Green synthesis of gold nanoparticles using aqueous extract of *Dillenia indica*

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**Abstract**

In this study, we report a novel method of gold nanoparticle (AuNP) synthesis using aqueous fruit extract of *Dillenia indica*. The phytochemicals present in the fruit extract act as an effective reducing and capping agent to synthesize AuNPs. The synthesized AuNPs were characterized by spectrophotometry, transmission electron microscopy (TEM), x-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. TEM studies revealed the particles of various sizes and mainly spherical in shape. Selected-area electron diffraction (SAED) patterns and high-resolution transmission electron microscopy (HRTEM) images confirmed the crystallinity of the particles. The XRD patterns showed peaks at (111), (200), (220) which exhibited preferential orientation of the AuNPs as face-centered cubic crystal. FTIR measurements confirmed the coating of phenolic compounds on the AuNPs indicating a possible role of biomolecules for the capping and efficient stabilization of the AuNPs. The synthesized AuNPs did not show any form of cytotoxicity in the normal fibroblast cell line L929.

**Keywords:** *Dillenia indica*, gold nanoparticle, green synthesis

**Classification numbers:** 4.02, 5.08

1. **Introduction**

In recent years, metallic nanoparticles, particularly gold and silver nanoparticles were used as theranostic agents for diagnosis and treatment of various disorders like diabetes, cancer, Parkinson’s, Alzheimer’s, HIV/AIDS, arthritis, hepatitis, cirrhosis, spinal cord injury, tuberculosis and cardiovascular diseases (CVD) due to their unique optoelectronic and physicochemical properties, and ease of synthesis, characterization and surface modification in the nanoscale range. Gold nanoparticles (AuNPs) have also gained significant interest for their various beneficial therapeutic, diagnostic and numerous technological applications. Nanoparticles are of immense interest due to their extremely miniscule size and high surface area to volume ratio, which lead to both chemical and physical differences in their properties (e.g. mechanical and biological properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point) compared to bulk of the same composition [1, 2]. Metallic nanoparticles exhibit various size- and shape-dependent optical properties, which are useful in various biomedical applications like the imaging of specific target cells and tissues, drug delivery, bio-sensing and catalysts to optics, etc [3]. Furthermore, there were diverse applications of nanoparticles as antimicrobial agents, computer transistors, chemical sensors, and magnetic nanoparticles as a contrasting agent in magnetic resonance imaging [4].

In particular, AuNPs have proved to be highly versatile for deep-tissue imaging, the detection of heavy metals [5], as
2.1. Preparation of Dillenia indica aqueous core extract

The skin from the D. indica fresh fruit was peeled off and the mucilaginous core was taken out (figure 1). Then the fruit core was chopped and dipped in 100 ml double-distilled water. After 30 min, the aqueous core extract (ACE) was filtered through Whatman 0.4 micron filter paper and the filtrate was collected. The filtrate was directly used for synthesis of the AuNPs or stored at 4 °C for further experiments.

2.2. Green synthesis of AuNPs

The varying volume of ACE of D. indica was mixed with 1 mM HAuCl4 in a 2 ml reaction volume. With the optimized quantity of ACE and 1 mM HAuCl4 aqueous solution, the reaction was kept under continuous stirring condition at 200 rpm (C-MAG-HS7, IKA®) at room temperature. The color change was visible within 15 min to verify the synthesis of the AuNPs and was further subjected for spectroscopic study. All reactions were carried out in closed glass vials and the final reaction volume made up to 1 ml with double-distilled water. The synthesis parameters were optimized with the gradient volume of ACE (5%–20%), (v/v) with 1 mM HAuCl4, keeping molar ratio constant.

2.3. Characterization of D. indica ACE-mediated AuNPs

The synthesized AuNPs were characterized by different techniques like UV-visible spectroscopy, transmission electron microscopy (TEM), x-ray diffraction (XRD) and by thermogravimetric analysis (TGA).

2.3.1. UV-visible spectroscopy. The ACE of D. indica was mixed with 1 mM gold chloride solution (HAuCl4) and the visible color change was observed due to the formation of nanoparticles. To optimize the concentration of the fruit extract, the experiment was carried out by varying the concentration of the extract against a fixed concentration of gold chloride. With the optimized quantity of ACE and 1 mM HAuCl4 aqueous solution, an additional reaction was carried out under continuous stirring condition at 200 rpm (C-MAG-HS7, IKA®) at room temperature. A color change was observed after 10–15 min. The synthesis of the AuNPs was characterized by spectroscopic (Cary 100 BIO UV–vis, Varian, CA, USA) study, the primary confirmatory tool for the detection of the surface plasmon resonance (SPR) property of AuNPs.

2.3.2. Transmission electron microscopy. The size and shape of the synthesized, optimized AuNPs were measured by TEM (JEM 2100, JEOL). To prepare the samples for TEM study, the synthesized particles were suspended in deionized water, and dispersed with ultra-sonication. Ultra-sonication is required to separate individual particles and to remove any form of particle aggregation. Then the sample was deposited on a copper grid followed by solvent evaporation using a suitable drying technique and was then used for TEM analysis.

2.3.3. XRD, FTIR, TGA/DSC analyses. The synthesized colloidal AuNPs were centrifuged at 20 000 rpm for 20 min, washed with double-distilled water 2–3 times and freeze dried which were then subjected to XRD analysis. The diffraction of the AuNPs was recorded with a Bruker D8 ADVANCE
X-ray powder diffractometer using Cu-Kα (λ = 1.54 Å) source in the region of 2θ from 30° to 75°.

For Fourier transform infrared (FTIR) spectroscopy measurements the AuNP solution was centrifuged at 20 000 rpm for 20 min. The pellet was washed with deionized water three times by centrifugation to remove the excess extracts not capped on the surface of the AuNPs. Then the pellet was re-dispersed in deionized water. The purified sample was lyophilized to obtain pure AuNPs in powder form. Pure AuNPs and ACE were mixed separately with potassium bromide (KBr) to obtain pellets and analyzed using FTIR spectrophotometer (Shimazdu, Kyoto, Japan) in the range of 450–4000 cm⁻¹.

The AuNPs were analyzed for thermogravitometric and differential thermal analyses (make Netzsch, model STA449F3A00). 5 mg of the sample was placed in platinum pans and heated under inert atmosphere at a rate of 40 °C/min to 100 °C and held for 30 min to remove residual solvents. The samples were then heated to 700 °C at a rate of 40 °C/min.

2.4. In-vitro cyto-compatibility assays

The mouse fibroblast cell line L929 and breast ductal carcinoma cell line MCF7 were obtained from NCCS, Pune, India, and maintained according to standard protocol in DMEM medium supplemented with 10% fetal bovine serum and 1% anti-mycotic antibiotic incubated at 37 °C in humidified atmosphere.

To assess the cyto-compatibility of the synthesized AuNPs, the MTT assay was carried out according to the protocol described by Mossman [19]. In brief, the confluent L929 cells at a density of 1 × 10⁴ cells were seeded in a 96 well culture plate and incubated for 24 h. After 24 h the cultured cells were treated with different concentrations of synthesized AuNPs (0–200 μg/ml) in serum-free media and incubated further for 24 h. After 24 h of incubation the media was discarded and the cells were treated with MTT dye at a concentration of (0.5 mg ml⁻¹) and incubated for 4 h. Finally,
100 μl of dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan precipitate, and the absorbance was measured at 570 nm using a microplate reader (Tecan Infinite I 200).

The cell viability was calculated using formula

\[
\text{cell viability (\%)} = \frac{A_t}{A_c} \times 100,
\]

\(A_t\) and \(A_c\) being the absorbance of test sample and control sample, respectively.

Figure 3. (a), (b) TEM, (c) HRTEM and (d) SAED pattern of AuNPs prepared by reacting with 20% ACE of D. indica with 1 mM gold chloride.

Figure 4. XRD pattern of synthesized AuNPs showing peaks of (111), (200), (220) confirming the crystallinity of the particles.
where $A_t$ is the absorbance of the treated sample and $A_c$ is the absorbance of the control.

Furthermore, to enumerate the dead cells, acridine orange (AO) and ethidium bromide (EB) staining was performed to both treated and untreated L929 cells. In this study, a 100 $\mu$g/ml concentration of synthesized AuNP was considered. The documentation of cell viability was carried out under an inverted fluorescence microscope equipped with a dual wavelength filter to capture both green and red signals from the live and dead cells.

3. Results and discussions

3.1. Reduction property of D. indica ACE

The reduction of AuCl$_4$ was visually evident from the color change and the stable ruby-red color indicated the formation of AuNPs. Synthesis of the AuNPs was confirmed by scanning the absorption maxima of the reacted mixture at the wavelength between 200–800 nm on a Cary 100 BIO UV–Vis spectrophotometer (Varian, CA, USA). Spectroscopic scanning of the colored solution exhibited distinct surface plasmon resonance (SPR) bands with an absorption peak centered at around 530–535 nm, characteristic of spherical AuNPs (figure 2). At the fruit extract concentration above 20%, the increase in SPR intensity was negligible, indicating an attainment of saturation in the bio-reduction of AuCl$_4$.
3.2. Structural and physical property analysis of synthesized AuNPs

3.2.1. Particle size analysis by TEM. The TEM images confirm the formation of AuNPs and it has been observed that the newly formed nanoparticles are polydispersed, consisting of various shapes like spherical, triangular, tetragonal, and pentagonal with irregular contours. Representative TEM images and the corresponding size-distribution histogram of the AuNPs obtained with the optimum mixture of gold salt and aqueous fruit extract showed that nearly spherical AuNPs were mostly dominant with a wide spectrum of particle size. The AuNPs obtained at 20% ACE confirmed the formation of particles with a size range of 5–50 nm. The ultra-high-resolution TEM (UHRTEM) images displayed clear lattice fringes on the particle surfaces. Selected-area electron diffraction pattern (SAED) of a single spherical particle confirmed the single crystalline nature of the AuNPs with the fcc phase. Rings were observed corresponding to (111), (200) and (220) planes of the fcc crystalline lattice of AuNPs (figure 3).

3.2.2. Crystallinity analysis of AuNPs by XRD. XRD patterns of the AuNPs synthesized with D. indica ACE concentration of 20% displayed Bragg reflections representative of the fcc structure of gold (figure 4). The intensity of the peak of (111) at 38.3° diffraction was much stronger than those peaks of (200) and (220) at 44.3° and 65.4°, respectively. The mean size of the AuNPs was also calculated using the Debye–Scherrer equation by determining the width of the (111) Bragg reflection. The mean size and shape of the AuNPs determined from XRD patterns confirmed the data analyzed from TEM images. In the case of the optimum aqueous core extract, the average diameter obtained from TEM and XRD analysis was in the range of 5–50 nm, respectively. As the Debye–Scherrer equation is best applicable to highly monodispersed nanoparticles, in the present study the average diameter of polydispersed AuNPs may be physically more relevant. Such deviation can be attributed to highly polydispersed AuNPs within adequate adaptation to a particular geometry [13, 20].

3.2.3. FTIR spectra analysis of synthesized AuNPs. The FTIR spectra (figure 5) of the fruit extract of D. indica showed characteristic bands for C-H alkanes, C=O carbonyl, N-H primary amines, N-O nitro group, C-C aromatic and C-O stretch for alcohols, carboxylic acids, esters and ethers at 2927 and 2908, 1768, 1652, 1558, 1477, and 1105 cm$^{-1}$, respectively [21, 22]. The corresponding peaks for the said spectra appeared due to the presence of various phytochemicals present in the crude extract. The FTIR analysis of the AuNPs revealed the presence of all bands common to the aqueous fruit extract of D. indica viz. C-H of alkane at 2947 cm$^{-1}$, C=O carbonyl at 1739 cm$^{-1}$, 1651 cm$^{-1}$ for N-H primary amines, 1456 cm$^{-1}$ for C-C aromatic and 1114 cm$^{-1}$ for alcohol, carboxylic acid, esters and ethers suggesting the presence of these phytochemicals on the surface of the AuNPs. The capping behavior and

Figure 8. Acridine orange/ethidium bromide staining of L929 cells treated with 100 μg/ml conc. of AuNPs.
stability of the synthesized AuNPs could be due to the presence of these phytochemicals in the crude extract.

3.2.4. Thermal property analysis of synthesized AuNPs. According to the TGA of the nanomaterials, the residual mass could be due to inorganic nanomaterials, residual metal catalysts from synthesis, or impurities within the sample. It was observed that 40% of organic components of the nanoparticles were degraded due to the higher temperature at 550 °C. The TGA spectrum of the AuNPs was recorded over a wide temperature range (100°C–800°C) which exhibited the significant weight loss (40%) of AuNPs (figure 6). This indicates that the bioactive primarily mucilaginous ingredients, polyphenolic compounds were capped on the synthesized AuNPs and were degraded due to the high temperature. Hence, it was confirmed that the AuNPs were capped with bioactive molecules of ACE of D. indica.

3.3. Cytotoxicity study of the synthesized AuNPs

The MTT dye-conversion assay is performed to assess mitochondrial dehydrogenase activity, which is correlated to cell viability for live cells. Metabolically active cells are able to produce an active mitochondrial dehydrogenase enzyme which can reduce the MTT tetrazolium salt to purple colored formazan crystals. But in the case of metabolically inactive cells the purple color change will not occur. After 24 h of treatment with AuNPs almost 90% of the cells retained their viability (figure 7). This revealed that the synthesized AuNPs were non-toxic and biocompatible to the normal cell line, providing an excellent platform for various biomedical applications.

Viability staining (acridine orange and ethidium bromide) showed the cell membrane integrity of the normal fibroblast L929 cell line based on the uptake or exclusion of a fluorescent dye from the cells (figure 8). AO is a membrane-permeable dye that stains both live and dead cells, while EB invades through the membrane of dead and dying cells in untreated as well as treated cells. AO stained cells emitted green fluorescence and EB stained cells fluoresce red. The untreated L929 cells gave intense green fluorescence and weak signals for red fluorescence, which was expected for the control experiment.

4. Conclusion

Nanobioscience has drawn increasing attention due to its avant-garde nature and the efficacy of nanoparticles in industrial, biomedical, and electronic applications such as a catalyst [23], for cancer detection [24, 25], and as bioimaging and theranostic agents. As an alternative to chemical reductants, green phytochemical reducing agents have gained much interest in the synthesis of nanoparticles due to their capability to promote sustainability initiatives. Green reductants consist of various biological entities ranging from bacteria and fungi to plant parts (fruits, leaves, petals), algae, etc [6, 26]. The high phenolic content of the aqueous core extract of D. indica having a strong anti-oxidant property helped in the reduction of gold cations to AuNPs. Characterization and TEM imaging of AuNPs revealed an average size range of 5–50 nm which is most promising for biological applications. This green and time-saving method for AuNP synthesis does not require any toxic or hazardous reducing chemical agent and thus has potential for use in biomedical applications. The various phytochemicals present in D. indica fruit mucilage extract mediate the surface capping of AuNPs which was evident from FTIR studies [27]. The crystalline nature of the AuNPs was confirmed from the SAED and XRD pattern. This eco-friendly green method for the synthesis of AuNPs provides an opportunity to use these AuNPs for application in drug delivery and molecular imaging.

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