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Original Research Article

Antibacterial and cytotoxicity effects of biogenic palladium nanoparticles synthesized using fruit extract of *Couroupita guianensis* Aubl.

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ABSTRACT

Herein, we report a facile route to synthesize palladium nanoparticles (CGPdNPs) using the aqueous fruit extract of *C. guianensis* Aubl. as a potent biological reducing agent. Reduction of PdCl2 solution into their Nano scale was confirmed with the formation of a black precipitate which gives a reduced absorbance in UV–vis spectroscopy. Fourier transform infrared spectroscopy (FTIR) reveals the active role of phenolic constituents from *C. guianensis* in reduction and surface functionalization of nanoparticles (NPs). Dynamic light scattering (DLS) and zeta potential analysis confirms the generation of polydispersity highly stable NPs with large negative zeta value (−17.7 mV). Interestingly, X-ray Diffraction (XRD) pattern shows that the synthesized CGPdNPs were face centered cubic crystalline in nature. The HRTEM micrographs of CGPdNPs display well-dispersed, spherical NPs in the size ranges between 5 and 15 nm with an average of 6 nm. It was also noticed that the synthesized CGPdNPs possess an effective antimicrobial activity against different bacterial human pathogens. On the other hand, *in vitro* cell viability (MTT) assays reveal that the synthesized CGPdNPs exhibited extraordinary anticancer properties. Eventually, hemocompatibility assay depicts the safe nature of synthesized NPs for biomedical application.

Introduction

The development of green chemistry route to obtain Nano scale values of noble metals with different chemical compositions, sizes, shapes and tunable properties have received considerable attention due to their unique applications in electronics, catalysis, environmental remediation, diagnosis, targeted drug/gene delivery and antimicrobials (Basavegowda et al., 2014). However, several physical and chemical methodologies such as radiation, ablation, thermal decomposition, electro and sonochemical methods existed for nanoparticles preparation. Most of these methods were associated with the utilization of toxic chemicals, intensive energy, and capital consumption (Govindarajan et al., 2017). As a low-cost eco-friendly alternative different biological entities such as bacteria (Klaus et al., 1999), fungi (Mukherjee et al., 2001), actinomycetes (Ahmad et al., 2003), yeast (Kowshik et al., 2002), algae (Xie et al., 2007) and plants (Sathishkumar et al., 2012) have been established to produce nanoparticles of noble metals. In compared with the microbial route, plant extract mediated NPs synthesis was receiving many preferences because of its reliability and easy scaling-up procedures (Mittal et al., 2013).

Moreover, an outbreak of deadliest diseases like malaria, cholera, tuberculosis and prevalence of multi-drug resistance (MDR) was the most important global health issues (Fayaz et al., 2010). Likewise, cancer continues to be the most potent scourges of mankind that causes major mortality rate every year worldwide (Misra et al., 2010). Lung cancer is one of the most common fatal cancers that causes severe mortality worldwide. According to

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International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), it was reported that around 1.8 million (13% of total) new lung cancer cases were diagnosed and about 1.6 million (19.4% of total) deaths occurred due to lung cancer (Ferlay et al., 2012).

Despite chemotherapy, early diagnosis and existence of targeted drugs lung cancer still remains the leading cause of mortality. Current cancer chemotherapy was suffers from certain limitations like poor drug distribution, solubility, insufficient drug concentrations, unbearable toxicity and inadequate ability to monitor therapeutic responses. This situation urges to design new strategies, tools, and drugs for the diagnosis and treatment of cancer (Torre et al., 2015).

Generally, nanomaterials of noble metals such as gold (Au), silver (Ag), copper (Cu), platinum (Pt), palladium (Pd), iron (Fe), zinc (Zn) and titanium (Ti) have gained colossal attention due to their indispensable unique physio-chemical properties relative to their macro scale counterparts (Parhi et al., 2012). Also, these nanomaterials have shown extraordinary pharmaceutical applications like drug delivery, imaging, cell labelling, Surface-Enhanced Raman Scattering (SERS) detection, antioxidant, anti-inflammatory, bactericidal and cancer theranostics (Kharissova et al., 2013). Especially, metal and metal oxide nanomaterials are known to possess high potential in cancer management based on the selective disruption of the mitochondrial respiratory chain leading to the production of reactive oxygen species (ROS) which in turn cause DNA damage (Mata et al., 2015; Sathishkumar et al., 2015). Among them, palladium nanoparticles (PdNPs) were recognized as a unanimous catalyst in many industrial applications. However, there are only very few studies have emphasized the distinct features of PdNPs for their applications as a drug delivery system, photothermal agents and anti-cancer/anti-microbial therapy (Dumas and Couvreur, 2015).

C. guianensis Aubl., commonly known as ayahuma and cannonball tree, belongs to *Lecythidaceae* family and possesses many medicinal properties such as antibiotic, antifungal, anticancer, antiulcer, antiseptic, and analgesic qualities. Extracts from this tree were used to cure colds and stomach aches and juice made from the leaves were used to cure malaria (Al-Dhabi et al., 2012). Hence, in the present study high phenolic enriched *C. guianensis* aqueous fruit extract was employed for the green synthesis of CGPdNPs towards valuable biomedical applications.

Materials and methods

Materials

Palladium (II) chloride (PdCl2) was purchased from Sigma-Aldrich Mumbai, India. Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), antibiotics, trypsin-EDTA, phosphate buffer saline, nutrient broth, agar-agar, streptomycin sulphate, sterile paper discs and other chemicals were purchased from Himedia Laboratories, India. Fresh and healthy cannonball fruits (*C. guianensis*) were collected from Gokarnesvarar temple, Pudukkottai town, Tamil Nadu State, India. A549 cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Bacterial pathogens used in this study *S. aureus* MTCC 96, *R. rhodochorous* MTCC 265, *E. coli* MTCC 1687, *P. mirabilis* MTCC 425, *P. aeruginosa* MTCC 1688, *V. cholerae* MTCC 3906, *B. cereus* MTCC 1272, *S. typhi* MTCC 3917, *M. luteus* MTCC 1809, *K. pneumonia* MTCC 530 were obtained from Microbial type culture and collection (MTCC), Chandigarh, India.

Preparation of fruit extracts (CGFE)

The collected fruit samples were cut into small pieces after removing their peel then washed thrice with distilled water to remove other impurities and allowed to dry in shade condition for 5–10 days. After that, the well dried fruit samples were finely grounded using stainless steel kitchen blender to obtain fine powder. Subsequently, extract for the synthesis of CGPdNPs was prepared by simple decoction method, for that 10 g of sterilized fruit powder was mixed with 200 ml of Milli-Q water and kept in boiling water bath (60 °C) for 20 min. Finally, the 5% extract was filtered through Whatmann no.1 filter paper and stored at 4 °C for further studies.

Synthesis of CGPdNPs

To synthesize CGPdNPs, the reaction mixture was prepared by blending 5 ml (5%) of CGFE in 95 ml of 1 mmol/l substrate (Pd Cl_2: 0.017 in 100 ml) and kept at room temperature under continuous stirring (Vimala et al., 2015). After that, the reaction mixture was checked for intense blackish colour formation and the absorbance maxima were monitored using UV–vis spectroscopy. Finally, the synthesized CGPdNPs was purified by repeated centrifugation at 16,000 rpm for 10 min at 4 °C and the unreacted ions and molecules colloidal in CGPdNPs suspension was then dialyzed repeatedly by using a cellulose tube (MWcutoff 12400D) against 1000 ml of deionized water for 9 h at 30 °C. Finally, the purified CGPdNPs were resuspended in Milli-Q water and then used for further characterization.

UV–visible spectroscopy

Initially, the reduction of Pd II ions was monitored based on the absorbance spectra in UV–vis Spectroscopy. For that, an aliquot of colloidal CGPdNPs was diluted with deionized water and the absorbance maxima were scanned in UV–vis spectrophotometer (Perkin-Elmer Lambda 2 UV198) at the wavelength of 300–700 nm.

FTIR and XRD analysis

FTIR analysis was achieved to determine the possible bio-compounds present in CGFE responsible for reduction and stabilization of CGPdNPs. Transmittance were measured for extract before and after addition of PdCl2, to perform FTIR the samples were ground well with KBr powder and pelletized, afterwards the spectra were recorded in JASCO 460 PLUS FTIR spectrometer at the wavelength of 4000 cm^{-1}–400 cm^{-1}. Further, the X-ray diffraction (XRD) study was performed at 40 kV and 30 mA using X-ray diffractometer (Ultima-III, Rigaku, Japan, λ = 1.54 Å) with Cu Ka radiation (k = 1.5406 Å) in the range of 20–80 at a scan speed of 2/min.

DLS, HRTEM, EDAX and SAED measurements

Hydrodynamic size distribution and surface charge of synthesized CGPdNPs were measured using dynamic light scattering (DLS) and zeta potential. To execute DLS, the colloidal CGPdNPs solution was further diluted and kept in water bath sonicator for 10–20 min to disperse the particles, size distribution measurements and zeta values were recorded using Malvern Zetasizer, Nano −ZS90 analyzer. The size and shape of synthesized CGPdNPs were measured with the micrographs obtained from JEOL JEM 2100 high resolution transmission electron microscope. For HRTEM analysis, the colloidal purified Pd solution allowed for sonication, a drop of this solution was used to make a thin film onto copper coated grid, allowed for drying under infrared lamp and micro graphical images of CGPdNPs were taken at different magnifications. Additionally, the presence of elemental palladium (Pd) in the mixture and their crystallinity were identified using an EDAX and SAED analysis.

Antimicrobial activity (Disc diffusion method)

Synthesized CGPdNPs was tested for its potential antimicrobial activity against bacterial human pathogens. To study antimicrobial

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