BIOSYNTHESIS OF GOLD NANOPARTICLES USING PIPER NIGURM AND THEIR ANTIBACTERIAL ACTIVITY

R. Devika¹, S. Elumalai*¹

¹PG and Research Dept of Plant Biology & Plant Biotechnology, Presidency College, Chennai-600005, India.

ABSTRACT
In this work, environmentally benign process of gold nanoparticles (Au-NPs) have developed using Piper nigrum leaf extract has been attempted. The bio reduction of Au⁺ ions in solution was monitored using UV-visible spectroscopy from zero reading and double distilled water was used as blank. The samples were withdrawn at various time intervals and the absorbance was measured. The gold nanoparticles synthesized using extracts of samples was confirmed by color changes and was characterized by UV-visible spectrophotometer; the UV-visible spectra showed a broad peak located at 535 nm for gold nanoparticles. The Scanning Electron Microscopy (SEM) analysis shows the surface morphology of Au-NPs. Thus gold nanoparticles has great potential as antimicrobial compound against pathogenic microorganisms.

Keywords: Gold nanoparticles, Piper nigrum, Antimicrobial Activity, Electron microscopes and Spectroscopic studies.

INTRODUCTION
In recent years noble metal nanoparticles have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. Their growing applications of nanomaterials in various fields such as in biosensors, bioremediation of radioactive wastes, functional electrical coating, synthesis of enzyme electrodes and particularly in medicine such as delivery of antigen for vaccination, gene delivery for treatment or prevention of genetic disorder, inspired the scientists to develop environment friendly...
procedures for the synthesis of nanoparticles and to avoid use of hazardous chemicals, Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant or plant extract have been suggested as possible eco friendly alternatives to chemical and physical methods\cite{8,9}. In this present research on nanomaterials, a rapid reduction of the gold ions was observed when the gold chloride solution was contacted with *Piper nigrum* leaf extract against microbial activities.

**MATERIAL AND METHODS**

**Extract Preparation**
The leaves of *Piper nigrum* was obtained from Kerala. About 30g of sample were thoroughly washed and finely cut leaves in a 300mL Erlenmeyer flask with 100 mL of sterile distilled water and then boiling the mixture for 5 min. The resulting extract was used for further experiments. The extract was treated with 3mM gold chloride solution. It is well known that gold nanoparticles exhibit red color in aqueous solution due to excitation of surface Plasmon vibrations in gold nanoparticles The appearance of the red color in solution containing the biomass was a clear indication of the formation of gold nanoparticles in the reaction mixture. The reddish dark color of the solution is due to the excitation of surface plasmon vibrations, which is essentially the vibration of the group conduction electrons in the gold nanoparticles

**SYNTHESIS AND CHARACTERIZATION**
For the synthesis of Au- NPs 5ml of *Piper nigrum* leaf extracts as test solution were incubated at room temperature for 1-2 hours. The gold nanoparticle solution thus obtained subjected for purification process. Then the supernatant is discarded and the pellet is dissolved in de ionised water. The gold nanoparticles were confirmed by color changes and qualitatively characterized by HITACHI U-2900 UV-Vis spectrophotometer.

**UV-Vis spectroscopy analysis**
UV- Vis spectral analysis was done by using HITACHI U-2900 UV-Vis spectrophotometer .The UV-Vis spectra reveals the formation of gold nanoparticles by showing surface plasmon resonance at 535 nm. UV-visible spectroscopy is one of the most widely used techniques for structural characterization of gold nanoparticles. The absorption spectrum (Fig. 1) of the pale red colour gold colloids prepared by hydrazine reduction showed a surface Plasmon absorption band with a maximum of 535nm indicating the presence of spherical or roughly spherical Au nanoparticles.
SEM Analysis of silver nanoparticles
Scanning Electron Microscopic (SEM) (Fig. 2) analysis was done using FEI QUANTA 200 FEG HR-SEM model. Thin films of the sample were prepared on a carbon coated, a very small amount of the specimen on the sample holder, extra solution was removed using a blotting paper, and then the film on the SEM allowed to dry by putting it under a mercury lamp for 5 min.

Antibacterial activity
Regarding antibacterial activity the Au-NPs showed activity against two bacteria (*Staphylococcus aureus* and *Vibrio cholera*) and the highest antibacterial effect on *Staphylococcus aureus* was found with zone of inhibition (19 mm) and lowest antibacterial effect in Au-NPs on *Vibrio cholera* (12 mm). Results were summarized in Figure 3. Between antibiotic the strongest antibacterial effect on *Staphylococcus aureus* at (09 mm) and the weakest activity was found in *Vibrio cholera* with zone of inhibition (07 mm). Comparison of the Au-NPs combined with antibiotics (Rifampicin) data obtained in this study, the maximum activity was observed in Au-NPs combined with antibiotics against *Staphylococcus aureus* and minimum activity was observed in Au-NPs combined with antibiotics against *Vibrio cholera* at inhibition variation was observed in the range of 12 mm.

RESULTS AND DISCUSSION
The color change showed the presence of gold nanoparticles in the *Piper nigrum* leaf extract and it was characterized by HITACHI U-2900 UV–visible spectrophotometer and monitored by taking readings at regular time intervals in spectrophotometer. The strong broad peak located at 535nm was observed for silver nanoparticles.

![UV-visible spectra of the *Piper nigrum* leaf extract](image)

Fig. 1: The UV-visible spectra of the *Piper nigrum* leaf extract shows the gold surface Plasmon resonance Peak.
Scanning Electron Microscope (SEM) surface morphology image showed relatively spherical shape gold nanoparticles formed with diameter range 100-200 nm in Figure [2].

**Fig. 2:** Scanning electron micrograph of gold nanoparticles from *Piper nigrum* leaf extract

Finally, the antimicrobial susceptibility of gold nanoparticles synthesized was investigated. The disk diffusion method was used as antimicrobial susceptibility testing method. Disposable plates inoculated with the tested bacteria, including highly multi resistant strains such as Rifampicin was used for the tests. Zones of inhibition were measured after 24 hr of incubation at 35 C. The comparative stability of discs containing. Rifampicin was made .Figure [3] shows plates to which a bacterial suspension was applied. The presence of nanoparticles at a certain level inhibited bacterial growth by more than 90%. The diameter of inhibition zones (in millimeters) around the different gold nanoparticles against test strain are shown in Figure [3]. Results were shown in (Table 1).

**Fig. 3:** Antibacterial activities of Au Nps synthesized by *Piper nigrum* 1)showing control 2)with Antibiotics(Rifampicin) 3)with Antibiotics+Nps 4) Nps
Table 1: Zone of inhibition of Au-NPs against pathogenic bacteria

<table>
<thead>
<tr>
<th>Study no.</th>
<th>Pathogenic bacteria</th>
<th>Zone of diameter in mm</th>
<th>Antibiotics</th>
<th>Nanoparticles</th>
<th>Antibiotics &amp; Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>09</td>
<td>12</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Vibrio cholera</em></td>
<td>07</td>
<td>09</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

**CONCLUSION**

In summary, gold nanoparticles were synthesized using gold chloride solution. The nanoparticles were characterized by UV/Vis and SEM. UV/Vis spectra show the characteristic plasmon absorption peak for the gold nanoparticles ranging from 525 to 535 nm. Additionally, the antibacterial activity of the nanoparticles dispersion was measured by Disc diffusion method. The results of this study clearly demonstrated that the colloidal gold nanoparticles inhibited the growth and multiplication of the tested bacteria, including highly multi resistant bacteria such as Rifampicin *Staphylococcus aureus* and *Vibrio cholera*. Such high antibacterial activity was observed at *Staphylococcus aureus*, with Rifampicin. Therefore it is proven from this study that the Au-NPs synthesized from *P. nigrum* leaf extract seems to be promising and effective antibacterial agent against infectious microbes.

**REFERENCES**