

PLANT MEDIATED SYNTHESIS OF GOLD NANOPARTICLES

R.Kirubha^{#1} and G.Alagumuthu^{#2}

#1 P.G & Research centre, Department of chemistry, Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi-627 412, India,

#2 P.G & Research centre, Department of chemistry, Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi-627 412, India,

ABSTRACT

Objective: To develop a reliable, eco-friendly and easy process for the synthesis of gold nanoparticles using flower extracts of medicinal plant '*Couroupita guianensis*' and evaluate its anti-microbial properties. **Methods:** The synthesis and characterization of gold nanoparticles was confirmed by UV-Visible, FR-IR, SEM, TEM, CV techniques and Disc diffusion assay method was used to confirm the antibacterial activity of gold nanoparticles. **Results:** UV-visible spectrum of the aqueous medium containing gold nanoparticles showed a peak around 536nm. FTIR analysis confirmed reduction of Au^{3+} ions to Au^0 ions in synthesized gold nanoparticles. The SEM and TEM analysis showed the particle size between 25-45nm, and spherical in structure. The gold nanoparticles have shown bactericidal effect against *E.Coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. **Conclusions:** The fruit extract of *C.guianensis* quickly reduces Au^{3+} to Au^0 and enhances synthesis of gold nanoparticles with anti-microbial activity.

Key words: Gold Nanoparticles; *Couroupita guianensis*; TEM; Antibacterial activity.

Corresponding Author: Kirubha.R

INTRODUCTION

Nanomaterials have received much attention because of their structure and properties differ significantly from those of atoms, molecules, and bulk materials [1]. The synthesis of metal nanoparticles has been widely discussed in the literature due to their small sizes, large surface area and unique physical and chemical properties, which have many potential applications [2-4]. The reducing agent, reaction medium, and stabilizer are the three key factors in the synthesis and stabilization of metallic nanoparticles [5]. Metal nanoparticles can be synthesized by physical, chemical and biological methods. Although the physical and chemical methods produce pure, well defined particles, these methods are not cost effective and ecofriendly. This drawback can be exhausted by biological method where the microorganism or plant extract or plant biomass is used as reducing agent [6-8]. Now-a-days biological synthesis of

metallic nanoparticles is gaining importance as it is reliable and ecofriendly. The formation of gold nanoparticles via green route is also studied by using *Hibiscus rosa sinensis* [9], *Camellia Sinensis* [10], *coriander leaves* [11], *Sugar beet pulp* [12] *Mentha piperita* [13] and *Aegle Marmelos* [14].

In our present study, we have demonstrated a suitable green route for the synthesis of gold nanoparticles using *Couroupita guianensis* flower extract as reducing agent. The antibacterial activity of gold nanoparticles has been tested against various pathogens.

MATERIALS AND METHODS

2.1. Materials:

Fresh flowers of *Couroupita guianensis* were identified and collected from Tamilnadu Agricultural University, Tirunelveli, and Tamilnadu, India and the taxonomic identification was made by Botanical Survey of India, Coimbatore. Chloro auric acid was obtained from the precision scientific co, Coimbatore, India.

2.2. Synthesis of gold nanoparticles:

The fresh flowers of *Couroupita guianensis* broth solution was prepared by taking 100 g of thoroughly washed and finely cut flowers in a 500 mL Erlenmeyer flask along with 200 mL of sterilized double distilled water and then boiling the mixture for 15 min before finally decanting it. The extract was filtered through Whatman filter paper no 1 and stored at -15°C and could be used within 1 week. The filtrate was treated with aqueous 1 mM HAuCl_4 solution in an Erlenmeyer flask and incubated at room temperature. As a result, a purple coloured solution was formed; indicating the formation of gold nanoparticles and it was further confirmed by UV-Vis spectrum analysis [15]. It showed that aqueous gold ions could be reduced by aqueous extract of plant parts to generate extremely stable gold nano particles in water (Figure 1).

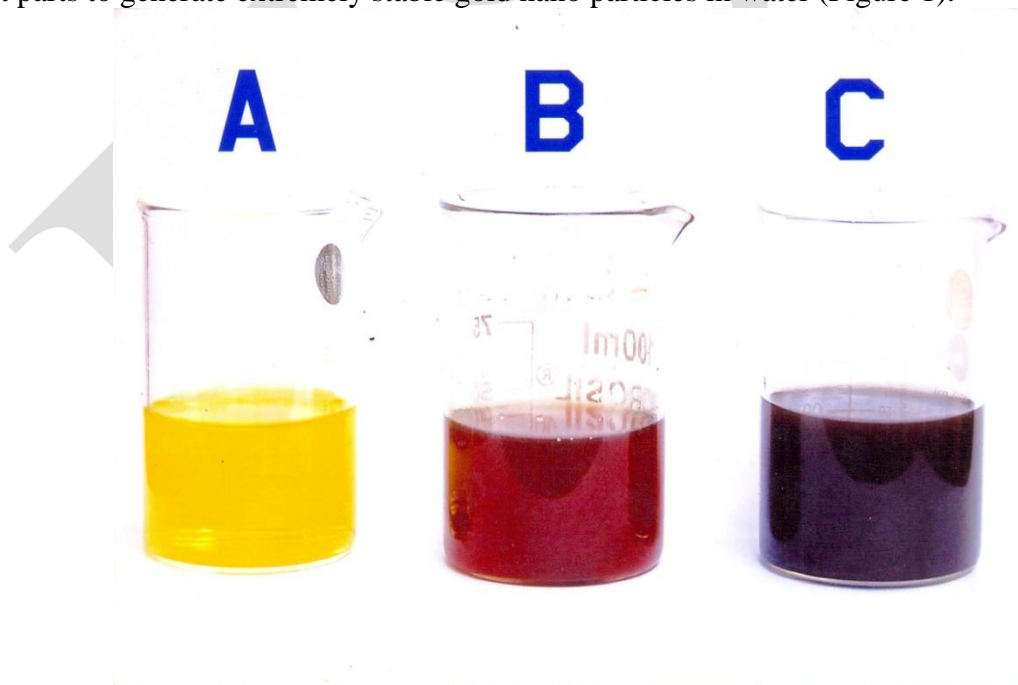


Figure 1: Photographs showing A) pure HAuCl_4 solution B) pure *Couroupita guianensis* flower Extract C) Colour changes after adding flower Extract with HAuCl_4 solution.

2.3. Characterization of the synthesized gold nanoparticles using UV-spectra:

Synthesis of gold nanoparticles solution with flowers extract may be easily observed by ultraviolet-visible (UV-Vis) spectroscopy. The bio-reduction of the Au^+ ions in solutions was monitored by periodic sampling of aliquots (1 mL) of the aqueous component and measuring the UV-Vis spectra of the solution. UV-Vis spectra of these aliquots were monitored as a function of time of reaction on a Vasco 1301 spectrophotometer in 400-600 nm range operated at a resolution of 1 nm.

2.4. Scanning electron microscopy (SEM):

The electronic images were made on Hitachi S-4500 SEM Analyzer.

2.5. Transmission electron microscopy (TEM) :

Transmission electron microscopy (TEM) (HITACHI, H-7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. Au nanoparticle image was formed from the interaction of the electrons transmitted through the specimen; the image of Au nanoparticles was magnified and focused onto an imaging device.

2.6. Cyclic Voltammetry analysis:

Analysis through cyclic voltammetry(CV) confirmed the presence of elemental gold signal of gold nanoparticles .The change in the oxidation state of the metal ion was studied by CV technique, using platinum electrode with fresh surface at the rate of 25mVs^{-1} in the potential range between -1.0 and 1.0V.

2.7. Antimicrobial activity study:

Antibacterial activities of the synthesized Au nanoparticles were determined, using the agar disc diffusion assay method. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petri plates. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. 100 mL of broth culture of each test organism (1105 cfu/mL) was used to prepare lawns. Agar of 5 mm diameter was prepared with the help of a sterilized stainless steel cork borer. Five plates were prepared in the agar plates. Ciprofloxacin was used as standard and positive controls. The plates containing the test organism and Au nanoparticles were incubated at 37°C for 24 - 48 h. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the plates. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

3. RESULTS:

3.1. UV-VIS spectra analysis:

Reduction of Au ion into gold nanoparticles during exposure to the plant extracts could be followed by color change. Au nanoparticle exhibit dark purple color in aqueous solution due to the surface plasmon resonance phenomenon. The result obtained in this investigation is very interesting in terms of identification of potential plants for synthesizing the Au nanoparticles. UV-Vis spectrograph of the colloidal solution of gold nanoparticles has been recorded as a function of time. Absorption spectra of gold nanoparticles formed in the reaction media at 10 min has absorbance peak at 536 nm, broadening of peak indicated that the particles are polydispersed (Figure 2).

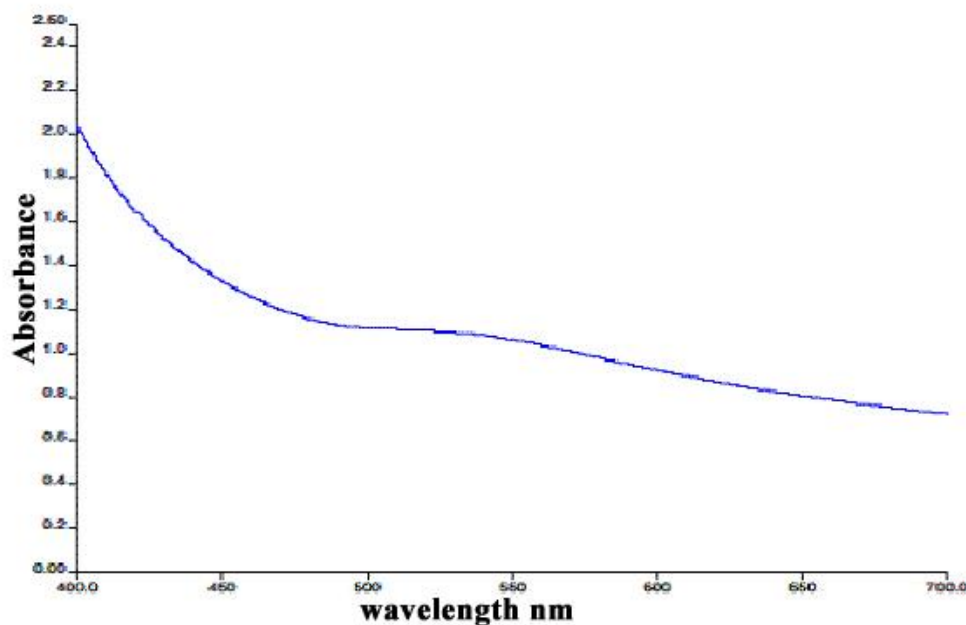


Figure.2. UV Spectra of gold nanoparticles

3.2 SEM Analysis of gold nanoparticle:

The diameter of synthesized nanoparticle was identified as 37.2nm using SEM as shown in figure 3.

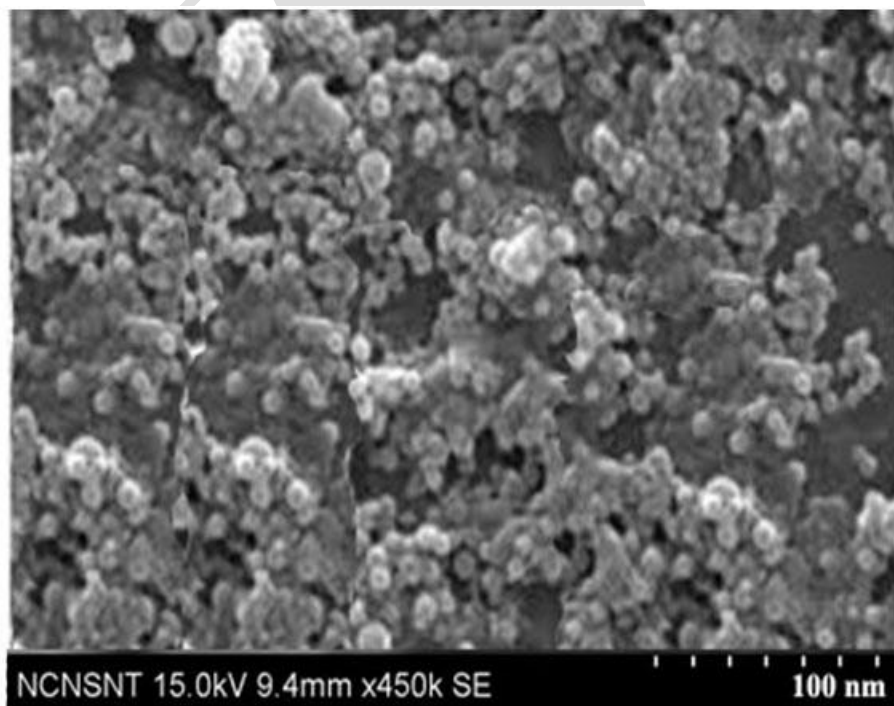


Figure .3. SEM image of gold nanoparticles using the flower extract of *Couroupita guianensis*.

3.3. TEM analysis of Au nanoparticles:

The gold nanoparticles synthesized with the help of *Couroupita guianensis* flower extract were scanned using TEM (HITACHI, H-7500) from which we can conclude that the average mean size of Au nanoparticles was 37.2 nm and seems to be spherical in morphology as shown in (Figure 4).

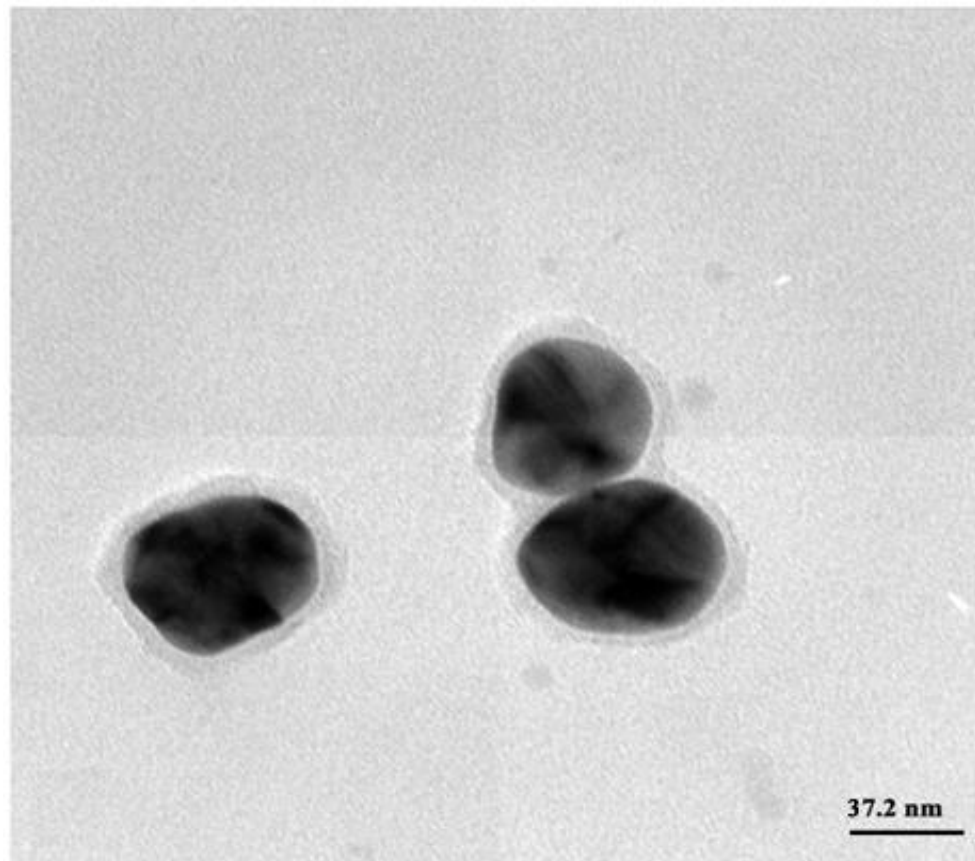


Figure .4. HR-TEM image of gold nanoparticles using the flower extract of *Couroupita guianensis*

3.4. Cyclic Voltammetry analysis:

In cyclic voltammetric analysis the *Couroupita guianensis* flower extract free solution makes all the metal ions are reduced to lower oxidation state, since there is no possibility for the formation of NPs. Upon addition of *Couroupita guianensis* extract in the reaction medium, the cathodic peak shifted towards the negative potential direction, implying that the reduced gold NPs are stabilized by *Couroupita guianensis* extract (Fig. 5). The extent of decrease in anodic peak current is greater than that of the cathodic peak current due to the fact that the rate of reduction of gold ion may be greater than its oxidation. This might be because of the electron donating methoxy, hydroxyl and amine groups containing *Couroupita guianensis* extract can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AuNPs shows the peaks observed at -0.54 and 0.59V.

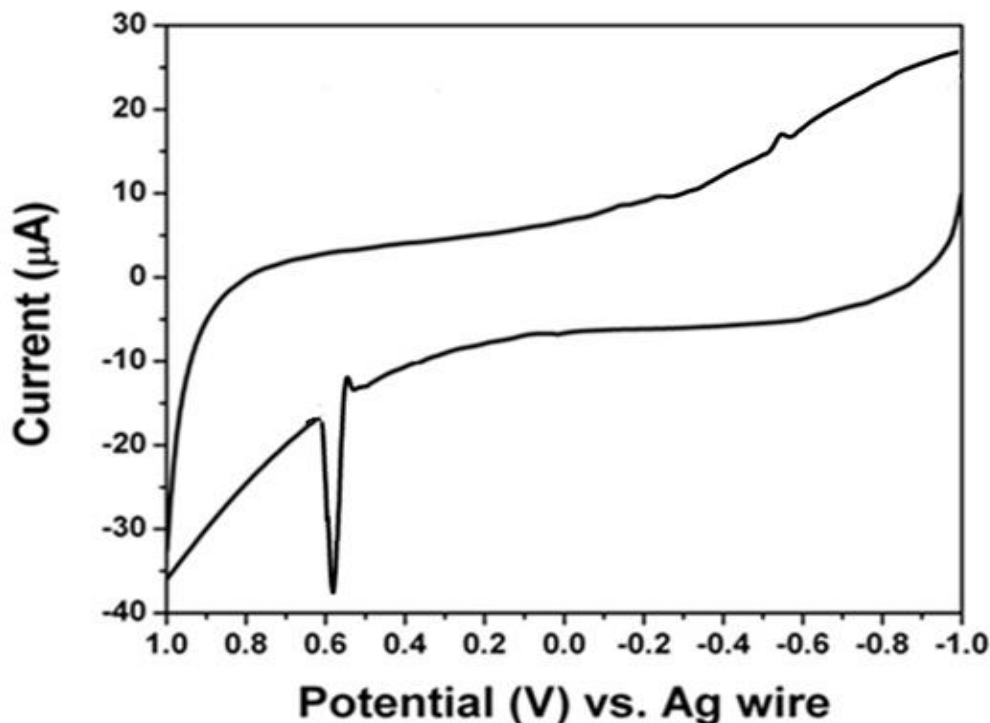


Fig.5.Cyclic voltammograms of gold nanoparticles

3.5. Antibacterial Activity study:

The antibacterial activity of gold nanoparticle was tested against the following microorganism, viz; *E.Coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Pseudomonas aeruginosa* by disc diffusion method and the results were tabulated in the table 1. The gold nanoparticle has shown antibacterial activity against all tested microorganism and maximum zone of inhibition was found against *Bacillus cereus*.(figure 6).

Table. 1. Antibacterial activity of gold Nanoparticles

Microorganism	Zone of inhibition in mm			Ciprofloxacin
	20μl	40μl	60μl	
<i>E.Coli</i>	11.00±0.64	12.54±0.46	13.33±0.33	14.67±1.20
<i>Bacillus cereus</i>	12.67±0.76	13.03±0.98	18.67±0.88	15.33±1.00
<i>Staphylococcus aureus</i>	8.67±0.34	9.56±0.87	10.33±0.56	12.00±0.78
<i>Pseudomonas aeruginosa</i>	9.00±0.12	10.35±0.12	12.50±0.33	14.67±1.87

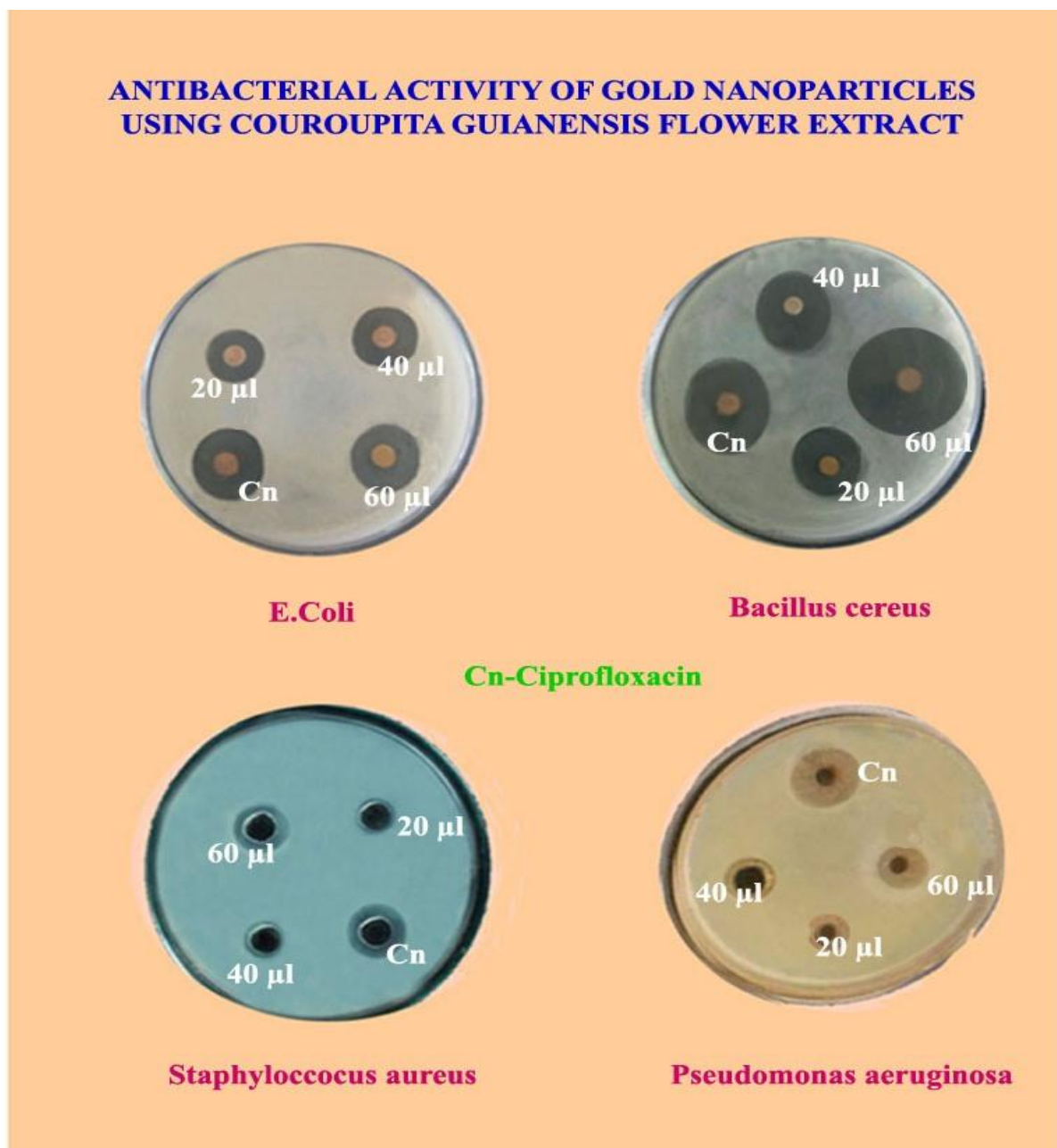


Figure.6. Antibacterial activity of Gold Nanoparticles using *Couroupita guianensis* flower extract.

4. DISCUSSION:

Antibiotic resistance by the pathogenic bacteria has been observed since last decade; hence, the researchers are focusing on the development of new antibacterial agents. In the present scenario, Au nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field[16]. The extremely small size of nanoparticles exhibits enhanced or different properties when compared with the bulk material. There are different physical and

chemical methods for the synthesis of nanoparticles, but there is always a need for the development of eco-friendly route for the synthesis process[17,18]. Hence, our current study proves to be an important step in this direction. Formation and stability of Au nanoparticles in aqueous colloidal solution are confirmed using UV-Vis spectral analysis. UV-vis spectra of aqueous component as a function of time variation of flower broth with 1 mM aqueous HAuCl_4 solution. Metal nanoparticles have free electrons, which give surface plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of gold colloids were observed at 536 nm. The intensity of absorption band increases with increasing time period of aqueous component and consequent colour changes were observed from intense yellow colour to dark purple colour. These characteristic colour variations is due to the excitation of the of the surface plasmon resonance in the metal nanoparticles.

SEM images provided information about the morphology and size of the biosynthesized gold nanoparticles. The gold nanoparticles were found to be cubic in shape. The diameter of synthesized nanoparticle was identified as 37.2nm and shown in figure 3. further SEM image showed the high density gold nanoparticles synthesized by the *Couroupita guianensis* flower extract. This confirms the development of gold nanostructures by the plant extract.

Transmission electron microscope image of gold nanoparticles obtained from *Couroupita guianensis* extract. The morphology of the nanoparticles was spherical in nature. The obtained nanoparticles are in the range of sizes 25-45 nm and few particles are agglomerated. The mean size of the gold nanoparticle was obtained as 37.2 nm from HR-TEM is a good agreement with the size obtained in the SEM measurements.

It is assumed that only the oxidized form Au^+ is present initially. Thus, a negative-going potential scan is chosen for the first halfcycle, starting from a value where no reduction occurred. As the applied potential approaches the characteristic E_o for the redox process, a cathodic current begins to increase, until a peak is reached. The sweep is reversed after traversing the potential region where the reduction process takes place. During the reverse scan, Au molecules are reoxidized back to Au^+ and it result in an anodic peak..

It is well known that Au ions and Au-based compounds have strong antimicrobial effects [19], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents [20-22]. These inorganic nanoparticles have a distinct advantage over conventional chemical antimicrobial agents. The most important problem caused by the chemical antimicrobial agents is multidrug resistance. Generally, the antimicrobial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism. Various microorganisms have evolved drug resistance over many generations. Thus far, these antimicrobial agents based on chemicals have been effective for therapy; however, they have been limited to use for medical devices and in prophylaxis in antimicrobial facilities. Therefore, an alternative way to overcome the drug resistance of various microorganisms is needed desperately, especially in medical devices, etc. Au ions and Au salts have been used for decades as antimicrobial agents in various fields because of their growth-inhibitory capacity against microorganisms. Also, many other researchers have tried to measure the activity of metal ions against microorganisms [23,24]. However, Au ions or salts has only limited usefulness as an antimicrobial agent for several reasons, including the interfering effects of salts and the antimicrobial mechanism of the continuous release of enough concentration of Au ion from the metalform. In contrast, these kinds of limitations can be overcome by the use of Au nanoparticles.

CONCLUSION

In this investigation, the bio-reduction of aqueous Au^+ ions by the flower extract of the plant *Couroupita guianensis* was studied and characterized by UV-Vis, SEM, HR-TEM and CV analysis. The potential antimicrobial activity of gold nanoparticles was performed and the maximum antibacterial activity was observed against *Bacillus cereus*. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic nanomaterials. Toxicity studies of gold nanoparticles on human pathogen open a door for a new range of antibacterial agents and anticancer agents.

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