IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

SYNTHESIS AND CHARACTERIZATION OF GOLD NANO PARTICLES USING HENNA AND ITS EVALUATION

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Abstract

Gold nano particles with mean sizes of 20-45 nm were successfully synthesized using Henna extract. The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AuNPs. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. AuNP functionalized with ampicillin (AuNP-AMP) were bactericidal. With the ever increasing resistant strains of microorganisms to the already available and synthesized antibiotics, this could be a potential alternative. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially stimulating for the large-scale synthesis. Toxicity studies of *Henna* mediated synthesized AuNPs are also underway.

Key Words: nanoparticles, ampicillin, *Henna.*

INTRODUCTION

Nanoparticle characterization is necessary to establish understanding and control of nanoparticle synthesis and applications. Characterization is done by using a variety of different techniques, mainly drawn from materials science. Gold nanoparticles have been known to have inhibitory and bactericidal effects and thus possess immense value as an antibacterial agent (Deitch *et al*., 1987; Silver, 2003; Law *et al*., 2008). The combined effect of gold nanoparticles with antibiotics has proven to be fruitful. Such effects were first observed in *Staphylococcus aureus* and *E. coli* using disk diffusion method. The antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin increased in the presence of gold nano particles against both test strains. The same effect was not observed in the case of antibiotics tested. The effects of gold nano particles on the antibacterial activity of the aforementioned antibiotics for *E. coli* were lower than *S. aureus*. In contrast, the most synergistic activity was observed with erythromycin against *S. aureus* (Hope *et al*., 1989). The antifungal effects of gold nanoparticles and their mode of action were investigated. Antifungal effects on fungi tested with low hemolytic effects against human erythrocytes were observed. Although antifungal drug resistance does not seem to be as much of a problem as resistance to antibacterial agents in bacteria, one long-term concern is that the number of fundamentally different types of antifungal agents that are available for treatment remains extremely limited. This is because fungi are eukaryotic organisms with a structure and metabolism that are similar to those of eukaryotic hosts. Therefore, there is an inevitable and urgent medical need for antibiotics with novel antimicrobial mechanisms. Though the biocidal effect and mode of action of silver ion are known, nevertheless, the antifungal effects and the mode of action of gold nano particles against fungi have remained mostly unknown. The ability of gold nano particles to disrupt the fungal envelope structure was documented using Transmission Electron Microscope (Yamanaka *et al*., 2005). Fungicides are chemicals or biological organisms used to kill or inhibit fungi and fungal spores. A fungicide is a specific type of pesticide that controls fungal disease by specifically inhibiting or killing the fungus causing

the disease. Pure Ampelomyces quisqualis is a colourless, white crystaline, odourless solid 250ºC. Technical grade is–which decomposes on melting at approximately 98%. *Ampelomyces quisqualis* is not corrosive. *Ampelomyces quisqualis* is stable at 35-50 ºC and can be stored at this temperature. It slowly decomposes in alkaline solution. It is stable in acid and forms water soluble salts. *Ampelomyces quisqualis* controls a wide range of fungal pathogens on cereals, fruits, cotton, tobacco, turf, ornamentals and vegetables. *Ampelomyces quisqualis* is said to be harmful to fish or other aquatic life. Resistance is a very serious problem. It has been seen in apple scab, eyespot and Botrytis. To combat resistance, *Ampelomyces quisqualis* is often combined with other fungicides with different modes of action and integrated plant disease management strategies are being developed. The biologically synthesized gold nanoparticles were tested for its potential antifungal activity and their synergistic studies were carried out with fungicide against selected fungal pathogens.

MATERIALS AND METHODS

Tetrachloroauric acid ($HAuCl_4 \cdot XH_2O$) was obtained from Sigma Aldrich. Aquaregia is used for the cleaning of glasswares. Freshly prepared triple distilled water was used throughout the experimental work. All experiments were conducted under laminar hood with strict aspectic conditions. Plants were collected. Primarily the leaves were washed and cleaned and shade dried. Then it was powdered and dispensed in 10 ml of sterile distilled water and boiled for 10 minutes at 70-80°C. Then the plants extract were filtered and centrifuge for 10 minutes at 5000 r.p.m. Now extracts were collected in separate conical flasks by standard sterilized filtration method and were stored at 4°C.

1mM solution was added to the extract with ratio of 2:1. Then it was placed in a magnetic stirrer .Within 30min colour changes from dark yellow to wine red indicating the presence of gold nanoparticles. The reduction of pure Au^{3+} ions to Au^0 was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of AuNPs solution diluting the sample in 3 ml Aqueouschloroauric acid (HAuCl4) distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 at the range of 300-600 nm and observed the absorption peaks at 530-550 nm regions due to the excitation of surface plasmon vibrations in the AuNPs solution, which are identical to the characteristics UV-visible spectrum of metallic gold and it was recorded.

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.4mg of ampicillin powder is dissolved in 4ml sterile distilled water. Nanoparticle –ampicillin conjugate was prepared by mixing (1:1) proportion. 5ml of aqueous gold nanoparticles was mixed with 5ml of antibiotic solution in a 20ml borosil beaker.

Antimicrobial activity was studied by the agar well diffusion method. Nutrient agar plates were prepared and plates were swabbed with different pathogens such as *E.coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhimurium.*With the help of well cutter, 5 holes were made equidistant from one another.40µl of ampicillin was loaded in first well.40µl of Au nanoparticle synthesized from *Henna* in second well and third well respectively. Final 2 wells were added with 40µl of ampicillin conjugated AuNPs synthesized using the plants.The wells were then incubated for 37ºC for 24-48 hrs.

The four fungal species such as: *Aspergillus clavatus, Aspergillus flavus, A. fumigatus*.and *Trichoderma asperellum* maintained in potato dextrose agar (PDA) slant at 27°C. Fungi cultures were inoculated into the PDA plates. The plates were maintained at room temperature for week. Fungal isolates were further subcultured to obtain pure culture. To prepare the biomass for biosynthesis, the fungus obtained were grown aerobically in Potato Dextrose Broth (PDB). The culture flasks were incubated on room temperature at 37°C for 2-6 days. The biomass was harvested after 120 hours of growth by sieving through a plastic sieve followed by extensive

washing with sterile double-distilled water to remove any medium components from the biomass and kept in shaker for 2 days. The fungal biomass was grown in culture flask. Typically 15 g of biomass (wet weight) were brought into contact with 100 ml sterile double-distilled water for 48 hours at 27°C in an Erlenmeyer flask and agitated 150rpm. After incubation the cell filtrate was filtered by Whatman filter paper No.1. Into these 100 ml of cell filtrate, a carefully weighed quantity of aqueouschloroauric acid was added to the Erlenmeyer flask to yield an overall Au+ ion concentration, and the reaction was carried out under dark conditions. The antifungal activity of gold Nanoparticle was done using microdilution method against pathogenic fungi, *Candida albicans* and *Zygomycetes rouxii*.

The micro dilution method was carried out in 96 well micro titer plate.10µl of 48 hours old *Candida albicans* was taken in each wells of A and B. *Zygomycetes rouxii* was taken in each wells of C and D along with 30µl of nutrient media was added. 30µl of sterile distilled water and 20µl of biologically synthesized AuNp with the concentration of 1:16 dilution was added in each wells of A and B. 20µl of non diluted gold nanoparticle was added in each well of C and D. The last two wells were taken as positive and negative control. In positive control only the media was added and in negative control, both media and organism was added in the well. The plate was sealed and kept in sterile condition for 72 hours of incubation at room temperature. After 72 hours incubation, 10µl of resazurin dye was added uniformly to all the wells. Microtiter plate was incubated for 2 hours and observes the color change from blue to pink. Synergistic activity with fungicide is tested by using mycelial inhibitory method. This method was used to test for the synergistic effect of gold nanoparticles with *Ampelomyces quisqualis* against the three fungi namely, *Aspergillus clavatus*, *Aspergillus flavus* and *A. fumigatus*.. The antifungal effect will be evaluated based on the inhibition of hyphal growth. Potato Dextrose Broth was prepared in 12 Erlenmeyer flasks. Three flasks were kept as control and the three cultures were inoculated respectively. 300 ppm of fungicide was dissolved in 15 ml of sterile water aseptically in a 100 ml conical flask. 5 ml fungicide solution was inoculated into PDB along with the first culture. This was repeated for the other two cultures. 5 ml of gold nanoparticle solution was inoculated into each of the next set of three flasks along with the three cultures. 300 ppm of *Ampelomyces quisqualis* was mixed with 15 ml of gold nanoparticles in a 100 ml conical flask. 5 ml of mixture was introduced into another PDB containing flask after inoculation of the first culture. This was repeated for the other two cultures. The 12 flasks were incubated in an orbital shaker for 48 hours and the broth was incubated at room temperature for another 3 days.On the 6th day, the contents in each of the flasks were filtered to separate the solids from the filtrate.The wet weights of the solids that contain hyphae were measured and stored in petri plates in hot air oven.The dry weights of the same were measured after 24 hours to confirm the results.

RESULTS AND DISCUSSION

Biosynthesis of gold nano particles

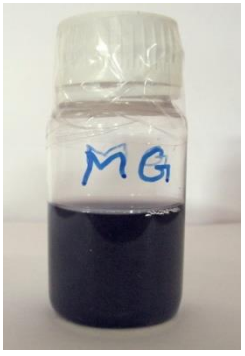

Figure3. Gold nanoparticles synthesized Using Henna

The plant extract was used to produce gold nano particles.The reduction of gold ions into gold particles during exposure to the plant extract is followed by colour change from yellow to different color depends on the plant extract**.**As the plant extract was mixed in the aqueous solution of the gold ion complex,it started to

change the color from yellowish to different colors due to reduction of gold ion,indicating the formation of gold nanoparticle

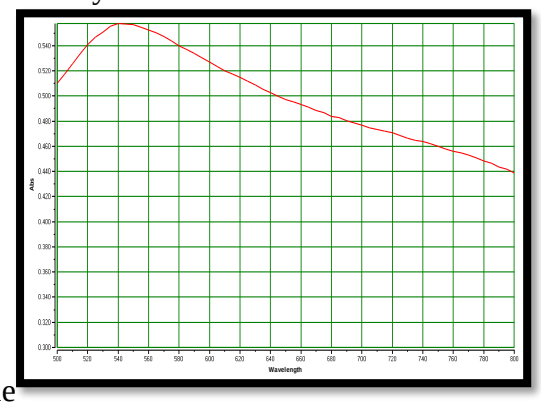

figure 4. UV-vis absorption spectra of AuNPs synthesized using *Henna*

The UV visible spectroscopy of the synthesized nano particles were in the range of 400-800 nm. Two plant extracts showed to synthesize the gold nanoparticles by the indication of suitable surface Plasmon resonance (SPR)with high band intensities and peaks under visible spectrum. A strong resonance at 540 nm is clearly seen in *Henna* and arises due to the excitation of surface plasmon vibrations in the gold nano particles.

Scanning electron microscopy of gold nano particles from Henna

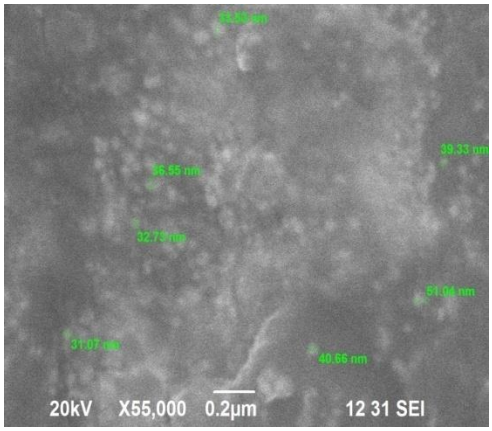

Figure 5.SEM photograph of AuNPs synthesized using *Henna*

The SEM photograph of gold nanoparticles is shown in figure 5. SEM photograph of gold nanoparticles clearly indicates that in the room temperature synthesized samples, the average size of the nanoparticles is ~37 nm for AuNPs using Henna with spherical and cubic shape.

Antimicrobial activity

Sl.no	**microorganism**	**Zone of inhibition(diameter in mm)**		
		Ampicillin alone	AuNPs Henna	Amp-AuNP Henna
Plate I	*E.coli*	26	-	24
Plate II	*Bacillus cereus*	21	-	19
Plate III	*S.aureus*	35	-	34
Plate IV	*S. typhimurium*	34	-	33

Table 1.Antimicrobial activity of gold nano particle a.AuNPs from Henna

c.Amp-AuNP from henna

Antimicrobial activity of gold nano particles alone and in conjugation with ampicillin is visible in the figure. The diameter of the inhibitory zone against each bacteria is given in Table.1.Gold nano particles do not have an adverse effect on bacteria. In contrast to AuNP alone, AuNP functionalized with ampicillin (AuNP-AMP) were bactericidal. The inhibitory action of plant extract was very close and identical in magnitude and is comparable with standard antibiotics.

Table 2.: Average weight of *fungal species* (Mean ± SD

Fungal Species	*Aspergillus clavatus*		*Aspergillus flavus*		*Aspergillus fumigatus.*	
	Wet weight Average (gms)	**Dry weight Average (gms)**	**Wet weight Average (gms)**	**Dry weight Average (gms)**	Wet weight Average (gms)	Dry weight Average (gms)
Control	7.94 ± 0.84	29.872 ± 0.76	6.40 ± 0.34	5.84 ± 0.78	54.33 ± 0.67	29.00 ± 0.66
Gold Nanoparticle	6.59 ± 0.57	25.67 ± 0.78	4.97 ± 0.56	4.69 ± 0.19	30.2 ± 0.65	7.44 ± 0.69
Fungicide	3.61 ± 0.22	5.59 ± 0.98	2.60 ± 0.66	2.36 ± 0.77	4.5 ± 0.47	2.77 ± 0.57
Coupled	3.19 ± 0.45	3.92 ± 0.45	0.91 ± 0.27	0.82 ± 0.56	3.9 ± 0.77	2 ± 0.54

Antifungal activity

In *A. fumigatus*, the wet weight of the mass of solids obtained after filtration of the culture with gold nanoparticles was reduced to 30.2gms when compared with the control in which the solids weighed 54.33gms. The dry weight reduced from 29gms to 7.44gms. The wet weight of the solids obtained after filtering the culture with Ampelomyces quisqualis was 4.5gms while the dry weight measured 2.77gms. When the culture with the goldnanoparticles coupled with Ampelomyces quisqualis was filtered and weighed, the wet weight obtained was 3gms and the dry weight 2gms.

CONCLUSION

Gold nano particles with mean sizes of 20-45 nm were successfully synthesized using Henna extract. The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AuNPs. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. AuNP functionalized with ampicillin (AuNP-AMP) were bactericidal. With the ever increasing resistant strains of microorganisms to the already available and synthesized antibiotics, this could be a potential alternative. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially stimulating for the large-scale synthesis. Toxicity studies of *Henna* mediated synthesized AuNPs are also underway.

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