Synthesis and Anti-bacterial Studies of Gold Nanoparticles from Eclipta prostrata

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Abstract - Nanotechnology has grown to be an important research field in all areas including medicinal chemistry. The size, orientation and physical properties of nanoparticles have reportedly shown to change the performance of any material. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. On the contrary, the green method of synthesizing nanoparticles is easy, efficient, and eco-friendly in comparison to chemical synthesis. The chemical synthesis involves toxic solvents, high pressure, energy and high temperature conversion. Since, green synthesis is the best option to opt for the synthesis of nanoparticles, therefore in the present study gold nanoparticles were synthesized by using aqueous leaf extract of *Eclipta Prostrata*. *Eclipta Prostrata leaf* extract was selected as it is of high medicinal value and it does not require any sample preparation and hence is cost-effective. The fixed ratio of plant extract and gold ions were mixed and kept at room temperature for reduction. The color change from golden yellow to lemon yellow confirmed the formation of nanoparticles. Further, the synthesized nanoparticles were characterized by UV, SEM, TEM and XRD data. The antimicrobial activity of synthesized nanoparticles has also been examined.
Keywords- *Gold Nanoparticles, Green synthesis, Eclipta Prostrata, Antibacterial activity*

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I. INTRODUCTION

The prospect of exploiting natural resources for metal nanoparticle synthesis has become to be a competent and environmentally benign approach [**1**]. Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles [**2**]. Metal nanoparticles have received significant attention in recent years owing to their unique properties and practical applications [**3, 4**]. In recent times, several groups have been reported to achieve success in the synthesis of Au, Ag and Pd nanoparticles obtained from extracts of plant parts, e.g., leaves [**5**], lemongrass [**6**], neem leaves [**7-8**] and others **[9]**. These researchers have not only been able to synthesize nanoparticles but also obtained particles of exotic shapes and morphologies [**7**]. The impressive success in this field has opened up avenues to develop "greener" methods of synthesizing metal nanoparticles with perfect structural properties using mild starting materials. Traditionally, the chemical and physical methods used to synthesize gold nanoparticles are expensive and often raise questions of environmental risk because of involving the use of toxic, hazardous chemicals. Nanoparticles may be synthesized either intracellularly or extracellularly employing yeast, fungi bacteria or plant materials which have been found to have diverse applications.

In the field of nanotechnology, green synthesis of AuNPs has gained much attention and emerged to be an active research area. Gold and AuNPs have found tremendous interest over last few decades due to possessing some remarkable novel properties such as intense plasmon resonance, electrical, magnetic, thermal conductivity, chemical and biostability, catalytic activity, anti-bacterial activity, anti-HIV activity, anti-angiogenesis activity, anti-malarial agent and anti-arthritic activity [**10, 11**]. Recent *in vitro* studies show that AuNPs do not cause cytotoxicity in human cell and therefore, AuNPs have received tremendous interest for modern biomedical sciences, including cancer photodiagnostics, photothermal therapy, biolabeling, nanodiagnostics, drug delivery, gene delivery, immunochromatographic identification of pathogens in clinical specimen [**11**]. In the field of nanotechnology the future application of AuNPs will open exciting possibility and they will be vital key materials in the 21^{st} century **[12]**.

AuNPs can be synthesized in different techniques such as citrate reduction of $HAuCl_4$ in water [**13, 14]**, seed-mediated growth method [**15]**, metal vapour synthesis, electrochemical method through gold ionization and reduction **[16]**. However, most of these physical and chemical methods involve the use of toxic chemicals, high temperature and pressure [**10, 13]**. Consequently, the researchers in the field of nanoparticles have to investigate some alternative bio-synthetic green approaches that utilize natural microorganisms and plant extracts for reduction of metal ions **[17]**.

In this work we report a plant mediated green synthesis approach for the synthesis of AuNPs by using ***Eclipta prostrata*** leaf extract which can act as a reducing, stabilizing or capping agent.

Eclipta prostrata (syn. Eclipta alba) commonly known as **false daisy**, **yerba de tago**, and **bhringraj**, is a species of plant in the family Asteraceae. Other common names include **kehraj** in Assamese and **karisalaankanni** in Tamil. This plant has cylindrical, grayish roots. The solitary flower

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heads are 6–8 mm in diameter, with white florets. This species grows commonly in moist places as a weed in warm temperate to tropical areas worldwide. It is widely distributed throughout India, China, Thailand, and Brazil. The plant has traditional uses in Ayurveda and is used to improve hair growthandcolour[**18-19]**.

Ecliptaprostrata contains wedelolactone and demethyl wedelolactone,polypeptides, polyacetylenes,thiophene derivatives, steroids, triterpenes and flavonoids.

II. EXPERIMENTAL

A. Materials

Chemicals used in the present study were of highest purity and purchased from Sigma-Aldrich (New Delhi, India); Merck and Himedia (Mumbai, India). ***Eclipta prostrata*** leaves were collected locally from SCET, Narsapur.

B. Preparation of plant extract

Plant leaf extract *of* ***Eclipta prostrata*** was prepared by taking 5 g of the leaves and properly washed in distilled water. They were then cut into fine pieces and taken in a 250 mL Erlenmeyer flask with 100 mL of sterile distilled water. The mixture was boiled for 5 min before finally filtering it. The extract thus obtained was stored at 4 °C and used within a week.

C. Synthesis of gold nanoparticles

The aqueous solution of 1 mM chloroauric acid ($HAuCl_4$) was prepared to synthesize AuNPs. 190 mL of aqueous solution of 1 mM $HAuCl_4$ was slowly added to10 mL of ***Eclipta prostrata*** aqueous leaf extract while stirring, for reduction into Au ions and kept at room temperature for 20 h.

D. UV-Vis spectra analysis

UV-Vis spectrum of the reaction medium recorded the reduction of pure Au^+ ions at different hours after diluting the sample with distilled water. UV-Vis spectral analysis was performed by using UV-Vis double beam spectrophotometer.

E. XRD (X-ray diffraction) measurement

The AuNPs solution was repeatedly centrifuged at 5000 rpm for 20 min, re-dispersed with distilled water and lyophilized to obtain pure AuNPs pellets. The dried mixture of AuNPs was collected to determine the formation of AuNPs by X'Pert Pro X-ray diffractometer (PANalytical BV, The Netherlands) operated at a voltage of 30 kV and a current of 30 mA with CuKα radiation in a θ- 2θ configuration.

F. SEM

SEM is a powerful instrument which permits the observation characterization of heterogeneous organic and inorganic materials. In this studies SEM is used to analyze micro structural characteristics of AuNPs. SEM images were obtained from JEOL JSM – 6610LV.

G. TEM

TEM technique was employed to visualize the size and shape of Au nanoparticles. The 200kV Ultra High Resolution Trans-mission Electron Microscope (JEOL-JEM2100F) has been used. TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp.

H. Antimicrobial Activities

Luria Bertani broth (Himedia), Luria Bertani agar (Himedia) standard antibiotic and ciprofloxacin (Himedia) were used in antimicrobial sensitivity testing. Briefly, Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculum (50 µl) of each culture were spread on to LB agar plates. Sterile paper discs of 5 mm diameter (containing 30 µl of AuNPs) along with the standard antibiotic, containing discs were placed in each plate. Antimicrobial activities of the synthesized AuNPs were determined, using the agar disc diffusion assay method [**20**].

III. RESULTS & DISCUSSIONS

A. UV -Visible studies

UV–Vis spectroscopy is an important technique to establish the formation and stability of metal nanoparticles in aqueous solution [**21**].The relationship between UV-visible radiation absorbance characteristics and the absorbate's size and shape is well-known. Consequently, shape and size of nanoparticles in aqueous suspension can be assessed by UV-visible absorbance studies.

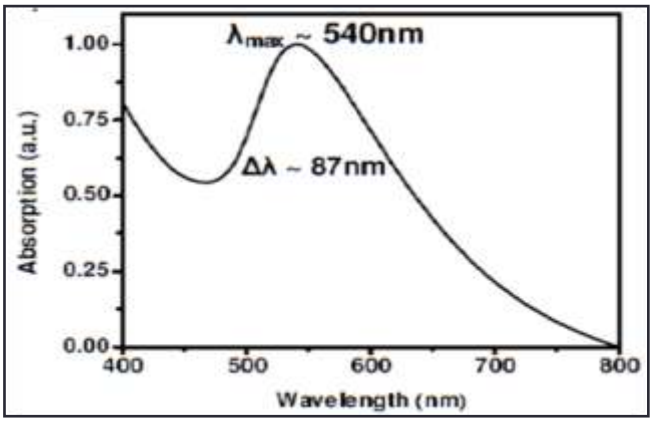

Figure 1. UV-Vis absorption spectrum of AuNPs synthesized by treating 1mM $HAuCl_4$ solution with *Eclipta prostrata* leaf extract.

Figure 1 depicts the absorbance spectra of reaction mixture containing aqueous chloroauric acid solution (1 mM) and ***Eclipta prostrata*** (prepared from 5 g leaf material). The absorption spectra obtained reveal the production of AuNPs. The optical absorption spectra of metal nanoparticles that are governed by surface plasmon resonances (SPR), move towards elongated wavelengths, with the increase in particle size. The absorption band position is also strongly dependent on dielectric constant of the medium and surface-adsorbed species [**22-23**].

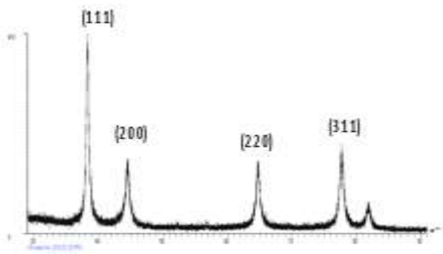

Figure 2**.** XRD-spectrum of purified sample of AuNPs.

B. XRD studies

The peaks observed in the spectrum at 2θ values 38.31, 44.60, 64.74, 77.72, 81.92 corresponds to (1 1 1), (2 0 0), (2 2 0), (3 1 1) planes of gold respectively **(Figure 2)** [**24**]. This clearly shows that the AuNPs are crystalline in nature, which are consistent with the FCC structure of the gold, due to reduction of Au^+ ions by ***Eclipta prostrata*** leaf extract. The AuNPs were centrifuged and redispersed in distilled water several times before XRD analysis. This excludes the possibility of any free compound/protein present that might lead to independent crystallization and thus, resulting in Bragg's reflections. The average size of the gold nanoparticles was calculated from the XRD data according to the line width of the maximum intensity reflection peak by using the Scherrer's equation and it is 20±2 nm.

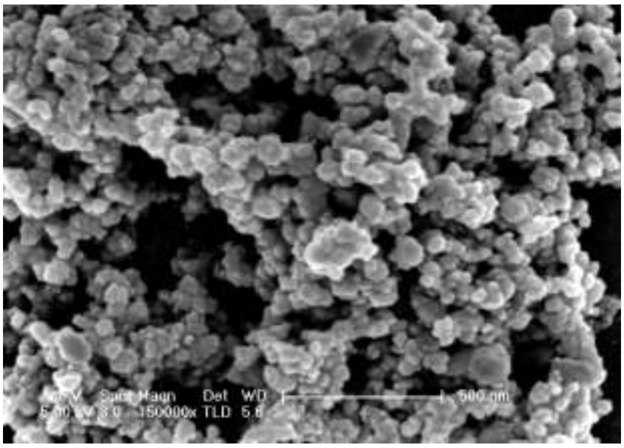

Figure 3**.** shows the *SEM image* of purified sample of AuNPs**.**

C. SEM Studies

The SEM (Scanning Electron Microscopy) image for gold nanoparticles was presented in **Figure 3**. Form this figure it was observed that the formed nanoparticles are crystalline in nature with certain degree of porosity. The schererr rings, which are characteristics of FCC for nanoparticles, were clearly observed. From the structures seen in SEM image reveals that synthesized nanoparticles are nanocrystalline in nature.

It was observed that gold nanoparticles were scattered over the surface and no aggregation was noticed.

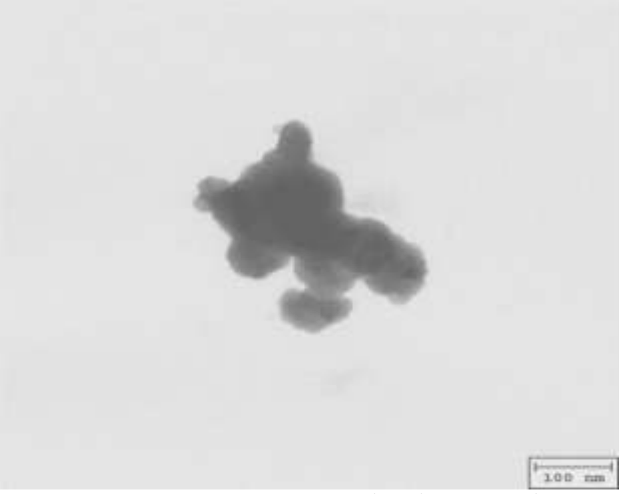

Figure 4 shows the *TEM image* of purified sample of AuNPs

D. TEM studies

Gold nanoparticles are further examined using transmission electron microscopy (TEM). The TEM image of synthesized gold nanoparticles was recorded and presented in **Figure 4**. The average size of gold nanoparticles was observed as 20±2 nm which was in agreement with Scherer's equation.

E. Antimicrobial Activities

TABLE. I. Zone of Inhibition of nano metals by agar well diffusion method

S. No	Human Pathogenic Bacteria	Disease	Zone of Inhibition (mm)*		
			Au	**extract**	**Antibiotic+**
1	*Salmonella typhi*	Typhoid	9	7	17
2	*Vibrio cholerae*	Cholera	10	7	18
3	*Shigelladysenteria*	Shigellosis	9	8	18
4	*Enterococcus faecalis*	Gastrointestinal infection	9	7	19

Well size 6mm, *zones at 30µg/ml concentration, +Ciprofloxacin.

The antibacterial activity of AuNPs was determined against human gastrointestinal pathogens viz., *S. typhi*, *V. cholerae*, *S. dysenteriae*, *E. faecalis* (**TABLE I**). The nanoparticles showed consistently moderate antibacterial activity when compared to ciprofloxacin. The zone of inhibitions of nano particles found to be in the range between 9-10mm. 10-100 mg/ml was determined minimum inhibitory concentration (MIC) of nano, which required to inhibit the bacterial growth. 10 mm was highest zone of inhibition of Au particles on *Vibrio cholerae* with lowest MIC (1mg/ml).

IV. CONCLUSIONS

The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AuNPs. The capping around each particle provides regular chemical environment formed by the bio-organic compound present in the ***Eclipta prostrata*** leaf extract, which may be chiefly responsible for the particles to become stabilized. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. From the nanotechnology point of view, this is a noteworthy development for synthesizing AuNPs economically. In conclusion, this green chemistry approach toward the synthesis of AuNPs possesses several advantages viz, easy process by which this may be scaled up, economic viability, etc. 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 of other inorganic materials, like nanomaterials.

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