# Green synthesis of gold nanoaprticles using black tea extract and their effect on the morphology and their antibacterial activity

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**Abstract:** Herbal extract stabilized green synthesis of nanoparticles is an alternative reducing agent for chemical synthesis. In this manuscript, green synthesis of gold nanoparticles (AuNPs) has been performed using aqueous extract of black tea. The effect of tea extract concentration on the morphology of the particles was studied. Formation, functional groups, crystalline phase, and morphology changes of the nanoparticles were characterized by UV-Vis spectrophotometer, Fourier transforms spectrometer, an X-ray diffraction pattern (XRD), scanning electron microscope (SEM), energy dispersive diffraction (EDS), and transmission electron microscopy (TEM) coupled with selected area diffraction. Antibacterial activity AuNPs were studied against bacteria. It was found that as the concentration of the tea extract increased, the shape of the particles changed and finally became spherical at high concentrations. The results of this research reveal the antibacterial activity of AuNPs.

**Keywords:** black tea extract; gold nanoparticles; antioxidant activity; morphology.

## 1. INTRODUCTION

Green synthesis of metal nanoparticles has drawn the attention of researchers due to their properties and applications (Subramanian *et al.*, 2013; Chinnadurai *et al.*, 2020). Among the metal nanoparticles, AuNPs are remarkable due to their applications in catalysis, optical sensing, and imaging (Shahzad *et al.*, 2017; Nazar Ul *et al.*, 2019; Abbasi *et al.*, 2015; Thakur *et al.*, 2022). The biosynthesis of nanoparticles has attracted scientists' attention because of the necessity to develop clean, cost-effective synthesis techniques. Among the natural reducing agents, plant extracts are considered necessary due to the ease of availability and simple procedure (Lee *et al.* 2020). Green synthesis of AuNPs can be carried out at room temperature. The reaction is simply scalable because varying reaction conditions can adjust the shape and size of NPs. Tea extract is rich in phenolic compounds. Biological activities such as radial scavenging, antibacterial and antifungal, and anticancer activity against cell lines have beenproven (Santhiya *et al.*, 2020).

Plant extracts stabilized green synthesis of AuNP is more beneficial than the microorganism and other reducing agents. Additionally, this method is better suited for industrial scale. A detailed understanding of bioactive nanomaterials served as the foundation for efforts to produce new green-methodology--based approaches for fabricating AuNPs. Many researchers have used plants' extract as

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reducing and' extract as reducing and' extract as reducing and' extract as reducing and capping agents to produce the AuNPs for various applications. Metal nanoparticle metals are extensively used in the environment, medicine, and engineering. At present, nanoparticles are synthesized mainly by chemical methods that have reverse effects on the environment, large energy consumption, and health problems. In reply to these challenges, green synthesis, which uses plant extracts instead of chemicals to reduce metal ions, has been proposed (Ying *et al.*, 2022).

Some the plant extracts used for the green synthesis of AuNPs are mentioned below. *Syzygium cuminil* (Vineet & Sudesh Kumar, 2012), *Mimosa tenuiflora* (Ericka *et al.* 2019), *Ziziphus zizyphus* (Aljabali *et al.* 2018), *Moringa oleifera* (Jayanta *et al.* 2021), *Cinnamon bark* (Omar *et al.* 2020), *Malva Verticillata* (Ismail *et al.* 2020), *Caulerpa racemosa* (Manikandakrishnan, 2019), *Simarouba glauca* (Thangamani & Bhuvaneshwari, 2019), *Annona muricata* (Aderonke *et al.*, 2019), *Melia azedarach* (Siwar *et al.*, 2020), *Mentha longifolia* (Rauf *et al.*, 2021), *Platycodon grandiflorum* (Anbu *et al.*, 2020), *Combretum indicum* (Gupta *et al.* 2021), and *Azhadirachta indica* (Patil *et al.*, 2022) have been used as green reducing agents for the synthesis of AuNPs. The synthesized AuNPs have been evaluated for antibacterial, antifungal, antioxidant, cytotoxicity, and photocatalytic activities (Lee *et al.*, 2020). Black tea is a valuable source of protein, minerals, and secondary plant metabolites such as tannins and saponins, polyphenols such as catechins and theaflavins. A major constituent of black tea is caffeine. Black tea is a wonder herbal that possesses antibacterial and antioxidant activities (Ramdani *et al.*, 2013; Turkmen *et al.*, 2007; Ramdani *et al.*, 2022; Eric *et al.*, 2011; Ochoa & Moyano, 2022). In the continuation of finding a new reducing agent, it was aimed to synthesize AuNPs using black tea extract and evaluate their morphology, and antibacterial activity

## 2. MATERIALS AND METHODS

### 2.1. Reagents

Auric chloride was purchased from LobaChemie, Mumbai, India. Double distilled water was used to prepare the solutions. Chemicals required for antibacterial activity were purchased from Ranchem, Mumbai, India. All the reagents were used as received without further purification.

### 2.2. Green synthesis of AuNPs

Various concentrations of black tea extract (0.2, 0.6 and 1.2 ml) were mixed with 10 ml of 1 mM auric chloride solution with constant stirring on a magnetic stirrer. A UV-Vis spectrophotometer monitored results. However, 1.2 ml of a black extract with auric chloride solution turned to dark pinkish red, revealing the formation of AuNPs. The color change may be due to the interaction between electromagnetic radiation and metallic nanoparticles, referred to as Surface Plasmon Resonance (SPR) (Smitha *et al.*, 2009). Hence, AuNPs synthesized using 1.2 ml were subjected to UV-Vis, FTIR, XRD, and SEM-EDS. Moreover, AuNPs synthesized using 0.2, 0.6 and 1.2 ml were subjected to TEM images to understand the effect of the concentration of black tea extract on morphology.

### 2.3. Characterization

Elico S3-159Elico S3-159 assessed the formation of AuNPs and photoluminescence assessed the formation of AuNPs and photoluminescence, UV-Visible spectrophotometer operated at a resolution of 1 nm from the range 200-800 nm. FTIR spectra of the samples were measured using Bruker-Tensor 27 FTIR spectrometer in the frequency range of 4000-400 $cm^{-1.}$ The XRD pattern was carried out on Shimadzu model Lab-X-XRD-6000 operated at a voltage of 40 kV and a current of 30 mA with Cu-Kα radiation at 1.5406A° wavelength. The SEM image analysis was taken on a JEOL model JSM-6390, at an accelerating voltage of 20 KeV. Energy dispersive X-ray (EDS) analysis was carried out on a JEOL JSM-6390 microscope coupled with an EDS analyzer. The transmission electron micrographs were recorded using Technai-G-10, an 80 KV TEM.

### 2.4. Antibacterial activity

The bactericidal activity of AuNPs was evaluated by agar well diffusion method against *Pseudomonas aeruginosa (P. aeruginosa)*, *Vibrio cholera (V. cholera)*, *Staphylococcus aureus (S. aureus)*, and *Escherichia coli (E. coli)*, according to the procedure described in the literature (Chinnadurai *et al.*, 2021). Appropriate quantities of nutrient agar medium were prepared by dissolving it in 100 ml of distilled water. The bacterial strain was suspended in Muller Hinton agar and diluted ($1\times10^5$ cfu/ml).

Wells having 5 ml diameter were cut using a sterile cork-borer and poured into the wells. The petri plates were incubated for 24 h at 37°C. The zone of inhibition was measured and expressed as diameter in mm. Gentamycin was used as a standard drug for comparison.

## 3. RESULTS AND DISCUSSION

### 3.1. UV-Visible study

The result expressed the green synthesis of AuNPs using the black tea extract. The black tea extract reduced an aqueous solution of auric chloride to nucleate AuNPs. The formation of AuNPs was noticed by color changes in the reaction mixture, where the $Au^{3+}$ ions was reduced by black tea extract into $Au^0$. Auric chloride is an aqueous solution remained yellow colored. After adding 1.2 ml of black tea extract, the pale yellow was changed to pale pink due to the plasmon resonance spectrum of AuNPs (Das *et al.* 2022). Progress of the reaction was monitored by UV-Vis spectrophotometer as a result of Surface Plasmon Resonance (SPR). The SPR is sensitive to the size and shape of the nanoparticles (Moores & Goettmann, 2006). Absorbance of the reaction mixture was increased with increasing the tea extract concentration concerning reaction time (0-5 h), which caused the red shift of the peak. The absorbance versus wavelength of AuNPs were recorded and plotted against time. Figure 1 shows the UV-Vis spectrum AuNPs colloids synthesized using black tea extract. The SPR band that appeared at 528 nm is characteristic of AuNPs.

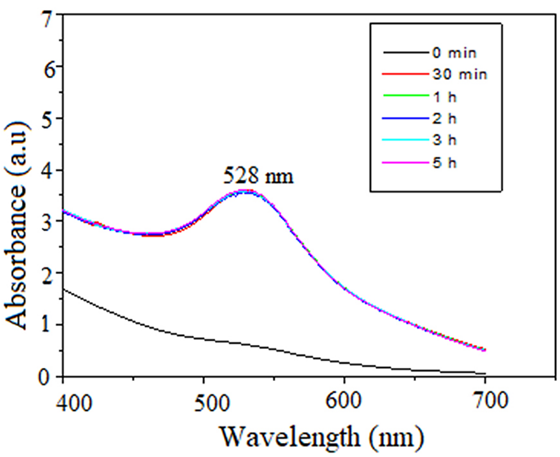


**Figure 1.** UV-Visible spectrum of gold colloid synthesized using 1.2 ml of black tea extract recorded at various time intervals

### 3.2. Functional group analysis

FTIR analysis is used to identify functional groups transformed after the synthesis of nanoparticles. The FTIR spectrum of the AuNPs synthesized using black tea extracts is shown in Figure 2. In FT-IR spectrum, a broadband appeared at 3322 $cm^{-1}$ assigned to O-H stretching vibrations of alcohol and moisture content. A band at 1626 $cm^{-1}$ may be present in the black tea extract due to C=O stretching vibration in the ketones, aldehydes, and carboxylic acid. The band at 1378 $cm^{-1}$ corresponds to C-N stretching vibration of aromatic amines. Thus, the bands that appeared at 1083 and 606 $cm^{-1}$ spectrum indicate the possibility that AuNPs are bound to protein through free amino acid groups (Kumar & Yadav, 2009; Castro *et al.*, 2010). From the FT-IR results, it is concluded that the phytochemicals present in the black tea extract adsorbed on the surface of AuNPs through amino ($-NH_2$) and carboxylic (-COOH) groups make AuNPs highly stable (24). We can believe that the proteins and other phenolic compounds, which are present in black tea extract, show characteristic peaks which could be to be responsible for the capping process. The phytochemicals present over the surface of AuNPs may be played a vital role in the reduction of gold ions to AuNPs and stabilization (Das *et al.* 2010).

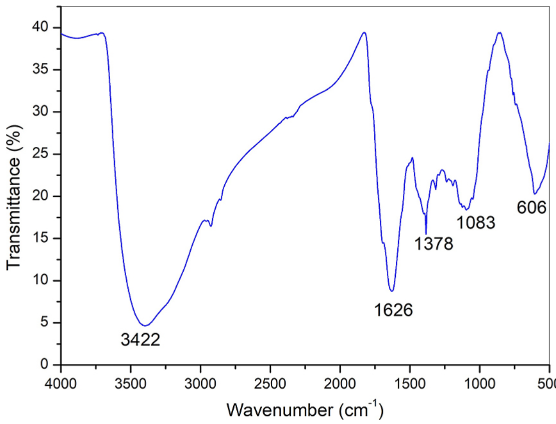


**Figure 2.** FT-IR Spectrum of AuNPs synthesized using 1.2 ml from black tea extract

### 3.3. XRD pattern analysis

The crystalline nature of AuNPs prepared using black tea extract was investigated by XRD pattern, and the corresponding diffraction pattern is shown in Figure 3. Several Bragg reflections are present which can be indexed based on the face-centered cubic structure of gold. The distinct diffraction peaks at 2θ values of about 38.4º, 44.5º, 64.6º, 77.9º and 82.03º can be indexed to the (111), (200), (220), (311), and (222) on fcc structure of metallic gold confirms the formation of AuNPs. XRD analysis of AuNPs exhibited clear peaks of cubic phases well-indexed with crystal data (JCPDS No. 03-0921) (Fazaludeen *et al.* 2012). From Figure 3, the XRD pattern for AuNPs reveals five distinctive peaks. When compared to previous results, a strong (111) diffraction peak shows noticeable nucleation of the particles along the (111) plane compared with the (200) plane (Kumar *et al.* 2014; Onitsuka *et al.*2019). The average crystallite size of nanoparticles was calculated using Debye-Scherrer's equation using (111) Bragg reflection (Sood *et al.* 2021; Narayanan and Sakthivel, 2008). The size of the nanoparticles was thus calculated to be about 12 nm. A few unassigned peaks were noticed in the vicinity of the characteristic peaks. These peaks might have been raised due to the stabilization of nanoparticles. Intense Bragg reflections suggest that strong X-ray scattering centers in the crystalline phase could be due to capping agents (Philip, 2010).

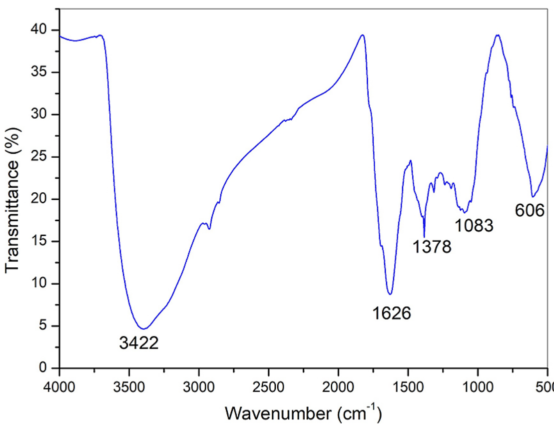


**Figure 3.** XRD pattern of Au nanoparticles synthesized using 1.2 ml black tea extract

## 3.4. SEM-EDS analysis

The SEM image was taken to analyze the size, shape, and surface morphology, showing that the synthesized AuNPs are spherical. Figure 4 (a) shows the synthesized AuNPs were uniform in size and shape. According to the SEM image, the particles' average size was 38 nm. Leemarose *et al.* (2021) reported spherical nanoparticles synthesized using *Anacardium occidentale* root extract. The SEM image exhibits discrete and larger spherical particles. Figure 4 (b) shows EDS spectrum of AuNPs, which confirms the presence of the elemental composition of AuNPs synthesized using black and green tea extracts. The strong signals of the gold atoms in the figure indicate that AuNPs are pure. The presence of weak signals of Cl, K, and O may be due to the contamination during the sample preparation.

## 3.5. TEM morphology study

Formation of nanoparticles and their growth kinetics, the shape and size of the AuNPs can be evaluated using TEM analysis. Figures 5 (a-d) show the TEM images of AuNPs synthesized using 02 ml of black tea extracts. The selected area of electron diffraction pattern with bright circular rings corresponding to (111), (200), (220), (311), and (222) planes reveals that AuNPs are highly crystalline. Figures 5 and 6 show hexagonal, triangle, pentagonal, and spherical particles. Similarly, spherical AuNPs have been reported using green tea extract as stabilizing agent (Onitsuka *et al.* 2019).

Figure 7 shows AuNPs synthesized using 1.2 ml black tea extracts found to be predominantly spherical particles. As the volume of tea extract increased, the shape of the particles gradually changed. Various fascinating shapes, such as trigonal, square, pentagonal, hexagonal, and octahedral, formed when the volume of black tea extract was 0.2 and 0.6 ml. When the volume increased to 1.2 ml, mostly spherical particles were formed. This may be due to the interaction between the biomolecules and $Au^{3+}$ ions during the formation of $Au^0$.

Prema *et al.* (2022) recently reported the biogenic synthesis of spherical AuNPs using green tea extract, and they also reported the anticancer activity against human prostate cancer cells. Interestingly, spherical, triangle, truncated triangles and decahedral AuNPs were reported by coriander leaf extract (Narayanan and Sakthivel, 2008). Similar shaped-AuNPs have been reported using the leaves extract of *Salvia officinalis, Lippia citriodora, Pelargonium graveolens* and *Punica granatum* (Elia *et al.* 2007). *Coleus amboinicus Lour* stabilized showed spherical, triangle, truncated triangle, hexagonal and decahedral AuNPs (Narayanan and Sakthivel, 2010).

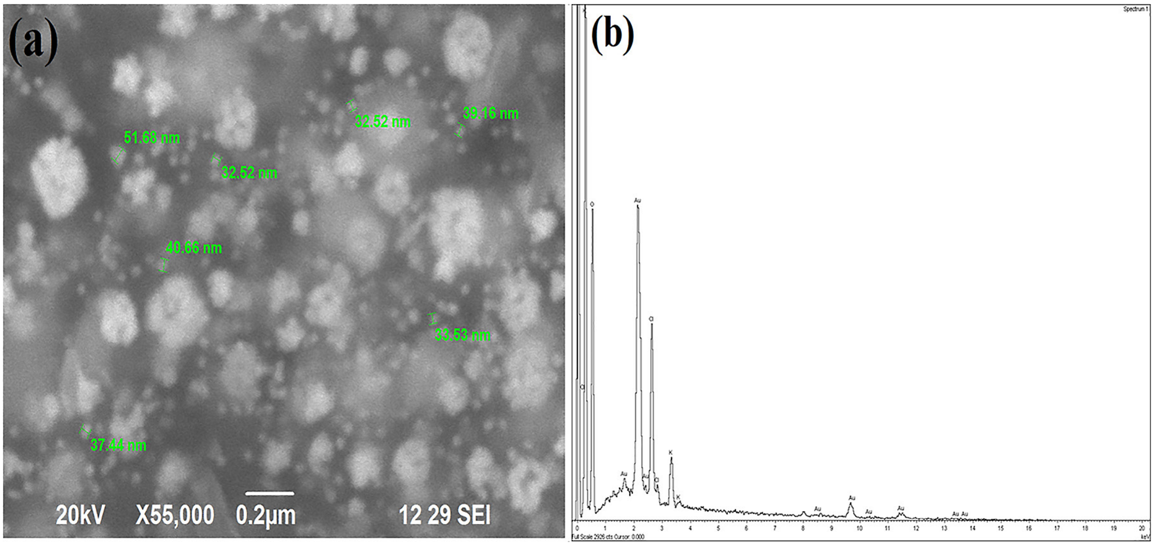


**Figure 4.** SEM images (a-b) and EDS (c) spectrum of AuNPs synthesized using 1.2 ml black tea extract.

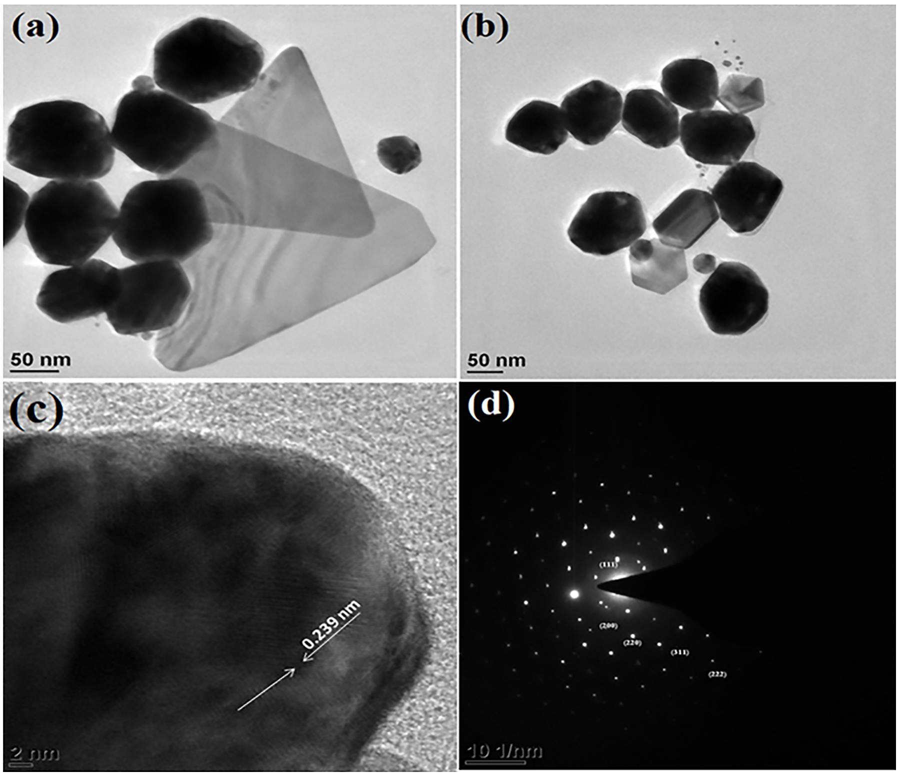


**Figure 5.** HR-TEM images of AuNPs synthesized using 0.2 ml black tea extract (a-c); (d) - selected area electron diffraction pattern.

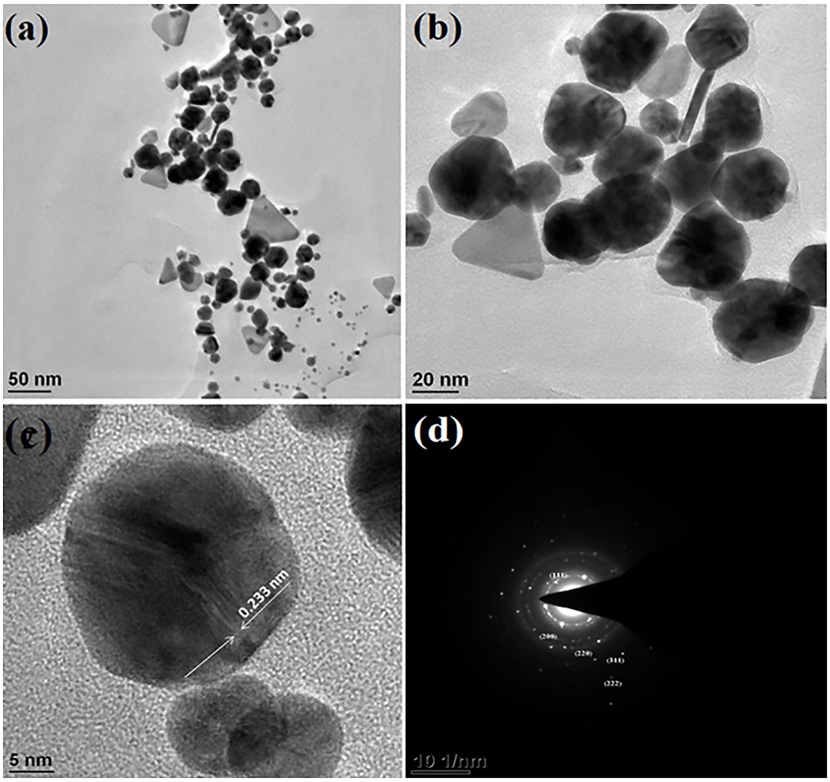


**Figure 6.** HR-TEM images of AuNPs synthesized using 0.6 ml black tea extract (a-c); (d)-selected area electron diffraction pattern.

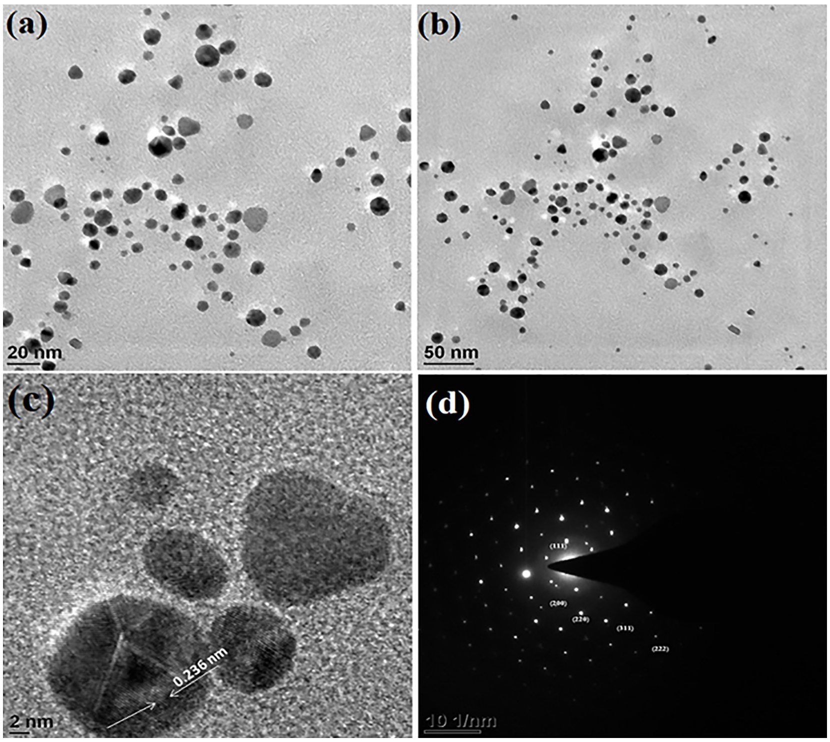


**Figure 7.** HR-TEM images of AuNPs synthesized using 1.2 ml black tea extract (a-c); (d)- selected area electron diffraction pattern

### 3.6. Photoluminescence study

In photoluminescence, a molecule absorbs a photon in the visible spectrums that excite one of its electrons to a higher electrical excited state and then emit a photon as the electron returns to a lower energy state. Figure 8 shows the photoluminescence spectra of AuNPs. The AuNPs synthesized using 1.2 ml black tea extract were luminescent with an emission at 572 nm for excitation at 530 nm. It was established that the interaction of fluorophores with AuNPs improves fluorescence emission intensity and photostability, which increases the emission peak intensity of the biomolecules bound to nanoparticles (Haick, 2007). The fluorescent properties of the AuNPs make them useful as fluorescent markers for cell labelling and biological sensing studies.

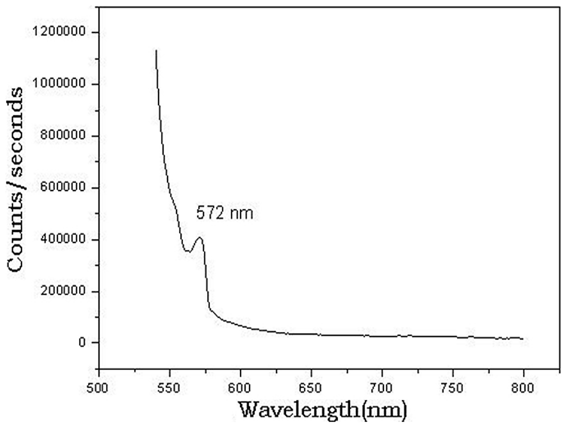


**Figure 8.** Photoluniscence spectrum of AuNPs synthesized from 1.2 ml black tea extract.

### 3.7. Antibacterial activity

The antibacterial activity of AuNPs against pathogenic bacteria was studiedThe antibacterial activity of AuNPs against pathogenic bacteria was studied using a standard zone of inhibition microbiology assay shown in Figure 9. Gentamycin, 10 mg/L,was used as a positive control for antibacterial activity. Chloroauric acid was used as negative control (100 µl). A maximum zone of inhibition of diameter 11 mm was found against the bacteria *S. aureus*, and a zone of 7 mm was found against *V. cholera*. Though the AuNPs exhibit zone of

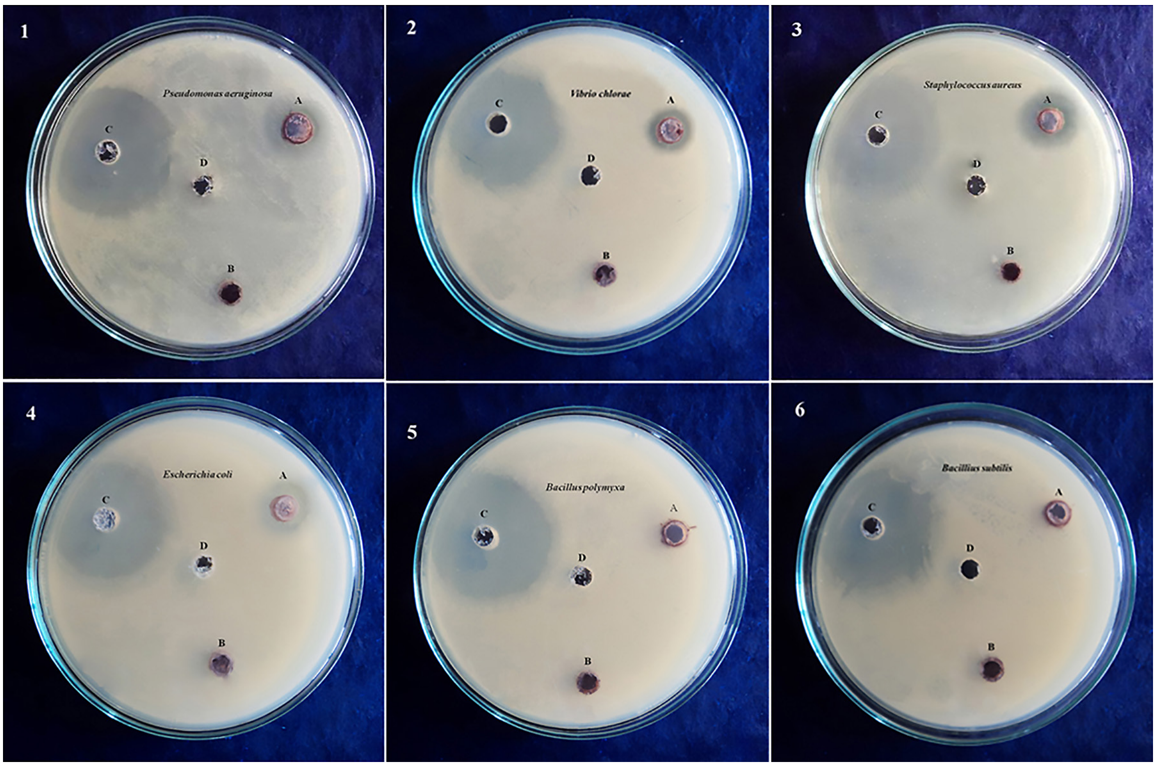


**Figure 9.** Antibacterial activity of nanoparticles against (1) *Pseudomonas aeruginosa* (2) *Vibrio cholera* (3) *Staphylococcus aureus* (4) *Escherichia coli coli* (5) *Bacillus polymyxa* (6) *Bacillus subtilis.*
A- Green tea mediated gold nanoparticles, C- Antibiotic (Gentamycin), D- Chloro auric acid.

inhibition against the tested bacteria, the results are not comparable with standard drugs (Srividhya, 2012). AuNPs synthesized using black tea extract have been used as a chemosensitizer and an anticancer agent to prevent colon cancer in HCT116 cells (Das *et al.* 2010; Quilambaqui & Muñoz, 2023). AuNPs showed less zone of inhibition. Since the antibacterial activity of AuNPs NPs strongly depends on the size and shape of the AuNPs.

| Name of the bacterial strains | Green tea stabilized gold nanoparticles | Antibiotic (Gentamycin) | Chloroauric acid |
|---|---|---|---|
| *Pseudomonas aeruginosa* | 9.1 ± 0.32 | 27.0 ± 0.22 | ND |
| *Vibrio cholerae* | 7.2 ± 0.10 | 26.2 ± 0.32 | ND |
| *Staphylococcus aureus* | 11.0 ± 0.12 | 27.4 ± 1.00 | ND |
| *Escherichia coli* | 10.3 ± 0.09 | 27.1 ± 0.17 | ND |
| *Bacillus polymyxa* | ND | 25.0 ± 1.26 | ND |
| *Bacillus subtilis* | ND | 24.6 ± 1.27 | ND |

ND: no zone of inhibition, Values are means of triplicate determination (n=3) ±SD

**Table 1.** Antibacterial activity of gold nanoparticles synthesized using green and black tea extracts

Antibacterial activities of AuNPs against *S. aureus* and *E. coli* have been reported (Sharmila *et al.* 2016). The maximum zone of inhibition was found to be 22 mm. Selvaraj and his coworkers found 27 mm for the zone of inhibition of *E. coli* with citrate-capped AuNPs (Selvaraj and Alagar, 2007), while Nazari *et al.* obtained 14 mm and 13 mm zone of inhibition against *E. coli* and *S. aureus* (Nazari *et al.* 2012). Black tea extracts stabilized AuNPs found inactive against *Bacillus polymyxa* and *Bacillus subtilis*. However, *P. aeruginosa, V. cholerae* and *S. aureus* exhibited less zone of inhibition against AuNPs. Moreover, further investigation is required to find out the proper reason for this lesser activity.

## 4. CONCLUSION

AuNPs have been synthesized using an efficient green chemistry methodology. It was demonstrated that the aqueous black tea extract could produce AuNPs at low concentrations. From the TEM morphology study, AuNPs produced with low concentrations of tea extract exhibit hexagonal, square, triangle, rectangular, and spherical shapes. However, spherical spherical-shaped particles were found to be predominant at high concentrations. The AuNPs were found to be active in terms of antibacterial activity against *Pseudomonas aeruginosa, Vibrio cholera, Staphylococcus aureus,* and *Escherichia coli.* Therefore, black tea extract can be used as a natural reducing agent to produce AuNPs, and these NPs can be used as an antibacterial drug to control pathogenic bacteria. As a future prospectus, extensive investigation of these nanoparticles for biological applications will be carried out.

## Conflict of interest

All authors declare that there are no conflicts of interest

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This manuscript is prepared from the thesis has been submitted to Periyar University, Salem, by B. Srividhya in 2012.

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