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Phytofabrication of gold nanoparticles assisted by leaves of *Suaeda monoica* and its free radical scavenging property

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ABSTRACT

Development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. An eco-friendly synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. In the present study, it is reported that *Suaeda monoica* leaf mediated synthesis of gold nanoparticles by the reduction of gold ions. The formation of gold nanoparticle was confirmed by color changes from turbid brown to deep purple violet color and a characteristic peak at 535 nm. The morphology and structure of synthesized gold nanoparticles were characterized on Scanning Electron Microscopy (SEM) equipped with a Thermo EDAX attachment, Transmission Electron Microscopy (TEM), X-ray diffraction (XRD), (FT-IR), Dynamic Light Scattering (DLS) which reveals that the Au nanoparticles are spherical and the average particle size is 12.96 nm. Crystalline nature of the nanoparticles is confirmed from the XRD pattern. FTIR spectrum indicates that the biomolecules of carboxyl, amine and hydroxyl functional groups involved in the reduction of gold nanoparticles. The biosynthesized gold nanoparticles displayed considerable antioxidant capacity.

1. Introduction

Nanotechnology has attracted a great interest in recent years due to its expected impact on many areas such as energy, medicine, and electronics. Nanoparticles have been synthesized by sol-process, micelle, chemical precipitation, hydrothermal method, pyrolysis, chemical vapor deposition, bio-based protocols, etc. [1]. Currently there is a growing need to develop environmentally benign colloidal nanoparticles synthesis process that does not involves any toxic chemicals in the synthesis protocol which raises great concern for environmental reasons [2]. Among the nanoparticles, gold nanoparticles have received major attention due to their unique and tunable Surface Plasmon Resonance (SPR) [3]. It has many effective applications in biomedical sciences including drug delivery, tissue/tumor imaging, photothermal therapy, and immunochromatographic identification of pathogens in clinical specimens [4]. Recently, the nanoparticles made from the noble metals like silver, gold, platinum and lead. Biosynthesis of gold nanoparticles using microorganisms like bacteria [5], fungi [6] and yeast [7] are already exploited. However, biosynthesis of nanoparticles by plant extracts is currently under exploitation. Further, synthesis of gold nanoparticles using extracts of various plants like *Plumeria alba* [8], *Sphearanthus amaranthoides* [9], *Macrotyloma uniflorum* [10], *Camellia sinensis* [11], *Allium cepa* [12] were reported.

Suaeda monoica, a coastal saltmarsh plant belongs to Chenopodiaceae family, is a shrubby perennial plant, occurring on coastal regions of India. The leaves were used as a medicine for hepatitis and wounds and possess antiviral activity because of the presence of triterpenoids and sterols [13]. Traditionally, mangrove and mangrove associate plants are rich in secondary metabolites viz, saponins, alkaloids, polyphenols which show antibacterial, antifungal, antiplasmodial and hepato-protective activities [14].

Our aim in the present contribution was to synthesize gold nanoparticles using leaf biomass of *S. monoica* and characterized them by UV–visible (UV–vis) spectroscopy, Scanning Electron Microscopy (SEM), Energy Dispersive X-ray Analysis (EDAX), Transmission Electron Microscopy (TEM) and Fourier Transform Infra-Red spectroscopy (FT-IR), X-ray Diffraction (XRD) and Dynamic Light Scattering (DLS) method and to evaluate their antioxidant potential.

2. Materials and methods

2.1. Chemicals

All chemicals used in this experiment were of analytical grade and were purchased from Hi-media (Mumbai, India).

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2.2. Collection and processing of plant materials

The leaves of *S. monoica* were collected from the coastal region of Vellar estuary, Parangipettai (Lat 11°29′N; Long 79°46′E), southeast coast of India. They were brought to the laboratory in an ice box, washed in distilled water and subsequently shade dried for a week days. Then they were kept in oven at 60 °C until constant weight obtained. The dried leaves were ground to powder and sieved through <0.5 mm.

2.3. Biosynthesis of gold nanoparticles from S. monoica leaves

Aqueous solution (1 mM) of hydrogen tetrachloroaureate ($HAuCl_4$) was prepared and used for the synthesis of gold nanoparticles. Biosynthesis of gold nanoparticles was evaluated [15]. Briefly, 250 mg of *S. monoica* biomass was added to 10 ml of $HAuCl_4$ solution and incubated in hot air oven.

2.4. Preliminary confirmation of gold nanoparticles synthesis

2.4.1. Visual observation

The reduction of metal ions was roughly monitored by visual observation of the solution. The color change appears after the completion of the reaction [16].

2.4.2. UV–visible spectroscopy

Synthesized gold nanoparticles were confirmed by UV–visible spectroscopy (Perkin Elmer Lambda 25) by sampling the aqueous component (2 ml), diluted to 20 times and measuring the UV–vis spectra of the solution. The light absorption pattern of the *S. monoica* biomass was kinetically monitored in the range of 400–800 nm [17].

2.5. Purification of gold nanoparticles

2.5.1. Obtaining dry powder of gold nanoparticles

The dry powder of gold nanoparticles were obtained in the following manner. After desired reaction period, the biomass of *S. monoica*–$HAuCl_4$ mixture solution containing the gold nanoparticles was centrifuged at 12,000 rpm for 15 min. The pellets were re-dispersed in Millipore water to get rid of any uninteracted biological molecules. This process of centrifugation was repeated thrice to ensure better separation of the gold nanoparticles. The purified pellets were then freeze dried and lyophilized. The purified dried powder were then used for the subsequent characterization studies.

2.6. Characterization of gold nanoparticles

2.6.1. Scanning Electron Microscopy

The surface morphology of gold nanoparticles was investigated by Scanning Electron Microscopy (SEM) and the samples were prepared by placing a drop over a carbon coated grid and allowing drying prior to measurement on a Hitachi S-3400 N. SEM instruments were operated at an accelerated voltage at 20 kV [18].

2.6.2. Energy dispersive analysis of X-ray (EDAX) measurements

EDAX gives qualitative as well as quantitative status of elements that may be involved in the formation of AuNPs [19]. EDAX analysis was carried out for dried powder of exposed *S. monoica* were drop coated on to carbon film and performed on SEM instrument equipped with a Thermo EDAX attachments. 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.

2.6.3. TEM analysis of gold nanoparticles

Morphology and size of the gold nanoparticles were investigated by TEM (Phillips, CM12) instrument. Thin film of the sample was prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid and drying under lamp.

2.6.4. X-ray diffraction analysis

The structural characterization and the crystalline nature of gold nanoparticles were determined by X-ray diffractometer (X′ Pert PAN analytical instrument) operating at a voltage of 40 kV and current of 20 mA with Cu Kα radiation [20].

2.6.5. Dynamic Light Scattering (DLS)

Particle size and charge measurements in solution were determined with Dynamic Light Scattering (DLS) and zeta potential measurements on a Malvern Instruments Zetasizer [21].

2.6.6. Fourier transform infrared spectroscopy

To identify the possible biomolecules responsible for the reduction of the Au ions and capping of the bioreduced gold nanoparticles synthesized by biomass of *S. monoica*, Fourier Transformed Infrared Radiation (FTIR) spectroscopy measurements were carried out (Perkin–Elmer 297 IR spectrophotometer) [22].

2.6.7. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical quenching assay

Free radical scavenging capacity of gold nanoparticles was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [23]. The reaction mixture was incubated at 37 °C for 30 min and the color change was measured spectrophotometrically at 517 nm. Percentage of DPPH scavenging activity was calculated by, DPPH radical scavenging activity (%) = $[(A_{control}–A_{sample})/A_{control}] \times 100$.

Where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of AuNPs by *S. monoica*.

3. Results and discussion

3.1. Visual observation

In the present study, the leaf extract of *S. monoica* was used as reducing agent for the synthesis of gold nanoparticles from aqueous $HAuCl_4{\cdot}3H_2O$ solution. The color of the gold reduced solutions gradually turned to purple violet from turbid brown indicating the generation of gold nanoparticles (Fig. 1). It is well known that gold nanoparticles exhibit dark purple or red wine color based on the shape and size of the colloidal nanoparticles of gold [24].

3.2. UV–vis spectroscopy

UV–vis spectra revealed the Surface Plasmon Resonance (SPR) of obtained nanoparticles. The characteristic absorption peak centered at 535 nm, indicating the formation of nanoparticles and are polydispersed (Fig. 2). This result is accordance with the results obtained from bioreduction of gold nanoparticles using *Justicia gendarussa*, which showed that a SPR gold band occurred at 536 nm [25].

3.3. Scanning Electron Microscopy and EDAX

Surface morphology of gold nanoparticles by SEM at higher magnification revealed that, the synthesized particles were predominantly spherical in shape with average size of <100 nm