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CELL VIABILITY STUDIES OF CUBIC GOLD NANOPARTICLES SYNTHESIZED USING THE EXTRACT OF *ALTERNANTHERA SESSILIS*

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ABSTRACT

Gold nanoparticles were synthesized using the extract of *Alternanthera sessilis* under three different conditions. The synthesized gold nanoparticles were characterized by UV-visible spectroscopy, XRD and SEM analysis. The particle size of the nanogold was confirmed by Debye-Scherrer's equation and SEM analysis. The phytoconstituents present in *Alternanthera sessilis* was found to act as a good reductant and capping agent. The cell viability testing on MCF-7 cell lines by MTT assay revealed gold nanoparticles to be non-toxic to the array of cells tested. Though not cytotoxic the non-toxic nature of the biogenic gold nanoparticles provides new opportunities for the safe application in molecular imaging and therapy.

Keywords: cubic nanogold, *Alternanthera sessilis*, XRD, SEM, cell viability.

1. INTRODUCTION

Nanotechnology is a budding and fast-growing field of generating nanomaterials with unique properties. These nanoparticles find applications in optoelectronic devices, [1] environment-friendly energy systems, biosensors, [2] biomedical imaging [3] and delivery purposes[4]. Enormous number of publications and patents are evidence for the exploitation of nanotechnology. Metal nanoparticles possess unique properties, much different from that of bulk material. Due to their distinct properties, nanoparticles form the basis for many technological and biological innovations in future. Gold nanoparticles are found to be

adaptable and useful due to its unreactive nature, not sensitive to air or light [5] and biocompatible [6].

Nanogold has received more attention due to its preclinical trials in the treatment of rheumatoid arthritis and has been fall under the mainstream as therapeutic agent. Gold nanoparticles can be synthesized in various shapes such as nanospheres, nanocages, nanorods, nanobelts and nanostars [7] and conjugated with biological molecules in a straightforward manner [8]. Several reports on biological synthesis of gold nanoparticles were found using stem extract of *Breynia rhamnoides*[9], sugar beet pulp [10], C*assia fistula* [11], leaf extract of *Memecylon edule* [12], *Justicia gendarussa* leaf [13], lemon grass extract [14], phyllanthin extract [15], algae extract of *Turbinaria conoides* [16], fruit extracts of *Ananas comosus* (L.) [17] and microbes like *Klebsiella pneumonia* [18].

In recent years, synthetic protocols of plant assisted nanoparticles have increased due to safe, eco-friendly nature and an alternative for most popular conventional methods which are bound with various implications. Biosynthesis of nanoparticles can be promoted to manipulate the commercial applications of these nanoparticles in the field of pharmaceuticals and other medical sciences which are limited factors for nanoparticles synthesized via conventional methods [19].

Alternanthera sessilis is a weed commonly known as joyweed, found in all parts of India. The plant has been scientifically proven to consist of chemical constituents like α-and β-spinasterols, lupeol isolated from roots and also contains β-sitosterol, stigmasterol etc. The leaves are used as traditional medicines for eye diseases, wounds, antidote to snake bite and skin diseases [20].

Hence in our present study, we have synthesized gold nanoparticles using the aqueous extract of *Alternanthera sessilis* and characterized by UV-visible spectroscopy and FTIR analysis. The crystallite size of the synthesized gold nanoparticles was confirmed by XRD and SEM analysis. Thus the facile method of synthesis of nanogold enhances the commercial applications compared to that chemical method of synthesis. To explore the use of synthesized nanogold, cell viability studies of normal cells and toxicity to cancer cells have been carried out on MCF-7 human breast cancer cell lines by MTT assay.

2. MATERIALS AND METHODS

The plant *Alternanthera sessilis* were collected from retail shop in Coimbatore. Gold chloride was purchased from s-d fine chemicals, India.

2.1 Preparation of the extract

Fresh plant (20 g) of *Alternanthera sessilis* was washed thoroughly with double distilled water and cut into small pieces, then boiled with 100 ml of double distilled water for 10 min. the prepared extract was filtered through Whatman filter paper and refrigerated.

2.3 Synthesis of gold nanoparticles

The gold chloride (3mM) solution (1 ml) was treated with different concentrations (1ml, 2ml, 3ml, 4ml, and 5ml) of aqueous extract of *Alternanthera sessilis* under various conditions i.e., room temperature (30ºC), higher temperature (75ºC) and sonication using sonic bath (Ultrasonic 1.5L(H)).

2.4 Characterization of gold nanoparticles

The formation of gold nanoparticles was confirmed by UV-Visible spectroscopy using Double beam spectrophotometer-2202 (Systronics). The FTIR spectra recorded using Fourier Transform Infrared spectroscopy 8400S (Shimadzu) confirms the reduction of gold chloride to nanogold by the phytoconstituents present in the aqueous extract of *Alternanthera sessilis*. The characterization of the crystalline gold nanoparticles of a drop coated film on a glass substrate after centrifugation was analyzed by X' pert powder (PANalytical) operated at a voltage of 45 kV and a current of 30 mA with Cu K*a* radiation. SEM analysis was carried out using TESCAN Electron microscope (Vega TC software) for the supernatant solutions of gold nanoparticles obtained after centrifugation.

2.5 Cell viability / toxicity studies of gold nanoparticles

2.5.1 Cell line

The human breast cancer cell line (MCF-7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity.

2.5.2 Cell treatment procedure

The monolayer cells were detached with trypsin-ethylene diamine tetraacetic acid

(EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dispersed in phosphate buffered saline (PBS) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five concentrations (0.1, 1, 10, 50, 100 µg/ml) of nanogold. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells which contain 100 µl of medium, resulting in the required final sample concentrations. Then, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The medium without samples served as control and triplicate study was carried out for all concentrations.

2.5.3 MTT assay

Succinate-dehydrogenase, a mitochondrial enzyme in living cells, cleaves the tetrazolium ring of 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT), converting it to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows:

% Cell viability = [A] Test / [A]control x 100

3. RESULTS AND DISCUSSION

The visual observation of the gold chloride solution treated with the different concentrations of aqueous extract of *Alternanthera sessilis* clearly confirms the change of greenish yellow to purple colour indicating the formation of gold nanoparticles within 3 h in room temperature (fig.1). Rapid reduction of gold ions to nanogold was achieved in 45 min at a temperature of 75ºC, whereas sonication resulted in nanogold formation in 15 min. Vankar and Bajpai, 2010 revealed the production of gold nanoparticles within 1h using *Mirabilis jalapa* flower extract [21].

Fig. 1 Visual colour change of gold nanoparticles at different concentrations of aqueous extract of *Alternanthera sessilis* with 1ml of gold chloride solution

It was observed that sonochemical technique, extremely activated by the ultrasonic irradiation is an easier method to prepare gold nanoparticles by the reduction of metallic ions. The process performed simply at room temperature is comparatively free of toxic chemical hazards. Ultrasonic assisted process has been proven to be a useful technique for the synthesis of novel materials. Significant and reproducible size differences was observed between sonicated and unsonicated samples, indicating that sonication is essential in the generation of smaller gold nanoparticles [22-24].

3.1 UV-visible spectroscopy

The UV-Visible spectra of the synthesized gold nanoparticles using *Alternanthera sessilis* showed two absorption peaks at 535 nm and 565 nm similar to that of the surface Plasmon resonance band of gold nanoparticles (fig. 2). The SPR bands revealed that the synthesized gold nanoparticles size may be of 30-100 nm. Literatures reported that shift in SPR bands towards longer wavelength (blue shift) and broader in shape are indication of formation of larger nanoparticles. The SPR band at 565 nm is due to the cube nanoparticles with slightly ellipsoidal shape of the cluster on the surface. The distinct blue shift is attributed to quantum size effects that come into play when the particle size becomes comparable to the mean-free path of electrons in the metal. The sharp absorption band of the synthesized nanogold indicates the presence of uniform size nanoparticles. The spectra are consistent with previous experimental results [25-28].

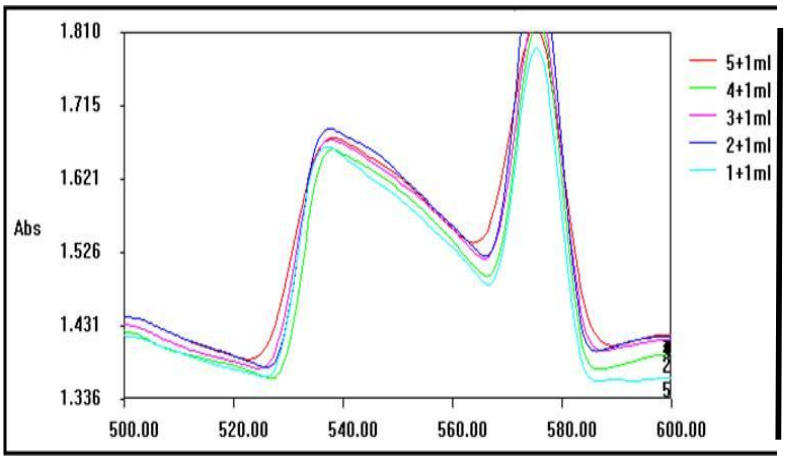

Fig. 2 UV-visible spectra of synthesized nanogold using the aqueous extract of *Alternanthera sessilis*

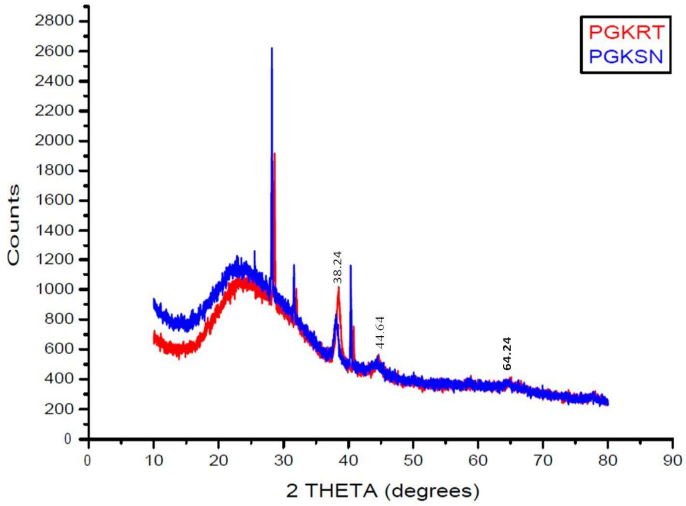

Fig. 3 XRD patterns of synthesized nanogold using the aqueous extract of *Alternanthera sessilis* under room temperature (PGKRT) and sonication (PGKSN)

3.2 X-ray diffraction analysis

Fig. 3 represents the XRD patterns of the synthesized gold nanoparticles using the aqueous extract of *Alternanthera sessilis* under room temperature and sonication. The XRD patterns of the synthesized gold nanoparticles revealed the presence of diffraction angles at 2θ= 38.24º, 44.64º, 64.24º, corresponds to the (111), (200), (220) facets of fcc of Bragg's reflection are similar under room temperature and sonication. The peaks other than the aforesaid one are due to the phytoconstituents present in the aqueous extract. The crystalline size of the synthesized nanogold by sonication was determined using the Debye-Scherrer's equation as given in the table 1.

Table 1 Determination of crystallite size of gold nanoparticles synthesized under sonication

S.No	2 θ (degrees)	Cos θ	β = π*FWHM/180 (radians)	D = k λ / β. Cosθ (nm)
1.	38.24	0.96068	0.00233	61.93
2.	44.64	0.92507	0.01400	10.70
3.	64.24	0.84693	0.00933	12.58

3.3 SEM analysis

The SEM micrograph of the synthesized gold nanoparticles is shown in fig. 4. It was observed that nanogold synthesized using aqueous extract of *Alternanthera sessilis* was well separated without agglomeration and cubic in shape. The size of the nanocubic gold particles was found to be less than 100 nm.

3.4 Cell proliferation study

The cytotoxic activity of the synthesized gold nanoparticles using the aqueous extract of *Alternanthera sessilis* under *in vitro* conditions was examined in terms of the effect of gold nanoparticles on cell proliferation by the MTT assay. The histogram plot of MTT assay results for cell viability studies of MCF-7 cell line after exposing to varying concentrations of nanogold was shown in fig. 5. The violet bars correspond to the % cell viability at the given concentration of gold nanoparticles. The plot clearly shows 99% cell viability for the cells that were treated with 0.1 µg concentration of gold nanoparticles for 24 h. It can be concluded that the gold salt ($AuCl_4^-$ ions) is highly toxic to the cells, [7] while the biogenic nanogold do not show acute toxicity even at high concentrations. Hence the biogenic gold nanoparticles are more biocompatible than the chemically synthesized gold nanoparticles.

The results revealed that the phytochemicals present in the *Alternanthera sessilis* provide non-toxic coating on AuNPs. This present results suggest that the gold nanoparticles do not show any cellular toxicity to cancerous as well as non-cancerous cells while the gold salt precursor showed acute toxicity to the cells. Connor et al, reported that citrated and biotinylated 18 nm gold nanoparticles did not induce toxicity in leukemic cells. [29] Similar result was obtained for the citrate, starch and gum Arabic- capped gold nanoparticles in the previous literature. [30] Cytotoxicity also depends on the type of cells used. The 33nm citrate-capped gold nanoparticles were found to be nontoxic to baby hamster kidney and human hepatocellular liver carcinoma cells but toxic to a human carcinoma lung cell lines. Hence the

AuNPs may have advantages over other metallic nanoparticles in terms of biocompatibility and non cytotoxicity. [31]

At each concentration, there was no significant cytotoxicity effect produced. The cell viability results indicate that gold nanoparticles are non-toxic to the array of cells tested. The non-toxic nature of the biogenic gold nanoparticles provides new opportunities for the safe application in molecular imaging and therapy. The incorporation of surface functionalities like amino acids renders these nanoparticles highly biocompatible. The cytotoxic activity of synthesized nanosilver using *Alternanthera sessilis* was studied against prostate cancer cells (PC3) and breast cancer cells (MCF-7) by MTT assay and found to show significant activity. [32-33] Thus the results demonstrate that the cytotoxicity depends upon the nature of the capping agents and also the metal.

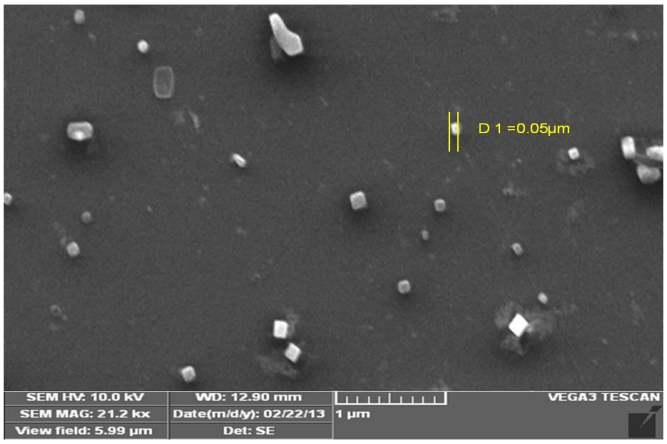

Fig. 4 SEM micrograph of nanogold synthesized under sonication by *Alternanthera sessilis* extract

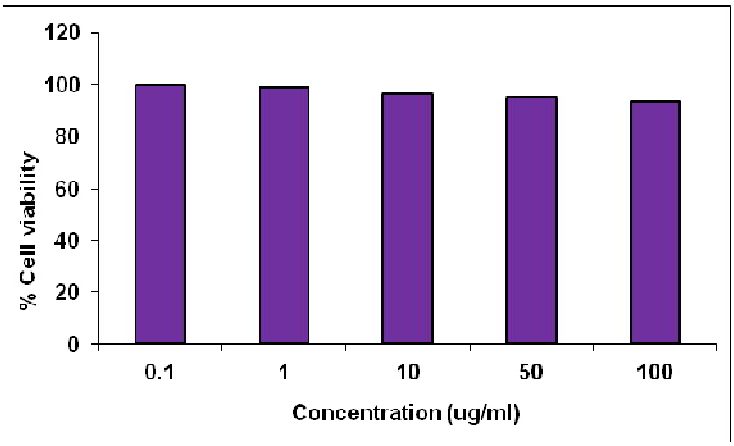

Fig. 5 Histogram of cell viability studies of MCF-7 cell line at various concentrations of nanogold

4. CONCLUSION

Facile method of synthesis of Gold nanoparticles was achieved using the aqueous extract of *Alternanthera sessilis* by means of sonication. The UV-Visible spectroscopy and X-ray diffraction analysis confirms the formation of nanogold. The shape and crystallite size of the gold nanoparticles were found to be cubic and less than 100 nm. The results of the cytotoxicity studies of the gold nanoparticles revealed that the biogenic nanogold does not cause any harm to the cancerous and non-cancerous cells even at higher concentrations. Hence capping agents and nature of the metal plays a significant role in the cytotoxic activity.

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