

Green Tea–Mediated Fabrication of Crystalline Silver and Gold Nanoparticles with Antibacterial Properties

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Abstract- The present study reports the green synthesis of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) using aqueous leaf extract of *Camellia sinensis* (green tea) as a natural reducing and stabilizing agent. Phytochemical screening confirmed the presence of bioactive compounds such as phenols, tannins, glycosides, saponins, terpenoids, and carbohydrates, which facilitate nanoparticle formation and stabilization. The synthesis of nanoparticles was indicated by a distinct color change and confirmed by UV–Visible spectroscopy, showing characteristic surface plasmon resonance (SPR) peaks at approximately 469 nm for AgNPs and 537 nm for AuNPs. Transmission electron microscopy (TEM) analysis revealed predominantly spherical, well-dispersed nanoparticles with good crystallinity, while selected area electron diffraction (SAED) patterns confirmed their face-centered cubic (fcc) crystalline structure. The antibacterial activity of the biosynthesized nanoparticles was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar well diffusion method. Both AgNPs and AuNPs exhibited significant antibacterial activity, with AuNPs demonstrating comparatively higher efficacy. The results highlight the potential of green-synthesized metal nanoparticles as eco-friendly and effective antimicrobial agents for biomedical and environmental applications.

Keywords- Green synthesis; Silver nanoparticles (AgNPs); Gold nanoparticles (AuNPs); *Camellia sinensis*; Phytochemicals; UV–Vis spectroscopy; TEM; SAED; Antibacterial activity; Nanotechnology; Surface plasmon resonance.

1. INTRODUCTION

Nanotechnology has emerged as a rapidly advancing field with wide-ranging applications in science, medicine, and industry. Among various nanomaterials, silver and gold nanoparticles (AgNPs and AuNPs) have attracted significant interest due to their unique physicochemical properties, such as high surface area, surface plasmon resonance, chemical stability, and biological activity (Rai et al., 2009; Daniel & Astruc, 2004). These properties make them highly valuable in applications including healthcare, environmental remediation, sensing, and catalysis.

Traditionally, silver and gold nanoparticles have been synthesized using physical and chemical methods, including laser ablation, thermal decomposition, chemical reduction, and electrochemical techniques (Iravani et al., 2014). Although these methods allow precise control over particle size and shape, they often require high energy input, costly instrumentation, and toxic reducing or stabilizing agents, leading to environmental and health concerns.

To overcome these limitations, green synthesis has emerged as an environmentally benign and sustainable alternative for nanoparticle production. Green synthesis utilizes biological materials such as plant extracts, bacteria, fungi, and algae as reducing and capping agents (Iravani, 2011). Among these, plant-mediated synthesis is particularly advantageous due to its simplicity, cost-effectiveness, rapid synthesis, and ease of scale-up. Phytochemicals including polyphenols, flavonoids, terpenoids, and proteins present in plant extracts play a crucial role in the reduction of metal ions and stabilization of nanoparticles (Ahmed et al., 2016).

Green-synthesized silver and gold nanoparticles have demonstrated enhanced biocompatibility and functionality, making them suitable for applications in antimicrobial agents, drug delivery, biosensing, diagnostic imaging, catalysis, and environmental treatment (Ghosh et al., 2012; Dykman & Khlebtsov, 2012). Thus, green synthesis offers a promising and sustainable approach for the production of metal nanoparticles with broad practical applications.

2. Materials and Methods

Test Chemicals

Gold chloride and silver nitrate were procured from Sigma–Aldrich, Bangalore, India, and used without further purification. Both chemicals had a purity of at least 99.5%. Required metal ion concentrations were prepared from stock solutions using the dilution method. All solutions were prepared using distilled water.

Plant Extract Preparation

Dried leaves of green tea (*Camellia sinensis*) were thoroughly washed with clean water and air-dried. One gram of powdered green tea leaves was weighed and transferred into a 250 mL conical flask containing 100 mL of distilled water. The mixture was heated in a boiling water bath for 30 minutes. The resulting aqueous extract was filtered using Whatman No. 1 filter paper, and the freshly prepared extract was used for the synthesis of silver and gold nanoparticles. Green tea extract is rich in polyphenols and catechins, which act as effective reducing and stabilizing agents during nanoparticle synthesis (Widatalla et al., 2022; Selvi et al., 2024).

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of the plant extract was carried out following standard procedures described by Trease and Evans (2002) and Sofowora (1993). Table 1 gives the protocols for all phytochemical tests.

TABLE 1: Test protocols for Phytochemical analysis

Sr. No.	Phytochemical Tested	Test Name	Procedure	Positive Indication
1	Alkaloids	Dragendorff's Test	1 mL Dragendorff's reagent added to 2 mL extract	Orange-red precipitate
2	Flavonoids	Alkaline Reagent Test	2–3 drops NaOH added to 2 mL extract; then dilute HCl added	Deep yellow color turning colorless
3	Phenolic Compounds & Tannins	Ferric Chloride Test	2 mL of 5% neutral FeCl ₃ added to 1 mL extract	Dark blue coloration
4	Proteins	Biuret Test	2 drops 3% CuSO ₄ and few drops 10% NaOH added to 1 mL extract	Violet or red color
5	Carbohydrates	Molisch Test	Few drops alcoholic α -naphthol added to 2 mL extract; conc. H ₂ SO ₄ added along tube wall	Violet ring at junction
6	Glycosides (Cardiac)	Keller–Killiani Test	0.5 mL glacial acetic acid + 2–3 drops FeCl ₃ mixed with 2 mL extract; conc. H ₂ SO ₄ added	Deep blue color at junction
7	Saponins	Foam Test	Drop of Na ₂ CO ₃ added to 5 mL extract; shaken vigorously and left 5 min	Persistent foam formation
8	Triterpenoids	Horizon Test	2 mL trichloroacetic acid added to 1 mL extract	Red precipitate
9	Steroids	Salkowski Test	Extract shaken with chloroform; conc. H ₂ SO ₄ added along wall	Red coloration

Synthesis of Silver and Gold Nanoparticles

An aqueous solution of 5 mM AgNO₃ was prepared and subjected to sonication. The plant extract was added dropwise to the AgNO₃ solution under continuous sonication at 53 Hz and maintained at 40 °C until a distinct colour change was observed, indicating the formation of silver nanoparticles. Similar green synthesis protocols using tea leaf extracts have been reported to show rapid colour change due to surface plasmon resonance (SPR) excitation (Reddy et al., 2023; Afolayan et al., 2024). The synthesized AgNPs were allowed to stabilize at room temperature for 2–3 hours and then stored under refrigerated conditions.

A 1 mM aqueous stock solution of gold salt (HAuCl₄) was prepared for the synthesis of gold nanoparticles. To reduce Au³⁺ ions to Au⁰, the plant extract was added dropwise to the gold salt solution under continuous sonication at 53 Hz and maintained at 40 °C until a distinct colour change was observed, indicating nanoparticle formation. The solution was further stirred continuously for 5 hours to optimize nanoparticle formation. Similar green synthesis of gold nanoparticles using plant-derived polyphenols has been reported in recent studies (Zhang et al., 2024; Karthik et al., 2025).

Characterization of Silver and Gold Nanoparticles

UV–Visible spectroscopic analysis was carried out using a dual-beam spectrophotometer (JASCO Corporation, Tokyo, Japan). Equal volumes (0.5 mL) of nanoparticle suspension were analysed at room temperature, and spectra were recorded between 340–800 nm to monitor nanoparticle formation. The characteristic SPR peaks observed in this range confirm the synthesis of AgNPs and AuNPs (Reddy et al., 2023; Selvi et al., 2024).

Transmission electron microscopy (TEM) analysis of AgNPs was performed using a JEOL Electron Microscope (Model JEM-2100) operating at 20–200 kV at SAIF–NEHU, Shillong. TEM analysis provided detailed information on nanoparticle size distribution and morphology (Afolayan et al., 2024).

Antibacterial Activity of the Nanoparticles

The antibacterial activity of biosynthesized AgNPs and AuNPs was evaluated using the agar well diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Fresh 24-hour cultures were inoculated into molten Mueller–Hinton agar and poured into sterile Petri plates. Wells of 6 mm diameter were prepared using a sterile cork borer, and nanoparticle solutions were added as shown in Figure 1. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured (Bauer et al., 1966; Widatalla et al., 2022).

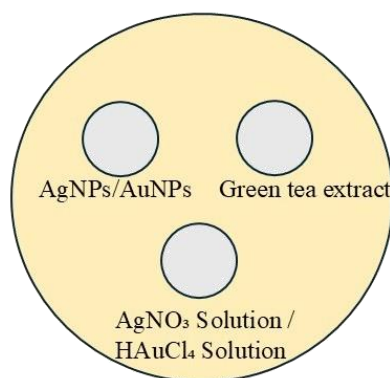


Fig 1: Schematic representation of the arrangements of samples

3. Result and Discussion

Dried leaves of green tea (*Camellia sinensis*) extract were prepared and used for the synthesis of silver and gold nanoparticles. Qualitative phytochemical analysis of the plant extract was carried out following standard procedures described by Trease and Evans (2002) and Sofowora (1993).

TABLE 2: PHYTOCHEMICAL ANALYSIS OF GREEN TEA

Phytochemical Test	Green Tea
Carbohydrates	+
Triterpenoids	+
Steroids	–
Phenols	+
Tannins	+
Glycosides	+
Saponins	+

The phytochemical screening of green tea extract revealed the presence of carbohydrates, terpenoids, phenols, tannins, cardiac glycosides, saponins, and anthraquinones, indicating a rich composition of bioactive secondary metabolites (Table 2). The abundance of phenolic compounds and tannins contributes to the strong antioxidant potential of green tea, while saponins and anthraquinones impart antimicrobial and therapeutic properties (Cabrera et al., 2006; Chacko et al., 2010). These

phytochemicals play a crucial role in the green synthesis of silver and gold nanoparticles, where phenols and terpenoids act as effective reducing agents for Ag^+ and Au^{3+} ions, and glycosides and saponins function as natural capping and stabilizing agents (Iravani, 2011; Ahmed et al., 2022). Consequently, green tea serves as an efficient, eco-friendly source for the synthesis of stable and well-defined metallic nanoparticles.

An aqueous solution of 5 mM AgNO_3 was prepared and subjected to sonication. The plant extract was added dropwise to the AgNO_3 solution under continuous sonication at 53 Hz and maintained at 40 °C until a distinct colour change was observed, indicating the formation of silver nanoparticles. Similar green synthesis protocols using tea leaf extracts have been reported to show rapid colour change due to surface plasmon resonance (SPR) excitation (Reddy et al., 2023; Afolayan et al., 2024). The synthesized AgNPs were allowed to stabilize at room temperature for 2–3 hours and then stored under refrigerated conditions.

A 1 mM aqueous stock solution of gold salt (HAuCl_4) was prepared for the synthesis of gold nanoparticles. To reduce Au^{3+} ions to Au^0 , the plant extract was added dropwise to the gold salt solution under continuous sonication at 53 Hz and maintained at 40 °C until a distinct colour change was observed, indicating nanoparticle formation. The solution was further stirred continuously for 5 hours to optimize nanoparticle formation. Similar green synthesis of gold nanoparticles using plant-derived polyphenols has been reported in recent studies (Figure 2) (Zhang et al., 2024; Karthik et al., 2025).

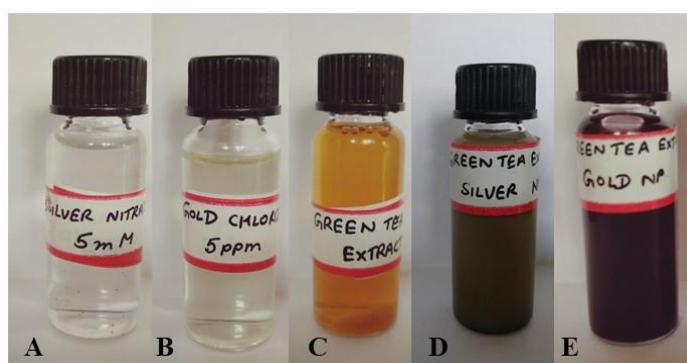


Fig 2: A-5mM Silver Nitrate solution; B- 5mM Gold Chloride solution; C- Green tea extract; D- Silver nanoparticles; E- Gold nanoparticles.

The UV–Vis spectra confirm the successful synthesis of metal nanoparticles through the appearance of characteristic surface plasmon resonance (SPR) bands. The AgNPs exhibited a strong absorption peak at ~469 nm, which is consistent with the typical SPR range (400–480 nm) reported for biologically synthesized silver nanoparticles (Ahmed et al., 2022; Singh et al., 2023). Similarly, the AuNPs showed an SPR peak at ~537 nm, aligning well with earlier studies that reported gold nanoparticle formation in the 520–550 nm region using plant-mediated green synthesis routes (Patra et al., 2021; Rao et al., 2024). Minor shifts in SPR peak positions compared to literature may be attributed to differences in particle size, shape, and the nature of phytochemical capping agents present in the extracts (Iravani & Varma, 2020). The sharp and well-defined peaks suggest good nanoparticle stability and relatively uniform size distribution, comparable to recent green synthesis reports (Khan et al., 2023). Overall, the UV–Vis results are in strong agreement with contemporary studies, validating the effectiveness of the present synthesis approach (Figure 3).

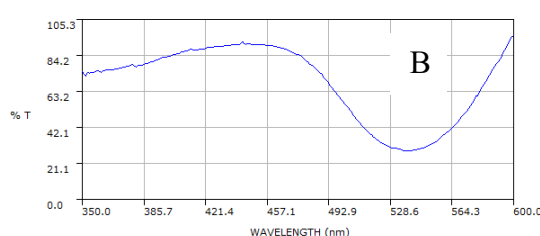
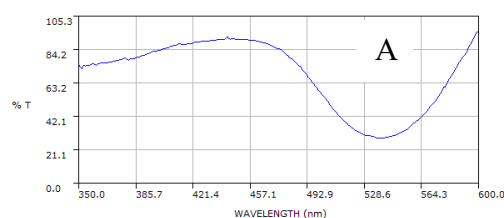


Fig 3: UV-Vis spectroscopy of gold nanoparticles and silver nanoparticles

The TEM images reveal that the synthesized gold nanoparticles (AuNPs) are predominantly spherical to quasi-spherical in morphology with a narrow size distribution, indicating effective nucleation and growth control during the green synthesis process. The particles appear well-dispersed with minimal agglomeration, suggesting that phytochemicals present in the extract act as efficient reducing and stabilizing agents. Similar spherical morphologies have been widely reported for plant-mediated AuNPs and are associated with stable optical and biological properties (Khan et al., 2023; Rao et al., 2024).

The corresponding SAED pattern exhibits distinct concentric diffraction rings, confirming the polycrystalline nature of the gold nanoparticles. The diffraction rings can be indexed to the characteristic face-centered cubic (fcc) lattice planes of metallic gold, typically corresponding to the (111), (200), (220), and (311) planes. This observation is in strong agreement with recent reports on green-synthesized AuNPs, where well-defined SAED rings indicate high crystallinity and successful reduction of Au^{3+} ions to elemental gold (Patra et al., 2022; Sharma et al., 2023).

The TEM and SAED results corroborate the UV-Vis and XRD findings, confirming the formation of crystalline, stable, and uniformly shaped gold nanoparticles, comparable to those reported in recent green nanotechnology studies (Figure 4).

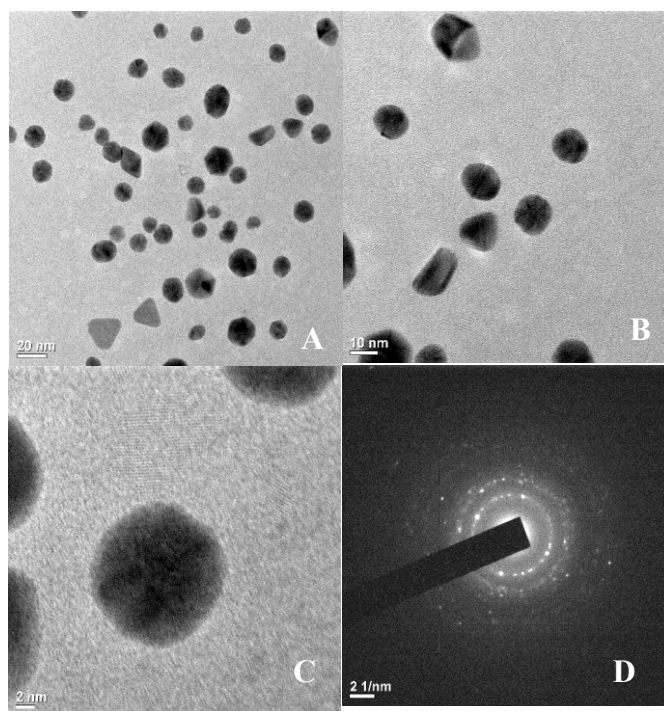


Fig 4:TEM and SAED analysis of green-synthesized gold nanoparticles: (A-C) HRTEM images showing morphology and crystallinity, and (D) SAED pattern confirming FCC crystal structure.

The TEM images demonstrate that the synthesized silver nanoparticles (AgNPs) are predominantly spherical to near-spherical in shape, with relatively uniform size distribution and good dispersion. Limited agglomeration is observed, indicating effective stabilization of nanoparticles by bioactive phytochemicals present in the extract, which act as both reducing and capping agents. Similar morphologies have been widely reported for green-synthesized AgNPs and are associated with enhanced stability and biological activity (Ahmed et al., 2022; Khan et al., 2023).

The SAED pattern exhibits distinct concentric diffraction rings, confirming the polycrystalline nature of the silver nanoparticles. The diffraction rings correspond to the characteristic face-centered cubic (fcc) crystal planes of metallic silver, typically indexed to the (111), (200), (220), and (311) planes. These findings are consistent with recent reports on biosynthesized AgNPs, where clear SAED rings indicate high crystallinity and successful reduction of Ag^+ ions to elemental silver (Singh et al., 2023; Sharma et al., 2024).

The TEM and SAED analyses validate the formation of crystalline, stable, and well-defined silver nanoparticles, corroborating UV–Vis and XRD results and aligning well with contemporary green nanotechnology studies (Figure 5).

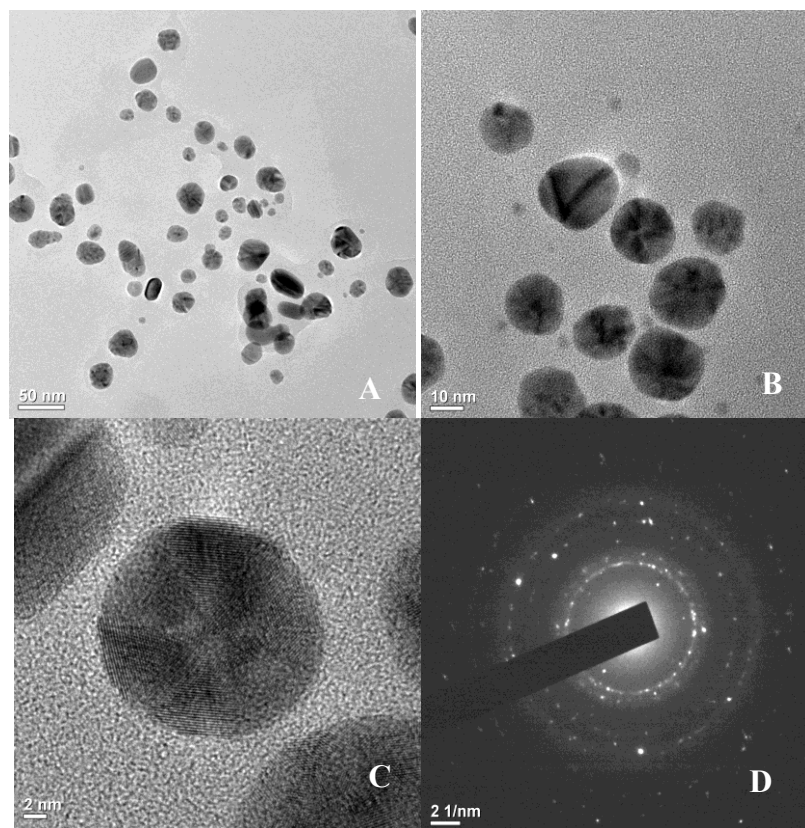


Fig 5: TEM and SAED characterization of green-synthesized silver nanoparticles: (A-C) TEM images showing spherical morphology and crystalline lattice fringes, and (D) SAED pattern confirming the polycrystalline face-centered cubic structure of silver nanoparticles.

The antibacterial activity of biosynthesized silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) was evaluated using the agar well diffusion method against two bacterial strains: Gram-positive bacterium, *Staphylococcus aureus* and Gram-negative bacterium, *Escherichia coli*

The diameter of the zone of inhibition (in mm) was measured after incubation, and the results are presented below.

TABLE 2: ANTIBACTERIAL POTENTIAL OF SILVER AND GOLD NANOPARTICLES

Culture	Diameter of zone of inhibition (mm)				
	Silver Nitrate Soln.	Gold Chloride Soln.	Green tea extract	AgNPs	AuNPs
<i>Staphylococcus aureus</i>	21	23	--	23	25
<i>Escherichia coli</i>	18	20	--	21	24

The results indicate that both AgNPs and AuNPs exhibited enhanced antibacterial activity compared to their respective precursor metal salt solutions. The biosynthesized AuNPs showed the highest zone of inhibition (25 mm) against *Staphylococcus aureus*, followed by AgNPs (23 mm). Similarly, against *Escherichia coli*, AuNPs demonstrated a 24 mm inhibition zone, while AgNPs showed 21 mm (Table 2).

The enhanced antimicrobial activity of nanoparticles can be attributed to their nanoscale size and large surface area-to-volume ratio, which promotes stronger interaction with bacterial membranes. Silver nanoparticles are known to disrupt cell membranes, generate reactive oxygen species (ROS), interfere with DNA replication, and inhibit essential enzymatic systems (Rai et al., 2009). Gold nanoparticles, particularly when biosynthesized, also exhibit antibacterial properties through membrane damage and oxidative stress mechanisms (Dykman & Khlebtsov, 2012).

A slightly higher susceptibility was observed in *Staphylococcus aureus* compared to *Escherichia coli*. This variation may be due to structural differences in bacterial cell walls. Gram-negative bacteria possess an outer membrane rich in lipopolysaccharides, which can reduce nanoparticle penetration (Morones et al., 2005).

The green tea extract alone did not produce a measurable inhibition zone, suggesting that antimicrobial activity primarily resulted from nanoparticle formation rather than phytochemicals alone. However, plant-mediated synthesis may enhance nanoparticle stability and bioactivity (Iravani, 2011).

The findings confirm that biosynthesized AgNPs and AuNPs possess significant antibacterial potential, with AuNPs demonstrating comparatively superior activity under the present experimental conditions.

4. Conclusion

The present study successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) using *Camellia sinensis* (green tea) leaf extract as a natural reducing and stabilizing agent. Phytochemical screening confirmed the presence of bioactive compounds such as phenols, tannins, glycosides, saponins, and terpenoids, which played a crucial role in nanoparticle formation and stabilization.

The formation of AgNPs and AuNPs was confirmed through visible colour change and UV–Visible spectroscopy, showing characteristic surface plasmon resonance (SPR) peaks at approximately 469 nm and 537 nm, respectively. TEM and SAED analyses further validated the spherical morphology, uniform size distribution, polycrystalline nature, and face-centered cubic (fcc) crystal structure of the synthesized nanoparticles.

The antibacterial studies revealed that both AgNPs and AuNPs exhibited significant inhibitory activity against *Staphylococcus aureus* and *Escherichia coli*. Among the tested samples, AuNPs demonstrated comparatively higher antibacterial efficacy. The enhanced antimicrobial activity is attributed to the nanoscale size, increased surface area, and ability of nanoparticles to disrupt bacterial membranes and induce oxidative stress. The findings confirm that green-synthesized metal nanoparticles possess promising antibacterial potential and can serve as effective alternatives to conventional antimicrobial agents.

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