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Phyllanthus emblica seed extract mediated synthesis of PdNPs against antibacterial, heamolytic and cytotoxic studies

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ABSTRACT

Ecofriendly synthesis of Palladium nanoparticles (PdNPs) were achieved using *Phyllanthus emblica* (*P. emblica*) seeds as reducing agent. Further the ecofriendly synthesized PdNPs were subjected for various analytical techniques like UV–Vis, FT-IR, XRD, Zeta potential, SEM and TEM. The results indicated that green synthesized PdNPs were spherical in shape with average particle size of 28 ± 2 nm with moderate stability. Further the synthesized PdNPs and extract were subjected for its antibacterial studies against various disease causing pathogens by agar well diffusion method. Seed extract resulted in 8.9 ± 1.46 mm against *B. subtilis* and PdNPs showed 9.6 ± 1.10 mm against *S. aureus* and synthesized PdNPs and extract were tested for hemolytic which resulted in 20% and 10% respectively. Toxicity studies were done against *Artemia salina* (*A. salina*). The LC_{50} value of green synthesized *P. emblica* capped PdNPs and the *P. emblica* seed extract were found to be less toxic for *A. salina* with a value of 1.00 μg/mL and 1.25 μg/mL. In addition samples were checked for *in vitro* cytotoxicity assays on HeLa cell lines.

1. Introduction

In the last decade, nanoparticles plays a versatile and valuable role in many fields because of its interdisciplinary approach [1]. The transition metal nanoparticles are focused according to recent technology due to their high potency. Mostly biosynthetic methods of nanoparticle have been considered as preferable one, when compared to physiochemical methods [2]. Due to the eco-friendly and non-toxicity nature to the environment, our interest focused on biological approach of synthesis. Recently many scientists have been focused towards biosynthetic approach for the production of Ag, Pd, Au, Pb, *etc.*, nanoparticles [3]. The utilization of plant mediated nanoparticles is more advantageous over microorganisms, since the latter involves in maintaining the microbial culture in an aseptic conditions [4]. The physiochemical methods used often are toxic, costly and non-eco-friendly [5]. Hence the researchers focused their method of synthesis towards the bio-reduction using fungi, plants and microorganism for the greater stability of nanoparticles which are non-toxic and also utilized as organic catalysts [6–16]. Most of the researchers focused on several plants and plant parts for synthesis of nanoparticles and utilized for several applicational studies [17–31].

The PdNPs are most probably used in dehydrogenation reaction, C—H olefination and Suzuki cross coupling reaction [32]. Recently PdNPs were used in diagnostic applications as they get bound to single stranded DNA without damaging their structure [33]. Biosensor for glucose estimation can be done when the palladium nanoparticles are doped to chitosan-graphene [34]. Palladium nanoparticles can be synthesized from the extracts of coffee, tea and custard apple peels, as they were capable to act as reducing agents at room temperature. The extracts of different plants may contain different secondary metabolites for conversion of metal starting material to metal nanoparticles [35]. Modified electrodes of PdNPs with multi-walled carbon nanotubes were utilized for electro catalytic oxidation [36]. Different catalyst systems were examined for ammonia borane hydrolysis, among which palladium-rhodium is an efficient catalyst for the hydrogen generation [37]. Palladium is used to detect hydrogen as a sensor [38]. The main advantage of palladium is high affinity to the hydrogen adsorption [39]. In the sensor application different methods has been adapted to synthesize PdNPs.

The impact of co-poisoning can be reduced significantly and enhances the activity by the help of palladium catalyst [40]. According to colloidal spray deposition one of the fabrication methods used for palladium nanoparticles to achieve the size of the nanoparticles that ranges

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between 100 and 300 nm [41]. Palladium was mostly used in industrial applications such as catalytic and reduction of automobile pollutants [42].

In Phyllanthacea; *P. emblica* is one of the major fruits used for medical purposes [43]. The *P. emblica* plant species were widely present in Asian regions. The *P. emblica* fruit is mostly utilized in the field of Unani and ayurvedic [44]. Fruit has the nature of cooling capacity, when the body suffers from heat; it has high content of Vitamin C [45–47].

In this study, antibacterial activity has been tested for both seed extract and the synthesized *P. emblica* capped PdNPs by well diffusion method. The *in vitro* toxicity has been tested in blood by haemolytic activity [48]. To know the level of toxicity of the synthesized *P. emblica* capped PdNPs and the methanolic seed extract, toxicological studies have been done against brine shrimp (*Artemia salina*). *Artemia salina* is one of the aquatic organisms mostly used for toxicological studies nowadays. *A. salina* is more versatile and capable to adapt any kind of environmental conditions [49]. Cytotoxicity was assessed by MTT assay and further evaluated against the HeLa cell lines [50].

Keeping the above points in mind, the focus of this study is to synthesize non-toxic *P. emblica* capped PdNPs using *P. emblica* seeds extract and to evaluate their potential biological applications such as toxicity studies which includes haemolytic activity, toxicity towards *A. salina* and cytotoxicity assay against HeLa cell lines and the antibacterial activity.

2. Material and Methods

2.1. Materials

The fruits of *P. emblica* were collected from in and around at state of Tamilnadu, Vellore City, Ambur town and its co-ordinates are (12.78°N 78.7°E). Further by the help of botanist Dr. P. Jayaraman we authenticated the fruit and authenticated number is PARC/2014/2058. Palladium acetate was purchased from Sigma Aldrich Chemicals Ltd., Mumbai, India. Methanol (Spectrochem research laboratories, Mumbai, India), Brine shrimp cysts (*Artemia salina*) were procured and cultured under laboratory conditions.

2.2. Extracts Preparation

Phyllanthus emblica fruits were collected and the seeds were separated from the fruit. The seeds were washed in running water, then in excess distilled water and shade dried for a week. After drying, the seeds were chopped and grounded by Deluxe Prestige high speed mechanical blender (Chennai, India). The seed powder was used for further studies. Initially, to remove hydrocarbons the dried seed powder was socked in petroleum ether and then extracted with methanol by immersion method. Further the solvent were distilled and utilized for further purpose (Fig. SI 1).

2.3. Green Synthesis of P. emblica Capped PdNPs

P. emblica capped PdNPs were prepared by using conventional method. One hundred mL of 1 M palladium acetate ($Pd(OAc)_2$) solution was prepared in sterile distilled water. The final solution was prepared by the mixture of 80 mL of $Pd(OAc)_2$ and 20 mL of *P. emblica* methanol extract with a final concentration of 0.87 M $Pd(OAc)_2$ in the solution. The reaction mixer was stirred at 60 °C for 3 h. UV–Visible spectroscopy results were observed at every 30 min time interval. After the visual indication of colour change in the solution, it was centrifuged at 3000 ppm for 20 min. The same procedure was repeated thrice for the settlement of *P. emblica* PdNPs. The *P. emblica* capped PdNPs pellets were separated and dried at 400 °C in furnace (Roopan et al. 2013).

2.4. Characterization of P. emblica Capped PdNPs

2.4.1. UV–Visible Investigation of PdNPs

The PdNPs were processed between the wavelength of 200–800 nm by using Shimadzu UVd-1800 PC, Japan. Further PdNPs absorbance were observed (Roopan et al., 2015).

2.4.2. X-ray Diffraction (XRD)

XRD analysis were processed by Advance Powder X-ray diffractometer D8, (Bruker, Germany) taken from 10 to 80°. The particles size can be calculated by Scherrer formula,

$$D = K\lambda/\beta\cos\theta$$

D = particle size, K = Scherer constant (0.94)λ = wavelength, β = Half Width Full Maximum, θ = diffraction angle.

2.4.3. PdNPs FTIR Analysis

FTIR analysis was performed using (Alpha T, Bruker, Germany) to identify the functional groups from the wavenumber of 400 to 4000 cm^{-1}.

2.4.4. Scanning Electron Microscope

The synthesized nanoparticle size analyzed using Hitachi H-7100 SEM voltage of120 kV, with the parameters to be set as EHT 19.79 kV, Extractor *V* 4.40 kV, Extractor I 157 μA, Fil I 2.410 A, Fil I Target 2.410 A, Extractor A Target 4.40 A, EHT Target 19.79 kV. Samples were submitted in powder form at room temperature.

2.4.5. Transmission Electron Microscope

The palladium nanoparticles samples were dispersed in water under ultrasonic treatment and then dropped on the carbon-coated copper grids (JEM-1230, JEOL, USA) at 80 kV.

2.4.6. Zeta Potential

To identify the stability of the synthesized PdNPs we subjected to Horiba Nano particle analyzer.

2.5. Antibacterial Study

In order to study antibacterial activity for the seed extract and PdNPs against four pathogens namely *P.aeruginosa*, *B. subtilis*, *S. aureus* and *P. mirabilis* was assessed by agar well diffusion method. Standard antibiotic streptomycin was used at the concentration of (50 μg/mL) and kept as a stock solution. Sterile distilled water was utilized as negative control. The medium (Muller Hinton agar) was autoclaved and poured in the Petri plates and allowed to solidify. The pure cultures were spread over the Petri plates by cotton swab. The wells were made by a sterile well puncher. The *P. emblica* seed extract and the PdNPs were prepared at three different concentrations 50, 75 and 100 mg/mL respectively by diluting it with sterile distilled water; in each well the prepared samples were added. The inoculated plates were incubated for 24 h at 37 °C. The incubated plates were then removed and examined for the zone of inhibition (mm) [5]. Triplicates were performed for the tests. In addition, Minimal Inhibitory Concentration (MIC) was performed for the samples by serial dilution method using nutrient broth in sterile test tubes. The samples were prepared at the range from 10 to 500 μg/mL. In each tube 100 μL of bacterial suspension were added and incubated overnight at 37 °C. The uncultured broths in test tubes were used as a negative control.

2.6. In Vitro Haemolytic Assay

The prepared *P. emblica* capped PdNPs and the extracts were evaluated for the haemolytic activity. According to [51], 4 mL of blood sample was collected from a healthy volunteer (B^{+ve}) aged 23 years old, in the