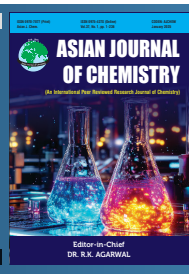
Asian Journal of Chemistry; Vol. 37, No. 6 (2025), 1496-1500

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33850



Synthesis, Characterization and Antimicrobial Activity of *Bombax ceiba* Fruit Extract based Palladium Nanoparticles

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Received: 10 April 2025; *Accepted*: 22 May 2025; *Published online*: 27 May 2025; AJC-22023

The synthesis of nanoparticles using different parts of plant extracts offers a cost-effective, efficient and environmental friendly option for applications in biomedical research, the environment and energy. This work presented a way to fabricate palladium nanoparticles (Pd NPs) using *Bombax ceiba* fruit extract in the non-calcined and calcined formed. Pd NPs have been applied as antimicrobial agents against a number of bacterial and fungal species after being investigated using XRD, FTIR, SEM, TEM and EDX methods. The synthetic approach was found to be cost-effective, efficient and safe. The antimicrobial study was conducted for both non-calcined as well as calcined Pd NPs. The findings of the antibacterial investigation demonstrated that Pd NPs were particularly effective against *A. niger* and *B. subtilis,* with zones of inhibition of 11.4 and 20.3 mm, respectively. The effectiveness of Pd NPs against bacterial and fungal species was found to be in the order of *B. subtilis* > *E. coli* > *S. aureus* and *A. niger* > *C. parapsilosis*, respectively.

Keywords: Palladium nanoparticles, *Bombax ceiba*, Antimicrobial study.

INTRODUCTION

The study of the fabrication, characterization and applications of materials and technologies at the nanoscale is known as nanotechnology. The basic characteristics of materials may be altered at the nanoscale to satisfy specific demands [1]. Globally and especially in industrialized nations, nanotechnology is quickly taking over a number of sectors [2]. The inorganic metallic nanoparticles have showed many improved physical, biological and chemical properties in different fields of science and technology [3,4]. Metal-based nanoparticles can be produced through physical, chemical and biological methods, which typically fall into top-down and bottom-up approaches. Physical vapour deposition, lithography, mechanical machining and thermal vapour deposition are examples of top-down methods, while sol-gel, chemical vapour deposition, microwave, hydrothermal and sonochemical are examples of bottom-up methods [5-7].

Among the metallic nanoparticles, palladium nanoparticles (Pd NPs) show many advanced physico-chemical properties, including excellent electronic, optical and plasmonic properties and strong chemical and thermal stability [8-10]. These nanoparticles can be easily synthesized and are less expensive. Palladium nanoparticles are particularly used in fuel cells, catalysis, hydrogen storage and sensors.

Green synthetic methods used in the synthesis of inorganic nanoparticles have many advantages such as being eco-friendly, simple, releasing fewer harmful chemicals and requiring less sophisticated equipment. The plant extract in this method is used for capping, reduction and stabilization [11,12]. *Bombax ceiba* is also known as Kapok tree, Moca and Semal. It belongs to the Bombacaceae family and has various medicinal uses in Indian traditional systems like Ayurveda, Siddha and Unani. Its pharmacological applications include antioxidant, antimicrobial, antiobesity, hypoglycemic and heptoprotective activities [13,14]. Thus, motivated with the enormous applications and green chemistry consideration, we aimed to synthesize Pd NPs from using *Bombax ceiba* fruit extract, characterized and finally evaluated as antimicrobial agent.

EXPERIMENTAL

Palladium(II) chloride, sodium hydroxide, Mueller-Hinton agar (MHA), ethanol, sabouraud dextrose agar (SDA) and double distilled water were used in this work.

Synthesis of palladium nanoparticles: The fresh *Bombax ceiba* fruits were collected, washed with distilled water and allowed to dry in sun for 2 weeks. The dried fruits were crushed into small pieces and ground into fine particles. In conical flask, 100 mL of double-distilled water was added with 5 g of finely powdered *B. ceiba* fruit. The mixture was kept at 70 ºC for 40 min while being continuously stirred on a magnetic stirrer. After adding 1 mL of ethanol, it boiled for a further 35 min. Filter the content once it has cooled to room temperature, then utilized the filtrate as an extract for the synthesis of Pd NPs. On mixing 100 mg of $PdCl_2$ with 100 mL of double-distilled water, the mixture was agitated for 30 min. After 30 min, 10 mL of extract was added and it was agitated for another 30 min at 60 ºC. After 30 min, allow it to cool to room temperature and added a few drops of NaOH. Again, stirred it for 30 min and after centrifugation (10000 rpm for 15 min) and complete washing with double-distilled water, the residue was collected and dried in hot air over. The powder of Pd NPs was then calcined 350 ºC for 2 h. Both calcined and non-calcined samples of Pd NPs were preserved in airtight bottles for characterization and antimicrobial studies. The characterization of Pd NPs was done using FTIR, TEM, EDX and XRD techniques.

Characterization: The Pd NPs was characterized using X-ray diffractometer (XRD; Rigaku, smart lab 2), scanning electron microscopy (SEM; Zeiss), energy dispersive X-ray spectroscopy (EDX; Zeiss), Fourier transform infrared spectroscopy (FTIR; Thermo Scientific iD1 transmission) and transmission electron microscopy (TEM; JEOL/JEM 2100) techniques.

Antimicrobial activity: Pd NPs were studied for their antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli via* the disc diffusion method [15]. A 20 mL of liquid media (MHA) was introduced on the sterilized petri plates and then solidified. The bacterial culture was spread on these plates and Pd NPs loaded on discs. Ciprofloxacin was used as a positive control. The petri dishes were incubated at 37 ºC for 24 h and after that the discs' zones of inhibition were examined. The antifungal studies was conducted against *Aspergillus niger* and *Candida parapsilosis*. A 20 mL of liquid media (SDA) was introduced on the sterilized petri plates. After solidification, the fungal culture was spread over these plates and Pd NPs loaded on discs. Amphotericin B (50 mg) was selected as positive control. For 48 h, the plates were incubated at 37 ºC. Observations of the zones of inhibition were made after this incubation period.

RESULTS AND DISCUSSION

FTIR spectral studies: The characteristic functional groups on the surface of Pd NPs were determined using FTIR technique. Fig. 1 depicts the FTIR spectra of Pd NPs (calcined and non-calcined). The characteristic FTIR peaks of non-calcined Pd NPs were obtained at 3358, 2883, 1637, 1451, 1043 and 583 cm^{-1}. These peaks are related to O-H (*str.*), $-CH_2$ (*str.*), C=C (*str.*), $-CH_2$ (scissoring), C-O (*str.*) and Pd-C or Pd-O bonds. Similarly, the FTIR peaks of calcined Pd NPs were obtained at 3372, 2881, 1444, 880, 655 and 595 cm^{-1}. These peaks are also related to O-H (*str.*), $-CH_2$ (*str.*), $-CH_2$ (scissoring), C-O (*str.*), Pd-C or Pd-O bonds, respectively [16-18].

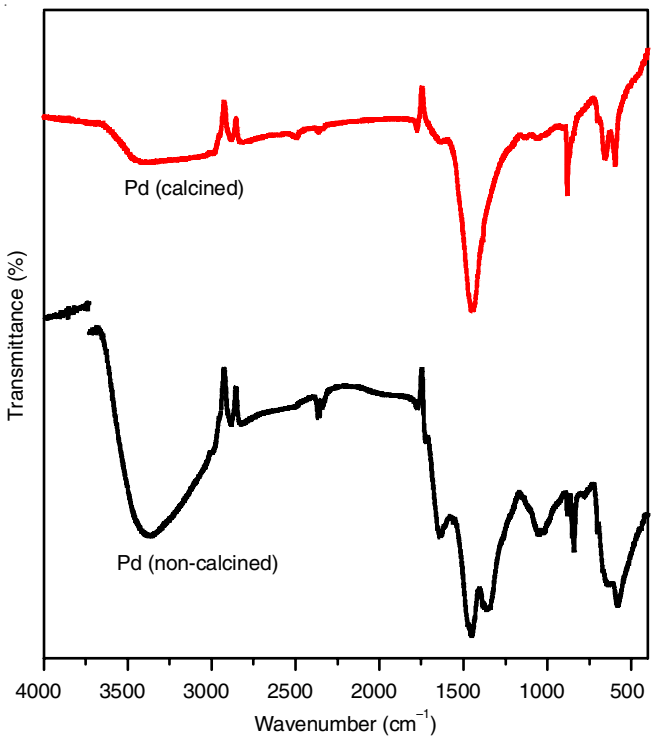

Fig. 1. FTIR spectra Pd NPs (non-calcined and calcined)

XRD studies: The XRD patterns of calcined and non-calcined Pd NPs based on *B. ceiba* fruit extract are illustrated in Fig. 2. The XRD peaks of non-calcined Pd have been assigned at 2θ = 34.2º, 40.1º, 46.5º and 68.01º. These all peaks are relevant to the 002, 111, 200 and 220 planes, respectively. The XRD peaks of calcined Pd NPs were obtained at 2θ = 34.1º, 40.1º, 46.6º, 54.7º, 60.2º and 71.4º, respectively. These peaks correspond to 002, 111, 200, 110, 112 and 211 planes [19-23].

Morphological studies: The morphological analysis of *B. ceiba* fruit extract-based non-calcined and calcined Pd NPs were performed using the SEM technique. Fig. 3a-b depict the SEM images of non-calcined and calcined Pd NPs. A thread like morphology of non-calcined Pd NPs has appeared, which transformed into irregular as well as semi-sphere morphology after calcination. Fig. 4 depicts TEM images of calcined Pd NPs, which also indicate irregular as well as semi-sphere morphology of calcined Pd NPs. The elemental composition of Pd NPs was observed *via* the EDX spectra and the elemental composition of these nanoparticles is also presented in Fig. 5.

Antimicrobial activity: Metal-based nanoparticles offer long-term antibacterial properties as well as the ability to differentiate bacterial cells from mammalian cells. Through different ways such as generation of ROS (reactive oxygen species), DNA damage, disturbances in nutrient uptake, metal ion release, destruction of cell membrane, *etc.* metal and metal oxide nanoparticles exert their antimicrobial behaviour against pathogenic

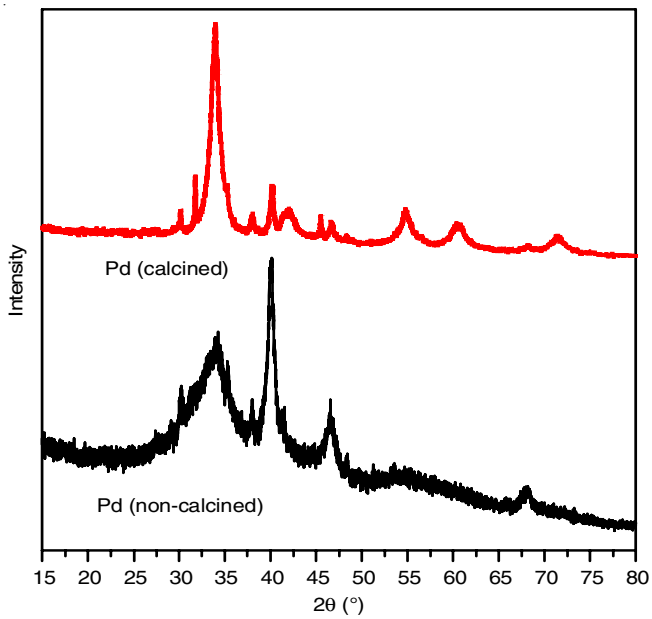

Fig. 2. XRD patterns of Pd NPs (non-calcined and calcined)

microbial cells [24-26]. Palladium nanoparticles exhibits the antimicrobial and anticancer activities due to the mechanical action of Pd^{2+} ions against different fungal and bacterial species. Pd NPs can bind with cell walls and disrupt cell growth and division [27].

The antibacterial activity of both calcined and non-calcined Pd NPs is illustrated in Fig. 6a-b. It has been confirmed that each bacterial and fungal strain has a minimum inhibitory concentration (MIC) of 5 mg/mL. The zones of inhibition were observed to be 6.0 mm for *E. coli*, 5.2 mm for *B. subtilis* and 5.1 mm for *S. aureus* at a concentration of 5 mg/mL of non-calcined Pd NPs. The results increased to 8.7 mm, 6.8 mm and 7.5 mm at 25 mg/mL, respectively. *E. coli*, *B. subtilis* and *S. aureus* all showed maximal zones of inhibition at 100 mg/mL, measuring 10.8 mm, 9.6 mm and 10.7 mm, respectively (Fig. 6a). In a comparable manner, the zones of inhibition against *E. coli*, *B. subtilis* and *S. aureus* were 2.1, 4.6 and 1.7 mm at the initial dosage of 5 mg/mL of calcined Pd NPs; these increased to 3.6, 7.8 and 4.1 mm at 25 mg/mL. At 50 mg/mL, the zones of inhibition against *E. coli*, *B. subtilis* and *S. aureus*

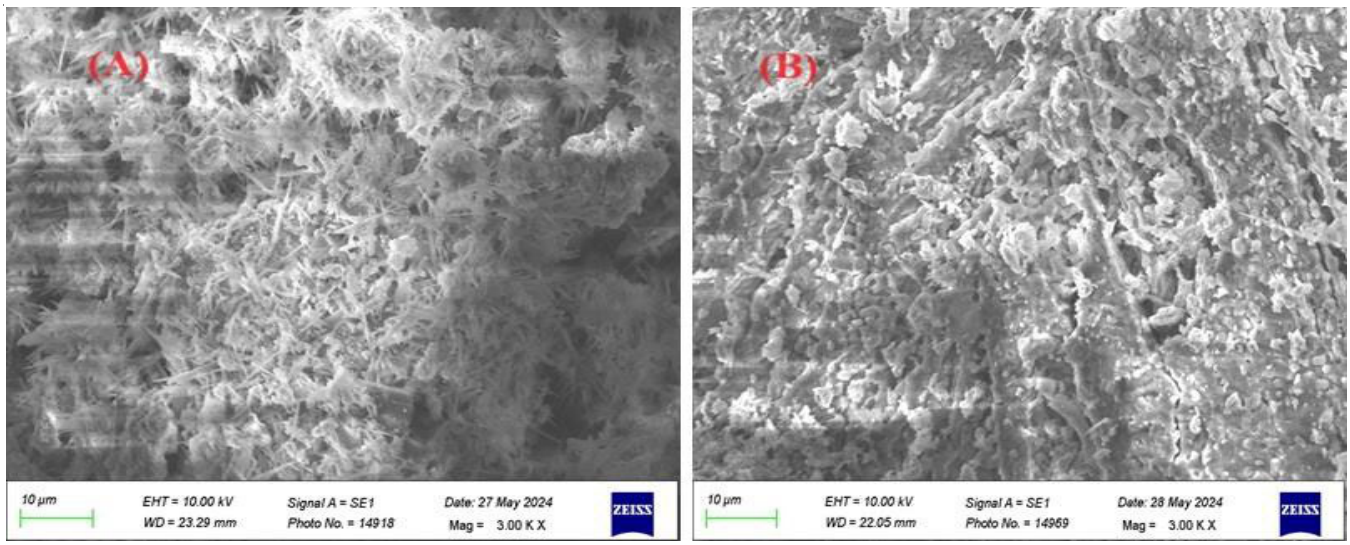

Fig. 3. SEM images of Pd NPs (a) non-calcined and (b) calcined

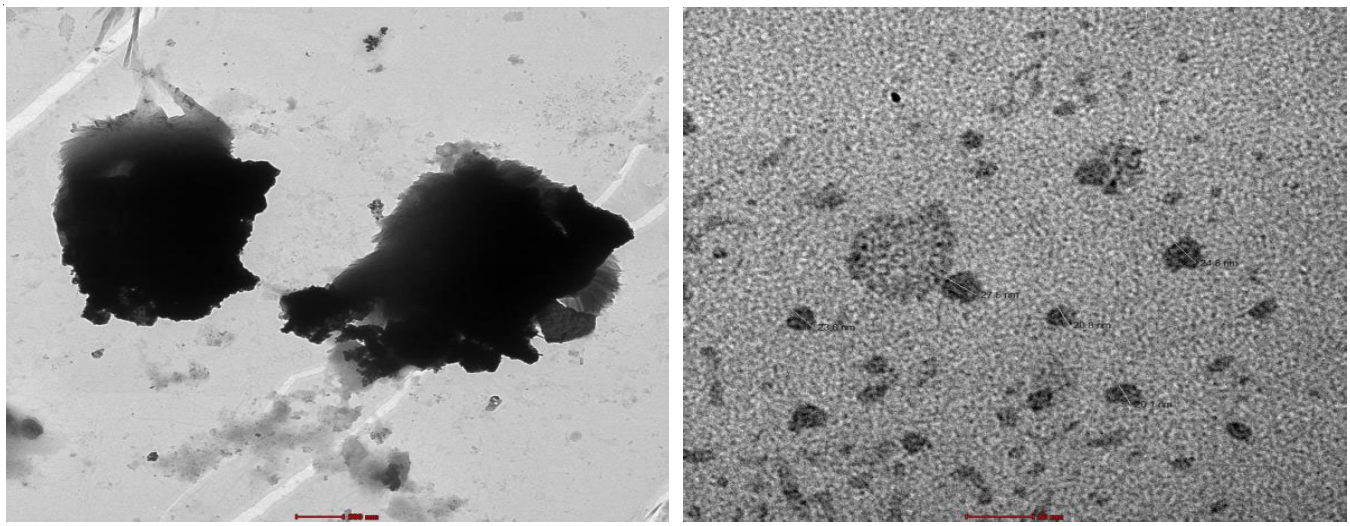

Fig. 4. TEM images of calcined Pd NPs

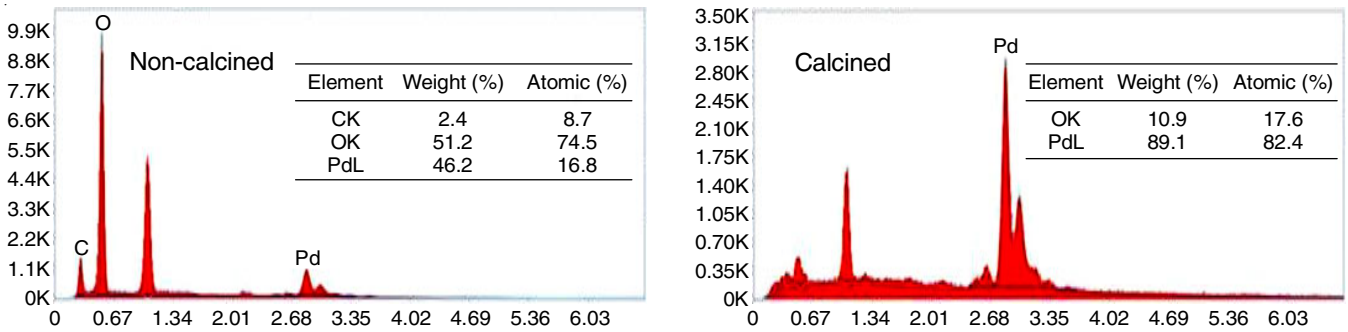

Fig. 5. EDX spectra and composition of Pd NPs

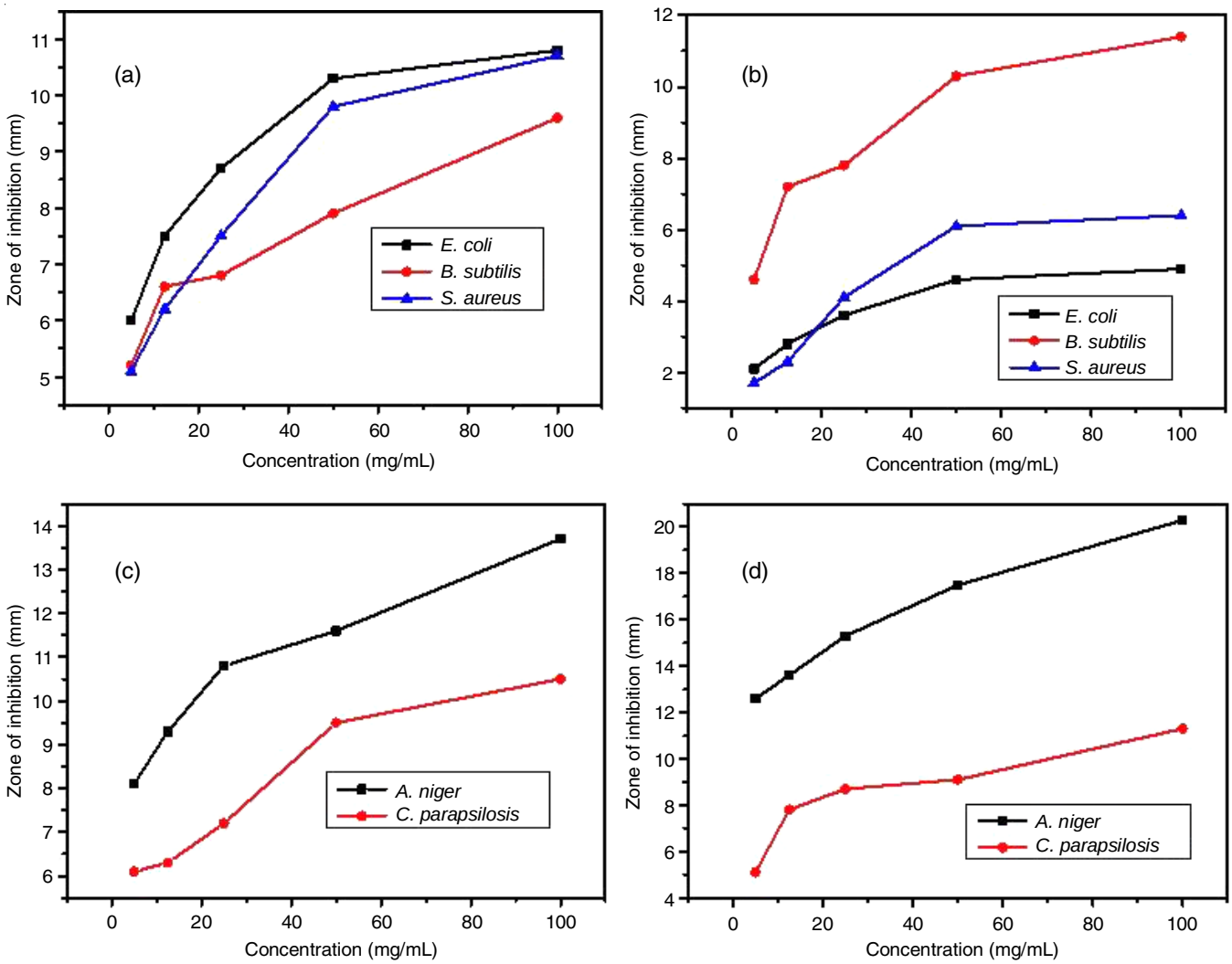

Fig. 6. (a & b) antibacterial activities and (c & d) antifungal activities of Pd NPs (non-calcined and calcined)

were 4.6, 10.3 and 6.1 mm, respectively. At 100 mg/mL, the highest zones of inhibition were found to be 4.9, 11.4 and 6.4 mm (Fig. 6b).

Metal-based nanoparticles react against fungi by gene regulation, releasing ions and generating oxidative stress. Fig. 6c-d illustrates the antifungal activity of both calcined and non-calcined Pd NPs. Both calcined and non-calcined Pd NPs had a MIC of 5 mg/mL. Zones of inhibition against *A. niger* and *C. parapsilosis* were 8.1 and 6.1 mm at 5 mg/mL for non-calcined Pd NPs, which increased to 10.8 and 7.2 mm at 25 mg/mL. At 100 mg/mL, the zones of inhibition were calculated to be 13.7 and 10.5, respectively (Fig. 6c). At a dosage of 5 mg/mL, the zones of inhibition for *A. niger* and *C. parapsilosis* were determined to be 12.6 and 5.1 mm, respectively, for calcined Pd NPs. The results were 15.3 and 8.7 mm at 25 mg/mL. At 100 mg/mL, the highest zones of inhibition were measured as 20.3 and 11.3 mm (Fig. 6d).

The comparison of Pd NPs with other materials is presented in Table-1 and found to be more effective as antimicrobial agent as compared to other reported metallic nanoparticles.

TABLE-1
COMPARISON OF PALLADIUM NANOPARTICLES WITH OTHER NANOPARTICLES

Nanoparticles	Zones of inhibition (mm)	Bacterial/fungal species	Ref.
Cr_2O_3	10, 10 and 11	*Bacillus cereus*, *S. aureus* and *E. coli*	[28]
CuO	14, 10, 15 and 12	*E. coli*, *Pseudomonas aeruginosa*, *B. subtilis* and *S. aureus*	[29]
Ag	7.33, 13.33 and 8	*S. aureus*, *P. aeruginosa* and *Streptococcus mutans*	[30]
Ag_2O	7, 8, 9 and 11	*B. subtilis*, *S. mutans*, *S. aureus* and *E. coli*	[31]
Pd	20.3, 11.3, 4.9, 11.4 and 6.4	*A. niger*, *C. parapsilosis*, *E. coli*, *B. subtilis* and *S. aureus*	Present study

Conclusion

The *Bombax ceiba*-based palladium nanoparticles (Pd NPs) was synthesized as an efficient antimicrobial agents. Various characterization methods to analyze the physico-chemical properties of Pd NPs, including their morphological characteristics, crystallinity and bonding type on their surface. The experimental data indicated that non-calcined Pd NPs were found effective for bacterial strains while calcined Pd NPs were effective for fungal strains. After incubation periods, the maximum zones of inhibition were found as 11.4 and 20.3 mm against *B. subtilis* and *A. niger*.

ACKNOWLEDGEMENTS

The present work was supported by the funded project (UCS&T/R&D-12/23-24/24556) by the Uttarakhand State Council for Science and Technology (UCS&T), India. The authors are thankful to Department of Chemistry, Graphic Era (Deemed to be University), Dehradun (India) for support and encouragement during the experimental work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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