



Biocompatible Synthesis of Palladium Nanoparticles and Their Impact on Fungal Species

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ABSTRACT

In recent years the utilization of secondary metabolites from plant extract has emerged as novel technology for the synthesis of various nanoparticles. In this manuscript synthesis of palladium nanoparticles (PdNPs) by using *Terminalia bellirica* fruit extract as reducing agent and capping agent. The synthesized PdNPs were characterized by using UV-visible spectroscopy. The particle morphology, distribution and size were characterized by HRTEM and particle size analyzer, and the particle size is found to be 30–45 nm, phase purity of synthesized PdNPs were characterized by X-ray diffraction patterns. The active bio molecules that are responsible for the synthesis and capping of PdNPs were characterized by Fourier transformed infrared (FTIR) spectroscopy. The stability of palladium nanoparticles were characterized by zeta potential. We also studied the comparison of EDS spectrum of synthesized PdNPs and *Terminalia bellirica* fruit extract, and its anti-fungal activity.

1. Introduction

Noble metal nanoparticles as silver, platinum, gold and palladium exhibits exceptional properties in the field of physical, chemical, optical and thermo dynamical properties in the nano scale [1, 2]. Because of these exceptional properties, metallic nanoparticles retain many applications in the field of catalysis, sensors [3], magnetic recording [4], photovoltaic cells [4], luminescent probes [5], biotechnology and in drug delivery [6]. Of all noble metal nanoparticles palladium nanoparticles are having an extensive application attributable to their high surface to volume ratio [7, 8]. At this moment in time PdNPs are synthesized by various methods such as sono chemical [9], electrochemical [10], polyol reduction [11], microwave assisted [12] and template assisted [13] methods. Nevertheless extensive use of hazardous chemicals in these processes has raised serious concerns regarding the possible adverse effect of the chemically synthesized metal nanoparticles on the environment and living cells [14]. To overcome this problem researchers have focused on the development of sustainable methods for the synthesis of metallic nanoparticles, bio fabrication of metal nanoparticles is one of the sustainable method.

Currently, increasing environmental concerns necessitate the development of new and eco-friendly techniques for the synthesis of nanoparticles. Accordingly intense research has been directed to the biosynthesis of nanoparticles using “nature factory” (viz. plants and microorganisms) [15–17], which are intrinsically greener. Various microorganisms such as bacteria, fungi, and yeasts have been suggested as nanofactories for synthesizing metal nanoparticles. But, the use of plants for the fabrication of nanoparticles has drawn the attention of researchers as a rapid, low cost, eco-friendly and a single step method for the biosynthesis process [18].

Biosynthesis of PdNPs is at emerging phase; sparingly few reports are available for the synthesis and capping agents of PdNPs using biological resources. The few recently reported biological resources for PdNPs synthesis by leaf extract of *Piper betel* [19], leaf extract of *Artemisia annua* (Sweet wormwood) [20], Sour Cherry tree Gum [21], leaf extract of *Hippophae rhamnoides* Linn [22], leaf extract of *Anacardium occidentale* [23]. In recent years bio synthesized PdNPs have been explored for various applications such as catalytic activity for the Suzuki–Miyauracoupling in water [22], catalytic activity by hydrogenation reaction for organic solvent

degradation [24], catalytic application in the ligand and copper free sonogashira coupling reaction under aerobic conditions [21] and antifungal activity [19]. There have been vast amount of applications are available at bio synthesized PdNPs.

Terminalia bellirica is commonly known as “Bahera” or Beleric or bastard myrobalan. It belongs to Combretaceae family, it is native of throughout deciduous forests of India. The main phyto constituents present in fruits are beta-sitosterol, gallic and ellagic acids, ethyl gallate, galloyl glucose, chebulagic acid and cardiac glycoside, bellaricanin. The ayurveda pharma-copoeia of India recommends the drug in powder form in emesis and worm infestation; in addition to other therapeutic applications the fleshy fruit pulp contains 21.4% tannin, both condensed and hydrolysable types [25].

At present scenario we synthesized PdNPs by using *Terminalia bellirica* fruit extract which is act as a reducing agent and capping agent. We are observed effect of temperature for the synthesis of PdNPs. The stability of PdNPs is observed by using zeta potential, the comparison of EDS confirms the elemental compositions that are mainly bounded on the surface of palladium nanoparticles. We are also studied anti-fungal study of palladium nanoparticles.

2. Experimental Methods

2.1 Materials

Terminalia bellirica were collected from Palakondalu hills, Kadapa, Andhra Pradesh, India. Palladium chloride (PdCl₂) was purchased from Sigma Aldrich Corporation (St. Louis, Mo, USA), throughout the experiment double distilled water was used.

2.2 Preparation of TB Fruit Extract

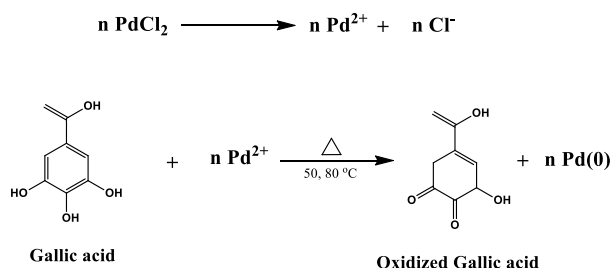
Aqueous extract of *Terminalia bellirica* was prepared by using freshly collected fruits from Palakondalu hills, Kadapa, Andhra Pradesh, India. The surface of the fruits was cleaned with double distilled water. These fruit pulp was separated and dried up to 10 days at room temperature under shade, we used two layered plastic mesh to avoid dust particles. Five grams of fruit pulp was pulverized with mortar and pestle. This fine powder was taken into 250 mL conical flask with 100 mL of double distilled water and then boiled up to 30 min under magnetic stirrer with constant stirring. The extract was filtered through Whatman No.1 filter paper. The filtrate was collected and then refrigerated for future use.

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2.3 Synthesis of Palladium Nanoparticles

The aqueous solution of 0.01 M palladium chloride (PdCl_2) was standardized in 100 mL of standard flask. 10 mL of the plant extract was mixed with 90 mL of Palladium Chloride solution under magnetic stirring at room temperature for the reduction of Pd^{+2} to Pd^0 , until the color changed from light yellow to brown. The appearance of brown color indicates formation of Palladium nanoparticles (PdNPs). This colloidal solution SPR (Surface Plasmon Resonance) is monitored at different temperature conditions (35, 50, 80 °C) by using UV-Visible spectrophotometry. The content was washed with double distilled water thrice by repeated centrifugation at 8000 rpm for 15min. The centrifuged PdNPs taken into petri plate, dried over night at 60 °C. Dried nanoparticles were collected, sealed and stored properly to further characterization and application. The hypothetical mechanism is shown in Scheme 1.



Scheme 1 Hypothetical mechanism showing formation PdNPs by the reduction of TB fruit extract which are contain contained polyphenols

2.4 Characterization of Palladium Nanoparticles

UV-Visible spectra were recorded as function of reaction time by Shimadzu-UV 1800 spectrophotometer operated at resolution of 1 nm. Fourier transform infrared spectroscopy (FTIR) spectrum of the sample was recorded by Perkin-Elmer, Two Model FT-IR spectrometer. The FTIR ranged from 4000 to 500 cm^{-1} at resolution of 4 cm^{-1} by making a KBr pellet with PdNPs and dried *Terminalia bellirica* fruit extract. The structure and morphology of the samples were characterized by powder X-ray diffractometry (XRD) Bruker D8 using $\text{CuK}\alpha 1$ (1.5406 Å) and $\text{K}\alpha 2$ (1.54439 Å) radiations, morphologies of as obtained products were studied with High resolution Transmission electron microscope (HR-TEM) measurements were performed on JEOL 3010 instrument operating at 200 kV energy dispersive X-ray spectroscopy (EDS) using a (SUPRA 55) -CARL ZEISS instrument operating at 5 to 20 kV. Particle size and zeta potential of PdNPs was measurement using HORIBA SZ-100.

2.5 Antifungal Activity of Palladium Nanoparticles

The palladium nanoparticles (PdNPs) synthesized from *Terminalia bellirica* fruit extract was tested for their antimicrobial activity by disc diffusion method against pathogenic organism like *Aspergillus niger*. The pure cultures of organism were sub cultured on sabouraud dextrose agar (SDA) plate. 5 mm discs were inserted on culture plates. Using micropipette, 50, 60, 70, 80 μg of the sample of nanoparticles solution were poured on discs. After incubation at 37 °C for 48 h, the different levels of zone of inhibition were measured.

2.6 EDS Comparison Study

The EDS comparison study done between dried *Terminalia bellirica* fruit extract (The 20 mL of fruit extract is dried with rotary evaporator by using Rotavapor R-215, and then after the compound is washed with methanol to dissolve dried compound in round bottom flask and the collected suspension is dried at 50 °C) and palladium nanoparticles. The EDS spectrums comparison between fruit extract and palladium nanoparticles is done with elemental composition data analysis. The data can give the clear idea about synthesis of palladium nanoparticles and which is capped on the surface of palladium nanoparticles.

3. Results and Discussion

The UV-Vis absorption spectra of the PdNPs were shown in Fig. 1. Absorption spectra of Pd^{+2} show an absorbance maximum at 420 nm. This broad peak disappears when time increases and the solution turns light yellow to brown color this indicates palladium ions formed into palladium nanoparticles, this indicates no absorption maximum for palladium nanoparticles. We are observed that the effect of temperature on palladium nanoparticles. At 35 °C there is no change in color this result

gives that PdNPs were not formed whereas at 50 and 80 °C color changes to brown in coloration clearly representing in Fig. 1 and the UV-Vis absorption spectrum is also indicating that the 420 nm maximum is disappeared after complete reduction of palladium ions. It is clearly indicates that the temperature is affects for the synthesis mechanism.

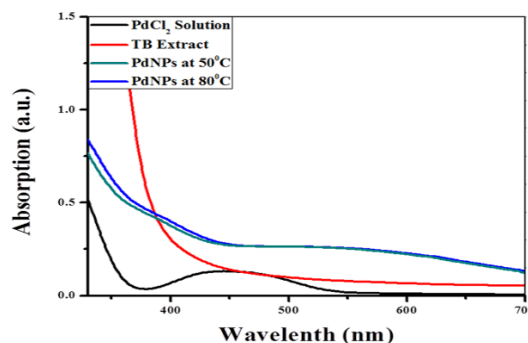


Fig. 1 UV-Visible spectra recorded as function of Temperature

To identify the functional groups of *Terminalia bellirica* fruit extract is responsible for the bio reduction of palladium chloride. FTIR study was carried out for fruit extract and dried Palladium nano particles shown in Fig. 2. The FTIR Spectra of *Terminalia bellirica* fruit extract showed absorption bands at 3391 and 2926 cm^{-1} representing O-H and C-H stretching of polyols the absorption peak located at 1615 cm^{-1} represented C=O stretching vibrations. The stretching vibration present at 1447 and 1209 cm^{-1} represented -CH and C-O vibrations of polyols respectively. A small band at 1350 and 1039 cm^{-1} represented C-F and C-O stretching vibration. This band indicates polyphenols, tannins, terpenoids, flavonoids and protein compounds present in *Terminalia bellirica* fruit extract. The synthesized palladium nanoparticles are analyzed by FTIR Spectroscopy, clear and significant bands were observed at 3436, 2926, 1627, 1053 cm^{-1} . This represents O-H, C-H, and C=C, C-O stretching vibration indicates different polyol compounds adsorbed on the surface of palladium nanoparticles.

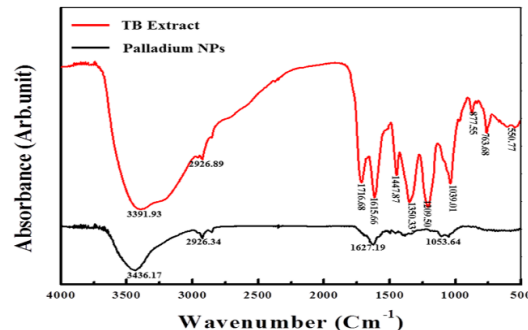


Fig. 2 FT-IR spectra of *Terminalia bellirica* fruit extract and palladium nanoparticles

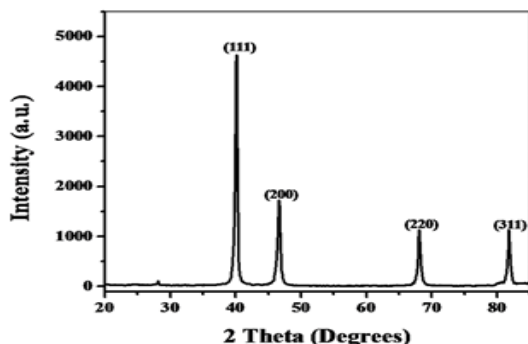


Fig. 3 XRD pattern of PdNPs synthesized by using *Terminalia bellirica* fruit extract

The structural determination of PdNPs was characterized with XRD patterns show that in Fig. 3. An XRD measurement is to determining the formation of new compound and phases. The phase compositions and crystal structure can be determined by comparison with report data (XRD) [26]. From the XRD patterns, strong Bragg's diffracted peaks were observed at 2θ values of 40.15, 46.61, 68.10, 81.80 that corresponds to the (111), (200), (220) and (311) Bragg reflections of face centered cubic (fcc) PdNPs. The resultant data matched with the database Joint Committee on

Powder Diffraction Standards (JCPDS) file no. 00-046-1043 [26]. No extra diffraction peaks in XRD pattern suggests that synthesized PdNPs were basically pure.

Fig. 4a and 4b displays the TEM and HRTEM images of PdNPs fabricated by using dried and crushed fruit at 80 °C. The particles were found to be triangular in shape with 30-45 nm range. The lattice fringes in the image are consistent planes of the fcc phase of metallic Pd, with a ~ 0.2344 nm periodicity. The white layer present around the palladium nanoparticles were observed that the phyto constituents of pseudo tannins that are present in *Terminalia bellirica* fruit extract function as capping agent.

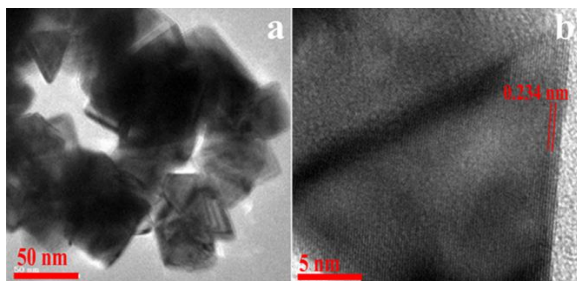


Fig. 4 (a) TEM image of PdNPs and (b) HRTEM image of PdNPs

The comparison study with energy dispersive spectroscopy (EDS) for dried PdNPs were shows the strong signal for Pd where as weak signal for carbon and oxygen in Fig. 5a and the elemental composition are represented in Table 1. Carbon and oxygen elements are showed in EDS that can have originated from the bio molecule bound to the surface of the palladium nanoparticles. In the same manner EDS analysis study for *Terminalia bellirica* fruit extract (Fig. 5b) is also shows different peaks such as C, O, Mg, S, Cl, K and Ca. But mainly C, O shows distinct peaks that are shown in PdNPs these results shows that palladium ions are affect by fruit extract and the phyto constituents that are present in the extract is responsible for capping agent. The remaining elements are present at less amounts of micro elements that are present in plant sample.

Table 1 Elemental composition of *Terminalia bellirica* fruit extract and PdNPs characterized with EDS

No.	Element composition of TB fruit extract	Element composition of PdNPs	Weight (%) TB extr	Weight (%) PdNPs	Atomic (%) TB extr	Atomic (%) PdNPs
1.	C	C	41.24	3.32	51.16	16.43
2.	O	O	46.62	8.58	45.62	32.83
3.	Mg	Pd	5.62	88.20	0.92	50.74
4.	S		1.52		0.32	
5.	Cl		1.21		0.78	
6.	K		2.43		1.02	
7.	Ca		1.36		0.18	

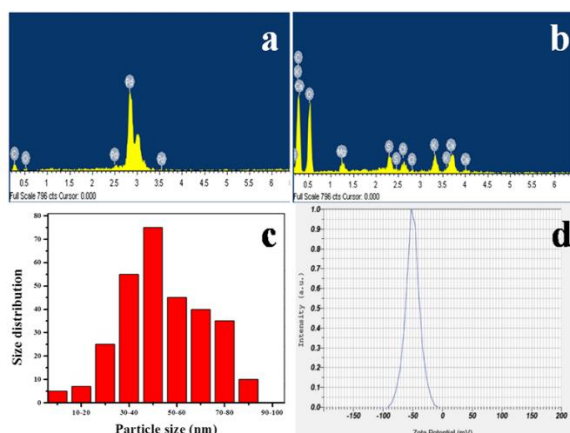


Fig. 5 (a) EDS spectrum for PdNPs (b) EDS spectrum for *Terminalia bellirica* fruit extract (c) Particle size determination of PdNPs and (d) Zeta potential determination of synthesized PdNPs

Particle size determinations of the synthesized PdNPs were shown under by size distribution. Histogram revealed that particles obtained are poly disperse mixture with the size ranging from 10 to 80 nm Fig. 5c. The

average diameter of the particles was found to be 30-45 nm. Fig. 5d shows the zeta potential of the synthesized PdNPs was determined in water as dispersant. The zeta potential was found to be -50.2 mV. The high negatives value confirms the repulsion among the particles and thereby increase in stability of the formulation.

The antifungal activity of green synthesized PdNPs was analyzed by disc diffusion method against *Aspergillus niger* [19]. The 72 hr grown fresh fungal culture was inoculated into the sabouraud dextrose agar (SDA) plate. 6 mm diameter disc was placed on SDA plate for disc diffusion method. PdNPs were dispersed in DMSO solution. In this method 5 μ L capacity discs with the concentration of the compound was designed which ranges from 50-80 μ g. The *Aspergillus niger* were spread on to the medium and the discs were placed and various volumes of PdNPs (50, 60, 70, 80 μ g) were added at the center of the disc. 5 μ L DMSO was added for negative control at the center of the plate and separate plate of 5 μ L of chloramphenicol is taken as positive control for the antifungal activity. The petri plates were incubated at 37 °C for 48 hr in which the antifungal activity was evidenced by the presence of a zone of inhibition (mm) around the disc; the results were shown in Fig. 6 and tabulated in Table 2.

Table 2 Inhibition zone of biosynthesized PdNPs against *Aspergillus niger*

Study no.	Pathogenic bacteria	Zone of diameter in mm (Mean of four replicates)
1.	Positive control (Chloramphenicol)	± 14
2.	Negative control (DMSO)	0
3.	<i>Aspergillus niger</i>	± 16

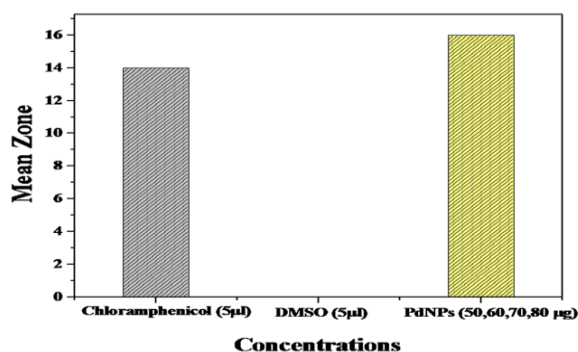


Fig. 6 Antifungal activity of PdNPs on *Aspergillus niger*. Here chloramphenicol as positive control and DMSO as negative control

4. Conclusion

In conclusion, we have demonstrated an eco-friendly, inexpensive and bio fabrication approach for the synthesis of PdNPs. *Terminalia bellirica* fruit extract as reducing agent and capping agent for the synthesis of PdNPs. Phenolic compounds present as the secondary metabolites were found to be responsible for reducing the palladium (II) to (0) valent ions. The shape of the PdNPs was found to be triangular and the size range at 30-45 nm. The zeta potential was found to be -50.2 mV. The synthesized PdNPs was studied for antifungal activity against the *Aspergillus nigeri* and showed good results. We concluded that the synthesis method we used is mostly strait, nontoxic and eco-friendly without side effects of human beings.

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