

Green synthesis of gold nanoparticles against pathogens and cancer cells

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Abstract

Nanotechnology is a most promising field for generating new applications in medicine. It is imperative to integrate nanoscience and medicine. The present investigation is highly warranted to throw more light upon the gold nanoparticles reduced from gold salt through the active principle of medicinal plant. The special emphasis of investigation is the active principle along with gold nanoparticles against for cancer cells. The 70 - 90 nm sized particles were synthesized by using *Diospyros ferrea* and this confirmed by SEM. These gold nanoparticles showed a characteristic absorption peak at 540 nm in UV spectra. The possibility of protein as a stabilizing material in gold nanoparticles is revealed by FTIR analysis. Remarkably, as a result of wide screening on the application of newly synthesized gold nanoparticles their anticancer potential has been discovered using MTT assay. The antimicrobial activity of AuNPs showed effective against bacteria than the fungal strains.

Keywords: Green synthesis, gold nanoparticles, *Diospyros ferrea*, anticancer activity, antimicrobial activity.

1. Introduction

Metal nanoparticles are of great importance due to their high surface area and a high fraction of surface atoms. Nanotechnology can be delineated as a researcher for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100nm. A young offshoot of nanotechnology is nanobiotechnology. Nanobiotechnology combines biological principles with physical and chemical processes to generate nano-sized particles with specific functions [1-3]. The scientific and technological significance of metal nanoparticles has made them the subject of intensive research, given their special chemical and physical properties. Nanotechnology represents an economic alternative for chemical and physical methods of nanoparticles formation.

In particular, gold nanoparticles are employed in many fields: biosensing, catalysis, electronics, enzyme electrodes, super conductors and cancer therapy among others [3]. Numerous methodologies are developed to synthesize noble metal nanoparticles of particular shape and size depending on specific requirements [5]. Biosynthesis of nanoparticles has an emerging highlight of the intersection of nanotechnology and biotechnology which has received increased attention to a growing need to develop environmentally benign technologies in material syntheses [6][7]. Biomolecules as reductants are found to have significant advantage over chemical reductants due to their non-biocompatible nature [8]. These methods of deduction can be divided on intracellular and extracellular [9].

Recently, metal nanoparticles have gained a great deal of attention due to their unique chemical, optical, magnetic, mechanical and electric magnetic properties [10]. Thus, metallic nanoparticles are used in different applications such as electronics, catalysis and photons [11][12]. Nanotechnology is dynamically developing as an important area of innovative research with potential effects in electronics and medicine [13][14]. Uncontrolled growth and spread of abnormal cells lead to cancer and finally results in death. Ethno-pharmacological process on the synthesis of nanoparticles is an amazing technology beneath construction symbiosis between nanoscience and medical sciences. The nanoparticle drug delivery system has the advantages of accumulating large amounts of therapeutic drugs in the tumor tissues through the passive and active targeting approach.

The green synthesized, characterized and bio-functionalized gold Nanoparticles from *Diospyros ferrea* were tested for in-vitro antimicrobial and anticancer activity against pathogens and human carcinoma cells, respectively. Our present findings clearly demonstrated that it is indeed possible to have a much greener way to synthesize Au-NPs without

compromising their medicinal properties and thus plant extracts may prove to be a good alternative to obtain Au-NPs with improved anticancer properties.

2. Materials and methods

2.1 Study area and sampling

The *Diospyros ferrea* plant materials were collected from Kolli hills, Namakkal district of Tamil Nadu in India during pre-monsoon 2015. The fine powder of the plant material was sterilized at 121°C for 15 min and weighed. Sterilized fine powder, 20 g each was taken each plant, mixed with 200 ml of Milli Q water and held in boiling water bath at 60°C for 10 minute. The extracts were filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C and it's used as test samples.

2.2 Biosynthesis of nanoparticles

Biosynthesis of gold nanoparticles, gold chloride prepared at the concentration of 10^{-3} M with pre-sterilized Milli Q water. A quantity of 10 ml plant extract was mixed with 90 ml of 10^{-3} M gold chloride for the synthesis of gold nanoparticles. Gold chloride has taken in similar quantities without adding plant extracts to main respective controls. The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations were recorded.

2.3 Characterization of nanoparticles

2.3.1 UV-VIS spectroscopy

The gold (Au) nanoparticles were characterized in a Perkin-Elmer UV-VIS spectrophotometer, Lambda-19 to know the kinetic behaviour of Au nanoparticles. The scanning range of the samples was 200-800 nm at a scan speed of 480 nm/min. Baseline correction of the spectrophotometer was carried out by using a blank reference.

2.3.2 Fourier transform-infra red (FT-IR) spectroscopy

The analysis of bio-reducing agent present in each of the extracts was measured by FT-IR. After the reaction, a small aliquot of the concentrated reaction mixture was measured in the transmittance mode at 400 to 4000 cm^{-1} . The spectra of the extracts taken after the biosynthesis of nanoparticles were analyzed.

2.3.3 Scanning electron microscope (SEM)

Electron microscopy is another commonly used method of characterization. Scanning electron microscopy and transmission electron microscopy are used for morphological characterization at the nanometer to micrometer scale. In this research work, Joel JSM-6480 LV SEM machine was used to characterize the mean particle size and morphology of nanoparticles.

2.4 Assay of antimicrobial activity

The antimicrobials activity of synthesized gold and silver nanoparticles was analyzed against the human pathogens such as *Bacillus cereus* (MTCC 430) (B1), *Klebsiella pneumoniae* (MTCC 432) (B2), fungal strains of *Candida albicans* (MTCC 227) (F1) and *Microsporum gypseum* (MTCC 2819) (F2). These microbes were procured from Microbial Type Culture Collection (MTCC), Chandigarh, and tested against biosynthesized gold nanoparticles following agar disc diffusion method [15-17]. The plant *Diospyros ferrea* derived gold nanoparticles solution was taken in this experiment. The two different concentrations of test sample (0.75 and 1.50 mg/disc) was used for study of antimicrobial activity on Muller Hinton agar (MHA) plates for bacteria and potato dextrose agar (PDA) plates for fungi [18][19]. The biosynthesized gold nanoparticles was incorporated in sterile disc and it was placed into plates by sterile forceps. Then, the culture plates were incubated at 37 °C for 24 h for bacterial strains and 28 °C for 48-72 h for fungal strain and then observations were recorded after incubation period. The ruler was used for measured the zone of inhibition and the mean value of the triplicate was recorded [5][20].

2.5 Testing of anticancer activity

For anticancer study, an in-vitro and AuNPs samples were dissolved in DMSO, diluted in culture medium and used to treat the chosen cell line (Hep G2) (obtained from NCCS) over a sample concentration (5 different concentrations – 1, 5, 10 25 and 50 $\mu\text{g/mL}$) range of 1 - 50 $\mu\text{g/mL}$ for a period of 24 h and 48 h. The DMSO solution was used as the solvent control. A miniaturized viability assay using 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl-2H-tetra-zolium bromide (MTT) was carried out according to the method described by standard procedure [17]. To each well, 20 μl of 5 mg/mL MTT in phosphate-buffer (PBS) was added and wrapped with aluminum foil, and incubated for 4 h at 37 °C. The purple formazan product was dissolved by addition of 100 μl of 100 % DMSO to each well. The absorbance was monitored at 570 nm (measurement) and 630 nm (reference) using a 96 well plate reader (Bio-Rad, Hercules, CA, USA). Data were collected for four replicates. Each and used to calculate the respective means. The percentage of inhibition was calculated, from this data, using the formula,

$$\frac{\text{Mean absorbance of untreated cells (control)} - \text{mean absorbance of treated cells (test)}}{\text{Mean absorbance of untreated cells (control)}} \times 100$$

3. Result and discussion

3.1 Biosynthesis of Au nanoparticles

The plant aqueous solution and gold chloride solutions were prepared separately. The plant extract was mixed with gold chloride for the synthesis of gold nanoparticles. During this fabrication process, color was changed from pale green to pink colour, suggested that formation of gold nanoparticles [21].

3.2 UV-VIS spectral analysis

The UV-VIS spectroscopy studies revealed the presence of beard peaks at 541 nm (Figure 1). The absorption spectra of Au nanoparticles formed in the reaction media have absorbance maxima at 540 nm. A remarkable broadening of peak at around 480 nm to 580 nm indicates that the particles are polydispersed. During each time interval, the peak became distinct and rising. This peak rising clearly denoted the increasing nanoparticles synthesis as the time increases.

3.3 Fourier transform infra-red (FTIR) spectroscopy

The synthesized gold nanoparticles were subjected to FT-IR analysis to find out the bioactive compounds synthesized by the plant and associated with the nanoparticles. The FTIR images of the Plant samples show a number of functional bonds associated with them which provide them with stability by capping them. From figure 2, 1638 cm⁻¹, 2076 cm⁻¹, 3435 cm⁻¹. The 1638 corresponds to the C=O bond, 2076 corresponds to the C-N bond, 3435 corresponds to the N-H bond. The high similarity between extract and colloidal solutions indicated that the same compounds existed in both media.

3.4 Scanning electron microscope (SEM)

The SEM image of gold nanoparticles synthesized by green synthesis process by using 5 % leaves extract and 1mM HAuCl₄ concentration it gave a clear image of highly dense gold nanoparticles. The SEM image showing gold nanoparticles synthesized using plant extract confirmed the growth of gold nanostructures (Figure 3).

3.5 Antimicrobial activity

The results of the antimicrobial activity was presented in figure 4. The two tested concentrations such as 0.75 and 1.50 mg/disc produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively. In the present study, higher (1.5 mg/disc) concentration of sample got greater sensitivity than (0.75 mg/disc) lower concentration in all the tested microorganisms. In bacteria, the test sample was most effective against B2 while moderate effect was noticed from B1. In fungi, which was effective against F2 whereas average effect was observed in F1. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (distilled water), reflecting that antimicrobial activity was directly related to the sample.

3.6 Anticancer activity

The cytotoxic effect of the AuNPs were examined on human cell lines (HeLa cells) for 24 h and 48 h (Sample conc. = 0.1 – 50 µL). The cytotoxicity effect is very high in biosynthesized AuNPs against HeLa cell lines (Figure 5). The AuNPs inhibited the growth of the cancer cells significantly, in a dose and duration dependent manner. The cytotoxic activity was finding according to the dose values of the exposure of the complex required to reduce survival to 50% (IC₅₀), compared to untreated cells. In AuNPs, the 50 µL sample is enough to control cancerous cell (Figure 6). The cytotoxic effect of the sample may be interpretable as due to its amphiphilic nature and, hence, would penetrate the cell membrane easily, reduce the energy status in tumors and also alter hypoxia status in the cancer cell. The cytotoxicity effect was compared with the standard anticancer drug 5-FU against HeLa cells and their LC₅₀ value was observed. Similarly cytotoxicity of chemically synthesized AuNPs was reported against HeLa cells by Miura and Shinohara. A large number of in vitro studies indicate that AuNPs are toxic to the mammalian cells. Interestingly, some studies have shown that AuNPs has the potential to intervene genes associated with cell cycle progression, also induce DNA damage and apoptosis in cancer cells. Indeed, the results of present study provide conclusive evidence for cytotoxic effect of AuNPs on cancer cell lines rather than normal cell lines.

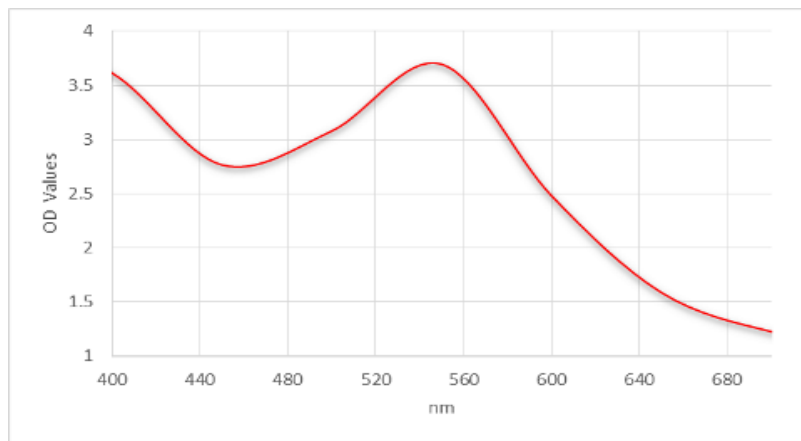


Figure 1: UV-Spectrum of AuNPs

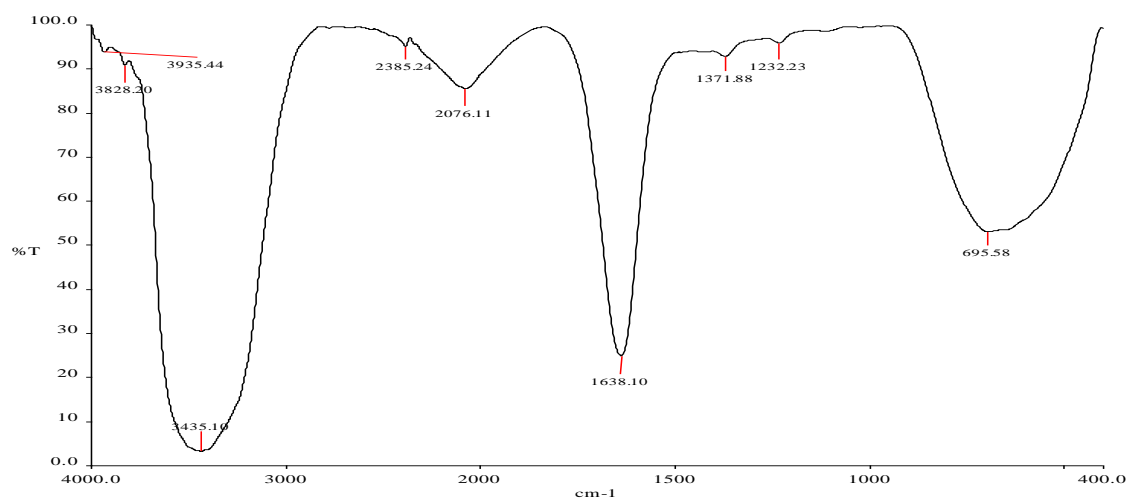


Figure 2: FTIR-Spectrum of AuNPs

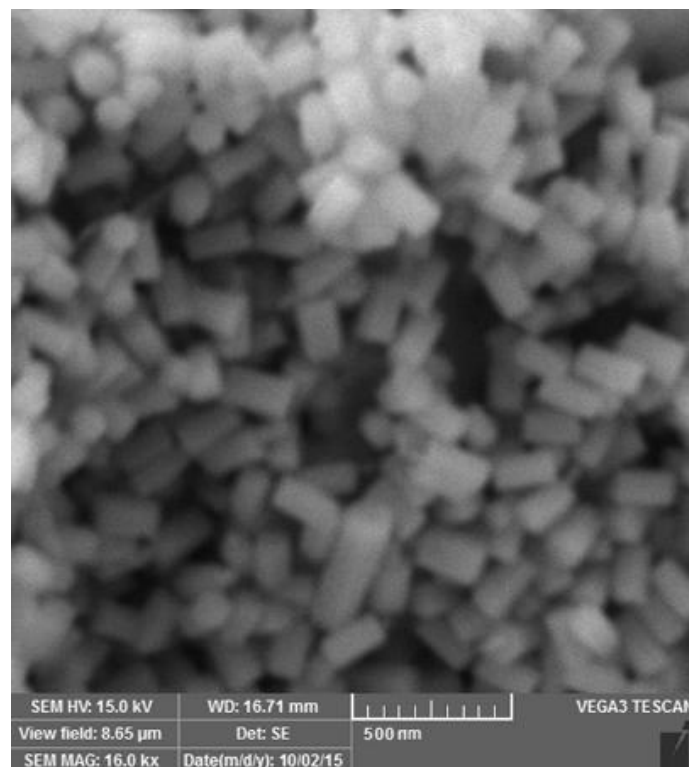


Figure 3: SEM Image of AuNPs

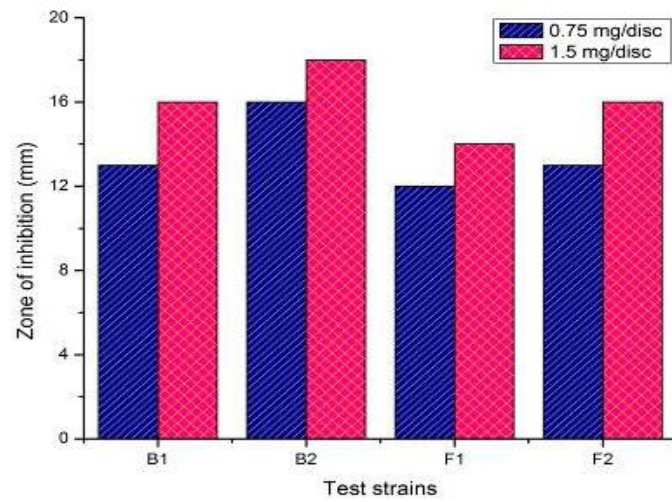


Figure 4: Antimicrobial activity of AuNPs

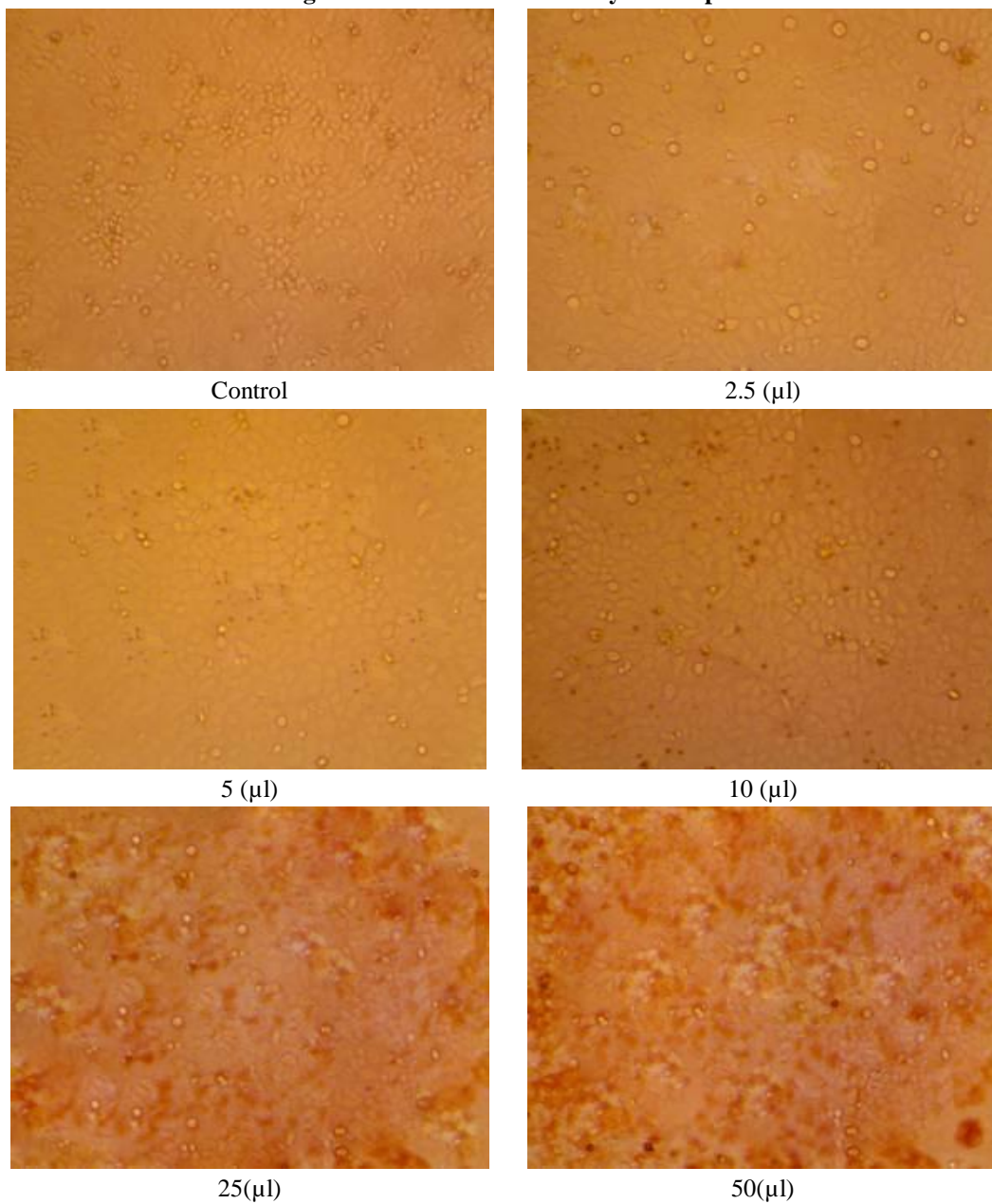


Figure 5: The photograph shows anticancer activity of AuNPs

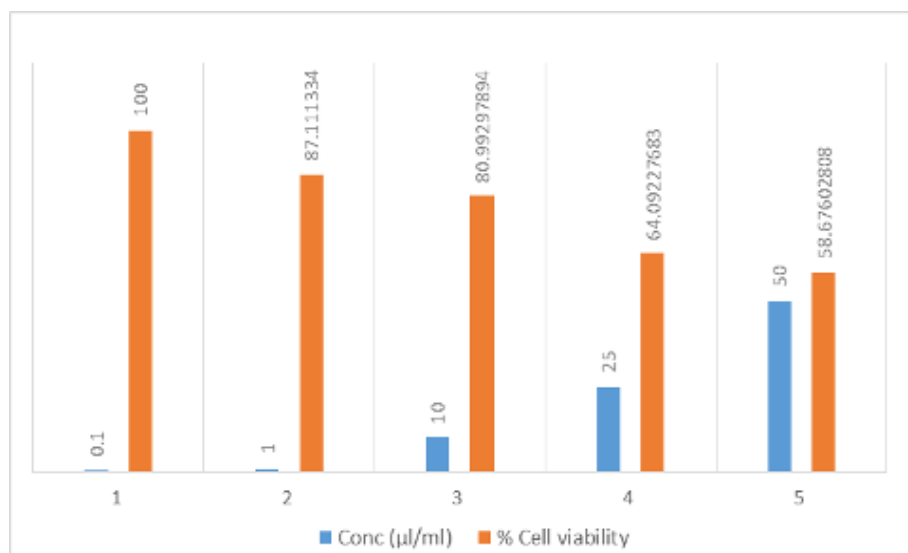


Figure 6: Anticancer activity of AuNPs with different concentrations

4. Conclusion

A green chemistry synthetic route has been used for gold nanoparticles synthesis. The reaction occurred at ambient temperature. Several plant biomass or plant extracts have been successfully used for extracellular biosynthesis of gold nanoparticles. Analytical techniques, such as ultraviolet-visible spectroscopy (UV-vis), and scanning electron microscopy (SEM) were applied to characterize the nanoparticles morphology. Recent studies have begun to reveal how AuNPs impinge on the structural/functional organization of cancer and normal cells. This knowledge is critical for two aspects of nanomedicine. First, it will help define the AuNP-induced events at the subcellular level. This will set the stage for the identification of new molecular targets for cancer therapy. Second, it will direct the design of AuNPs with physico-chemical properties that overcome the current limitations these particles face in basic research, diagnosis and therapy

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