Research Paper www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2013, Sep., Vol. 4 (3)

ISSN: 0976-9390
IJCPS

Synthesis of gold phyto nanoparticles and their antibacterial efficacy

Alagumuthu G* and Kirubha R.

P.G & Research centre, Department of chemistry, Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi, India

***Corresponding Author:** E-Mail: alagupathi@yahoo.co.in

ABSTRACT

The aim of this work was the biosynthesis of gold nanoparticles using *Morinda Tinctoria* plant extract and evaluation of its antimicrobial potentials. The resulting gold nano particle was characterized by using UV–Visible, FT-IR, SEM, TEM, and CV techniques. UV-visible spectrum of the aqueous medium containing gold nanoparticles showed a peak around 520.05 nm. The SEM analysis revealed that the particles were gold nanoparticles and mean size of 20.1 nm with a face centered cubic structure. The TEM image confirms that the particles are spherical in shape and its size is varied in the range of 18-36 nm. Furthermore, the antibacterial activity of obtained gold nanoparticles significantly control the growth of gram positive and gram negative bacteria at the lower rate of 60µg/ml as minimum inhibitory concentration.

Keywords: Nanoparticles; *Morinda Tinctoria*; TEM; Gold nanoparticle; Antibacterial activity.

1. 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] and *Mentha piperita*[13], *Aegle marmelos*[14].

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

2. MATERIALS AND METHODS

2.1. Materials

Fresh plants of *Morinda Tinctoria* 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 plant of *Morinda Tinctoria* broth solution was prepared by taking 100 g of thoroughly washed and finely cut plants 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^{0}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).

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

Synthesis of gold nanoparticles solution with plants 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.

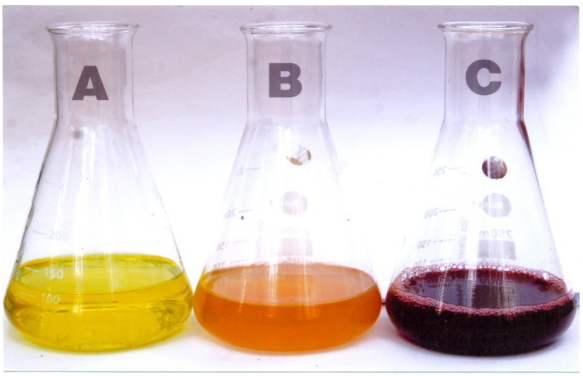

Figure - 1: Photographs showing A) pure $HAuCl_4$ solution B) pure *Morinda tinctoria* plant Extract C) Colour changes after adding plant Extract with $HAuCl_4$ solution.

2.4. FT-IR Spectroscopy

FT-IR measurements is undertaken in order to confirm the formation of crystalline nanocrystals and identify adsorbed species onto the crystal surface. Generally, FT-IR is recorded using Nicolet FT-IR spectrometer mode impact 400. The spectra were recorded at wave number in the range of 400 and 4000cm^{-1}.

2.5. Scanning electron microscopy (SEM)

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

2.6. 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.

2.7. 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.

2.8. 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 ^{o}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 AND DISCUSSION

3.1. UV-VIS spectra analysis

In the present scenario, Au nanoparticles as antimicrobial agents have come up as a promising candidate in the medical field[16]. Reduction of Au ion into gold nanoparticles during exposure to the plant extracts could be followed by color change.

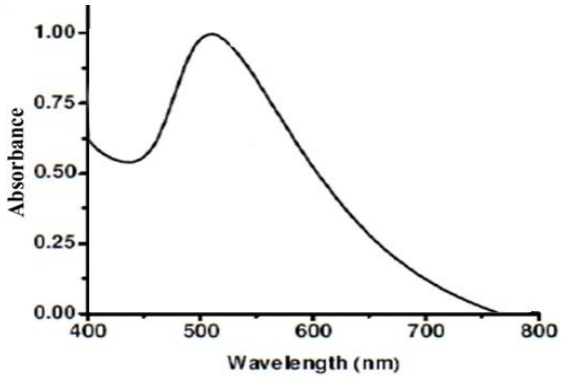

Figure - 2: UV Spectra of gold nanoparticles

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 520.05 nm, broadening of peak indicated that the particles are polydispersed (Figure 2).

3.2. FT-IR studies

FTIR analysis was carried out to identify the possible interaction between the biomolecule and Au^+ during the biogenric reduction reactions. The FTIR data for pure $HAuCl_4$ (3a) and the gold nanoparticles containing *Morinda Tinctoria* plant extract (3b) are shown in figure 3. The band at 3513cm^{-1} is assigned for O-H stretching vibration of alcohol and phenol compounds and bands observed at 1410,1050 and 1710cm^{-1} are due to the C-O stretching and C=O stretching mode of the carbonyl functional groups in alcohol,ethers,acids and esters. The carbonyl bands at 1710cm^{-1} was shifted to 1608cm^{-1} during the formation of gold nanoparticles. The shifts in bands at 1710,1050 and 1410cm^{-1} were clearly indicating the coordination of carboxylic acid with gold nanoparticles.

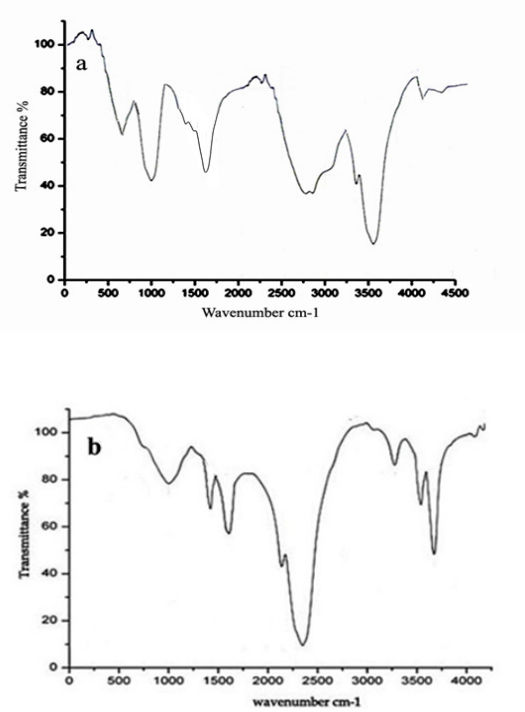

Figure - 3: FT-IR spectra of 3(a) pure Chloro Auric acid and 3(b) biosynthesized gold nanoparticles using the plant extract of *Morinda tinctoria.*

From the analysis of FTIR studies, we revealed that the carbonyl group from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e., capping of gold nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of gold nanoparticles in the aqueous medium. These results imply that proteins, sugars and amino acid present in *Morinda Tinctoria* plant extract are play a major role on reduction of Au^+.

3.3 SEM analysis of gold nanoparticle

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 20.1 nm and shown in figure 4. further SEM image showed the high density gold nanoparticles synthesized by the *Morinda tinctoria* plant extract. This confirms the development of gold nanostructures by the plant extract.

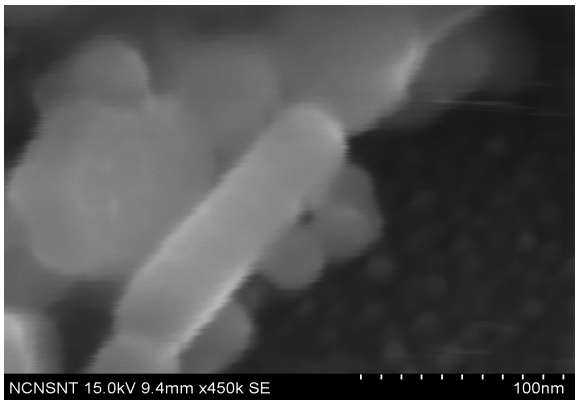

Figure - 4: SEM image of gold nanoparticles using the plant extract of *Morinda tinctoria.*

3.4. TEM analysis of Au nanoparticles

The resulting gold nanoparticles was analysed with TEM techniques and conclude that the average mean size of Au nanoparticles was 20.1 nm, which seems to be spherical in morphology as shown in (Figure 5). HR-TEM is a good agreement with the size obtained in the SEM measurements.

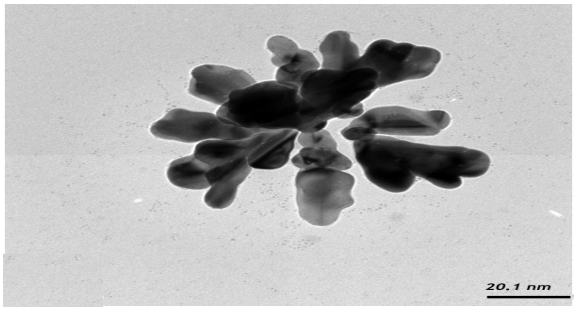

Figure - 5: HR-TEM image of gold nanoparticles using the plant extract of *Morinda tinctoria*

3.5. Cyclic voltammetry analysis

In cyclic voltammetric analysis the *Morinda Tinctoria* plant 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 *Morinda Tinctoria* extract in the reaction medium, the cathodic peak shifted towards the negative potential direction, implying that the reduced gold NPs are stabilized by *Morinda Tinctoria* extract (Fig. 6). 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 *Morinda Tinctoria* extract can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AuNPs shows the peaks observed at -0.86 and 0.94V.

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 *Eo* 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..

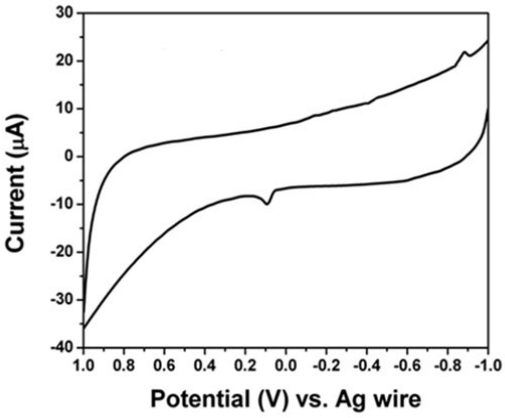

Figure - 6: Cyclic voltammograms of gold nanoparticles.

3.6. Antibacterial Activity study

The antibacterial activity of gold nanoparticle was tested against the following microorganism, viz; *E.Coli, Staphylococus aureus, Bascillus 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 *Bascillus cereus.*(figure 7)

It is well known that Au ions and Au-based compounds have strong antimicrobial effects[17], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents[18-20]. 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[21,22]. 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.

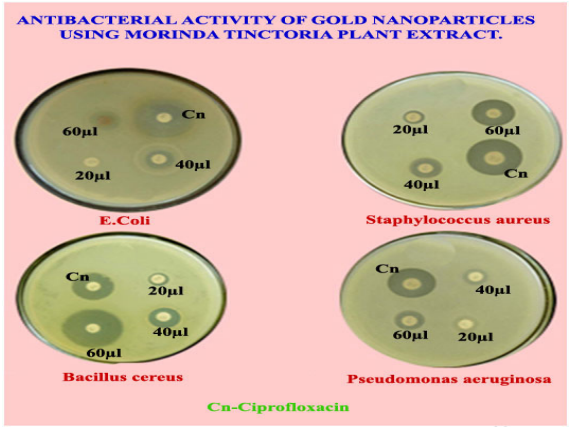

Figure - 7: Antibacterial activity of Gold Nanoparticles using *Morinda Tinctoria* plant extract.

Table - 1: Antibacterial activity of gold Nanoparticles				
Microorganism	**Zone of inhibition in mm**			
	20µL	**40 µL**	**60 µL**	**Cifrofloxacin**
E.Coli	8.00±0.64	12.54±0.46	14.33±0.33	16.67±1.20
Staphylococcus aureus	9.67±0.76	13.03±0.98	15.67±0.88	17.33±1.00
Bacillus cereus	9.67±0.34	13.56±0.87	18.33±0.56	14.00±0.78
Pseudomonas aeruginosa	10.00±0.12	12.35±0.12	13.50±0.33	15.67±1.87

4. CONCLUSION

In this investigation, the bio-reduction of aqueous Au^{+} ions by the *Morinda Tinctoria* plant extract was studied and characterized by UV-Vis, FT-IR, SEM, HR-TEM and CV analysis. The potential antimicrobial activity of gold nanoparticles was performed and the maximum antibacterial activity was observed against *Bascillus 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.

6. REFERENCES

1. Rosei F. Nanostructured surfaces: challenges and frontiers in nanotechnology. **J Phys Condens Matter.** 2004; 16:S1373–S1436.
2. Papp S, Patakfalvi R and Dekany I. Metal nanoparticle formation on layer silicate lamellae. **Colloid Polym Sci.,** 2008; 286: 3–14.
3. Seoudi R and Said RA. Studies on the effect of the capping materials on the spherical gold nanoparticles catalytic activity. **World. J. Nano Sci and Engg.,** 2011; 1: 51-61
4. Foglia S, Suber L and Righini M. Size tailoring of CdS nanoparticles by different colloidal chemical techniques, **Colloids Surf. A.,** 2001; 177(1):3-12.
5. Liu J, He F, Gunn T M, Zhao D and Roberts C B. Precise seed-mediated growth and size-controlled synthesis of palladium nanoparticles using a green chemistry approach**. Langmuir,** 2009; 25:7116–7128.
6. Mohanpuria P, Rana Nisha K and Yadav SK. Biosynthesis of nanoparticles: technological concepts and future applications, **J. Nanopart. Res.,** 2008; 10:507-517.
7. Liu C, Liu Z, Tsao R and Liu S. Identification of phenylethanoid glycosides in plant extract of *Plantago asiatica* L. by liquid chromatography-electrospray ionization mass spectrometry, **Chinese J. Chem.,** 2009; 27:541-545.
8. Padmadhas R and Ragunathan R. Effect of lead nano particle present in the leaf of *Calotrphis Gigantea* which result in the loss of the painted grass hoppe of the Western ghats species in India. **Int, J. Nanotechnol. Appl.,** 2009; 3: 89-96.
9. Daizy Philip. Green synthesis of gold and silver nanoparticles using Hibiscus rosa sinensis. **Physica E.,** 2010; 42: 1417-14242.
10. Vilchis-Nestor A San chez-Mendieta V, Camacho-Lo pez M, Go mez-Espinosa R, Camacho-Lo pez M and Arenas-Alatorre. Solventless synthesis and optical properties of Au and Ag nanoparticles using *Camellia sinensis* extract. **J. Mater. Lett.**, 2008; 62: 3103.
11. Narayana KB and Sakthive NJ. Coriander leaf mediated biosynthesis of gold nanoparticles. **Mater. Lett.,** 2008; 68:4588-4590.
12. Laura Castro, Luisa Blazquez M, Jesus A Munoz. Felisa Gonzalez, Camino Garcia-Balboa and Antonio Ballester. Biosynthesis of gold nanowires using sugar beet pulp, **Process Biochemistry,** 2011: 46:1076-1082.
13. Mubarakali D, Thajuddin N, Jeganathan K and Gunasekaran M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens, Colloids Surf. B. 2011:85:360-365.
14. Alagumuthu G and Kirubha R. Biofabrication and antibacterial activities of gold nanoparticles using *Aegle Marmelos* fruit extract, **Inter. J. Adv. Sci and Technol,** 2013; 6(6): 73-95.
15. Mona Safaepour, Ahmad Reza Shahverdi , Hamid Reza Shahverdi, Mohammad Reza Khorramizadeh and Ahmad Reza Gohari. Green Synthesis of Small Silver Nanoparticles Using Geraniol and Its Cytotoxicity against

Fibrosarcoma, **Avicenna J Med Biotech,** 2009; 2:111-115.

16. Duran N, Alves OL, De Souza GIH, Esposito E and Marcato PD. Mechanistic aspects of biosynthesis of silver nanoparticles by several Fusarium oxysporum strains. **J Biomed Nanotechnol**, 2007; 3: 203-208.
17. Enrique Lima, Roberto Guerra, Víctor Lara and Ariel Guzmán, Gold nanoparticles as efficient antimicrobial agents for Escherichia coli and Salmonella typhi, **Chem. Central J,** 2013; 7: 11.
18. Garry G, Graham G, Champion D and Ziegler J. The cellular metabolism and Effects of gold complexes, **Metal. Based Drugs,** 1994; 1: 395–404.
19. Hamouda J Myc A, Donovan B, Shih A, Reuter JD and Baker JR. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. **Microbiol Res**., 2000; 156: 1 -7.
20. Fricker X. Medicinal chemistry and pharmacology of gold compounds, **Transit Met Chem.,** 1996; 21: 377–383.
21. Rreddy GR, Jayakumar C, Morais AB, Sreenivasn D and Gandhi NN. Green synthesis characterization and in-vitro antibacterial activity of polyshaped gold nanoparticles by using sennasiamea (lam.) Plant leaf extract. **Int J Green Chem Bioproces,** 2012; 2(1): 1-5.
22. Marsh PD. Microbiological aspects of the chemical control of plaque and gingivitis. **J Dent Res.,** 1992; 71:1431- 8.