Research Paper

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Synthesis and Characterization of Gold Nanoparticles Embedded with Extract of the Plant *Panicum maximum* with Enhanced Antioxidant Behavior

Chemistry

KEYWORDS : Gold Nanoparticle, Panicum maximum, Bioassay, Enhanced antioxidant behavior.

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ABSTRACT

The present piece of work deals with the green synthesis of gold nanoparticles embedded with ethyl acetate extract of plant Panicum maximum. The best parameters used for the synthesis includes NaAuCl4 salt solution (1 ml; 1 mM) and ethyl acetate extract of Panicum maximum (5 ml; 125 µg/ml). The results were confirmed by X-ray diffraction analysis, Scanning electron microscopy, Atomic force microscopy and UV-Vis spectroscopy. Antioxidant activities were determined using DPPH and Fenton bioassays. Gold nanoparticles embedded with ethyl acetate extract of Panicum maximum shows 36.2% enhancement in the antioxidant behaviour.

Introduction

Nanotechnology is an interdisciplinary research area involving chemistry, engineering, biology and medicine and has great potential for designing excellent procedures and products for sustainable development of environment and human mankind (Cai et al. 2008). Recently, inorganic nanoparticles due to their small size have been associated with toxicity and potential side effect issues particularly in biological system. One approach to tackle such issues associated to sustainability is to incorporate organic nanomaterials of plant origin for the development of non-toxic biodegradable nanoparticles for medicinal applications (Das et al. 2011). Plants contain various phytochemicals present in the human food chain and are non-toxic to living organisms and environment (Holst 2008). Plants secondary metabolites like alkaloids, terpenoids, flavonoids, steroids, etc. have been found to exhibit several pharmacological properties (Siddhuraju & Becker, 2003). These secondary metabolites of pharmacological importance can be coated on the freshly generated nanoparticles, providing robust shielding from aggregations and with added medicinal properties. The utility of phytochemicals in the overall synthesis and architecture of nanoparticle embedded phytoproducts brings an important symbiosis between Plant Sciences and Nanotechnology and referred to **Green nanotechnology** (Katti et al. 2009, Roy 2006). It explores an unprecedented process of the production and stabilization of gold nanoparticles in a singular green process, made from more ecofriendly materials including plants, agricultural wastes and crops with wide applications in a myriad of applications in Nanomedicine and Environmental Science and Technology. The reduction capabilities of cocktail of phytochemicals present in plants chemically reduce gold (III) salts to gold (0). Chemical partnership of various phytochemicals that contain bio functional groups such as (COOH, C=O, NH_2, SH and OH) etc provide powerful synergistic chemical reduction of gold salts into their corresponding nanoparticles.

The present piece of work deals with the synthesis and characterization of gold nanoparticles using extract of *Panicum maximum* (Guinea grass), followed by the estimation of their antioxidant behavior with the belief that bioefficacy is enhanced by gold nanoparticles embedded with phytochemical responsible for that particular bioefficacy .

Panicum maximum, commonly known as **Guinea grass**, is a perennial, tufted grass with a short, creeping rhizome, native to Africa, belongs to the family **Poaceae** and known to have phytochemicals exhibiting antioxidant behavior (Gibbs *et al.* 1990).

Materials and Methods

Chemicals and Reagents

Saplings of the plant *Panicum maximum* were obtained from "Indian grassland and fodder research institute", Jhansi and grown in botanical garden of the institute. DPPH (2, 2-diphenyl-1-picryl hydrazyl) (0.3mM in methanol), Methanol, Deoxyribose (3mM), Ferric chloride (0.1mM), Ascorbic acid (0.1Mm), EDTA (0.1mM), H_2O_2 (1mM), Thiobarbituric acid (1% in 100 ml NaOH; 0.05 N), Trichloroacetic acid (5 % in water), Phosphate Buffer Saline (pH 7.4), α-tocopherol were from Sigma Aldrich Pvt. Ltd, USA.

Method of extraction

Aerial and root parts (400g) of the plant material were washed with running water and dried through filter paper. Plant material was refluxed using Soxhlet assembly in Ethyl acetate for 48 hrs. The extract was distilled under vacuum using Rota vapor and the dried mass of extract was collected.

Synthesis of gold nanoparticles embedded extract of ***Panicum maximum***

Aqueous sodium tetrachloroaurate ($NaAuCl_4$) solution (1 ml; 1 mM) was added to extract (5 ml; 125µg/ml) and 5 ml of distilled water with ratio of 1:5 and sonicate for 5 minutes. Within 5 minutes, change in color from light yellow to pinkish red was observed, indicating the formation of nanoparticles.

Antioxidant activity evaluation

DPPH (2, 2-diphenyl-1-picryl hydrazyl) assay

The reaction mixtures of dilution series (5-100 µg/ml) of ethyl acetate extract of *Panicum maximum* were incubated with methanolic DPPH (3.0 ml; 0.15 mM). The solution was allowed to stand for 20 min at room temperature. Extracts when reacted with DPPH, a stable purple colored free radical was converted into colorless compound (α-α diphenyl β-picryl hydrazine). The extent of discoloration indicates the amount of DPPH scavenged (Huang et al. 2005). The absorbance was measured at 517nm using α-tocopherol as a reference antioxidant. The percent inhibition of DPPH was calculated using the formula: **[(C-T)/C] X 100,** where **C** is the absorbance of control and **T** for test sample.

Fenton's assay:

The reaction mixtures containing different dilution series of ethyl acetate extract of *Panicum maximum* were incubated with Deoxyribose, H_2O_2, $FeCl_3$, Ethylene Diamine Tetra Acetic acid (EDTA) Phosphate buffer (pH 7.4). The reaction was terminated by Thiobarbituric acid (1ml) and Trichloroacetic acid by boiling in water bath for 15 min. The pink chromogen was formed resulting in the formation of thiobarbituric acid reactive substance (TBARS) (Yang and Guo, 2001) α-tocopherol was used as a reference antioxidant. The percent inhibition of hydroxyl radical generation was calculated using the same formula, mentioned above.

Characterization of gold nanoparticles

Gold nanoparticles prepared from ethyl acetate extract of *Panicum maximum* were characterized by X-ray diffraction

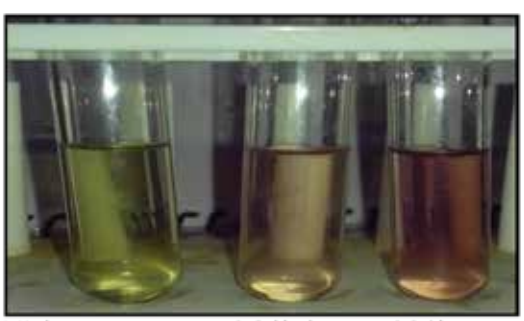
analysis, Atomic force microscopy, Scanning electron microscopy, UV- Visible Spectral analysis. X-ray diffractometer (Bruker AXS D8 Advance, Germany) was used to determine the crystallinity and the lattice properties operated at 40KV, current of 30 mA with Cu Kα radiation. Scattered radiation was detected in the range 2θ = 30^{o}- 80^{o}, at a speed of 2^{o} per min. Atomic force microscope (Nanosurf easy scan Switzerland; Version 1.8) was used for the morphological observation of biosynthesized gold nanoparticles. All scans were performed in air with commercial Si nanoprobe tips. Height and phase images were obtained simultaneously in tapping mode at the fundamental resonance frequency of the cantilever with a scan rate of 0.5–0.8 Hz. The oscillating amplitude was 0.5 V. The morphological characteristics of gold nanoparticles were also evaluated using Table tops SNE-3200M Scanning Electron Microscope at working voltage 30 KV with 700X magnification. UV–Vis spectroscopy (Shimadzu 2450 UV-Vis spectrometer) is also one of the most important techniques to characterize gold nanoparticles. The SPR band of gold nanoparticles at scanning range between 200-800 nm was observed.

Results and Discussions

Synthesis of gold nanoparticles

Gold nanoparticles were synthesized from sodium tetra chloroaurate solution ($NaAuCl_4$) by treating with the ethyl acetate extract. The color of the solution changed to pinkish red within 5 min of reaction (by sonication). The appearance of the pinkish red color indicated formation of gold nanoparticles.

Fig. 1: Formation of gold nanoparticles embedded with extract



Characterization of gold nanoparticles

1. X-ray Diffraction (XRD)

X-ray diffraction spectra show a number of Bragg's reflections which can be indexed on the basis of the face centred cubic (fcc) structure of gold. The diffraction peaks at 2θ = 38.15° (1 1 1), 44° (2 0 0) obtained are identical with those reported for the standard gold metal (Joint Committee on Powder Diffraction Standards-JCPDS, USA).

The crystallite size was estimated using Scherrer's equation

$$B = \sqrt{(FWHM)^2 - (0.045)^2} = x$$

$$B_{radian} = \frac{x\,\pi}{180} = y$$

$$t = \frac{K\lambda}{yCos\theta}$$

Where B is the breadth of the peak of a specific phase (2θ=38.15 in our case), K is a constant that varies with the method of taking breadth (K=0.94), λ is the wavelength of incident X-rays (λ=0.15418nm), θ is the centre angle of the peak, and L is the crystallite length (size).The crystalline size comes out to be **40.57nm.**

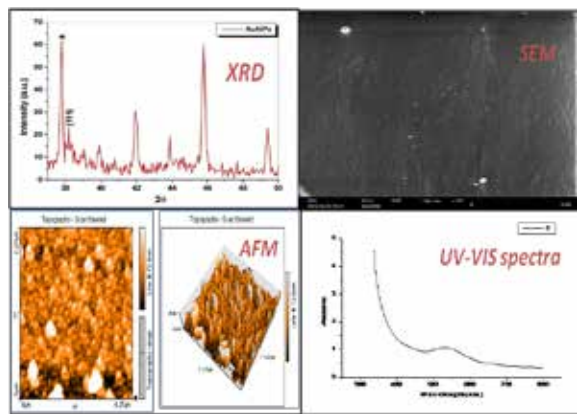
2. Scanning Electron Microscopy

Information on the morphology of gold nanoparticles obtained from SEM measurements, the image was taken at 3 um dimensions. SEM image reveals that nanoparticles are not in physical contact but are separated.

3. Atomic Force Microscopy

AFM is an important biophysical technique to study the morphology of nanoparticles. From the topographical view, it is evident that the most of the nanoparticles are in spherical shape. The average roughness of the particles is **14.28 nm.**

Fig. 2: Characterization of gold nanoparticles embedded with extract



4. UV-Vis analysis

The UV- visible light absorption of gold nanoparticles was monitored in the range of 200-800nm. In case of gold ions reduction, the bands corresponding to the Surface Plasmon Resonance (SPR) occurred at **540 nm**.

DPPH and Fenton bioassays of *Panicum maximum*

Ethyl acetate extract was examined for the antioxidant behavior against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging effect taking reference compound α-tocopherol as a positive control. The free radicals were scavenged and solution becomes colorless because of formation of α,α-diphenyl-β-picryl hydrazine. The extract depicted percentage inhibition of 89.1% for DPPH radicals at the concentration range 50 µg/ml respectively. Reference antioxidant α-tocopherol inhibited 94.6% of DPPH radicals for the same concentration. IC_{50} value calculated was 9.8µg/ml using DPPH assay. Similarly, the extract was examined for antioxidant behavior against OH radicals taking reference compound α-tocopherol as a positive control, using Fenton's bioassay. The percentage inhibition was 88.7% for OH radicals at the concentration range 50 µg/ml respectively. IC_{50} value calculated was 9.1µg/ml against OH radicals. Gold nanoparticles embedded with phytoextract exhibited percentage inhibition of DPPH radicals 91.4%.

Enhancement in antioxidant activity of gold nanoparticles embedded *Panicum maximum* **plant extract**

The antioxidant behaviour of gold nanoparticles embedded with ethyl acetate extract in terms of IC_{50} value compared to native ethyl acetate extract of the plant *Panicum maximum* shows 36.2% enhancement in the antioxidant behaviour.

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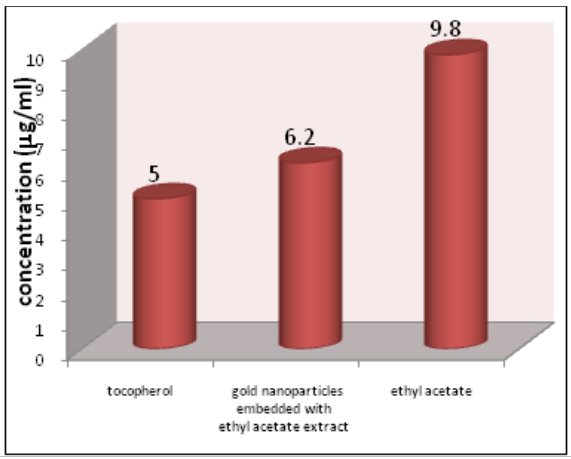
Fig. 3: IC50 values of native extract and gold nanoparticles embedded with extract



A possible explanation to the observed enhancement in the antioxidant behaviour of gold nanoparticles embedded with ethyl acetate extract can be ascribed to the fact that nanoparticles have advantages of their unique interaction with light compared to bulk materials due to their surface plasmon resonance in which cross-sections of metal nanoparticles are enhanced by five orders of magnitude, increasing the bioefficacy (Eustis et al. 2006).

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