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Green Synthesis and Characterization of Biocompatible Gold Nanoparticles Using *Solanum indicum* Fruits

Regular Paper

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Abstract This paper reports on the eco-friendly synthesis of gold nanoparticles (AuNPs) using *Solanum indicum* fruit extract (SFE). We have evaluated various parameters for synthesis of AuNPs such as SFE (0.03%), $HAuCl_4$ (0.5 mM) and reaction time (20 seconds). The synthesized AuNPs were characterized with different physical techniques such as transmission electron microscopy (TEM), Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) and energy dispersive X-ray spectroscopy (EDX). TEM experiments showed that AuNPs presented an anisotropic shape and size ranging from 5-50nm. FT-IR spectroscopy revealed that biomolecules containing an amine group (–NH2), a carbonyl group, –OH groups and other stabilizing functional groups were adsorbed on the surface of the synthesized AuNPs. EDX showed the presence of the elements on the surface of the AuNPs. The cytotoxicity of the synthesized AuNPs were tested on two different human cancer cell lines, HeLa and MCF-7 and were found to be nontoxic, thus providing an opportunity to be used in biomedical applications.

Keywords *Solanum indicum*, Gold nanoparticles, Crystal growths, electron microscopy, Fourier transform infrared spectroscopy, nanostructure, X-ray diffraction

1. Introduction

The synthesis of metallic nanoparticles has gained a great significance due to its remarkable chemical and physical properties. In particular, gold nanoparticles (AuNPs) can be used in many fields such as biosensors, cosmetics, electronics, catalysis, semiconductors, drug delivery and tumour imaging, as they possess size and shape dependent unique properties [1, 2]. Different physical and chemical methods have been used to synthesize AuNPs including radiation [3], thermal decomposition [4, 5], vapour deposition [6], reduction in microemulsion [7] and electro- and photo- chemical processes [8-11]. In these conventional methods, toxic synthetic chemicals are used as reducing and stabilizing agents. Synthetic reducing agents are not favoured for producing AuNPs for biomedical applications, as traces of such chemicals

left unreacted in the process can be harmful. Therefore, there is a considerable interest in developing new procedures for AuNP synthesis, which are simple, rapid and environmentally benign. In this context, microorganisms have been used to synthesize and stabilize AuNPs without the involvement of synthetic chemicals [12]. However, a major limitation in using microorganisms is the intricacy of control over the shape, size and crystallinity of AuNPs [13-14]. However, using plant and fruit extracts for AuNP synthesis has the potential to address the drawbacks associated with microorganisms [15-17].

In a continuous effort to search for desirable plant material for the eco-friendly synthesis of AuNPs, we found *Solanum indium* fruits, which have both reducing and stabilizing capabilities. *S. indicum* is an indigenous plant grown widely in India and Taiwan and has many medical applications such as an anti-inflammatory, wound-healing and analgesic and is used for the treatment of rhinitis, coughs and breast cancer. In Assam and Thailand, fruits of *S. indicum* are used as food [18, 19].

Here we report the use of microwaves for the rapid synthesis of the AuNPs using *Solenum indicum* Fruit Extract (SFE). Microwaves are electromagnetic waves, which can generate enormous amounts of heat by molecular dipole moment interactions with high frequency electromagnetic radiations. The heat generated by microwaves assists in rapid and uniform synthesis of AuNPs. In contrast to conventional methods, microwaves also have the advantages of homogeneous heating that directly influences the nucleation process of AuNP synthesis [20-22].

2. Experimental Details

2.1 Materials

S. indium was grown in the experimental field and healthy leaves were harvested for AuNP synthesis. All reagents were purchased from Sisco Research Laboratories (Mumbai, India) and Merck (Mumbai, India). Cell culture related plastic ware and MTT (3, 4, 5- dimethylthiazol-2-yl-2-5-diphenyltetrazolium bromide) were obtained from the Sigma–Aldrich (Bangalore, India). Cell lines were obtained from National Centre for Cell Sciences (Pune, India).

2.2 Preparation of aqueous Solanum indicum fruits extract

S. indicum fruits were washed in deionised water to remove adsorbed dirt. To 25ml of distilled water, 50g of chopped fruit was added and grinded (Bajaj Model GX 11, India) for 15 minutes (min). The grinding mixture was filtered with Whatman filter paper (50mm) and the filtrate was stored at 4^0C for various experiments.

2.3 Biochemical test for flavonoids and phenolic compounds

To 5ml of diluted ammonium solution, 2ml of SFE was added, followed by several drops of concentrated sulphuric acid. The appearance of a yellowish colour indicated the presence of flavonoids.
To 2ml of SFE, several drops of 5% ferric chloride were added. The appearance of a dark green colour indicates the presence of poly phenolic compounds.

2.4 Syntheses of AuNPs

The syntheses of AuNPs were carried out by varying the SFE concentration (0.005-0.05%) against 0.5mM $HAuCl_4$ and the final volume was made up to 2ml with double distilled water. The desired percentage of the SFE was obtained by dissolving the dried powder of SFE in distilled water to make 0.1% (W/V) of the stock solution from which the working solution was obtained by further dilutions. To the whole resulting mixture, we applied microwave irradiation for 15 seconds. To find out the optimum concentration of $HAuCl_4$, experiments were carried out by varying the gold solution (0.1-1mM) concentration against 0.03% of the SFE. 30ml of 0.1% SFE was mixed with 10ml of the 5mM $HAuCl_4$ solution and the final volume was made up to 100ml with the distilled water to obtain the concentrations of 0.03% SFE and 0.5mM $HAuCl_4$ (working solution). To determine the optimum time for the AuNPs, a fixed amount of the working solution (2ml) was taken and exposed to different microwave irradiation times from 5-25 seconds with an interval of 3 seconds.

2.5 Characterization of AuNPs

2.5.1 UV-visible spectroscopy

UV-visible spectroscopic measurements of the synthesised AuNPs were carried out on a Cary 100 BIO UV–Vis spectrophotometer (Varian, CA, USA).

2.5.2 Transmission Electron Microscope (TEM)

A volume of 10ml of AuNP solution was centrifuged at 20000rpm for 20 minutes. The resulting pellet was resuspended in 3ml of distilled water and centrifuged at 20000rpm for 20 minutes and the process was repeated three times. The obtained pellet was finally resuspended in 1ml of distilled water. Several drops of the redispersed colloidal solution were placed over a carbon coated copper grid and the water was evaporated in a hot air oven (Daihan Labtech Co. Ltd. model LDO-150F) at 65^oC for four hours. TEM measurements were performed on a Transmission Electron Microscope (TEM-JEOL model 2100, 200kV) operated at a voltage of 190kV.

2.5.3 XRD, FT-IR and EDX analysis

30ml of AuNP colloidal solution was centrifuged at 20000rpm and the resulting pellet was resuspended in 4ml of double distilled water. This process was repeated three times to obtain pure nanoparticles. The pellet obtained was redispersed in 5ml of double distilled water and freeze dried in a lyophiliser (Christ Gefriertrocknungsanlagen GmbH Model 1-4) for 16 hours. The fine dried powder was analysed with the help of an XRD instrument (Bruker Advance D8 XRD machine) with a Cu source at 1.5406 A° wavelength in thin film mode. The infrared spectra were recorded using a FT-IR spectroscope (Spectrum One, Perkin Elmer, MA, USA), from 4000/cm to 450/cm, with a resolution of 2cm and five scans/sample by using a KBr pellet containing 1mg of dried AuNPs.

The elemental composition of the as-prepared AuNPs was obtained by using Energy-Dispersive X-ray Spectroscopy (LEO 1430 VP) at variable pressures with a scanning electron microscope equipped with an INCA Oxford EDX detector, by using an acceleration voltage of 10keV

2.6 Cytotoxicity studies

The Minimal Essential Medium (MEM) containing 1.0mM $C_3H_3NaO_3$, 0.1mM nonessential amino acids, 1.5g/l $NaHCO_3$, 2mM L-glutamine supplemented with 10% FBS (heat inactivated) and 1% antibiotic-antimycotic solution (1000U/mL penicillin G, 10mg/mL streptomycin sulphate, 5mg/mL gentamycin and 25μg/mL amphotericin B) was used to maintain the HeLa (Human cervical cancer) and MCF-7 (Human breast cancer) cells. The cells were cultured at 37°C in a humidified incubator (Heal Force, HF 160W, China) supplemented with 5% CO_2.

The MTT assay was used to test the cytotoxicity of AuNPs on cancer cells. Monocultures of the HeLa and MCF-7 cells were incubated with increasing filter concentrations (0.2 microns) sterilized the AuNPs for 24 hours. The cell viability was estimated by an MTT dye conversion assay. Cells not exposed to AuNPs were considered as referring samples. In a 96-well plate (Cell Bind, Corning), approximately 1 × 10^4 cells were seeded and maintained using MEM medium containing serum. After 24 hours of incubation, the medium was replaced with the serum free medium containing various concentrations of AuNPs (10-100µM). The media were removed after 24 hours of treatment and cells were washed with phosphate-buffered saline (PBS, 0.01M, pH-7.2) followed by the addition of 100µl of MTT (0.5mg/mL), prepared in a serum free medium. The media were then incubated for four hours in an incubator. Subsequently, medium was removed and 100µl of dimethyl sulphoxide (DMSO) was added to each well to solubilise the formazan crystals. The concentration of formazan was determined using a multiwell plate reader (Tecon micro plate reader, model 680, CA, USA) at 470nm absorbance. The cell viability was calculated with the following equation.

$$\text{Cell viability (\%)} = (A_{\text{treated}} / A_{\text{control}}) \times 100$$

where $A_{treated}$ and $A_{control}$ are the absorbance of the treated and untreated cells, respectively.

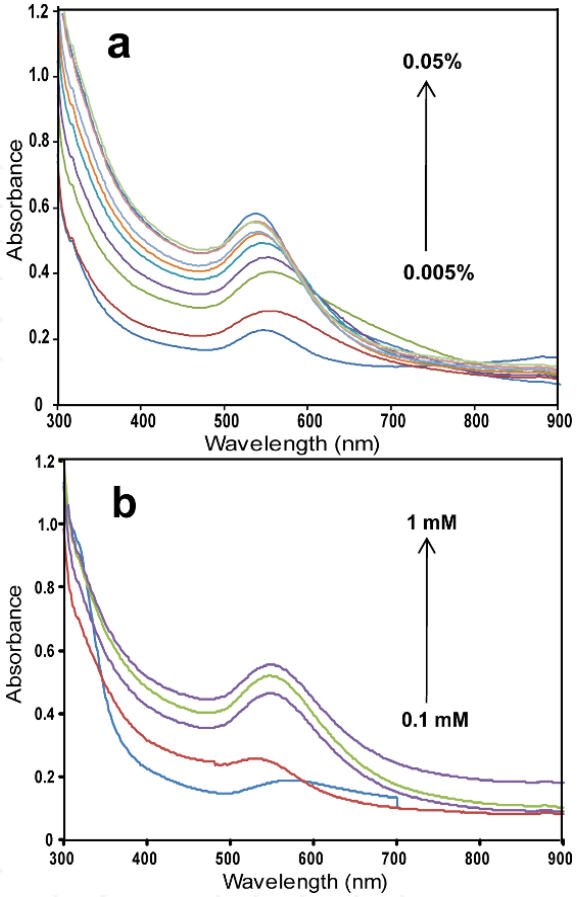

Figure 1. UV-Visible absorption spectra of AuNPs synthesized by (A) different concentrations of SFE (0.005-0.05%) for 15 seconds against 0.5mM $HAuCl_4$ (B) varying concentrations of $HAuCl_4$ (0.1- 1mM) against to the 0.03% SFE.

3. Results and discussion

3.1 SFE catalysed synthesis yields diverse shaped AuNPs under optimum conditions

The reaction mixtures (0.005-0.025%) showed a gradual increment in the formation of the AuNPs, whereas the reaction mixtures above 0.03% of SFE showed flattened peaks. This revealed that 0.03% of SFE was sufficient to reduce 0.5mM of $HAuCl_4$. Thus, 0.03% of SFE was considered as optimum for AuNP synthesis (Fig. 1a). UV-Vis spectra of products obtained by reacting 0.03% SFE with varying concentrations (0.1-1mM) displayed an intense peak at 547nm for 0.5mM $HAuCl_4$. However, the peaks of the Surface Plasmon Resonance (SPR) displayed

by 0.1 and 0.3mM $HAuCl_4$ were suboptimal, whereas those of 0.7 and 1mM $HAuCl_4$ were broadened, indicating an insignificant formation of the AuNPs. Thus, 0.5mM $HAuCl_4$ provided the optimum conditions for the synthesis of the AuNPs (Fig 1b).

Several experiments were performed to determine the optimum time for the synthesis of the AuNPs by incubating 0.03% SFE and 0.5mM $HAuCl_4$ solution at different MI time intervals from 5-25 seconds. We observed broadened bands above 20 seconds indicating no further significant formation of AuNPs. The peaks below 20 seconds were considered as suboptimal because of their low intensity. This revealed that 20 seconds was sufficient to reduce 0.5mM of gold solution using 0.03% SFE for AuNPs synthesis (Fig 2).

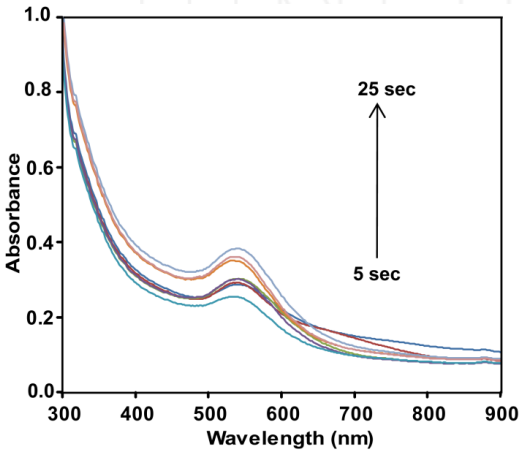

Figure 2. MW irradiation time (5-25 seconds) with 0.03% SFE and 0.5mM $HAuCl_4$.

3.2 TEM and XRD studies confirmed the crystalline nature of AuNPs

We employed a TEM technique to determine the shape and size of the AuNPs formed. A typical bright-field TEM image of AuNPs obtained under optimum reaction conditions showed the formation of anisotropic AuNPs (Fig. 3a). The AuNPs with different shapes and were found as shown in the insert of Fig. 3b, where the pie chart presents the distribution of the different shapes (circular, hexagonal, triangular and rod). The size of the synthesized AuNPs with a circular shape ranged between 5 and 50nm, while the average size was found to be 7.4nm (Fig. 3b).

The crystalline nature of the synthesized AuNPs was confirmed from the selected area electron diffraction (SAED) pattern with bright circular rings corresponding to the (1 1 1), (2 0 0) and (2 2 0) planes (Fig. 4a). The HRTEM image showed lattice fringes of 0.22nm, revealing that the growth of the AuNPs occurred preferentially on the (1 1 1) plane (Fig. 4b). The inter plane distance of the Au (1 1 1) plane was in agreement with the (1 1 1) d-spacing of bulk Au (0.2255nm) [22-24].

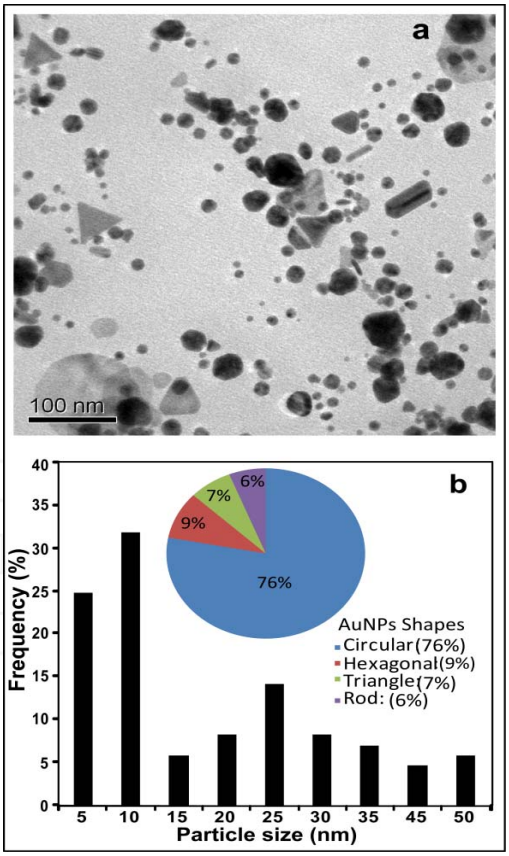

Figure 3. Transmission Electron Microscope of (a) AuNPs synthesized with 0.03% and 0.5mM $HAuCl_4$ for 20 seconds (b) size distribution histogram.

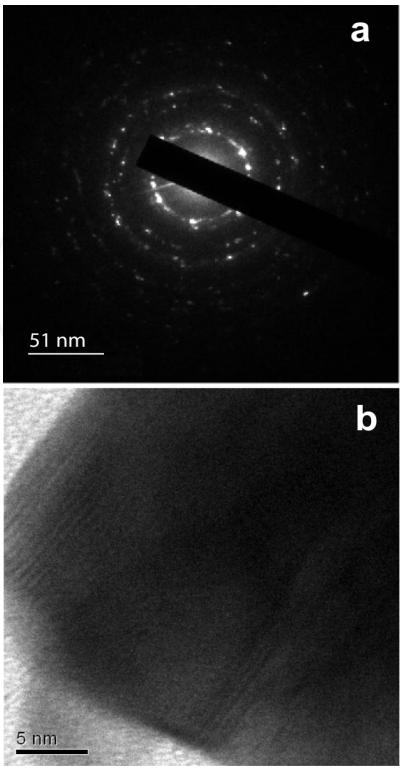

Figure 4. (a) SAED pattern of the Au NPs, the white marker represents $51nm^{-1}$. (b) HRTEM of AuNPs synthesized with optimized parameters (0.03% PLE, 0.5mM HAuCl4 and 20 seconds).

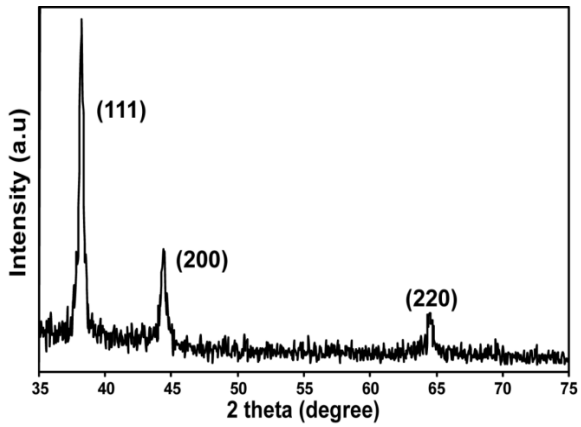

Figure 5. XRD pattern of AuNPs synthesized with optimized parameters.

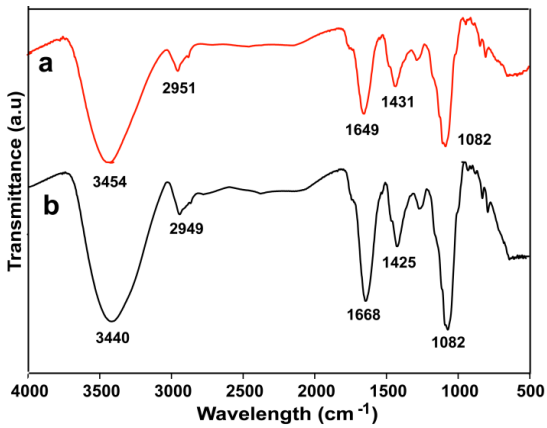

Figure 6. FT-IR spectra of lyophilized SFE (spectrum a) and AuNPs (spectrum b)

The XRD pattern revealed prominent Bragg reflections, which were indexed on the basis of the fcc structure of gold. The intensity of the (1 1 1), (2 0 0) and (2 2 0) diffraction peaks corresponding to 38.1°, 44.5° and 64.8° respectively, confirmed that the synthesized AuNPs were of crystalline nature [20, 22]. The ratio between the intensity of the (2 0 0) and (1 1 1) diffraction peaks was much lower than the usual value (0.52) suggesting that the (1 1 1) plane was the predominant among the three diffraction peaks (Fig. 5).

3.3 FTIR and EDX revealed adsorption of biomolecules on the AuNPs

The FT-IR spectra of AuNPs synthesized under optimized conditions indicated the presence of the biomolecules on the AuNPs. FT-IR spectra of SFE showed strong signals at 3454, 2951, 1649, 1431 and 1082cm^{-1}, corresponding to the hydroxyl group arising from alcohols and phenolic compounds, secondary amine, amide I bond of proteins, asymmetric deformation of CH_3 from alkenes and C–N stretching vibrations of the amide bond, respectively (Fig. 6a). The FT-IR spectra of AuNPs showed strong signals at 3440cm^{-1} (hydroxyl group of alcohols or phenols), 2949cm^{-1} (secondary amine), 1668cm–1 (amide I bond of proteins), 1425cm^{-1} (deformation of the -CH3 from alkanes) and 1082cm^{-1} (stretching vibrations of C–N from amide bond) (Fig. 6b) [25]. This confirmed the presence of bioactive molecules in the SFE and their adsorption onto AuNPs. The adsorption pattern (200-290nm) of the SFE after biochemical tests shows the presence of phenolic compounds and flavonoids [26]. These bioactive molecules (phenolic compounds and flavonoids) reduced the Au^{3+} ion into Au^{o} nanoparticles by oxidizing hydroxyl (R-OH) to carbonyl groups (R-C=O) or transfer their π-electrons. During the microwave irradiation process the bioactive molecules were adsorbed onto the AuNPs and stabilized the synthesized AuNPs.

The EDX profile of AuNPs presents a strong signal for gold and copper along with very strong carbon and oxygen peaks (Fig 7). The signal for copper was due to the grid used for the TEM observations. We found molecular carbon and oxygen, which may originate from flavonoids or phenolic or organic biomolecules bound to the surface of the AuNPs. The EDX suggested the presence of biomolecules on the surface of the synthesized AuNPs.

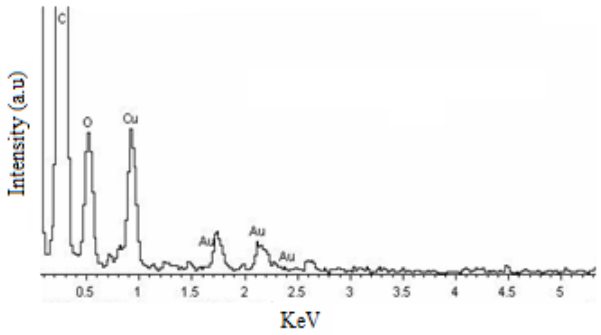

Figure 7. EDX pattern of AuNPs synthesized with 0.03% FLE, 0.5mM $HAuCl_4$ and 20 seconds of MW irradiation time.

3.4 SFE provided a nontoxic coating on the surface of AuNPs

The AuNPs synthesized were tested on the HeLa and MCF-7 cells and were found to be biocompatible. The cellular morphologies of the two cell lines were unaltered after treatment with AuNPs suggesting that treatment with AuNPs did not induce any cytotoxic effect causing significant damage or death of the treated cells. Both HeLa and MCF-7 cells lines showed excellent viability up to 100μmol of AuNPs/L of MEM after 24 hours post treatment (Fig. 8). HeLa cells showed more resistance to the synthesized AuNPs, compared to the MCF-7 cells. This indicated that the SFE provided a nontoxic coating on the surface of the AuNPs. Therefore, the rapid and eco-friendly method presented in this work for the synthesis of AuNPs provides an effective solution for applications in drug delivery and molecular imaging.

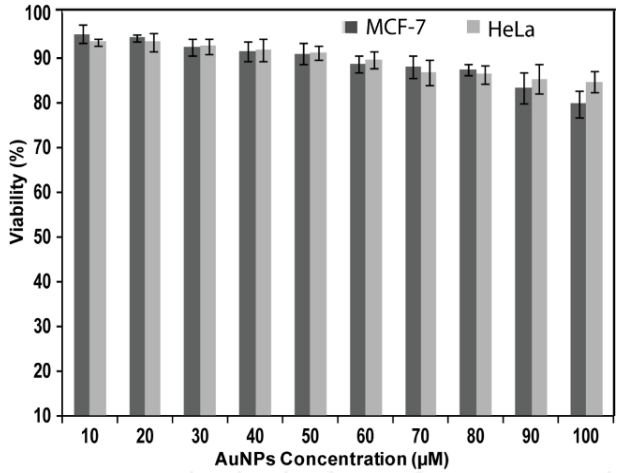

Figure 8. Cytotoxicity assay: Cell viability of HeLa and MCF-7 cells exposed to different concentrations of AuNPs (10–100µM) over a period of 24 hours treatment.

4. Conclusion

We have successfully synthesized AuNPs using an eco-friendly method using *Solanum indicum* fruit extract under optimized conditions (0.03% SFE, 0.5mM $HAuCl_4$ and 16 seconds). TEM observations showed that the synthesized AuNPs were anisotropic. HRTEM and XRD analysis confirmed the crystalline nature of AuNPs. Also, FTIR and EDX studies revealed the adsorption of bioactive molecules on the surface of AuNPs. Moreover, cytotoxic studies showed that the synthesized AuNPs were non toxic on human cancer cell lines, indicating their biocompatibility and thus their potential use in several biomedical applications. The method presented in this work has the advantages of eco-friendliness, rapid synthesis, cost effectiveness and most importantly producing biocompatible AuNPs.

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