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STUDIES OF THE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF GOLD NANOPARTICLES

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ABSTRACT

Due to the multi-drug resistant bacteria's increased involvement in the spread of numerous diseases, the development of antibacterial medicines is attracting more and more attention. One of the novel and important materials to be produced as antibacterial agents in the realm of nanotechnology is metallic nanoparticles, particularly gold nanoparticles. *Uncaria gambir Roxb* leaf extract has been used to successfully create nanoparticles as a bio reducing agent and triethanolamine is used as a capping agent when a flavonoid molecule in the leaf extract reduces Au^{+3}. The effects of concentrations of triethanolamine and hydrogen tetrachloroaurate (III) acid on the stability and size of nanoparticles were studied. The stability of colloidal gold was successfully maintained by the addition of triethanolamine 1% as a capping agent. 100ppm of hydrogen tetrachloroaurate (III) acid is present in nanoparticles. Analysis of X-Ray Diffraction exhibited peak patterns with a crystallite size of 32.52 nm that were in accordance with the metallic gold standard. Transmission Particle study using an electron microscope revealed that they were triangular, hexagonal, and spherical, with the

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smallest Particles were 7 nm in size. Gold nanoparticles that were created shown excellent antibacterial action against *S. aureus* and *E coli*. Gold nanoparticles made by synthesis were shown to be stable and antimicrobial, indicating they have the potential to be developed in applications in biomedicine
Keywords: Triethanolamine, bioreducing agent, *Uncaria gambir Roxb*., gold nanoparticles, green synthesis, and biomedical applications.

INTRODUCTION

Infections in people are brought on by pathogenic bacteria, which can be found in water, food, and clothing. To treat some disorders caused by bacteria, medical treatments—mostly drugs—are utilised and created. But now days, instead of treating illnesses, people choose to prevent bacterial infections by adding specific antibacterial agents to items like food containers, cosmetics, clothing, and surgical supplies (Abbasi et al., 2016). Development of antibacterial agents is currently receiving some interest for this reason. At current time, metal nanoparticles' exceptional qualities can be developed and modified through the use of nanotechnology to achieve biomedical application objectives (Franci et al., 2015).

Nanotechnology is the study of the synthesis, planning, and manipulation of materials with a size between one and one hundred nanometers (nm) (Iravani, 2011; Moodley et al., 2018). Due to its exceptional features, which are fundamentally different from those of bulk materials in terms of chemistry, physics, and biology, nanotechnology has recently attracted a lot of attention (Heiligtag and Niederberger, 2013). Metallic nanoparticles are one of the most advanced fields and have been used extensively in medicine, including in body tissue repair, gene delivery, drug delivery, immunodiagnostic, optics, the food industry, environment, and imaging (Ahmed and Ikram, 2015; He et al., 2019; Kim et al., 2012; Marza et al., 2019). Because of their high surface to volume ratio and nanoscale size, metallic nanoparticles have the potential to operate as antibacterial agents (Khalil et al., 2013). Due to their enormous potential as antibacterial agents, silver and gold are the two metals that have undergone the most development (Dykman and Khlebtsov, 2012). Additionally, it is because of the material's high degree of functionalization, nontoxicity, simplicity of detection, and photothermal activity (Dizaj et al., 2014). According to several research, the toxic-free metal ion that forms on the surface of metal nanoparticles and enters bacteria cells is the basis for the antibacterial action of these particles. The cell membrane was then disrupted by the accumulation of these harmful metal ions the cell's metabolism, which causes cell death. Additionally, this may be because the metal particles are nanosized, allowing for simple entry into the cell (Dizaj et al., 2014; Labanni et al., 2019; Parham et al., 2016).

A number of techniques, including the reduction approach (Heidari et al., 2014), hydrothermal (Tippayawat et al., 2016), microwave (Ngo et al., 2016), laser irradiation (Gonzalez-Rubio et al., 2016), and many more, can be utilised to create gold nanoparticles. Chemical reduction is the most advantageous technique because it is straightforward and uses little energy (Elia et al., 2014). However, using potentially dangerous chemical reagents can endanger both the environment and living things. In order to lessen the potentially hazardous effects, researchers are now developing an environmentally friendly synthesis procedure. Using

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this technique, certain living things including fungi, algae, and plants whose active chemicals were utilised as reducing agents (Shah et al., 2015).

Due to the presence of up to 33% catechin, which is categorised as a flavonoid compound and is utilised in items like tanner base material for dyeing, insecticide, adhesive plywood, and mainly as medicine to treat illnesses, it has been used for a long time (Fauza, 2014). In this study, a bioreducing agent called *Uncaria gambir Roxb*. leaf extract was employed to create gold nanoparticles. In our earlier research, *Uncaria gambir Roxb* was successfully used as a bioreducing agent in the hydrothermal technique in water solvent (Arief et al., 2015), in isopropanol solvent (Arief et al., 2017), and also by reduction method in the presence of diethanolamine (Arief et al., 2018). (Labanni et al., 2018). To the best of our knowledge, this is the first report of the investigation into the bioreducing agent *Uncaria gambir Roxb*. used in the manufacture of gold nanoparticles.

Additionally, a stabilising agent must be introduced to the reaction to regulate particle development and size to create stable colloidal nanoparticles (Patel et al., 2017). Given that the antibacterial activity is greatly influenced by the size, shape, and concentration of the nanoparticles, this control is important for the medicinal use (Franci et al., 2015). As a result, triethanolamine is used as a capping agent in this work. To examine the impact of concentration on the characteristics of gold nanoparticles, the concentration of HAuCl4 as a precursor was also changed.

MATERIALS AND METHODS

Triethanolamine (TEA) was used as the capping agent in the synthesis, and materials from Merck included $HAuCl_4$ as the precursor. We collected fresh *Uncaria gambir Roxb*. Leaves from organic garden, Tirupattur.

Making leaf extract from *Uncaria gambir Roxb*.

To eliminate dirt, *Uncaria gambir Roxb* leaves was first washed with tap water, after which they were dried in the shade for a week at room temperature. In addition, gambir leaves were ground into a powder using a grinder. The powder was then safeguarded and kept in a dark, sealed container for storage to avoid degradation and harm Plant extracts were made by weighing 10 g of plant powder, adding 100 ml of distilled water, and heating the mixture for two hours at 60 °C. The extract was then obtained by filtering the mixture. For later usage, the extract was kept in the refrigerator.

Gold nanoparticle synthesis

For a total volume of 50 ml, 25 ml of HAuCl4 solution with concentrations of 20 and 100 ppm was combined with 10 ml of 1% TEA and 15 ml of 6% *Uncaria gambir Roxb* extract. Then, these combinations were mixed at room temperature for six hours. Using an ultraviolet-visible (UV-Vis) spectrophotometer, the growth and synthesis of colloidal gold nanoparticles were periodically observed (510000 SECOMAM). The same precursor concentration was also used to create the colloidal gold nanoparticles without TEA serving as a capping agent. The gold nanoparticles that had been stabilised with TEA were subsequently designated AuNps-1 and AuNps-2 for use with 20 and 100 ppm HAuCl4, respectively. For concentrations of 20 and 100 ppm HAuCl4, the unstabilized gold nanoparticles were coded as AuNps-3 and AuNps-4,

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respectively.

Characterization

X-ray diffraction (XRD) Phillips X'pert Powder PAN analysis with radiation of CuK(= 1,5406) operated at 30 kV and 30 A was used to investigate the crystallinity of AuNps. Colloidal AuNps was precipitated to get the AuNps powder sample. To create the powder AuNps, the obtained solid was removed from the filtrate, rinsed with distilled water, and dried in a hot air oven. Transmission electron microscope (TEM) JEOL JEM 1400 was used to examine the size distribution and morphology of colloidal AuNps.

Test for bacterial activity

Using the disc diffusion or Kirby Bauer methods, the as-synthesized *Uncaria gambir Roxb*. Mediated AuNps were evaluated against *Escherichia coli* and *Staphylococcus aureus* bacteria. The bacteria were grown on nutrient agar medium. On nutrient agar (NA) agar plates, 100 l of fresh overnight bacterial culture inoculum was applied. Each plate also contained a positive control and a negative control along with sterile paper discs measuring 5 mm in diameter and containing 50 g/ml AuNps. Water and amoxicillin were employed as the test's positive and negative controls, respectively. The samples' inhibitory zone was assessed following a 24-hour incubation period at 37°C. All statistics presented are average results because the test was done twice.

RESULTS AND DISCUSSION

Spectrophotometer research using UV-Vis

Then, based on the surface plasmon resonance (SPR) phenomenon, the creation of gold nanoparticles was validated using a UV-Vis spectrophotometer at a wavelength of 515–570 nm. A spectrum of the *Uncaria gambir Roxb* extract is shown in the UV-Vis spectrophotometer result (Fig. 1), and it exhibits a peak at 279 nm, known as catechin. When reacting with $HAuCl_4$, the absorbance peak changed to a wavelength of 534 nm, confirming the creation of gold nanoparticles and demonstrating that the catechin in *Uncaria gambir Roxb* leaf extract has successfully reduced Au3+ ions to Au0. This is consistent with the earlier synthesis by Yu et al. (2016) that utilised Citrus maxima aqueous extract and Paul et al. (2015) demonstrated the local surface plasmon resonance (LSPR) band of gold nanoparticles at 535-538 nm and 510-560 nm, respectively.

It was discovered by spectrophotometry research that the concentration and application of capping agents had a significant impact on the optical characteristics of the gold nanoparticles. TEA-capped AuNps demonstrated greater stability than unstabilized AuNps at HAuCl4 concentrations of 20 ppm (AuNps-1) and 100 ppm (AuNps-2), respectively, and at a concentration of 20 ppm (AuNps-3) and 100 ppm, respectively (AuNps-4). Precipitation did not appear in AuNps-1 and AuNps-2 until the reaction time of 168 hours, but it did in AuNps-3 and AuNps-4 after the reaction time of 24 hours. The agglomeration causes the nanoparticles to precipitate. It is brought on by the interaction between the nanoparticles' van der walls and growth (Sinha and Mukherjee, 2014). By reducing the interaction between nanoparticles, a capping agent or stabiliser can stop the excessive development of nanoparticles that leads to agglomeration (Chu et al., 2018). According to the findings of this study, the use of TEA as a

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capping agent effectively avoided or postponed the agglomeration and deposition of nanoparticles by up to seven times The outcomes further showed that the speed of nanoparticle aggregation and precipitation can be influenced by the concentration of the HAuCl4 precursor. AuNps-3 and AuNps-4 both precipitated in the TEA-unstabilized gold nanoparticles, with AuNps-3 occurring before and AuNps-4 occurring after 24 hours. The TEA-stabilized AuNps-1 and AuNps-2, on the other hand, displayed excellent stability and did not experience precipitation until 168 hours. These findings demonstrated that even with a 5-precursor concentration, TEA can preserve the stability of gold nanoparticles.

On the basis of the Tyndall effect, laser beam radiation was also used to study the production of nanoparticles. The laser beam radiation on AuNps-1 was depicted in Figure 1's inset image in the time reactions of 0, 2, and 24 hours. It was discovered that light was scattered within the colloid, confirming that the particles were scattering light. After some time, the colloidal nanoparticles' hue changed to a thick shade, confirming that their number continued to rise during the reaction.

Table 1 lists the wavelength and absorbance values of gold nanoparticles produced by *Uncaria gambir Roxb* as determined by a UV-Vis spectrophotometer. It demonstrates that as the precursor concentration climbed from 20 to 100 ppm, the absorbance of the nanoparticles increased as well.

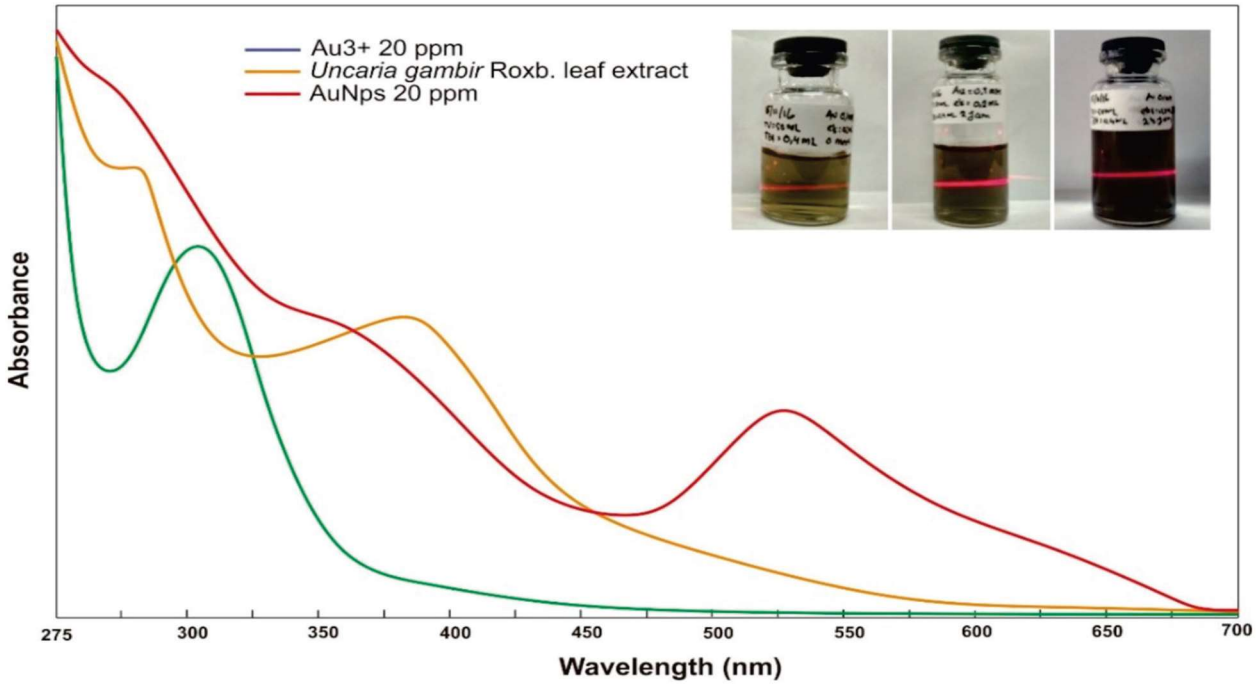

Table 1. Absorbance and wavelength value of U. gambir Roxb. mediated colloidal gold nanoparticles.

Reaction Time (h)	Wavelength (nm)				Absorbance			
	AuNps-1	AuNps-2	AuNps-3	AuNps-4	AuNps-1	AuNps-2	AuNps-3	AuNps-4

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0.5	-	-	-	545	-	-	-	1.688
1	-	-	515	543	-	-	0.338	1.939
2	541	555	515	538	0.271	1.158	0.339	2.055
4	541	560	515	538	0.318	1.241	0.346	2.258
6	544	565	515	538	0.331	1.447	0.352	2.295
24	534	570	520	535	0.426	0.577	0.376	2.355
168	534	-	522	534	0.407	-	0.402	2.313

Gold nanoparticles both stabilised and destabilised by TEA. This data revealed that a larger precursor concentration causes a greater production of nanoparticles during the reaction. Additionally, comparable outcomes were seen in the wavelength values, where a higher concentration corresponds to a larger wavelength value

Crystallinity study

By using XRD analysis, the crystalline makeup of the produced Au nanoparticles was further investigated. The XRD pattern of powdered Au nanoparticles is displayed in Figure 2. Based on the International Center for Diffraction Data (ICDD) standard 039065-2870, five different peaks at 38.22° (111), 44.41° (200), 64.62° (220), 77.73° (311), and 81.77° (222) were found to correspond to well-crystalline face-centered cubic (FCC) gold nanoparticles. This outcome was consistent with earlier research that used extracts from the leaves of *Pogestemonbenghalensis, Stevia rebaudiana, Lantana camara, Citrus maxima*, Paul et al. (2016), and *Stevia rebaudiana* (Dash et al., 2015). By analysing the gold nanoparticles produced by the extreme bacterium *Deinococcus Radiodurans*, Li et al. (2016) stated that the crystallite size of the gold was reported to be 36.28 nm. Based on the Debye-Scherrer formula, it was calculated that the crystallite size of the gold was 32.5 nm.

A morphology studies

The TEM analysis' findings the synthesised AuNPs-1, AuNPs-2, AuNPs-3, and AuNPs-4 are depicted in Figure 3. It was found that the synthetic gold nanoparticles come in a variety of forms, primarily round but also hexagonal and triangular. This TEM result demonstrated how the particle size was impacted by using different doses of TEA as a capping agent. Figures 4a–4d show, accordingly, the particle size distribution of AuNps-1, AuNps-2, AuNps-3, and AuNps-4.

The average particle sizes of AuNps-1, AuNps-2, AuNps-3, and AuNps-4 were 29, 11, 23, and 31 nm, respectively. The particle size ranges of AuNps-1, AuNps-2, and AuNps-3 were 13-82 nm, 4-16 nm, 10-56 nm, and 11-62 nm, respectively. According to the TEA unstabilized gold nanoparticles, an increase in precursor concentration results in the size. The increase in concentration had no impact on the particle size, unlike the TEA stabilised gold nanoparticles. These findings revealed that even in higher concentration synthesis, TEA was able to retain the particle size of gold nanoparticles. It is anticipated that the nitrogen component of TEA will be essential to the capping mechanism of gold nanoparticles. A protective monolayer is created on the surface of gold nanoparticles as a result of the interaction between positively charged

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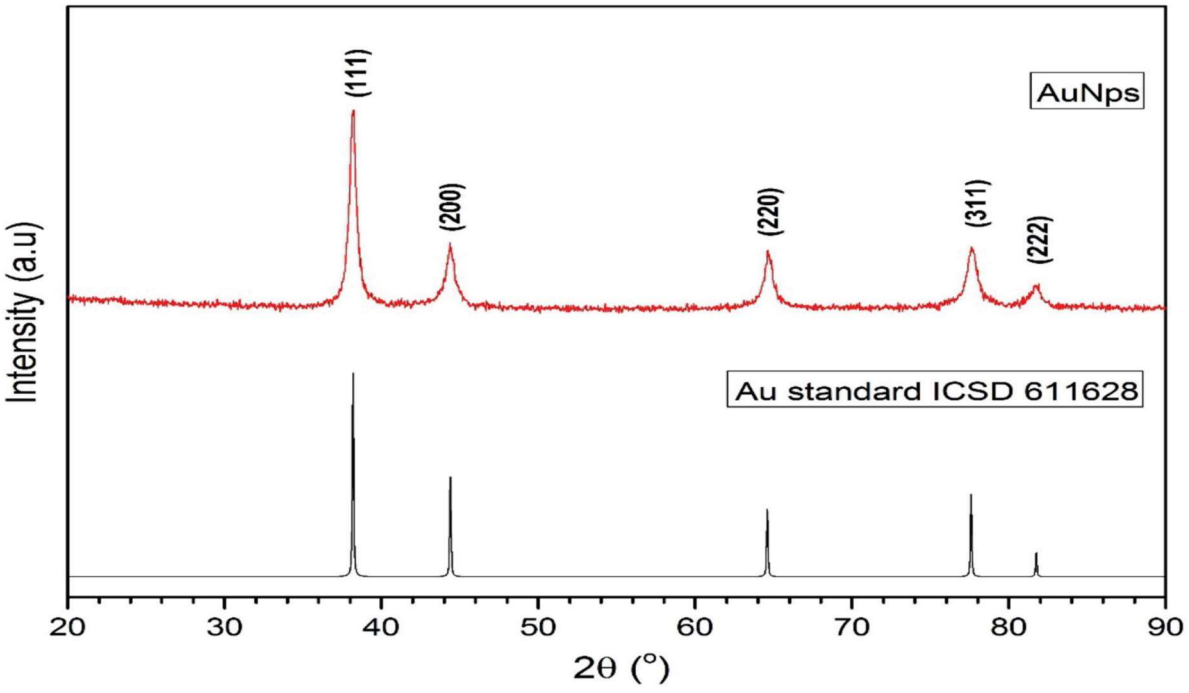
nitrogen and positively charged gold nanoparticles. This protective monolayer keeps the gold nanoparticles from interacting too much with one another, which inhibits aggregation and limits the proliferation of gold nanoparticles (Yamamoto et al., 2006).

Study of gold nanoparticles produced by *Uncaria gambir Roxb*:

As examples of gram-positive and gram-negative bacteria, *S. aureus* (a) and *E. coli* (b) are shown in Figure 5 with clearly defined zones of gold nanoparticle inhibition. Table 2 displays the sample's inhibition zone. All of the data are average results because the test was done twice. Inhibitory zones for *S. aureus* and *E. coli* were 6, 7, 6, and 6 mm for AuNps-1, AuNps-2, AuNps-3, and AuNps-4, respectively. Amoxicillin, used as a positive control, has a 4 mm against *S. aureus* and a 5 nm against *E. coli* measurement. The test against E. coli showed a somewhat greater inhibition zone of AuNps than the test against *S. aureus*. This is because bacteria belonging to positive strains and negative strains have different cell wall compositions. Positive strain bacteria have a thicker peptidoglycan coating than do negative strain bacteria, which allows AuNps to more readily enter the cytoplasm. Currently being explored, the precise mechanism underlying AuNps antibacterial action will be revealed in the following study.



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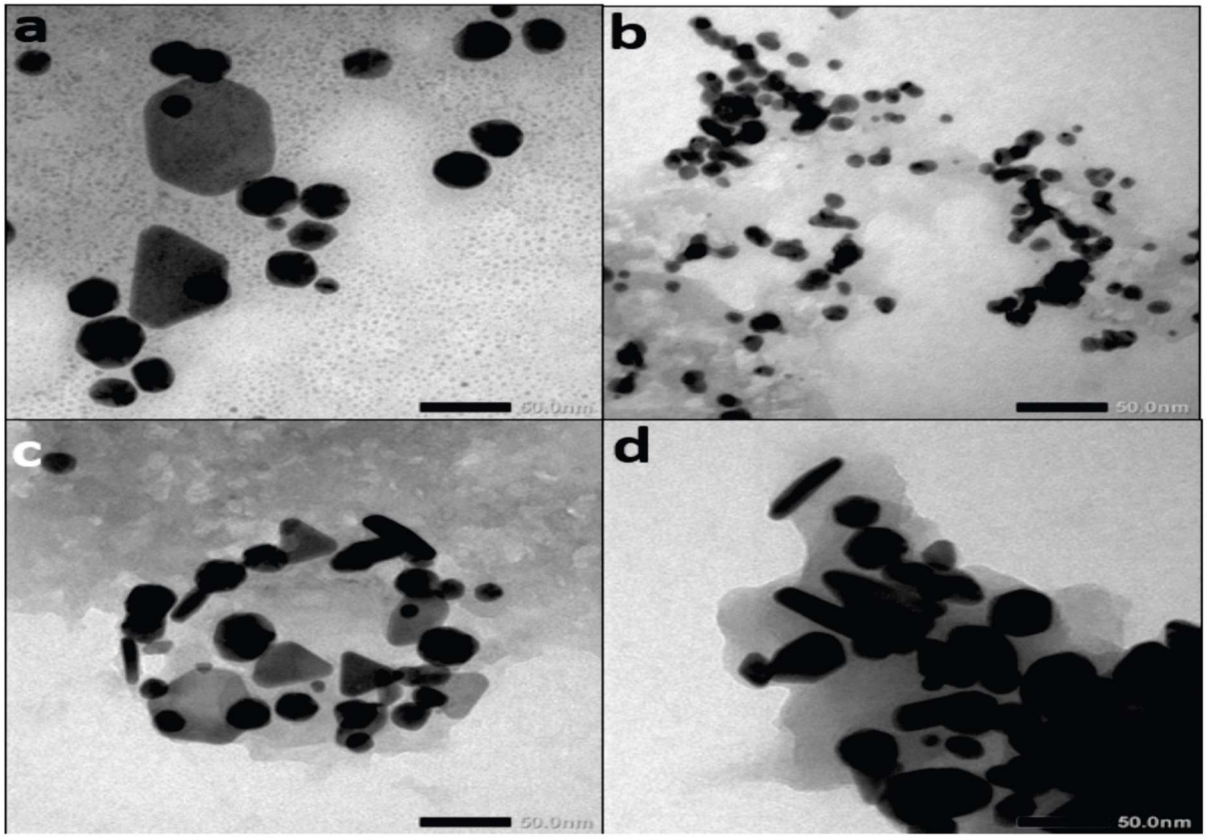

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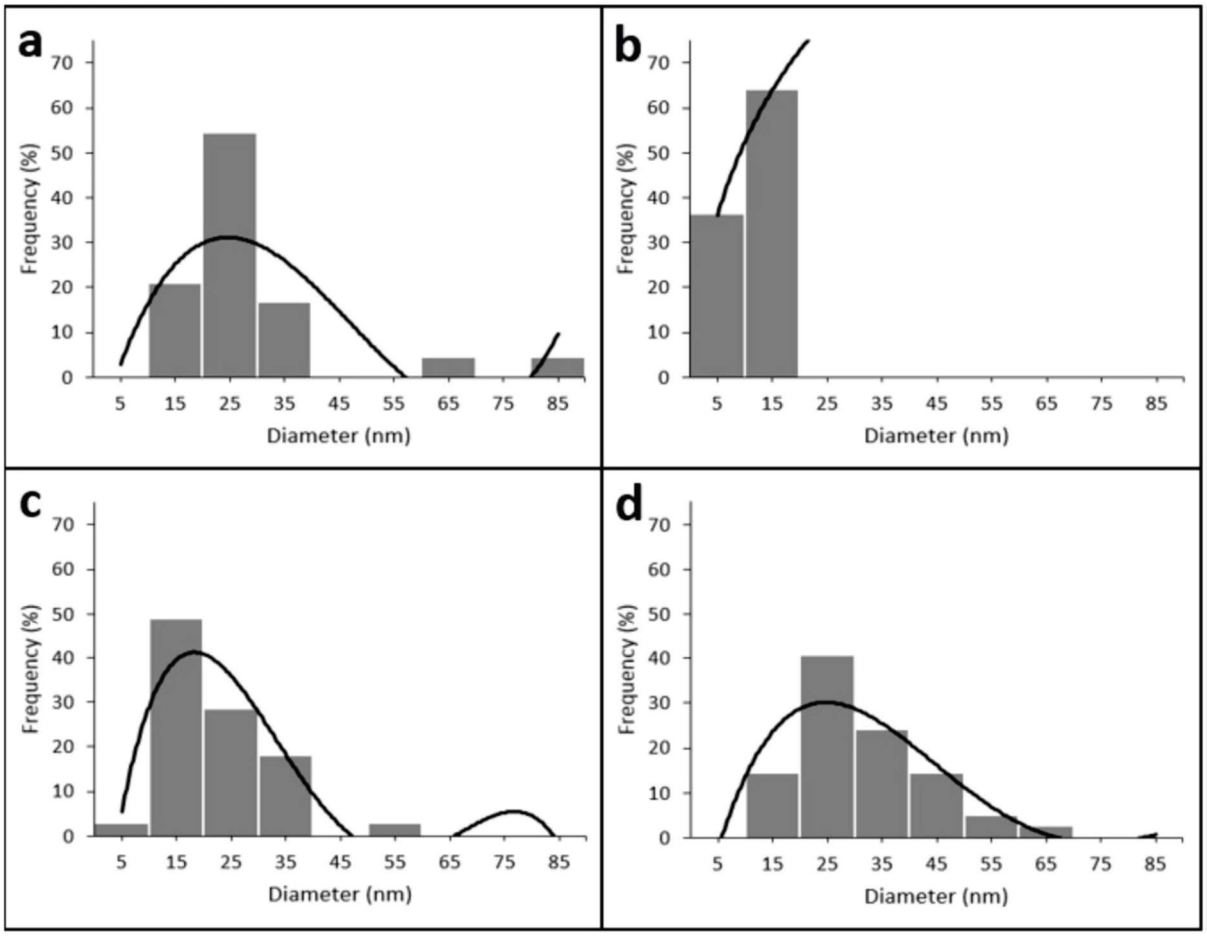

Figure 4. Particles size distribution of a) AuNps-1, b) AuNps-2, c) AuNps-3, and d) AuNps-4.

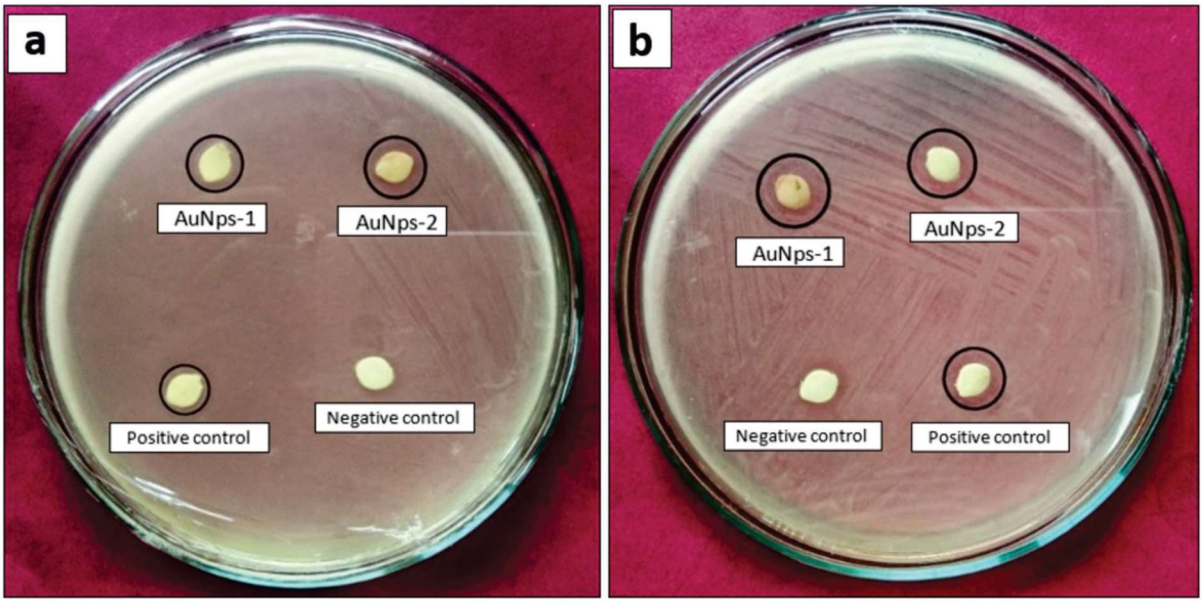

Figure 5 Antibacterial activity of gold nanoparticles against a) *S. aureus* and b) *E. Coli*.

Table 2 Inhibition zone of gold nanoparticles.

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Bacteria	Inhibition zone (mm)					
	(-) control	(+) control	AuNPs-1	AuNPs-2	AuNPs-3	AuNPs-4
S. aureus	-	4	6	7	6	6
E. coli	-	5	8	9	7	6

The findings demonstrated that varying HAuCl4 concentrations used to create gold nanoparticles had no discernible impact on the inhibitory zone. Additionally, AuNps-2 demonstrated a greater inhibitory zone against both *S. aureus* and *E. coli*. This might be as a result of AuNps' size, which made it possible for it to penetrate the cell's cytoplasm with ease. AuNps induced the growth of biofilm and interacted with a component of the cell wall, causing the structure to change, the cell to degrade, and ultimately the cell to die. While interacting, AuNps became stuck in the biofilm and let off certain chemicals, which deformed the cell wall. Table 3 displays some earlier research on greenly produced gold nanoparticles and their antibacterial activity in contrast to our samples. These findings demonstrated the potential for *Uncaria gambir Roxb*-mediated gold nanoparticles with TEA as capping agents to be created as antibacterial agents for biomedical applications.

Table 3. Comparison of particle size and inhibition zone of green synthesized gold nanoparticles.

Extract	Average particles size (nm)	Bacteria strains	Inhibition zone (mm)	References
Gloriosa superb leaf extract	20	*B. subtilis* *E. coli*	- -	(Gopinath *et al*., 2016)
Deinococcusradioduransbacteria	43.75	*S. aureus* *E. coli*	8.95 ± 0.17 9.21 ± 0.20	(Li *et al*., 2016)
*Nephenteskhasiana*leaf extract	50	*Bacillus sp.* *E. coli*	10.0 8.0	(Bhau*et al*., 2015)
U. gambir Roxb leaf extract	11	*S. aureus* *E. coli*	7 9	Present Research work

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CONCLUSION

By adding TEA and adjusting the concentration, it was possible to successfully create gold nanoparticles of various shapes and sizes from U. gambir leaf extract. The production of AuNps by SPR absorption at a wavelength of 515–570 nm was confirmed by the UV–Visible spectrophotometry study. The findings of the XRD examination demonstrated the development of high-crystalline AuNps with an FCC structure. The results of the TEM investigation revealed that the produced AuNps were triangular, hexagonal, and spherical with sizes ranging from 4-82 nm. Even at a 5-precursor concentration, the particle size can be maintained by using TEA as a capping agent. Against *S. aureus* and *E. coli*, the produced AuNps shown remarkable antibacterial action.

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