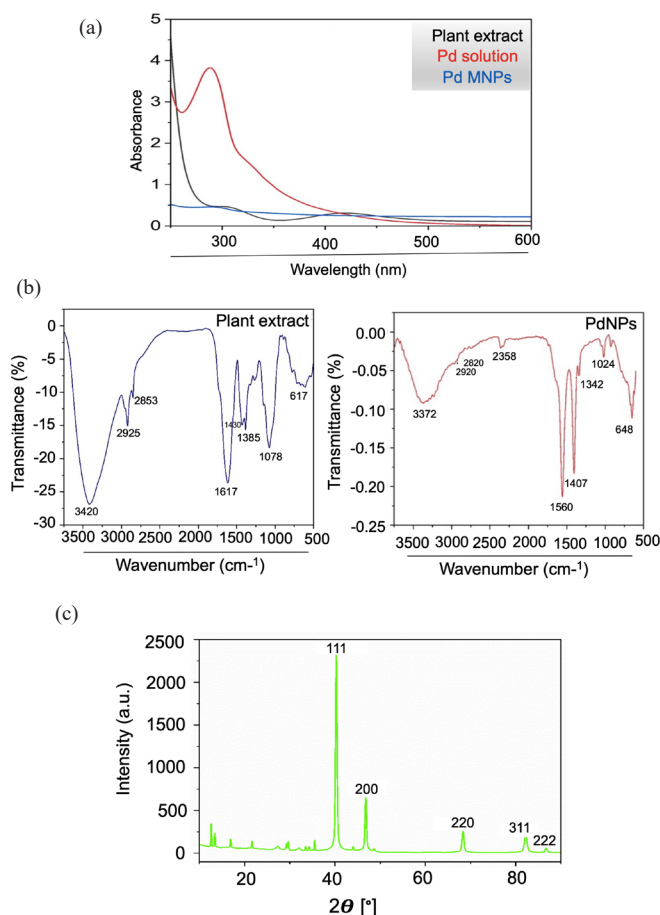


Fig. 2. (a) UV Visible spectrum of Plant extract, Pd<sup>2+</sup> solution, and PdNPs. (b) FT-IR spectra of plant extract and PdNPs. (c) Powder XRD pattern of PdNPs.

crystallographic planes of metallic palladium (Pd0) nanoparticles' fcc crystalline structure (JCPDS No: 89–4897) (111), (200), (220), (311), and (222) (Sarmah et al., 2019; Wang et al., 2015). In the determination of the average nanocrystalline size, the Debye–Scherrer method was employed (Al-Fakeh et al., 2021). The formula  $D = \lambda k / \beta \cos \theta$  was utilized, where D represents the crystal size, k is a constant with a value of 1,  $\lambda$  represents the X-ray wavelength (0.1541 nm),  $\beta$  is the full width at half maximum and  $\theta$  corresponds to the diffraction angle related to the lattice plane (111). Application of the Debye–Scherrer equation revealed an average crystallite size of 5.58 nm.

### 3.4 FESEM and EDS analysis of PdNPs

Scanning electron microscopy (SEM) is a helpful technique utilized in materials science and numerous other fields to achieve high-resolution images of surfaces at the nanoscale. FESEM (Field Emission Scanning Electron Microscopy) is further enhanced with energy-dispersive X-ray spectroscopy (EDS) detectors, allowing for elemental analysis of the sample. According to the SEM images displayed in Fig. 3(a), it was observed that the PdNPs were almost spherical, even at higher resolutions. Additionally, these particles were evenly distributed on the surface with minimal clustering or agglomeration. The EDS spectrum detected unique signals based on the analysis of the elemental composition of PdNPs synthesized using *P. amboinicus* leaves extract. In Fig. 3(b), the absorption peaks ranging from 0.277 to 2.83 keV were attributed to forming PdNPs in the EDS spectra of PdNPs. In the EDX analysis, a strong signal for Pd was identified at 2.8 keV with a 57.92 weight percentage, indicating palladium PdNPs in the sample. In contrast, signals for C, O, N, and S were also present at 0.27, 0.52, 0.39, and 2.30 keV with 16.17, 16.60, 2.61, and 8.70 weight percentages, respectively, which is likely due to the plant leaf extract and conductive coating.

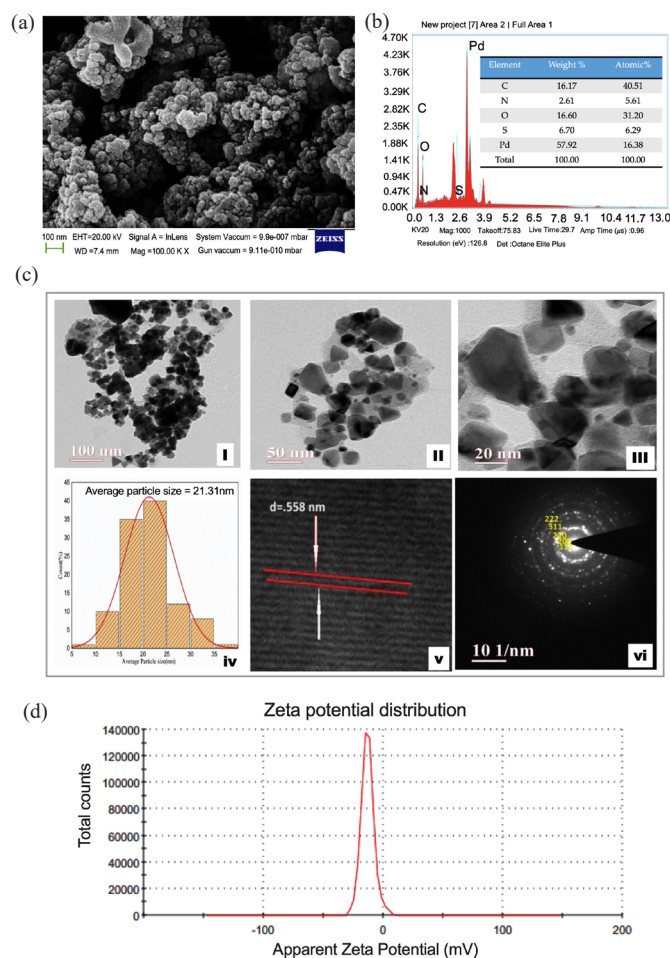


Fig. 3. (a) FESEM image and (b) EDX spectra of biosynthesized PdNPs. TEM image of PdNPs at 100 nm (c) HRTEM images of PdNPs at different magnifications (i) 50 nm and 20 nm magnification (ii-iii). Particle size distribution curve (iv). d-spacing of PdNPs (v). SAED pattern (vi). (d) Zeta potential of synthesized PdNPs. EDX: Energy dispersive X-ray spectroscopy, TEM: Transmission electron microscopy, HRTEM: High-resolution transmission electron microscopy.

### 3.5 TEM – HRTEM – SAED analysis

The particle size and structure of bio-inspired PdNPs were assessed by transmission electron microscopy (TEM), and the crystallinity was evaluated via selected area electron diffraction (SAED) pattern. According to the TEM – HRTEM images of PdNPs, they are triangular and rectangular shaped with particle sizes ranging from 5 to 40 nm displayed in Fig. 3c(i-III). The TEM image analyzed using ImageJ software revealed that the PdNPs have an average particle size of around 21.31 nm (Fig. 3c(iv)). Additionally, the lattice space was determined through HR-TEM, showing a d-space value of approximately 0.558 nm, as illustrated in Fig. 3c(v). The crystalline structure of plant-mediated PdNPs was established using the SAED pattern. Circular dots in the SAED pattern indicated the interplanar distances corresponding to the fcc crystalline structure of the PdNPs, including planes 111, 200, 220, and 311, confirming their crystalline nature (Fig. 3c(vi)). The PdNP sample's selected-area electron diffraction analysis displays clear concentric circles with bright intermittent spots, confirming the outstanding crystalline purity of the PdNPs. The diffraction patterns are categorized according to the PdNP crystallinity and are in line with JCPDS card No. 89-4897. The diffracted rings correspond to the crystallographic planes (111), (200), (220), (311), and (222) of the fcc PdNPs, and the results align with the XRD lattice plane of the PdNPs.

### 3.6 Zeta potential analysis

Zeta potential is paramount in colloidal systems as it measures the electrical charge at the interface between a particle surface and a liquid.

It determines the stability and behavior of the system, making it vital in industrial and medical applications. The stability of PdNPs synthesized by the green method was determined by zeta potential parameters. Fig. 3(d) shows the zeta potential value for PdNPs is -12.9 mV. The zeta potential value of PdNPs suspension revealed even distribution and assessed its potential stability of particles (Aarzo et al., 2021; Al-Fakeh et al., 2021). The synthesized PdNPs surfaces have a negative charge (-12.9 mV) and stable particle suspensions generally have a zeta potential range of +30 to -30 mV, as per published research (Han et al., 2019).

### 3.7 Antibacterial assay

The synthesized PdNPs were assessed for their antibacterial efficiency against gram-positive and -negative bacteria. The results, as illustrated in Fig. 4(a), revealed that PdNPs have higher effectiveness than the plant extract and palladium chloride. Increase in the conc. of plant extract, Pd salt and PdNPs increased the zones of inhibition (Fig. 4b). The highest antibacterial effect with inhibition zone  $14 \pm 0.3$  mm was observed against *Escherichia coli* with a minimum inhibitory concentration (MIC) of  $5 \mu\text{g/mL}$ . In contrast, the lower inhibition zone were observed against *B. subtilis*, *S. aureus* and *P. aeruginosa* with an MIC around  $5 \pm 0.3$ ,  $5 \pm 0.6$  and  $5 \pm 0.3 \mu\text{g/mL}$  with inhibition zone  $11 \pm 0.6$ ,  $11 \pm 0.6$  and  $11 \pm 0.4$  mm respectively (Table 1). These findings suggest that PdNPs have a potent antibacterial effect, attributed to their ability to inhibit a broad range of bacterial strains (Gangwar et al., 2023b; Jayakumar et al., 2023; Sadalage and Pawar, 2023).

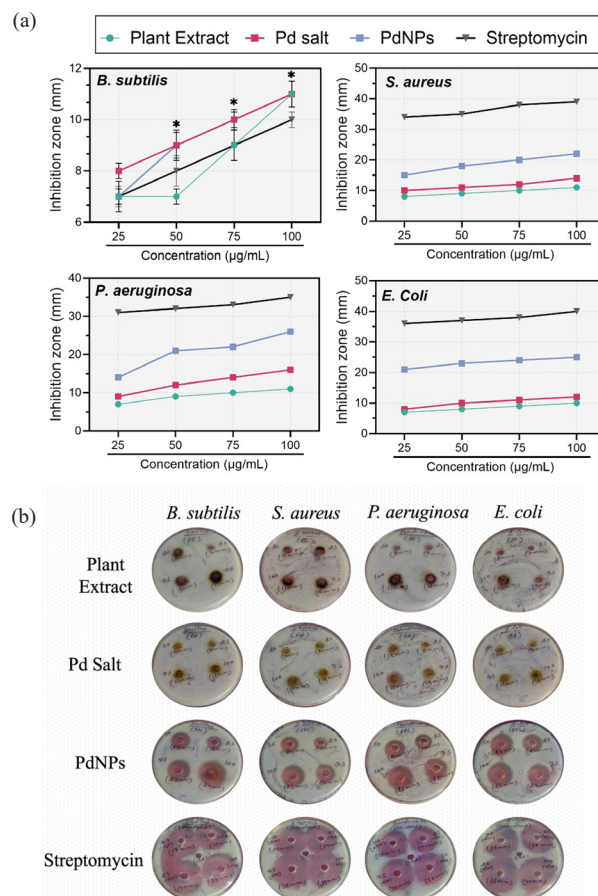


Fig. 4. Antimicrobial susceptibility of plant extracts, palladium chloride and PdNPs and streptomycin by disk diffusion method. (a) Antibacterial activity of plant extract, palladium chloride, PdNPs and streptomycin, (b) Zones of inhibition of plant extract, palladium chloride, PdNPs and streptomycin against the pathogenic strains *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. Results are shown as means  $\pm$  S.D for triplicate with error bars indicating statistical significance at  $p < 0.05$ .

**Table 1.**  
Comparison of MIC and IZ for Plant extract, Pd salt and PdNPs.

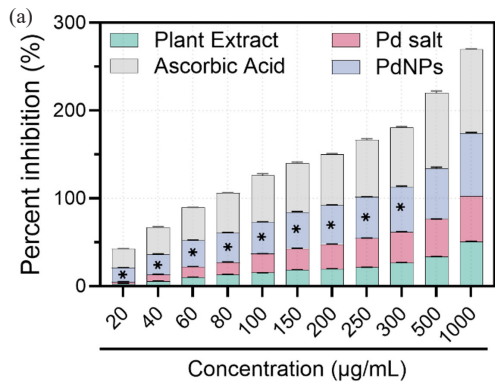
| Bacteria strains | Minimum inhibitory concentration (MIC) with inhibition zone (IZ) |         |             |         |             |          |
|------------------|------------------------------------------------------------------|---------|-------------|---------|-------------|----------|
|                  | Plant extract                                                    |         | Pd salt     |         | PdNPs       |          |
|                  | MIC (µg/mL)                                                      | IZ (mm) | MIC (µg/mL) | IZ (mm) | MIC (µg/mL) | IZ (mm)  |
| B. subtilis      | 25 ± 0.3                                                         | 7 ± 0.4 | 18 ± 0.3    | 7 ± 0.5 | 5 ± 0.3     | 11 ± 0.6 |
| S. aureus        | 18 ± 0.4                                                         | 7 ± 0.6 | 15 ± 0.6    | 7 ± 0.4 | 5 ± 0.6     | 11 ± 0.6 |
| P. areuginosa    | 25 ± 0.4                                                         | 7 ± 0.4 | 15 ± 0.4    | 7 ± 0.3 | 5 ± 0.3     | 11 ± 0.4 |
| E. coli          | 20 ± 0.6                                                         | 7 ± 0.3 | 20 ± 0.3    | 7 ± 0.3 | 5 ± 0.6     | 14 ± 0.3 |

Pd salt: Palladium salt

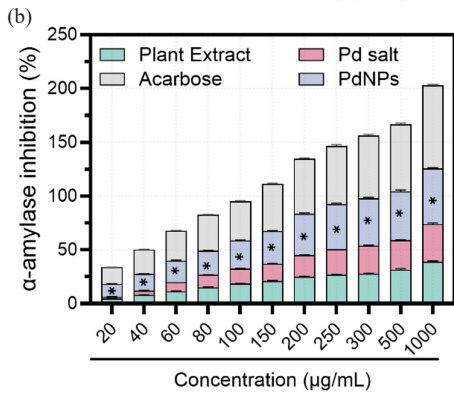
Once inside, Pd<sup>2+</sup> ions bind to phosphorus and sulfur in proteins, or nucleic acid can ultimately destroy bacterial function (Skłodowski et al., 2023; Tahir et al., 2016).

3.8 Antioxidant assay

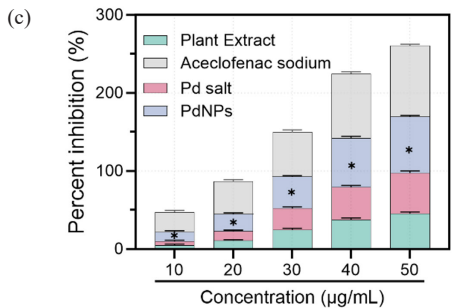
The *in vitro* antioxidant efficiency of PdCl<sub>2</sub>, *P. amboinicus* leaves extract, and PdNPs was estimated via a DPPH assay. The samples were measured for their absorbance against the DPPH radical at 517 nm using a UV–vis spectrophotometer. The findings demonstrated that the samples' capacity to scavenge DPPH radicals increased in a dose-responsive manner (Fig. 5a). Both the precursor salt PdCl<sub>2</sub> and *P. amboinicus* leaves exhibited the highest anti-DPPH scavenging activities at 1000 µg/mL, with values of 52.35 ± 0.08 and 50.50 ± 0.53, respectively. Meanwhile, DPPH radical scavenging percentages of PdNPs and ascorbic acid at 1000 µg/mL were 71.41 ± 0.94 and 95.27 ± 0.88, respectively. The IC<sub>50</sub> values of PdCl<sub>2</sub>, *P. amboinicus* leave extract, and PdNPs were 773.81, 910.72, and 425.26 µg/mL, respectively (Fig. 5a). However, the IC<sub>50</sub> values of precursor salt were much higher than those of the PdNPs and the positive control. The DPPH analysis indicates that the *P. amboinicus* leaves extract has less DPPH scavenging potency than PdNPs and ascorbic acid. Previous research has shown that PdNPs exhibit excellent antioxidant activity compared to precursor salts due to free charge transfer from the containing PdNPs to the DPPH radical (Gangwar et al., 2023a; Tiri et al., 2024).



| Concentration (µg/mL)    | % scavenging activity (mean ± SD) against DPPH radical |               |              |               |
|--------------------------|--------------------------------------------------------|---------------|--------------|---------------|
|                          | Pd salt                                                | Plant extract | PdNPs        | Ascorbic acid |
| 20                       | 2.07 ± 0.63                                            | 2.44 ± 0.63   | 16.49 ± 0.23 | 21.67 ± 0.08  |
| 40                       | 7.66 ± 0.31                                            | 5.54 ± 0.33   | 23.20 ± 0.37 | 30.97 ± 0.43  |
| 60                       | 12.07 ± 0.28                                           | 10.07 ± 0.25  | 30.21 ± 0.27 | 37.32 ± 0.23  |
| 80                       | 14.26 ± 0.26                                           | 12.98 ± 0.42  | 33.75 ± 0.28 | 45.13 ± 0.53  |
| 100                      | 21.78 ± 0.16                                           | 15.25 ± 0.23  | 36.23 ± 0.11 | 53.02 ± 1.76  |
| 150                      | 24.26 ± 0.36                                           | 18.71 ± 0.27  | 41.38 ± 0.69 | 56.05 ± 0.99  |
| 200                      | 28.17 ± 0.23                                           | 19.70 ± 0.18  | 44.40 ± 0.39 | 57.91 ± 1.04  |
| 250                      | 33.20 ± 0.17                                           | 21.41 ± 0.14  | 46.96 ± 0.28 | 64.99 ± 1.40  |
| 300                      | 35.28 ± 0.21                                           | 26.56 ± 0.26  | 51.30 ± 1.04 | 67.40 ± 1.42  |
| 500                      | 43.05 ± 0.11                                           | 33.53 ± 0.27  | 57.91 ± 1.01 | 85.93 ± 1.90  |
| 1000                     | 52.35 ± 0.08                                           | 50.50 ± 0.53  | 71.41 ± 0.94 | 95.27 ± 0.88  |
| IC <sub>50</sub> (µg/mL) | 773.81                                                 | 910.72        | 425.26       | 158.26        |



| Concentration (µg/mL)    | % alpha-amylase inhibition (mean ± SD) |               |              |              |
|--------------------------|----------------------------------------|---------------|--------------|--------------|
|                          | Pd salt                                | Plant extract | PdNPs        | Acarbose     |
| 20                       | 1.65 ± 0.25                            | 4.83 ± 0.21   | 12.05 ± 0.14 | 15.31 ± 0.15 |
| 40                       | 4.17 ± 0.20                            | 8.09 ± 0.17   | 15.35 ± 0.21 | 22.71 ± 0.33 |
| 60                       | 8.63 ± 0.06                            | 11.52 ± 0.25  | 19.98 ± 0.62 | 27.82 ± 0.15 |
| 80                       | 11.52 ± 0.24                           | 15.19 ± 0.33  | 22.5 ± 0.42  | 33.69 ± 0.16 |
| 100                      | 13.75 ± 0.39                           | 18.62 ± 0.3   | 26.47 ± 0.32 | 36.33 ± 0.59 |
| 150                      | 16.1 ± 0.22                            | 21.18 ± 0.33  | 30.3 ± 0.41  | 43.81 ± 0.67 |
| 200                      | 19.9 ± 0.3                             | 25.31 ± 0.15  | 38.4 ± 0.83  | 51.2 ± 0.63  |
| 250                      | 23.78 ± 0.06                           | 26.68 ± 0.43  | 42.36 ± 0.7  | 53.76 ± 1.22 |
| 300                      | 26.02 ± 0.61                           | 27.87 ± 0.38  | 43.98 ± 0.97 | 58.67 ± 0.9  |
| 500                      | 27.62 ± 0.25                           | 31.54 ± 0.93  | 45.18 ± 1.24 | 62.27 ± 1.24 |
| 1000                     | 34.89 ± 0.86                           | 39.11 ± 0.73  | 51.78 ± 0.6  | 77.25 ± 0.79 |
| IC <sub>50</sub> (µg/mL) | 1009.53                                | 817.31        | 547.75       | 272.59       |



| Concentration (µg/mL)    | % Bovine serum albumin inhibition (mean ± SD) |               |              |                    |
|--------------------------|-----------------------------------------------|---------------|--------------|--------------------|
|                          | Pd salt                                       | Plant extract | PdNPs        | Aceclofenac sodium |
| 10                       | 4.85 ± 1.02                                   | 5.32 ± 1.63   | 11.60 ± 1.58 | 25.49 ± 2.23       |
| 20                       | 10.70 ± 1.09                                  | 12.53 ± 1.40  | 21.85 ± 1.42 | 41.54 ± 2.27       |
| 30                       | 24.90 ± 1.50                                  | 27.51 ± 1.59  | 40.53 ± 1.06 | 56.70 ± 2.79       |
| 40                       | 37.60 ± 2.13                                  | 41.84 ± 2.10  | 62.32 ± 2.61 | 82.93 ± 2.66       |
| 50                       | 45.71 ± 2.34                                  | 51.89 ± 1.99  | 72.00 ± 1.76 | 90.90 ± 1.91       |
| IC <sub>50</sub> (µg/mL) | 53.24                                         | 48.12         | 35.17        | 24.47              |

**Fig. 5.** (a) DPPH radical scavenging activities of Pd salt, plant extract, PdNPs and ascorbic acid, (b) Anti-diabetic assay of Pd salt, plant extract, PdNPs and acarbose, (c) Anti-inflammatory activity of Pd salt, plant extract, PdNPs and aceclofenac sodium by analysing the percentage bovine serum albumin inhibition of respective groups. Results are shown as means ± S.D for triplicate with error bars through one-way analysis of variance (ANOVA) and Tukey multiple comparison test indicating statistical significance at \*p < 0.05. Pd salt: Palladium salt, IC<sub>50</sub>: Half-maximal inhibitory concentration.

**Table 2.**  
Comparison of metal and metal oxide nanoparticles' DPPH scavenging capabilities.

| Nano-particles | Average size (nm) | Concentration | DPPH scavenging (%) | References            |
|----------------|-------------------|---------------|---------------------|-----------------------|
| PdNPs          | 21.31             | 1000 µg/mL    | 71.41               | Present study         |
| AgNPs          | 9.1               | 250 µg/mL     | 80.00               | Gur et al., 2025      |
| AuNPs          | 5-23              | 300 µg/mL     | 57.70               | Hosny et al., 2021    |
| PtNPs          | 1-3               | 50 µg/mL      | 72.00               | Eltaweil et al., 2022 |
| PdNPs          | 7.44              | 500 mg/L      | 79.60               | Gulbagca et al., 2021 |
| CuO-NPs        | 35.8-49.2         | 500 µg/mL     | 29.30               | Das et al., 2020      |
| ZnO-NPs        | 8-12              | 100 µg/mL     | 81.92               | Ghareib et al., 2019  |

AgNPs: Silver nanoparticles, AuNPs: Gold nanoparticles, CuO-NPs: Copper oxide nanoparticles, ZnO-NPs: Zinc oxide nanoparticles.

A study of phytochemical-synthesized AgNPs (9.1 nm) showed DPPH scavenging activity of 80% at 250 µg/mL (Gur et al., 2025). Another study on biosynthesized AuNPs (5-23 nm) indicated 57.7% scavenging at 300 µg/mL (Hosny et al., 2021). Research on plant-mediated PtNPs (1-3 nm) and PdNPs (7.4 nm) found scavenging activities of 72.0% at 50 µg/mL and 79.6% at 500 mg/mL, respectively (Eltaweil et al., 2022; Gulbagca et al., 2021). Additionally, studies on CuO-NPs (35.8-49.2 nm) and ZnO-NPs (8-12 nm) revealed scavenging activities of 29.30% at 500 µg/mL and 81.92% at 100 µg/mL (Das et al., 2020; Ghareib et al., 2019). The findings reveal that both particle size and concentration play a notable role in modifying antioxidant activity. To provide a more robust evaluation of our results, we performed a comparative analysis alongside the findings of other metal and metal oxide nanoparticles, as outlined in Table 2. Therefore, the biosynthesized PdNPs from the *P. amboinicus* leaves extract could be a promising antioxidant drug for oxidative stress-related ailments.

### 3.9 Anti-diabetic assay

$\alpha$ -amylase is an enzyme that has a critical function in converting complex carbohydrates into simpler sugars. Inhibiting  $\alpha$ -amylase can be beneficial in managing blood sugar levels in individuals with diabetes, or as a potential strategy for weight control. In this study, biosynthesized PdNPs were evaluated for their anti-diabetic activity by measuring the inhibition of  $\alpha$ -amylase. When this enzyme is inhibited, the absorption rate of glucose can be reduced. Acarbose was used as a control to serve as a reference point, and the results showed a 77.00% inhibition at the conc. of 1000 µg/mL. The biosynthesized PdNPs showed a more substantial inhibition of  $\alpha$ -amylase than the PdCl<sub>2</sub> and plant extract. Fig. 5(b) provides the percentage inhibition and IC<sub>50</sub> values for the PdNPs, plant extract, and control. The results indicated that biosynthesized PdNPs exhibited higher inhibition of alpha-amylase than the *P. amboinicus* leaves extract and the palladium salt. A maximum inhibition (51.78%) of alpha-amylase was observed at 1000 µg/mL by biosynthesized PdNPs. Fig. 5(b) shows a graphical representation of alpha-amylase inhibition.

A comparison of biosynthesized PdNPs with other metal and metal oxide nanoparticles, including AgNPs (Rehman et al., 2023), AuNPs (Rokkarukala et al., 2023), CuO-NPs (Ameena et al., 2022), and ZnO-NPs (Rehman et al., 2023) has been illustrated in Table 3, highlighting the moderate anti-diabetic properties of biosynthesized PdNPs.

### 3.10 Anti-inflammatory assay

The results of the anti-inflammatory assay suggest that the biosynthesized PdNPs were more effective in inhibiting bovine serum albumin than the *P. amboinicus* leaves extract and the palladium salt (Fig. 5c). The analysis showed a significant relationship between PdNPs and protein denaturation (72.00% inhibition at 50 µg/mL and the *P. amboinicus* leaves extract (51.89% inhibition). The Pd salt and *P. amboinicus* leaves extract showed inhibition ranging from 4.85% to 45.71% and 5.32 % to 51.89 % respectively and biosynthesized PdNPs showed anti-inflammatory activity ranging from 11.60% to 72.00% at

**Table 3.**  
Comparison of the anti-diabetic efficiencies of metal nanoparticles and metal oxide nanoparticles.

| Nano-particles | Average size (nm) | Concentration | Inhibition of alpha-amylase (%) | References               |
|----------------|-------------------|---------------|---------------------------------|--------------------------|
| PdNPs          | 21.31             | 1000 µg/mL    | 51.78                           | Present study            |
| AgNPs          | 34.43             | 100 µg/mL     | 75.00                           | Rehman et al., 2023      |
| AuNPs          | 5-50              | 100 µg/mL     | 68.00                           | Rokkarukala et al., 2023 |
| CuO-NPs        | 63.46             | 100 µg/mL     | 64.50                           | Ameena et al., 2022      |
| ZnO-NPs        | 16-28             | 100 µg/mL     | 71.90                           | Rehman et al., 2023      |

**Table 4.**  
Comparison of the anti-inflammatory activity of metal nanoparticles and metal oxide nanoparticles.

| Nano-particles | Average size (nm) | Concentration | Protein denaturation (%) | References               |
|----------------|-------------------|---------------|--------------------------|--------------------------|
| PdNPs          | 21.31             | 50 µg/mL      | 72.00                    | Present study            |
| AgNPs          | 15.96             | 20 mg/kg      | 57.08                    | Sharifi-Rad et al., 2020 |
| AuNPs          | 34.2              | 500 µg/mL     | 82.00                    | Khuda et al., 2021       |
| CuO-NPs        | 6.89              | 500 µg/mL     | 75.16                    | Manasa et al., 2021      |
| ZnO-NPs        | 70.37             | 50 µg/mL      | 77.89                    | Nandhini et al., 2025    |

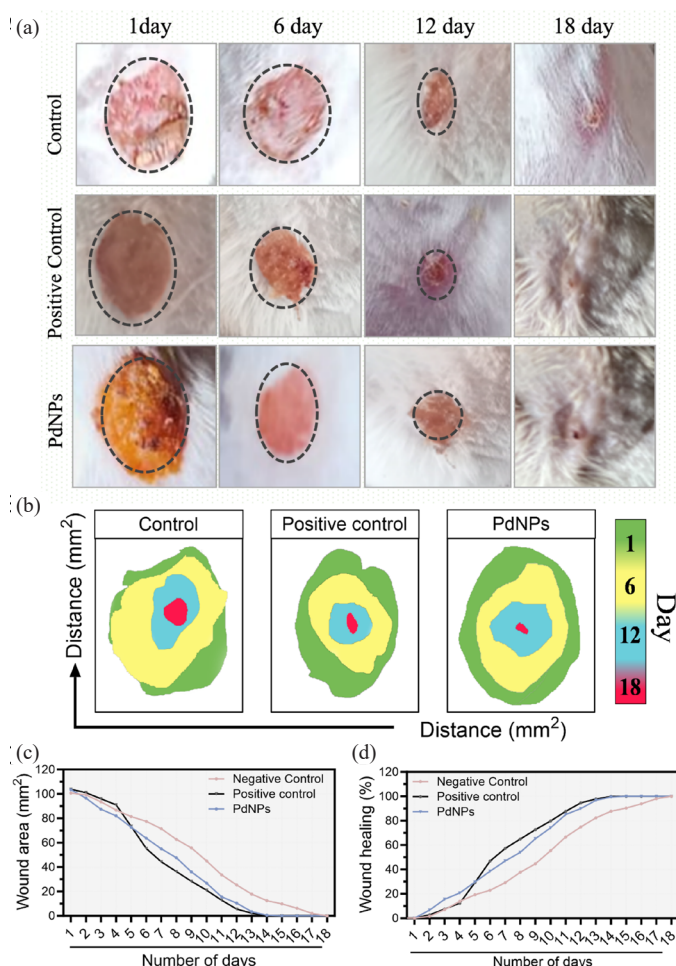
concentration 10-50 µg/mL (Fig. 5c). These findings align with other studies using PdNPs synthesized from diverse medicinal plants (Bi and Srivastava, 2024; Sandhya et al., 2024).

A study on phytochemicals used to synthesize AgNPs (34.2 nm) showed an anti-inflammatory activity of 82% at 500 µg/mL (Sharifi-Rad et al., 2020). Biosynthesized AuNPs (15.96 nm) had an anti-inflammatory activity of 57.7% at 20 mg/kg (Khuda et al., 2021). CuO-NPs (6.89 nm) and ZnO-NPs (70.37 nm) exhibited anti-inflammatory activities of 75.16% at 500 µg/mL and 77.89% at 50 µg/mL (Manasa et al., 2021; Nandhini et al., 2025). A comparison between biosynthesized PdNPs and other nanoparticles (AgNPs, AuNPs, CuO-NPs and ZnO-NPs) to suggest the higher protein denaturation efficacy of biosynthesized PdNPs has been presented in Table 4. The eco-friendly synthesis of PdNPs demonstrated excellent anti-inflammatory properties without any negative impacts typically associated with chemically synthesized medications.

### 3.11 Wound healing efficacy

In addition to its antibacterial properties, PdNPs also exhibit anti-inflammatory activity, which collectively contributes to their remarkable wound-healing efficacy. In this study, all the animals treated with PdNPs showed a sizable increase in the percentage of healed wounds in comparison with the control groups during the 18-day experiment using the excision wound model. Application of the PdNPs for 10 days led to 74.76% wound being healed compared to 55.56 % of negative control animals and 80.77 % of the positive control group i.e. nitrofurazone (0.2%w/w) ointment treated. The area of the wound expressed in mm<sup>2</sup> reduced significantly faster in the PdNPs treated and positive control group compared to control group. It has taken 14 days to heal completely using the PdNPs compared to 18 days in the negative control group and 14 days in the positive control group. Photographs taken on the 1, 6, 12, and 18<sup>th</sup> day also reflect the same (Fig. 6a). The efficiency of PdNPs in facilitating wound healing is reflected in both the percentage of wound contraction(mm<sup>2</sup>) and the faster reduction in wound area observed in this study compared to control have been depicted in Fig. (6a-d), respectively (Arumugam et al., 2024; Hamid et al., 2024; He et al., 2022; Yin et al., 2023).

Nanoparticles play a critical role in wound healing by moderating biological mechanisms like cellular migration, proliferation, angiogenesis, and antimicrobial defense. PdNPs exhibit promising wound-healing potential due to their ability to generate ROS in



**Fig. 6.** *In vivo* wound healing efficacy study in mice. (a) Representative photographs capturing the wound healing process at specific timepoints (days 0, 6, 12, and 18) in different treatment groups: control (untreated), PdNPs-treated, and positive control (nitrofurazone 0.2% w/w ointment). (b) Simulation analysis photographs of wound healing with respective treatments, (c) Effect of treatments on wound contraction (mm<sup>2</sup>) over time across the treatment groups. (d) A comparison of the Percentage of wound healed between the PdNPs, positive control compared to control with  $p < 0.05$  statistically significant difference in comparison with untreated group.

controlled amounts (Mubarak-Ali et al., 2023), which act as signaling molecules to promote angiogenesis and fibroblast migration (Liu et al., 2024). Additionally, PdNPs modulate oxidative stress by balancing ROS levels, creating an optimal environment for tissue repair. They can also initiate key signaling mechanisms such as PI3K/AKT and MAPK (Kumar and Sood, 2020), which regulate keratinocyte proliferation and migration, as well as upregulate vascular endothelial growth factor (VEGF) to enhance angiogenesis and collagen synthesis (Khosravi and Khamari, 2019). These mechanisms make PdNPs a valuable candidate for wound-healing applications, particularly when incorporated into advanced nano scaffolds or drug delivery systems.

Compared to other nanoparticles, PdNPs are uniquely advantageous in their recyclability and catalytic properties, which can be leveraged for sustained therapeutic effects (Liu and Zheng, 2017). AgNPs excel in antimicrobial activity, disrupting microbial membranes and generating ROS (Rai et al., 2009), while AuNPs are highly biocompatible and effective in promoting keratinocyte growth and angiogenesis at low concentrations (Dykman and Khlebtsov, 2012). Cerium oxide nanoparticles (CeO<sub>2</sub> NPs) function as redox modulators, scavenging excessive ROS and activating angiogenic pathways such as hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) (Sreejith et al., 2016). Although PdNPs may require functionalization to enhance their antimicrobial efficacy, they provide a versatile and cost-effective alternative for wound-healing applications due to their superior catalytic efficiency and balanced ROS modulation (Zhang and Liu, 2019).

**Table 5.**

A comparison of wound healing activity of biosynthesized PdNPs alongside various other types of nanoparticles.

| Nano-particles     | Types of wound healing agents | Dosages/concentration | Wound contraction (%) | Exposure to wound area | References              |
|--------------------|-------------------------------|-----------------------|-----------------------|------------------------|-------------------------|
| PdNPs              | Ointment                      | 1% w/w                | 74.76                 | 10 days                | Present study           |
| AgNPs              | Hydrogel                      | 30% w/v               | 60.42                 | 10 days                | Chinnasamy et al., 2021 |
| AuNPs              | Ointment                      | 30 $\mu$ g/kg         | 80.00                 | 14 days                | Soliman et al., 2022    |
| PEG coated ZnO-NPs | Spray                         | 10 mg /500 $\mu$ L    | 89.00                 | 10 days                | Shoukani et al., 2024   |

w/w: Weight/Weight, w/v: Weight/Volume, PEG: Polyethylene glycol.

A comparison of wound healing activity of biosynthesized PdNPs alongside various other types of nanoparticles, including AgNPs (Chinnasamy et al., 2021), AuNPs (Soliman et al., 2022) and ZnO-NPs (Shoukani et al., 2024), has been provided in Table 5. This comparison underscores the significant wound healing properties demonstrated by the biosynthesized PdNPs, showcasing their potential use in wound healing dressing.

Conventional nanoparticle synthesis methods often use toxic solvents, require high energy input, and generate hazardous byproducts, endangering the environment and human health. Eco-friendly synthesis methods using plant extracts or green chemistry present a promising alternative to reduce environmental costs and have the prospective to lead to more economical and ecologically sustainable production in the long run. MNPs, including silver, gold, and zinc oxide, present significant safety concerns primarily because of their small size and distinct physicochemical features. These nanoparticles can easily penetrate biological barriers such as cell membranes, leading to potential cytotoxic effects. This cytotoxicity can stem from various mechanisms, including ROS production, which contribute to oxidative stress within cells. Oxidative stress can disrupt cellular homeostasis, leading to inflammation as the immune system responds to the perceived threat of these foreign particles.

In Table 6, a comparison is made between the safety, sustainability, and cost-effectiveness of biosynthesized PdNPs and various other types of nanoparticles, including AgNPs (Bharathi et al., 2024), AuNPs (Khan et al., 2024), PtNPs (Shabani et al., 2023), CuO-NPs (Khairy et al., 2024), and ZnO-NPs (Anehia et al., 2022). This comparison highlights the biosynthesis method for producing PdNPs using aqueous leaf extract from *P. amboinicus*, which is not only sustainable and more economical but also uses less time and energy compared to the synthesis of other nanoparticles. Oxidative stress may result from an excess of ROS production, impairing the cells' ability to maintain normal physiological redox-regulated functions. This disruption to cell function and development can result in oxidative modifications of proteins, the generation of protein radicals, DNA strand breaks, and nucleic acid modifications, ultimately leading to cell death and genotoxic effects. Additionally, in contrast to other metal and metal oxide nanoparticles, biosynthesized PdNPs demonstrate lower toxicity toward *E. coli* bacterial cells. The toxicity is affected by the type of nanomaterial, its shape, size, surface charge, and the specific bacterial strain involved, as shown in Table 6.

#### 4 Conclusion

In conclusion, this study showcases the successful green and bio-inspired synthesis of PdNPs using biogenic reduction through aqueous leaf extract of *P. amboinicus*. Numerous characterization methods were utilized, including UV-Vis spectroscopy, FTIR, FESEM, EDS, high-resolution TEM, XRD, and zeta potential analysis. The synthesized PdNPs demonstrated the structural and morphological properties of the PdNPs, while biological assays demonstrated their multifunctional therapeutic potential, including significant antibacterial activity. Notably, enhanced wound healing efficacy in

**Table 6.**  
A comparison of sustainability cost-effectiveness and safety for metal and metal oxide nanoparticles.

| Nanoparticles | Sustainability                                                     | Cost-effectiveness  | Safety                 |                            |                   |                                         | References                            |
|---------------|--------------------------------------------------------------------|---------------------|------------------------|----------------------------|-------------------|-----------------------------------------|---------------------------------------|
|               | Synthesis method/<br>Biological entity                             | Reaction conditions | (ROS-related toxicity) |                            |                   |                                         |                                       |
|               |                                                                    |                     | Average size (nm)      | Shape of particles         | Dosages/<br>Conc. | Inhibition zone (mm) for <i>E. coli</i> |                                       |
| PdNPs         | Green Synthesis / <i>Plectranthus amboinicus</i> leaf extract      | 80°C, 30 min.       | 21.31                  | Triangular and rectangular | 100 µg/mL         | 14.03                                   | Present study                         |
| AgNPs         | Green Synthesis / kiwi fruit peel extract                          | 27°C, 3 h           | 10-70                  | Cubic                      | 50 µg/mL          | 16.00                                   | <a href="#">Bharathi et al., 2024</a> |
| AuNPs         | Green Synthesis / <i>Callistemon viminalis</i> extract             | 80°C, 24 h          | 100                    | Circular                   | 0.2 mg/mL         | 11.50                                   | <a href="#">Khan et al., 2024</a>     |
| PtNPs         | Green Synthesis / <i>Penicillium pinophilum</i> cell-free filtrate | 25°C, 24 h          | 2–30                   | Spherical                  | 100 µg/mL         | 19.20                                   | <a href="#">Shabani et al., 2023</a>  |
| CuO-NPs       | Green Synthesis / <i>Azadirachta indica</i> leaf extract           | 30°C, 24 h          | 30.9                   | Semispherical              | 100 µg/mL         | 19.00                                   | <a href="#">Khairy et al., 2024</a>   |
| ZnO-NPs       | Green Synthesis / pomegranate peel aqueous extract                 | 25°C, 90 min.       | 30.34                  | Spherical                  | 100 mg/mL         | 27.00                                   | <a href="#">Anehia et al., 2022</a>   |

ROS: Reactive oxygen species.

laboratory mice was observed within a period of 14 days emphasizing their suitability for diverse biomedical applications. Moving forward, future research may focus on conducting in-depth *in vivo* studies to further validate the mechanistic activities of PdNPs and their potential in drug delivery. This study offers an excellent starting point for scientists interested in the field of nanobiotechnology and nanomedicine, providing insight into nanocarrier systems and drug delivery metabolism.

The future prospects of biosynthesized PdNPs for wound healing are highly promising due to their ability to modulate oxidative stress, enhance angiogenesis, and promote cellular proliferation through ROS-mediated signaling pathways. Advancements in functionalization and integration with biomaterials could further optimize their therapeutic efficacy, biocompatibility, and targeted delivery, paving the way for innovative wound-care solutions.

**CRediT authorship contribution statement**

**Ashwini Singhal:** Investigation, Formal analysis, Writing – original draft, Visualization. **Gyan Prakash Meghwal:** Investigation, Formal analysis. **Apurva Jaiswal:** Investigation, Formal analysis. **Neha Kaushik:** Investigation, Formal analysis. **Anita Kumari:** Investigation, Formal analysis. **Nighat Fahmi:** Investigation, Validation, Formal analysis. **Rizwan Wahab:** Writing – review, editing & Funding acquisition. **Dev Dutt Patel:** Investigation, Validation, Formal analysis. **Abdulaziz A. Al-Khedhairy:** Writing – review, editing & Funding acquisition. **Priyadarshi Meena:** Supervision, Resources, Writing – review & editing. **Nagendra Kumar Kaushik:** Supervision, Resources, Writing – review, editing & Funding acquisition. **Ramhari Meena:** Conceptualization, Methodology, Supervision, Resources, Writing – review & editing, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Declaration of Generative AI and AI-assisted technologies in the writing process**

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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