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BIOSYNTHESIS OF GOLD NANOPARTICLES USING *PIPER NIGURM* SEED EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

A novel environmentally benign bioprocess of gold nanoparticles (Au-NPs) have developed using *Piper nigrum* seed extract. 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 was measured at various time intervals and the absorbance were noted. The gold nanoparticles synthesized using seed 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 540 nm for gold nanoparticles. The Scanning Electron Microscopy (SEM) analysis shows the surface morphology of Au-NPs. Therefore these gold nanoparticles are potential as antimicrobial compound against infectious pathogens.

KEYWORDS: Gold nanoparticles, *Piper nigrum*, Antimicrobial Activity and Spectroscopic studies.

INTRODUCTION

A bio inspired material was developed using environment friendly procedures for the synthesis of nanoparticles . Biological methods of nanoparticles synthesis using microorganism, enzyme, and plant extract have been suggested as possible eco friendly alternatives to chemical and physical methods [1,2]. Ag-particles have found tremendous applications in the field of high sensitivity bio-molecular detection and diagnostics [3], antimicrobials and therapeutics [4,5]. As part of our work, we have observed that aqueous gold ions, when exposed to the seed extract, are reduced in solution, thereby leading to the formation of an extremely stable gold nanoparticle. The Au-NPs are in the range of 30 nm in

dimensions, and are stabilized in the solution. One approach that shows immense potential is based on the biosynthesis of nanoparticles using biological organisms such as harmful bacteria.

MATERIAL AND METHODS

Extract Preparation

The seed extract of *Piper nigrum* was obtained from Kerala. About 30g of seeds were powdered finely. Then experiment started with seed extract with 100 mL of sterile distilled water. The resulting extract was used for further experiments. The extract was treated with 2mM 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 nanopartilcles.

SYNTHESIS AND CHARACTERIZATION

For the synthesis of Au- NPs 5ml of *Piper nigrum* seed extracts used as test solution and were incubated at room temperature for 1-2 hours. The gold nanoparticle solution thus obtained was purified. 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 540 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 reduction showed a surface Plasmon absorption band with a maximum of 540 nm 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 (20 mm) and lowest antibacterial effect in Au-NPs on *Vibrio cholera* (13 mm). Results were summarized in Figure 3.Between antibiotic the strongest antibacterial effect *on Staphylococcus aureus* at (10 mm) and the weakest activity was found in *Vibrio cholera* with zone of inhibition (08 mm). Comparison of the Au-NPs combined with antibiotics (Tetracyclin) 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 13 mm.

RESULTS AND DISCUSSION

The color change showed the presence of gold nanoparticles in the *Piper nigrum* seed 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 540 nm was observed for silver nanoparticles.

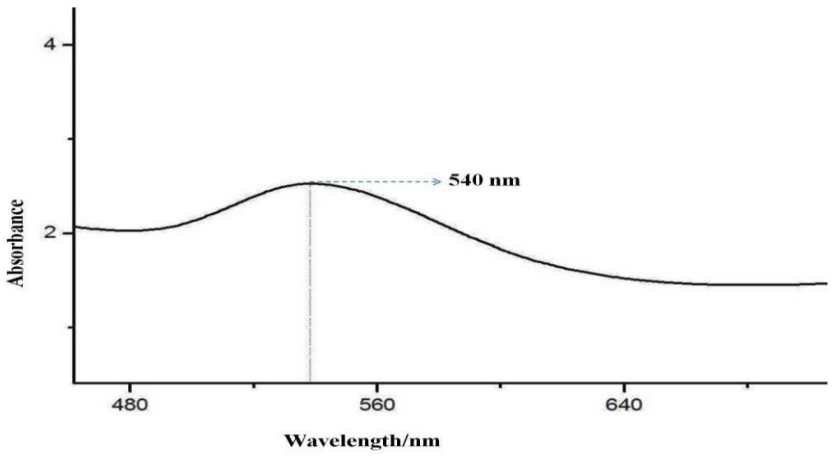

Fig. 1: The UV-visible spectra of the Piper nigrum seed extract shows the gold surface Plasmon resonance band.

Scanning Electron Microscope (SEM) surface morphology image showed relatively spherical shape gold nanoparticles formed with diameter range 100-200 nm in Figure [2].

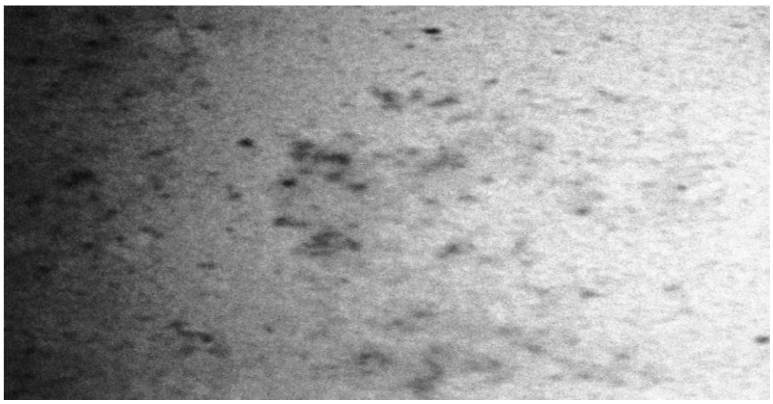

Fig. 2: Scanning electron micrograph of gold nanopartilcles from *Piper nigrum* seed extract

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 Tetracyclin was used for the tests. Zones of inhibition were measured after 24 hr of incubation at 35 C. The comparative stability of discs containing. Tetracyclin 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

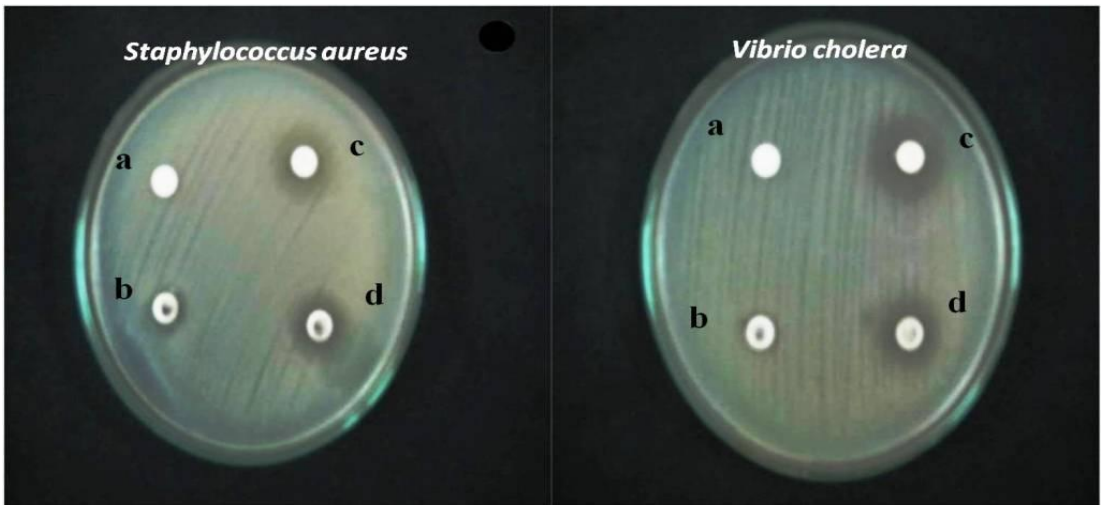

Fig. 3: Antibacterial activities of Au Nps synthesized by Piper nigrum a)showing control b)with Antibiotics(Tetracyclin) c)with Antibiotics+Nps d) Np

shown in Figure [3]. Results were shown in (Table 1).

Table 1: Zone of inhibition of Au-NPs against pathogenic bacteria

Study no.	Pathogenic bacteria	Zone of diameter in mm		
		Antibiotics	Nanoparticles	Antibiotics & Nanoparticles
1	*Staphylococcus aureus*	10	13	20
2	*Vibrio cholera*	08	10	13

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 530 to 540 nm. Additionally, the antibacterial activity of the nanopartilcles 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 Tetracyclin. Thus it is proven from this study that the Au-NPs synthesized from *P. nigrum seed* extract seems to be promising and effective antibacterial agent .

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