International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 5(2): March-April 2015

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Pharmaceutical Sciences **Research Article……!!!**

Received: 21-02-2015; Revised: 28-02-2015; Accepted: 01-03-2015

SYNTHESIS OF PLANT MEDIATED GOLD NANOPARTICLES USING *POUTERIA CAMPECHIANA* AND IT'S EFFECTS ON PATHOGENS

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Keywords:

Gold Nanoparticles; *Pouteria campechiana;* TEM; Antibacterial activity

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ABSTRACT

In this study, we present a facile and cost-effective method for the synthesis of gold nanoparticles (Au- NPs) using fruit *Pouteria campechiana* extract. The results recorded from UV–Vis spectroscopy, X-ray diffraction (XRD), High Resolution Transmission Electron Microscopy (HRTEM) and Cyclic voltammetry (CV) support the biosynthesis and characterization of gold nanoparticles. From XRD analysis, the average size of the gold nanoparticles was measured 49.6 nm. Further, the antibacterial activity of synthesized gold nanoparticles showed effective inhibitory activity against the pathogens namely: *Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Pseudomonas aeruginosa*.

1. INTRODUCTION

Nanoparticles are being viewed as elementary building blocks of nanotechnology. The most important and distinct property of nanoparticles is that they show evidence of larger surface area to volume ratio. The most successfully studied nanoparticles today are those made from noble metals, in particular Ag, Pt, Au and Pd. Metal nanoparticles have marvelous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices. [1]. Conventionally, nanomaterials are synthesized using either chemical or physical methods which include sol process, micelle, chemical precipitation, hydrothermal method, pyrolysis, and chemical vapour deposition [2]. Some of these methods are easy and provide control over crystallite size by restoring the reaction environment. But problem still exists with the general stability of the product and in achieving monodisperse nanosize using these methods [3]. Moreover, many of the conventional techniques have been found to be capital intensive and inefficient in materials and energy use [4].

Biological methods have emerged as an alternative to the conventional methods for synthesis of NPs. Synthesis of inorganic nanoparticles by biological systems makes nanoparticles more biocompatible and environmentally benign [5]. Many plants such as Geranium leaf [6], Alfalfa [7], *Azadirachta indica* [8], *Lemon grass* [9], *Aloe vera* [10], *Cinnamomum camphora* [11], *Emblica officinalis* [12], *Capsicum annuum* [13],*Diospyros kaki* [14], *Carica papaya* [15], *Coriandrum sp.*[16], *Boswellia ovalifoliolata*[17], *Tridax procumbens, Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium* [18], *Aegle marmelos*[19], *Cissus quadrangularis*[20] and *Morinda tinctoria*[21] have shown the potential of reducing nature for the formation of NPs. The present study aims at the synthesis of gold nanoparticles from the aqueous extract of *Pouteria campechiana* fruits. We also attempt to combine the inherent antimicrobial activities of gold nanoparticles for enhanced antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Materials:

Fresh plants of *Pouteria campechiana* were identified and collected from Tamilnadu Agricultural University, Tirunelveli, 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 *Pouteria campechiana* broth solution was prepared by taking 120 g of thoroughly washed and finely cut plants in a 500 mL Erlenmeyer flask along with 250 mL of

sterilized double distilled water and then boiling the mixture for 20 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 [22]. 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).

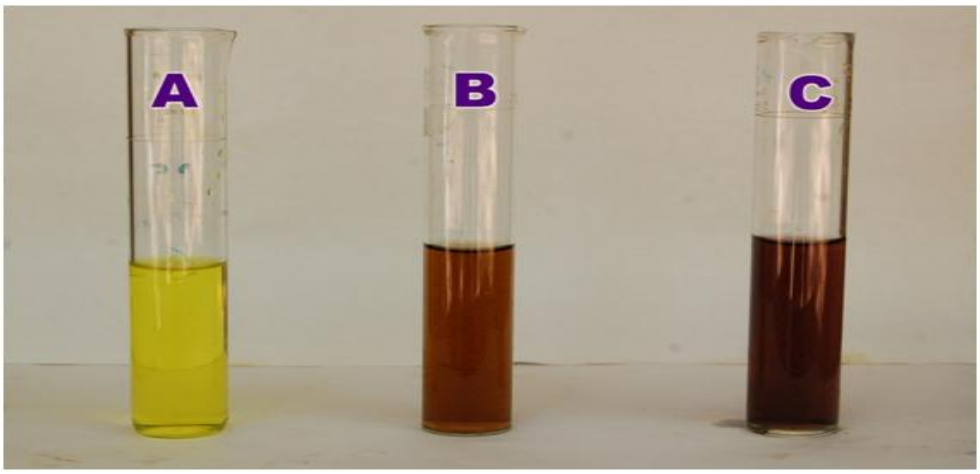

Figure 1: Photographs showing A) pure $HAuCl_4$ solution B) pure *Pouteria campechiana* plant Extract C) Colour changes after adding plant Extract with $HAuCl_4$ solution.

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^{3+} 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 . X-ray diffraction (XRD) analysis:

The particle size and nature of the gold nanoparticle were determined using XRD. This was carried out using Shimadzu XRD-6000/6100 model with 30 kv, 30 mA with Cu k α radians at 2 θ angle. The analyzed material is finely ground, and average bulk composition is determined. The particle or grain size of the particles on the gold nanoparticles was determined using Debye Scherer's equation. $D = 0.94\ \lambda / ß \cos \theta$.

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 Muller Hinton agar media 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 was used to prepare lawns. Agar of 5 mm diameter was prepared with the help of a sterilized stainless steel cork borer. Four 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

The spectra were recorded after at 0, 2, 4, 6, 8 and 10 min. The effect of the reaction time on AuNPs synthesis was also evaluated with UV-Visible spectra and with time, the peak become sharper. The SPR band of gold nanoparticles appears at 532.15 nm and even after 10 min of incubation only slight variation was observed (fig 2). The increase in intensity could be due to increasing the number of nanoparticles formed as a result of the reduction of gold ions present in the aqueous solutions[23].

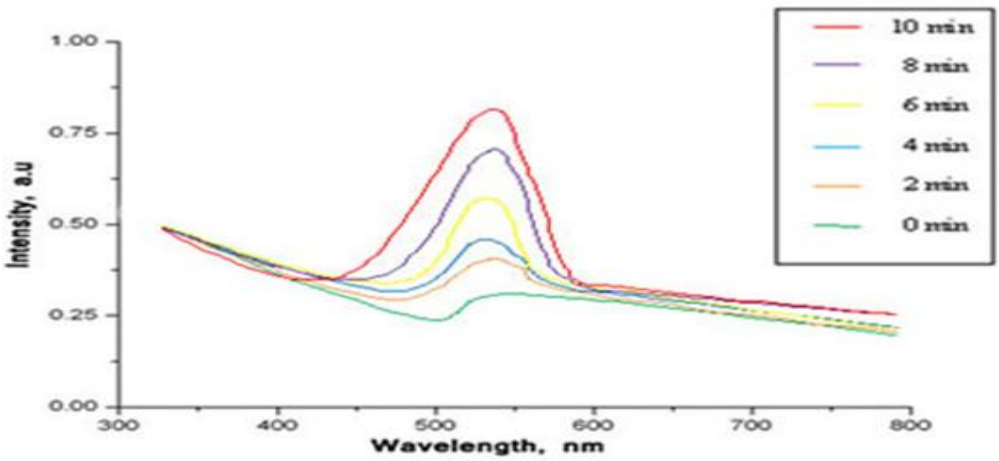

Figure.2. UV Spectra of gold nanoparticles

3.2.. XRD studies

The XRD spectrum (Figure 3) has three distinct diffraction peaks at 37.96°, 46.7° and 63.5°, which are indexed (111), (200) and (220) of the face-centered- cubic(fcc) gold, respectively(figure .3). The obtained data was matched with the JCPDS file No.03- 0921 and the same results were also reported earlier [24]. The average grain size of the gold nanoparticles formed in the process was estimated from the Debye-Scherrer equation and the mean size of the particle was 49.6 nm.

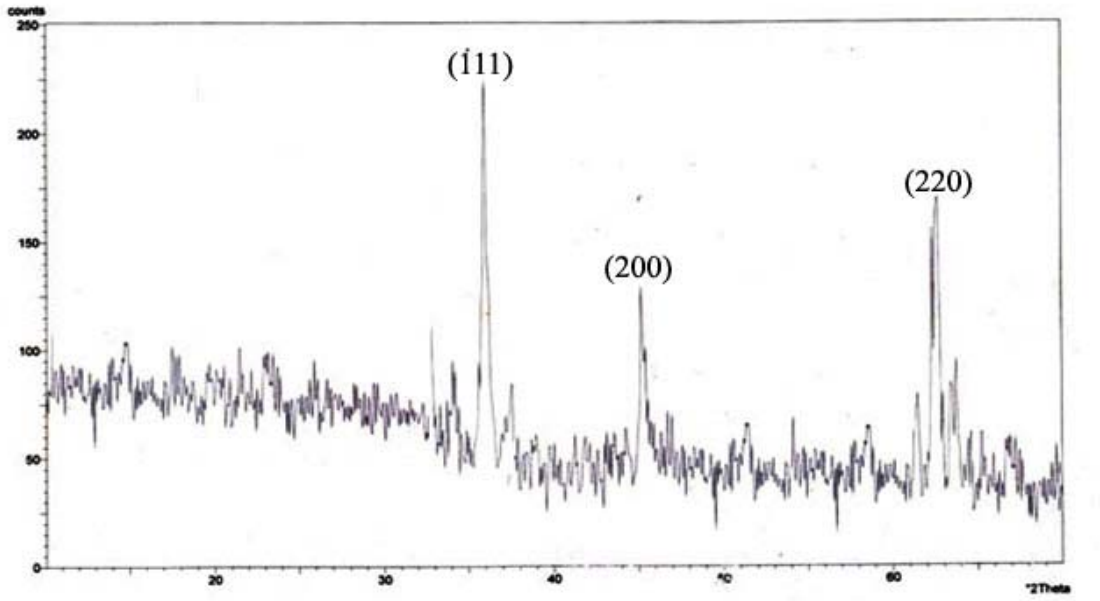

Figure.3. XRD of biosynthesized gold nanoparticles using the plant extract of *Pouteria campechiana*

3.3. 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 49.6nm, which seems to be spherical in morphology as shown in (Figure 4).

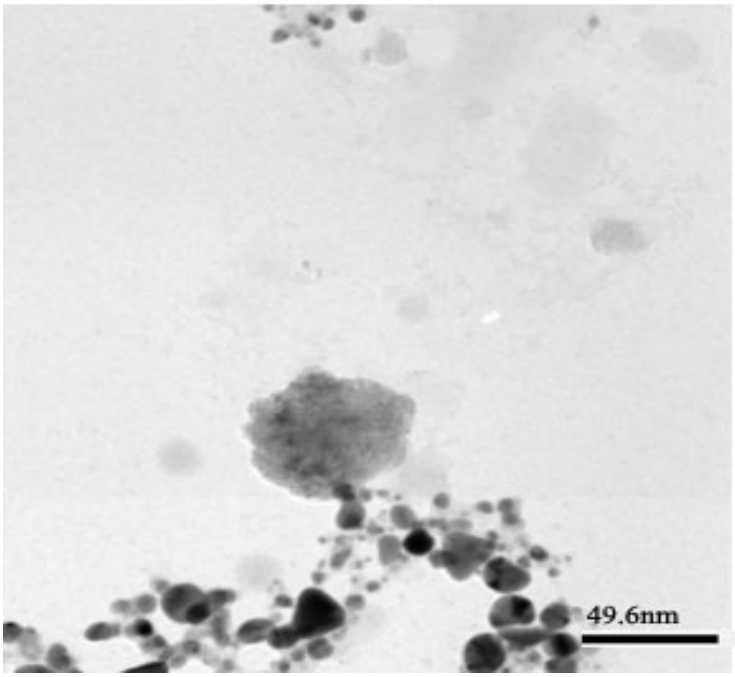

Figure .4. HR-TEM image of gold nanoparticles using the plant extract of *Pouteria campechiana*

3.4. Cyclic Voltammetry analysis:

In cyclic voltammetric analysis the *Pouteria campechiana* 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 *Pouteria campechiana* extract in the reaction medium, the cathodic peak shifted towards the negative potential direction, implying that the reduced gold NPs are stabilized by *Pouteria campechiana* 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 *Pouteria campechiana* extract can provide a suitable environment for the formation of nanoparticles. The cyclic voltammogram of AuNPs shows the peaks observed at -0.90 and 0.14V.

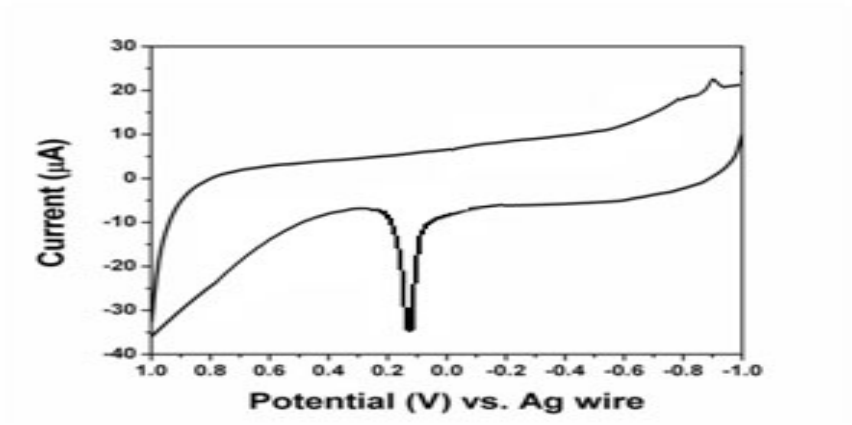

Figure.5.Cyclic voltammograms of gold nanoparticles

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 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^{3+} and it result in an anodic peak.

3.5. 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 6). It is well known that Au ions and Au-based compounds have strong antimicrobial effects [25], and many investigators are interested in using other inorganic nanoparticles as antibacterial agents [26-28]. 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 microorganism [29, 30]. 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.

Table. 1. Antibacterial activity of gold Nanoparticles

Microorganism	Zone of inhibition in mm			
	20ml	40ml	60ml	Cifrofloxacin
E.Coli	8.66±0.58	10.25±0.99	12.54±0.21	16.35±1.02
Staphylococcus aureus	8.33±0.75	9.05±0.65	12.59±1.55	14.36±0.15
Bacillus cereus	8.33±0.65	9.46±0.15	15.63±0.63	11.08±0.45
Pseudomonas aeruginosa	9.74±0.33	10.98±0.85	12.66 ±1.22	14.98±0.11

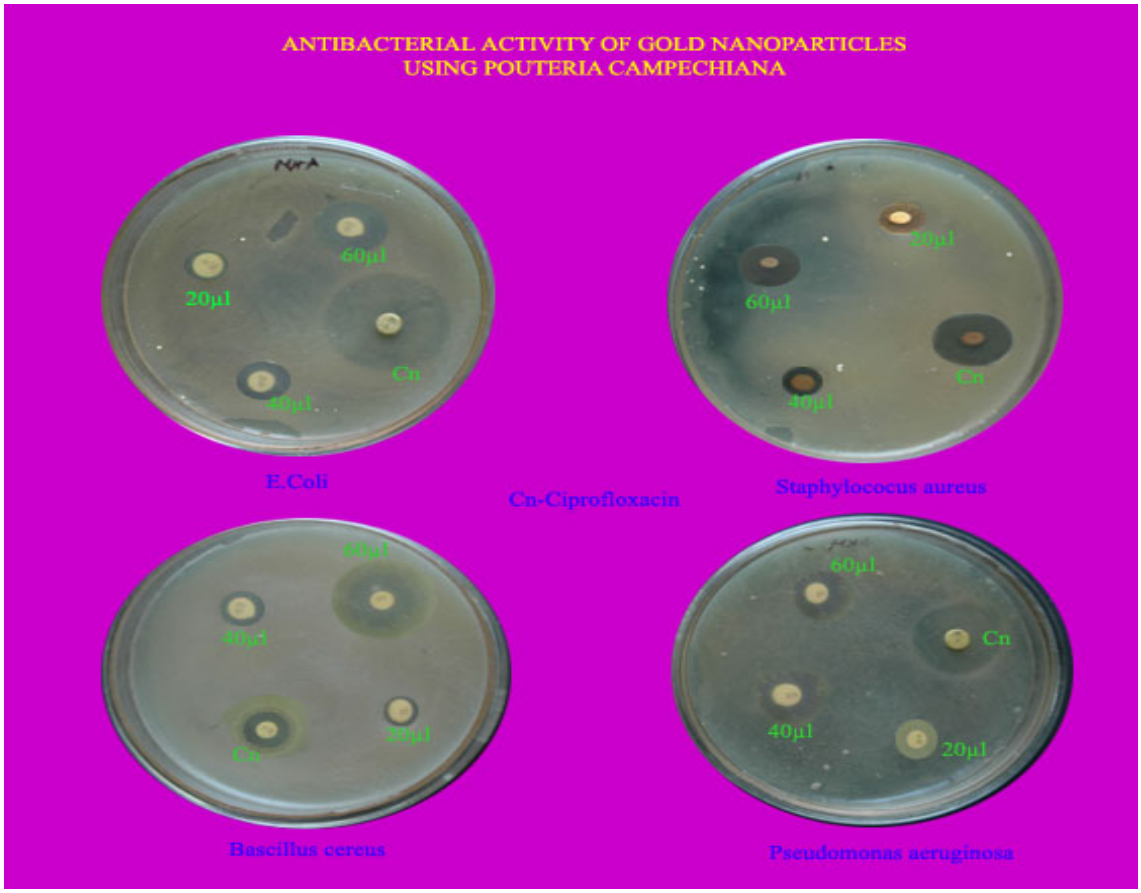

Figure.6. Antibacterial activity of Gold Nanoparticles using *Pouteria campechiana* plant extract.

4. CONCLUSION

In this investigation, the biosynthesized Gold nanoparticles were characterized by UV-Vis, HR-TEM, XRD and CV measurements. This green synthesis method is alternative to chemical method, since it is cheap, pollutant free and eco-friendly. 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.

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