Original Article

Biogenic fabrication of gold nanoparticles using Camellia japonica L. leaf extract and its biological evaluation

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ABSTRACT

Development of green technique for the fabrication of noble metal nanoparticles is of great importance in order to avoid the usage of toxic chemicals. In this strategy, gold nanoparticles (AuNPs) are synthesized at room temperature by using Camellia japonica leaf extract under room temperature. The successful formation of AuNPs was confirmed by various spectroscopic techniques including UV, FTIR, XRD and SEM studies. The resulting antimicrobial activity of the synthesized AuNPs stabilized in C. japonica is tested against seven different microbial strains such as Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Candida albicans. The present study opens a new window for future synthesis of AuNPs via green technique.

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1. Introduction

Nanotechnology has recently advanced as a great interdisciplinary area of research, developing new nanoscale structures, properties, particle size and morphology. Metal nanoparticles have unique physical and chemical properties that are significantly different from bulk properties. Noble metal nanoparticles such as Ag, Au, Pd and Pt possess great advantages in the field of physics, chemistry and biological aspects [1–6]. Among them, gold nanoparticles (AuNPs) have been considered for many applications in biomedical science including drug delivery, tissue/tumor imaging, photothermal therapy, catalysis and biochemical sensors due to their extraordinary physicochemical properties [7–13]. Therefore, various researchers [14–18] have developed different methods for the synthesis of AuNPs by using different chemicals (sodium citrate, elemental hydrogen, LiAlH4, etc.), which act as reducing agents.
The continuous usage of hazardous chemicals enters into the aquatic and soil ecosystem, causing numerous diseases to the living organisms. In addition, the aforementioned chemical technique needs high temperature, costly chemicals and generates hazardous by-products [19]. Therefore, the development of simple, green and cost-effective method is an important concern. Recently, the synthesis of AuNPs via green route using fungi, algae and plant extracts has gained much attention among the researchers due to its non-toxic as well as eco-friendly nature [20–23]. Camellia japonica L. plant belongs to the family of Theaceae an annual and perennial herb. Its stems are glabrous, the leaves are deeply lobed and irregularly toothed.

In this study, the synthesis of AuNPs by using C. japonica L. leaves extract at room temperature was, for the first time, performed without using any chemicals. The as-prepared AuNPs were characterized by various analytical and spectroscopic techniques. Furthermore, the AuNPs showed an excellent antibacterial activity against human pathogenic bacteria.

### 2. Experimental

#### 2.1. Materials and methods

The fresh plant leaves of C. japonica were collected from Virudhunagar Hindu Nadars’ Senthikumara Nadar College campus voucher number SPGH 316 to plant identified in herbarium specimen. Chlorauric acid (HAuCl₄) was received from Sigma–Aldrich Company. All other chemicals and reagents are analytical grade and were used without further purification.

#### 2.2. Preparation of C. japonica L. aqueous leaves extract

The collected fresh leaves of C. japonica were washed with double distilled (DD) water twice to remove the dust particles and other impurities on the surface of the leaves. After that, it was allowed to dry at room temperature in shadows.
Afterward, the dried leaves were cut into very small fine pieces. Weighed quantity (5 g) of the fine pieces of dried leaves was transferred into 500 mL beaker containing 200 mL water. The mixture was boiled for about 10 min. The pale yellow colored slurry obtained was filtered through Whatman No. 1 filter paper. The filtrate was used as reducing and stabilizing agent for the preparation of AuNPs. The filtrate was stored in the refrigerator at 4 °C for further experiments.

2.3. Green synthesis of AuNPs using C. japonica leaves extract

In a typical experimental method, 10 mL of C. japonica leaf extract was added with 50 mL of 0.5 mM of gold solution taken in a 100 mL beaker at room temperature with constant stirring. Change of color of the solution from pale yellow to wine red was observed in 40 min. It confirmed the successful formation of AuNPs and it was further confirmed by the characteristic UV–vis spectroscopy. Further, the AuNPs formed were collected as residue by centrifugation at the rate of 6000 rpm/min for about 30 min. The residue thus obtained was washed several times with water, dried at room temperature and stored in amber color bottle. AuNPs synthesized by this greener way was characterized by physicochemical methods.

2.4. Characterization of the AuNPs

The preliminary formation of green synthesized AuNPs were monitored by UV–vis spectroscopy (jasco V-770 spectrophotometer) in the windows potential range of 200 to 800 nm. The IR spectrum was recorded before and after the addition of C. japonica plant leaves extract using Shimadzu FT-IR-8201PC instrument. The powder X-Ray diffraction (XRD) studies were recorded on a PAN analytical X’pert powder instrument.

2.5. Antimicrobial activity

The antimicrobial activities were done by seven microbial strains such as Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Candida albicans by standard agar well diffusion method. The antimicrobial activity of the C. japonica leaves extract reduced AuNPs were determined by well diffusion method. Agar medium was used to cultivate bacteria. Overnight cultures of inoculums (20 mL) were spread on to agar plates. Then the Gram positive and negative strains of microorganisms like B. subtilis, S. aureus, S. faecalis, K. pneumoniae, P. aeruginosa, E. coli and C. albicans were swabbed on the plates. Wells were formed by using a cork borer of 6 mm diameter. Leaves extract, HAuCl₄ and synthesized gold nanoparticles at different concentrations (100 µg/ml, 150 µg/ml, 200 µg/ml) were poured into each well. The doped plates were kept for incubation at 37 °C for 24 h. The antimicrobial activity was assayed by measuring the zones of inhibition formed around the well by using Streptomycin as a positive control.

3. Results and discussion

AuNPs shows a characteristic band at 539 nm in its Surface Plasmon Resonance (SPR). The UV–vis band centered on 539 nm was observed for the synthesized AuNPs (Fig. 1A), thus confirming the formation of AuNPs [17]. The spectra recorded for the aliquots of reaction mixtures with varying concentrations of HAuCl₄ is given in Fig. 1B and the spectra recorded for the aliquots of reaction mixtures isolated at different time intervals of the bio-reduction reaction is given in Fig. 1C. The formation of the AuNPs was principally identified by the color change from the yellow to wine red color within 40 min after the addition of C. japonica leaf extract to 1 mM aqueous HAuCl₄. It also indicates the stability of the particles at physiological conditions.

The functionality of the bio-reducing biomolecules in the C. japonica leaves extract are responsible for the bio-reduction of Au³⁺ into Au has been arrived from the FT-IR spectroscopic study. The FT-IR spectrum of the C. japonica leaves extract (curve ‘a’ of Fig. 1D) shows an intense band at 1070 cm⁻¹ corresponding to the C–OH stretching vibrations of secondary alcoholic functionality [24] present in the extract. The peaks obtained both at 1398 and 1634 cm⁻¹ corresponds to the C–N stretching vibrations of aromatic amines and the amide (I) group arises due to the carbonyl stretching vibrations in the amide linkages of the proteins [25]. The broad peaks at around 3427 (curve a) cm⁻¹ corresponds to NH stretching vibrations of amide (II) group [16]. After the reduction of chloroaurate

![Fig. 2 – (A) XRD pattern of the AuNPs and (B) Camellia japonica plant.](image-url)
ion (curve b), the peak shifts from 3427 cm\(^{-1}\) to 3440 cm\(^{-1}\) indicating the N–H functional group of C. japonica as a reducing agent in the synthesis of AuNPs. Thus, the FTIR analysis clearly shows that capping and reducing functionality of the biomolecules present in leaf extract of C. japonica for the synthesis AuNPs is also responsible for its prolonged stability.

The crystalline nature and purity of the synthesized AuNPs were determined by X-ray diffraction analysis as presented in Fig. 2A. The pronounced diffraction peaks in the 2\(\theta\) values at 38.12°, 44.12°, 63.34° and 79° corresponds to (111), (200), (220) and (311) lattice planes of face centered cubic structure of AuNPs, respectively. The main lattice plane (111) was observed by its high peak intensity than the other planes. There are no other discernible peaks present which confirm the purity of the synthesized AuNPs. The obtained results are in good agreement with the JCPDS file number (00-004-0783) and previous literatures [26–29]. In addition, the average particle size was calculated using Scherer’s equation as follows:

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

where \(k\) is the constant, \(\lambda\) is the wavelength (\(\lambda = 1.5406\ \text{Å}\)), \(\beta\) is full width at half maximum (FWHM) of the high intensity plane, \(\theta\) is Bragg’s angle. The estimated average crystalline size of the synthesized AuNPs is 24 nm.

Fig. 3A and B shows the SEM micrographs of the prepared AuNPs showing the close attachment of the sphere-like structure with smooth surfaces and the spheres with each other. Moreover, the detailed surface structure of the prepared

![Fig. 3](https://example.com/fig3)

**Fig. 3** – (A and B) SEM micrographs of AuNPs; (C) HRTEM image of AuNPs; (D) the selected area electron diffraction (SAED) image of AuNPs; (E) particle size distribution of AuNPs.
Fig. 4 – Antimicrobial activity of AuNPs against (1) Bacillus subtilis, (2) Staphylococcus aureus, (3) Streptococcus faecalis, (4) Klebsiella pneumoniae, (5) Pseudomonas aeruginosa, (6) Escherichia coli, (7) Candida albicans (a – Camellia japonica leaves extract, b – HAuCl₄, c–e – different concentrations of AuNPs, f – streptomycin).

Fig. 5 – Antimicrobial activity of synthesized AuNPs against different microbial strains.
Table 1 – Antimicrobial evolution of AuNPs in different dose rates against selected microbial strains in terms of diameter of zone of inhibition (mm).

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>AuNPs</th>
<th>Streptomycin (10 μg/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>100 μg/ml</td>
<td>150 μg/ml</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>11 ± 0.82</td>
<td>14 ± 1.41</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>11 ± 0.82</td>
<td>12 ± 0.82</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>10 ± 0.82</td>
<td>12 ± 0.82</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10 ± 0.82</td>
<td>13 ± 1.41</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12 ± 0.82</td>
<td>15 ± 0.82</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>11 ± 0.82</td>
<td>12 ± 0.82</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10 ± 0.82</td>
<td>11 ± 0.82</td>
</tr>
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</table>

Each value represents mean ± standard deviation of triplicates.

AuNPs was analyzed using transmission electron microscopy (TEM) analysis and the results are shown in Fig. 3C and D. Fig. 3C clearly confirms the sphere-like surface structure with the average particle size 20 nm and also in accordance with powder XRD data. The SAED pattern in Fig. 3D and E revealed a high crystalline nature and particle size distribution of the prepared AuNPs.

The gold nanoparticles were studied for antimicrobial activity against seven different pathogens namely B. subtilis, S. aureus, S. faecalis, K. pneumoniae, P. aeruginosa, E. coli and C. albicans and the results are represented in Fig. 4.

The diameter of the zone of inhibition (ZOI) in and around each well in different concentrations levels of AuNPs were presented in Fig. 5 and the average diameter of ZOI as a result of triplicate measurements are given in Table 1. AuNPs show maximum antibacterial effect against the strains such as B. subtilis (15 ± 0.82 mm) and P. aeruginosa (15 ± 0.82 mm).

The antimicrobial action of gold NPs against Gram-positive and Gram-negative bacterial strains were explained by Shamaila et al. [30]. For the Gram positive bacteria the structure of the membrane, i.e., the peptidoglycan layer's thickness, which is an important part of pathogenic bacteria, is 50% higher than in Gram-negative bacteria. Thus, larger doses of nanoparticles are required for Gram-positive bacteria. This reflects in our study that the AuNPs were found to be active against the bacterial strains only in high dose rates (200 μg/mL).

4. Conclusion

Herein, a green method for the synthesis of gold nanoparticles (AuNPs) has been reported where C. japonica leaves extract were used as a reducing agent. The formation of nanoparticles were confirmed by various spectroscopic and physical techniques such as UV–vis, FT-IR, XRD, SEM and HRTEM. Furthermore, antimicrobial screening was performed with the synthesized AuNPs against various Gram-positive and Gram-negative pathogens including B. subtilis, S. aureus, S. faecalis, K. pneumoniae, P. aeruginosa, E. coli and C. albicans. The AuNPs showed a moderate activity at the dosage rate of 200 μg/mL.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

[10] Almirall JR, Furton KG, Wu IJ. Optimization of solid-phase microextraction (SPME) for the recovery of explosives from aqueous and post-explosion debris followed by gas and


