Green Synthesis of Gold Nanoparticles Using Neem (*Azadirachta indica* L.) Leaf Extract and Its Biomedical Applications

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**ABSTRACT**

Eco-friendly green synthesis is one of the promising branches of nanoscience for applications in different biomedical fields. It makes more attractive potential option due to non-toxic and very low cost of synthesis. This paper deals with the synthesis of eco-friendly and cost-effective gold nanoparticles by using leaf extract of neem (*Azadirachta indica*) as a reducing agent. The SEM image shows that the gold nanoparticles are predominantly spherical in morphology whereas TEM image shows different shapes like hexagonal, triangular and spherical. DLS results indicated that the particle and dispersity of gold nanoparticles represented effective diameter of about 15:1 nm and polydispersity index (PDI) 0.643. Thus, this rapid, eco-friendly and economical route can be used to synthesize HAuCl₄ with a wide range of biomedical applications.

**Keywords:** Biosynthesis, Gold Nanoparticles (HAuCl₄), *Azadirachta indica*, Green Gold.

**INTRODUCTION**

It is well known that phytochemicals in the synthesis of nanoparticles is one of the significant relationship among nanotechnology and green chemistry [1, 2, 3]. It is very important to build up ‘nano-naturo’ which associates among nanotechnology and green domains of the natural world like unfolds of nano revolution. This is a great concern to make nontoxic conditions of nanoparticles for technological and medical applications [4, 5, 6]. It is also well known that the role of phytochemicals which instigate different types of transformations chemically in biological systems [5, 7, 8, 9].

Gold nanoparticles are of interest because of the unique properties (e.g., size and shape depending optical, electrical, and magnetic properties) [10] which can be incorporated into anticancer properties, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products and electronic components. Several physical and chemical methods have been used for synthesizing and stabilizing gold nanoparticles [11-15]. Recently, nanoparticle synthesis is among the most interesting scientific areas of inquiry, and there is growing attention to produce nanoparticles using environmentally friendly methods (green chemistry) [16].
Recent research provides much more emphasis on biological or plant mediated synthesis of nanoparticles as it is eco-friendly and also very simple process. Though it has already been reported the plant mediated synthesis or biosynthesis of gold nanoparticles such as Allium cepa [17], Terminalia catappa [18], tea [19], Cymbopogon citratus [20], but still it is in primary stage, so, it needs improvement particularly for reduction of time for synthesis of nanoparticles, modulation of size and shape and also improve the monodispersity of the nanoparticles through bio-based processes. Really, it is very interesting study to know the nature of nanoparticles which is produced by using extracts of various parts of a plant. The selection of neem (Azadirachta indica) plant extracts for this study because of its various activities such as antibacterial, antioxidant and anticancer and so on [21].

In this study, Azadirachta indica leaves were used because of availability, widely cultivated and fast growing trees in all over the India. It has versatile applications in pharmaceuticals and cosmetics as it grows in all climatic conditions including tropical region. The activated neem leaves consists of mainly three dissimilar kinds of phenolic compounds such as 4-chlorophenol (4-CP), 4-nitrophenol (4-NP) (Figure 1B) and phenol (P) was studied from simulated waste water in batch as well as fixed bed mode [22]. This work aimed to synthesis of gold nanoparticle (Figure 2) using neem (Azadirachta indica) and which was characterized through Uv-visible, XRD, FTIR, DLS, SEM and TEM techniques.

**EXPERIMENTAL**

**REAGENTS AND CHEMICALS**

HAuCl₄·xH₂O (Tetrachloroauric acid) was obtained from Sigma Aldrich. Milipore water was used for conduct experimental works.

**Ethanolic Extract Preparation**

Neem leaves or sticks (Figure 1A); 300gm were selected, washed, cut into small pieces and dried in an oven (45-50°C) for 3 days. Neems leaves or sticks were blended with blender and then extracted using 96% ethanol 10h (Figure 1C). The extraction was carried out using soxhlet apparatus (60- 80°C; 1:50, w/v) until the last extract was colourless. The combined extract was filtered and the filtrate was concentrated and evaporated under the same condition as described before to afford the soxhlet extract [23].

**Biological Synthesis of Gold Nanoparticles**

The broth was used for reduction of Au³⁺ ions to Au⁰ by using finely cut neem leaves (10g) in a 500 ml Erlenmeyer flask with 40 ml of sterile distilled water and it was boiled upto15min. In this research, 0.2 ml of broth was added to 50 ml of 10⁻³ M aqueous chloroauric acid (HAuCl₄) solution. The colour of the solution (Figure 1C) was changed to cherry red colour within an hour (50 minutes) [17].

![Figure 1: (A) Leaves of Azadirachta indica (B) Structure of 4-Nitrophenol (C) Picture of ethanolic extract solution of Azadirachta indica with 10⁻³ mM HAuCl₄](image)
**UV-vis Spectroscopy Studies**

UV-vis spectroscopy (UV-1601 pc Shimadzu) was used to know the absorption spectroscopy in the UV-visible spectral region. It uses light in the visible and adjacent near-UV and near-infrared (NIR) ranges. The absorption during the observable range directly affects the perceived colour of the concerned chemicals. In this region of the electromagnetic spectrum, molecules undergo electronic transitions.

**XRD Measurements**

XRD of Phillips PW 1830 instrument working at a voltage of 40 KV and current of 20 mA with Cu-K radiation was used for creation of bio-reduced gold nanoparticles in this study.

**FTIR Spectroscopy Measurements**

BIORAD-FTS-7PC type FTIR spectrophotometer was used for synthesis of gold nanoparticles following green technology to make it powder form for this investigation. Both the gold and bio-reduced chloroauric acid solutions were centrifuged at 10,000 rpm for 15 minutes and then washed the pellet was washed with 20 ml of deionized water at least three times. It was analysed with FTIR of finally dried resultant purified suspension after the ground with KBr pellets. A total of 512 scans were recorded in order to obtain a good signal.

**SEM Measurements**

SEM measurement was carried out by using Hitachi S - 4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

**TEM Measurements**

The instrument of JEOL model 1200EX TEM at an accelerating voltage at 80 kV was used for TEM measurements. It is a microscopy performance in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through.

After the interaction of electrons transmitted through the specimen, an image was formed which was then magnified and focused on top of an imaging device.

**DLS Measurements**

This is one of the most popular techniques which were used to determine the size of particles. Shining a monochromatic light beam, such as a laser, onto a solution with spherical particles in Brownian motion causes a Doppler Shift when the light hits the moving particle, changing the wavelength of the incoming light. This change was related to the size of the particle. From DLS (Zetasizer, Malvern) it was possible to compute the sphere size distribution and give a description of the particle’s motion in the medium, measuring the diffusion coefficient of the particle and using the auto correlation function.

**RESULTS AND DISCUSSIONS**

**UV-vis near-infrared spectra**

The UV-vis near infrared absorption spectra was used in aqueous suspension for synthesis of gold nanoparticles and optical properties were exhibited closely associated with their shapes of such aqueous suspension metal nanoparticles. The ultraviolet visible absorption spectra of the gold nanoparticles harvested after 24 hours of reaction (Figure 3A) showed two major patterns with an increase in the concentration of the extract in the reaction mixture. The first was that the transverse plasmon resonance band that appeared at 540 nm shifted towards a higher wavelength, confirming a red shift along with amplified absorbance intensity; the second was that the longitudinal plasmon resonance band appearing in the near infrared region of the electromagnetic spectrum at 823 nm showed a red shift. The ultraviolet-visible spectrum (Figure 3A) also showed a very broad band towards the longer wavelength (infrared) region [24-26].
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Figure 3A: Ultraviolet-visible-near infrared spectra of gold nanoparticles synthesized by exposing various amounts of *Azadirachta indica* extract to a fixed volume (5 ml) of HAuCl₄ solution (10⁻³ M) for 24 hours.

Figure 3B: Representative ultraviolet-visible-near infrared spectra depicting kinetics of the reaction of 1 ml of *Azadirachta indica* extract with 10 ml of aqueous HAuCl₄ solution for specified time periods.

Figure 3C: Colour development as a function of surface plasmon resonance in *Azadirachta indica* extract-mediated synthesis of gold nanoparticles. (A) HAuCl₄ aqueous solution, (B) Incubation of 5 ml of HAuCl₄ aqueous solution with 1 ml of *Azadirachta indica* extract keeping the final volume of reaction mixture at 10 ml, (C) Incubation of HAuCl₄ aqueous solution (5 ml) with 3 ml of *Azadirachta indica* extract, making the final volume of reaction mixture 10 ml by adding 2 ml of deionized water.

Figure 3B shows the time kinetics of the reaction in terms of ultraviolet-visible near-infrared spectra recorded from the reaction mixture consisting of 1 ml of *Azadirachta indica* extract and aqueous HAuCl₄ (10⁻³ M), bringing the total volume of reaction mixture to 10 ml. It was observed that, as time progressed, the peak at 543 nm remained fixed, but the absorbance of the reaction mixture steadily increased to saturation, with no further appearance of any other band at any wavelength. In contrast, the longitudinal plasmon resonance band was seen to change its position with time [25].

4-Nitrophenol and phenolic compounds present in plant extracts bind to nanoparticles via either free amine groups. Phenolic compounds and quinones from *Azadirachta indica* capped the gold nanoparticles, thereby stabilizing them. To conclude, water-soluble fractions comprised of 4-Nitrophenol, phenolic compounds in the aqueous plant extract helped in the bio-reduction of gold ions (Figure 3C) [27].

X-RAY DIFFRACTION STUDIES

Figure 4: A Representative XRD profile of Gold Nanoparticles.
The phase formation of the synthesized AuNPs was analysed employing X-ray diffraction which confirmed that the bio-reduced metal nanoparticles are of elemental gold (Figure 4). Existence of peaks (111), (200), (220), (311) and (222) matched with the standard Joint Committee for Powder Diffraction Set (JCPDS) data-04784. This confirmed face centered cubic structured AuNPs formation. Peak broadening indicated restricted particle size. Enlarged pattern of (111) peak is shown in the inset of XRD plot. The crystallite size was calculated using Scherrer’s formula.

\[ D = \frac{0.9 \lambda}{\beta \cos \theta} \]

Here 0.9 is the shape factor, generally taken for a cubic system, \( \lambda \) is the x-ray wavelength, typically 1.54 Å, \( \beta \) is the full width at half the maximum intensity (FWHM) in radians, and \( \theta \) is the Bragg angle. Using the above formula the crystallite size calculated is \(~15\) nm [26].

**Fourier Transform Infrared Spectroscopy (FTIR) Analysis**

![FTIR spectra of (a) Azadirachta indica extract before and (b) After gold reduction.](image)

*Figure 5: FTIR spectra of (a) Azadirachta indica extract before and (b) After gold reduction.*

Functional groups involved in the biosynthesis of gold nanoparticles, the nature of the biomolecules involved in the reduction and formation of gold nanoparticles was studied by FTIR analysis of the biomass before and after reduction and of the nanoparticles (Figure 5). The FTIR spectrum of the un-reacted Azadirachta indica extract shows bands at 1742 and 1636 cm\(^{-1}\) (Figure 5a). The first band is characteristic of stretching vibrations of the carbonyl functional group in ketones, aldehydes and carboxylic acids. The second absorption at 1636 cm\(^{-1}\) corresponds to the amide I band. The intense broad absorbance at 3412 cm\(^{-1}\) is attributed to the O–H stretching modes of vibration in hydroxyl functional group in alcohols and N–H stretching vibrations in amides and amines. Moreover, the 1059 cm\(^{-1}\) band can be assigned to C–O stretching vibrations. The absorption peak at 2930 cm\(^{-1}\) (Figure 5b) corresponds to C–H stretching vibration modes in the hydrocarbon chains. The main difference between both spectra is that the treated extract exhibits peaks of less intensity for the amide band. This would suggest those phenol compounds are responsible for Au (III) reduction and gold nanoparticles stabilization [25, 28].

**SEM (Scanning Electron Microscopy)**

Figure 6 shows the green synthesized gold nanoparticles are predominantly spherical in morphology, obtained by the reaction of tetrachloroauric acid with aqueous extract of Azadirachta indica, respectively, within 1 hour of time at room temperature condition [29].

![SEM images of the gold nanoparticles synthesized using aqueous extract of Azadirachta indica.](image)

*Figure 6: SEM images of the gold nanoparticles synthesized using aqueous extract of Azadirachta indica.*
Particle Size Analysis

Particle size and dispersity of gold nanoparticles generated and capped using aqueous extract of *Azadirachta indica*, were determined based on DLS (Dynamic Light Scattering) phenomena represents effective diameter of about 18.4nm and polydispersity index (PDI) 0.643 as shown in Figure -7 [30].

![Figure 7: Shows distribution of different diameter of gold nanoparticles in graph of lognormal size distribution](image)

TEM (Transmission Electron Microscopy)

Figure 8 represents a transmission electron micrograph of gold nanoparticles synthesized by the reaction of aqueous chloroaurate ions with different amounts of extract of *Azadirachta indica* after 48 hours of reaction. This is in concordance with the shift in the ultraviolet spectra of the gold nanoparticles. Hexagonal, triangular, and spherical nanoparticles could be seen in the transmission electron micrographs. At a higher concentration of extract (5ml), a large number of isomorphic spherical gold nanoparticles of 15-18nm in size could be seen in the transmission electron micrographs (Figure 8) [25, 26].

![Figure 8: Transmission electron micrographs of gold nanoparticles synthesized using Azadirachta indica extract.](image)

CONCLUSION

The present study demonstrates bioreductive synthesis of nanosized gold particles using *Azadirachta indica*. The *Azadirachta indica* aqueous leaf extract appears to be environmentally friendly, so that this protocol could be used for rapid production of gold nanoparticles. The size of the nanoparticles can be easily adjusted by using different amounts of leaf extract. Synthesis of AuNPs has been demonstrated to be a rapid and environmentally benign route. Bioreduced AuNPs showed excellent catalytic properties in a reduction reaction of 4-nitrophenol. Thus this rapid, eco-friendly and economical route can be used to synthesize AuNPs with wide biotechnological and chemical applications.

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REFERENCES


