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Green Synthesis, Characterization and Preliminary Biological Evaluation of Gold Nanoparticles Using Azadirachta Indica Leaf Extract

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Abstract: ***Green synthesis of gold nanoparticles (AuNPs) using plant extracts is an attractive approach because phytochemicals participate in the reduction and stabilization of metal ions. In the present study, Azadirachta indica leaf extract was used for the synthesis and characterization of AuNPs from 1 mM $HAuCl_4$. A reaction mixture containing 90 mL $HAuCl_4$ and 10 mL leaf extract was maintained under continuous stirring at 60–80 °C for 60 min. Formation of AuNPs was initially indicated by a change in colour from pale yellow to reddish-purple and confirmed by a characteristic surface plasmon resonance (SPR) band at 535 nm. The UV–visible dataset showed progressive development of the SPR band with reaction time, reaching a maximum absorbance of 1.21 AU at 60 min. The strongest response among the tested extract volumes was observed at 10 mL. Comprehensive characterization demonstrated phytochemical capping, crystalline face-centred-cubic gold, nanoscale particle morphology (~ 18.4 nm core size), elemental Au as the major component, a hydrodynamic diameter of ~ 78 nm, and a moderately negative surface charge (-24.6 mV).***

Keywords: *Azadirachta indica*; gold nanoparticles; green synthesis; UV–visible spectroscopy; FTIR; XRD; TEM; EDX; DLS; zeta potential

1. Introduction

Gold nanoparticles have received extensive attention because their optical, catalytic, sensing, and biological properties depend strongly on particle size, morphology, surface chemistry, and aggregation state [1–4]. Conventional physical and chemical synthesis routes often involve high energy input, organic solvents, strong reducing agents, or other reagents that generate undesirable hazardous residues. Plant-mediated synthesis has therefore become an important green-nanotechnology strategy, in which plant metabolites act as reducing, capping, and stabilizing components [5–8]. *Azadirachta indica* A. Juss. (neem) is rich in diverse phytochemicals, including phenolic compounds, flavonoids, terpenoids, and proteins that contribute to metal-ion reduction and nanoparticle stabilization. Earlier work demonstrated rapid formation of Au and Ag nanoparticles using neem leaf broth, establishing the feasibility of neem-mediated noble-metal nanoparticle synthesis [9]. Recent literature emphasizes the importance of systematic characterization, as the biological and physicochemical behaviour of plant-derived nanoparticles depends heavily on synthesis conditions and surface-associated phytochemicals [5–8]. The present work focuses on the green synthesis of AuNPs using *A. indica* leaf extract and provides a consolidated characterization framework. The experimental dataset includes UV–visible evaluation of the synthesis process along with complete FTIR, XRD, TEM, EDX, DLS, and zeta-potential evaluations.

2. Materials and Methods

2.1 Plant Material and Extract Preparation

Fresh leaves of *Azadirachta indica* were collected from Jalna district, Maharashtra, India. Leaves were washed thoroughly with distilled water, shade-dried at room temperature, and ground into a fine powder. An aqueous leaf extract was prepared by heating 10 g of powdered plant material in 100 mL of double-distilled water at 80 °C for 20 min. The resulting mixture was filtered through Whatman No. 1 filter paper and stored at 4 °C prior to use.

2.2 Gold Nanoparticle Synthesis

The optimized reaction conditions for the green synthesis of AuNPs are summarized in Table 1. A control solution containing 1 mM $HAuCl_4$ without plant extract was maintained under identical conditions.

Table 1: Experimental reaction parameters for AuNP synthesis.

Parameter	Condition
$HAuCl_4$ concentration	1 mM
$HAuCl_4$ volume	90 mL
Leaf extract volume	10 mL
Total reaction volume	100 mL
Reaction temperature	60–80 °C
Reaction time	60 min
Stirring speed	Continuous
Control	$HAuCl_4$ solution without extract

2.3 Characterization Techniques

- **UV–Visible Spectroscopy:** The optical response was monitored in the 450–700 nm range using a UV-Vis spectrophotometer.
- **FTIR Spectroscopy:** Surface-bound functional groups were analyzed in the range of 4000–400 cm^{-1}.
- **X-Ray Diffraction (XRD):** Crystalline structure and phase identification were evaluated using Cu Kα radiation ($\lambda = 0.15406$ nm).
- **TEM and EDX Analysis:** Nanoparticle morphology, core size, and elemental composition were determined via transmission electron microscopy and energy-dispersive X-ray spectroscopy.

- **DLS and Zeta Potential:** Hydrodynamic diameter, polydispersity index (PDI), and surface electrical charge were measured via dynamic light scattering.

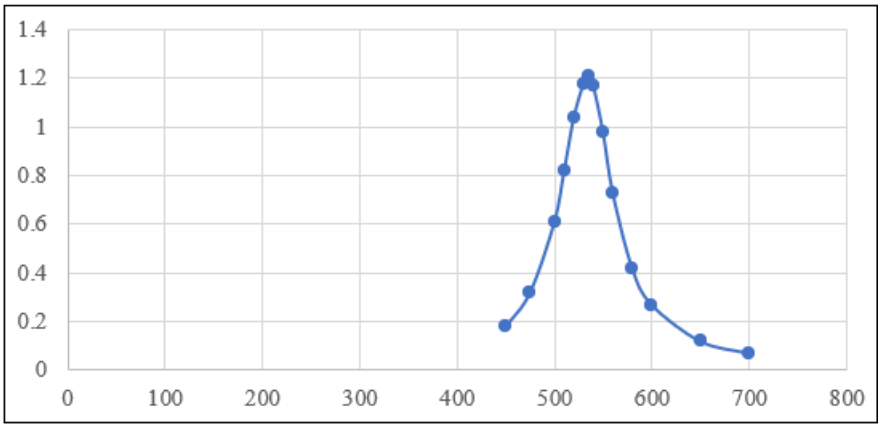
3. Results

3.1 Visual Confirmation and UV–Visible Spectral Characterization

Upon reaction with the neem leaf extract, the solution underwent a distinct colour change from pale yellow to reddish-purple, confirming the formation of colloidal gold nanoparticles via localized surface plasmon resonance (LSPR). The spectral profile (Table 2, Figure 1) shows an absorption maximum (λ_{max}) at 535 nm with an absorbance of 1.21 AU.

Table 2: UV–Visible absorption spectrum profile of synthesized AuNPs.

Wavelength (nm)	Absorbance (AU)	Wavelength (nm)	Absorbance (AU)
450	0.18	540	1.17
475	0.32	550	0.98
500	0.61	560	0.73
510	0.82	580	0.42
520	1.04	600	0.27
530	1.18	650	0.12
535	**1.21**	700	0.07



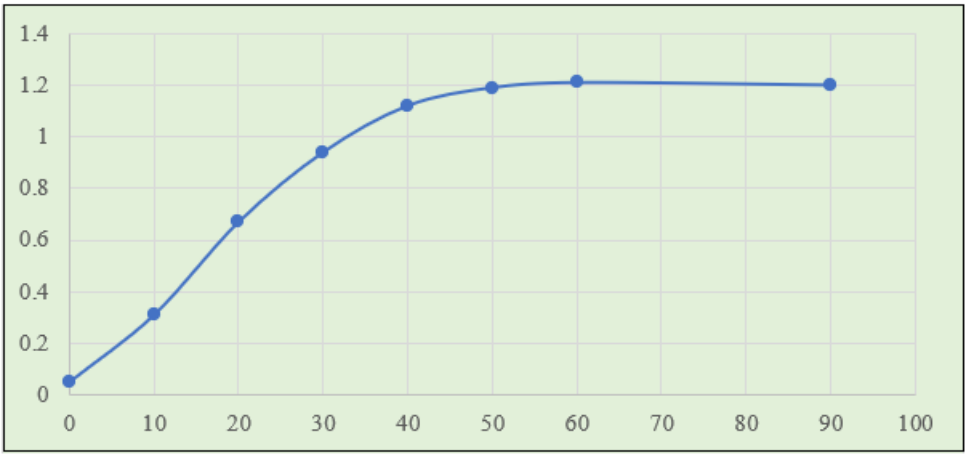
Graph 2: Showing the UV–Visible absorption spectrum profile of synthesized AuNPs

3.2 Effect of Reaction Time

Monitoring the reaction over time (Table 3, Figure 2) showed a steady increase in SPR absorbance up to 60 min (1.21 AU). Extending the reaction time to 90 min yielded no further increase (1.20 AU), demonstrating completion of the reaction within 60 min.

Table 3: Time-course monitoring of AuNP synthesis.

Time (min)	λmax (nm)	Absorbance (AU)
0	—	0.05
10	528	0.31
20	532	0.67
30	534	0.94
40	535	1.12
50	535	1.19
60	535	1.21
90	537	1.20



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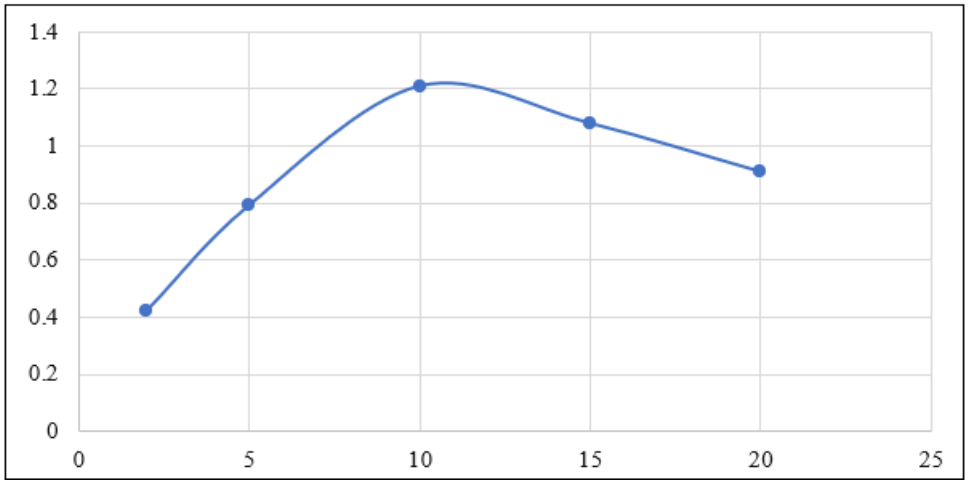
3.3 Effect of Plant Extract Volume

The volume of *A. indica* leaf extract was varied from 2 mL to 20 mL (Table 4, Figure 3). An extract volume of 10 mL produced the maximum absorbance (1.21 AU) with a sharp SPR peak. Higher extract volumes (15–20 mL) resulted in lower absorbance and redshifted λ_{max}, likely due to over-reduction or changes in the optical environment.

Table 4: Influence of leaf extract volume on AuNP optical density

Extract Volume (mL)	λmax (nm)	Absorbance (AU)
2	520	0.42
5	528	0.79
10	535	1.21
15	540	1.08
20	548	0.91



Graph 4: Showing the Influence of leaf extract volume on AuNP optical density

3.4 FTIR Spectral Analysis

FTIR analysis identified key functional groups involved in reducing $Au^{3+}$and capping the resulting AuNPs (Table 5).

Table 5: FTIR peak assignments for capped AuNPs.

Peak (cm^{-1})	Assignment	Interpretation
~ 3420	O–H / N–H stretching	Hydroxyl and amine groups from phenolics/proteins
~ 2922	Aliphatic C–H stretching	Hydrocarbon chains of phytochemical capping agents
~ 1635	C=O amide / aromatic vibration	Protein backbones and polyphenolic complexes
~ 1384	C–N phenolic vibration	Phytochemical nitrogen-containing metabolites DOCX
~ 1045	C–O / C–N stretching	Alcohols, carbohydrates, or ether linkages
~ 618	Metal-organic region	Surface interaction between gold and bio-molecules

3.5-Structural and Morphological Characterization (XRD, TEM, EDX, DLS):

1) **XRD Crystalline Phase**: Peaks observed at $2\theta = 38.2°, 44.4°, 64.6°$, and $77.5°$correspond to the (111), (200), (220), and (311) planes of face-centred-cubic (fcc) metallic gold. Application of the Scherrer equation ($D = \frac{K\lambda}{\beta \cos\theta}$) to the primary (111) peak indicates a crystalline domain size of approximately 20.0 nm.
2) **TEM Morphology:** TEM micrographs show predominantly spherical to quasi-spherical particles. Statistical analysis of particle size distributions gives a mean core diameter of 18.4 ± 5.6 nm (range: 8–31 nm). EDX Composition: Elemental analysis confirms elemental gold as the primary constituent (~ 82.0 wt%), alongside carbon (~ 7.0 wt%) and oxygen (~ 10.0 wt%) originating from the organic capping layer.
3) **DLS and Zeta Potential:** The hydrodynamic diameter determined by DLS is ~ 78 nmwith a Polydispersity Index (PDI) of 0.31. The larger DLS size relative to TEM core size is attributed to the hydrated phytochemical corona. The zeta potential of -24.6 mVindicates moderate electrostatic repulsion contributing to colloidal stability.

Table 6: Consolidated characterization summary of synthesized AuNPs.

Analytical Technique	Primary Observation	Physical Conclusion
UV–Vis	Peak at $\lambda_{max} = 535$ nm	Surface Plasmon Resonance of colloidal AuNPs
FTIR	Shifts at 3420, 2922, 1635, 1045 cm^{-1}	Phytochemical bio-capping layer
XRD	Reflections at $38.2°, 44.4°, 64.6°, 77.5°$	Crystalline face-centred-cubic (fcc) Au
TEM	Mean size: 18.4 ± 5.6 nm	Spherical to quasi-spherical core morphology
EDX	Au signal dominant (~ 82.0%)	High-purity metallic gold core
DLS	Size: ~ 78 nm(PDI: 0.31)	Hydrodynamic diameter in aqueous suspension
Zeta Potential	-24.6 mV	Moderately negative surface charge

4. Discussion

The optical absorption peak at 535 nm provides strong evidence for the successful green synthesis of gold nanoparticles. Plant-mediated AuNPs display characteristic LSPR bands whose intensity and position depend on particle size, shape, dielectric environment, and state of aggregation [2,3,8]. Time-course studies revealed rapid growth kinetics during the first 60 min, after which the reaction plateaued, confirming completion of salt reduction.

Varying the extract volume demonstrated that 10 mL provides the optimal concentration of reducing and capping metabolites. Excess plant extract lowered the overall absorbance, likely due to alternated nucleation-growth rates or altered dielectric properties of the medium. FTIR spectroscopy confirmed that plant metabolites specifically hydroxyl, amine, carbonyl, and aromatic compounds- are directly involved in reducing $Au^{3+}$to $Au^{0}$and capping the resulting nanoparticle surfaces [5–8].

Structural analysis by XRD verified the fcc metallic structure of gold. The larger hydrodynamic diameter (~ 78 nm) measured by DLS compared to the TEM core diameter (18.4 nm) reflects the solvation layer and organic capping mantle surrounding the particles in aqueous suspension. Finally, the negative surface charge (-24.6 mV) provides electrostatic stability, preventing rapid aggregation.

5. Conclusion

A successful green synthesis of stable gold nanoparticles using *Azadirachta indica* leaf extract was established. The reaction optimized at 10 mL leaf extract and 60 min reaction time at 60–80 °C, yielding spherical AuNPs with a characteristic SPR band at 535 nm. The particles exhibit a core diameter of 18.4 ± 5.6 nm, an fcc crystalline structure, a hydrodynamic diameter of ~ 78 nm, and surface stabilization provided by plant phytochemicals.

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