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Development of colloidal bio-nano drug for anticancer studies

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Abstract

The exploitation of bio (plant) materials for the biosynthesis of nanoparticles is considered a green technology as it does not involve any harmful chemicals. The present study reports the raw plant extraction and synthesis of gold (Au) nanoparticles from $HAuCl_4$ using the powder of novel *C. guianensis*. The secondary metabolites were responsible for the reduction of gold metal to nano-sized Au nanoparticles. The 50% (IC_{50}) value of cytotoxic activity was observed in 50 µL concentration of sample and was enough to control the cancerous HeLa cells.

Keywords: *Couroupita guianensis*, Anticancer activity, DLS, zeta potential.

1. Introduction

The World Health Organization estimates that 80% of the people in developing countries of the world rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts. This means that about 3.5 to 4 billion people in the world rely on plants as sources of drugs [1]. Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases [2][3]. It is estimated that 12.5% of the population dies due to cancer. The disease is widely prevalent, and in the West, almost a third of the population develops cancer at some point of time during their life. Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at the molecular level [4][5].

Moreover, it is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases [3]-[7]. All cells are exposed to oxidative stress, and thus, oxidation and free radicals may be important in carcinogenesis at multiple tumor sites.

Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would prevent, slow, or reverse cancer development. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents [8][9]. Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents.

In recent years, numerous methodologies are developed to synthesize noble metal nanoparticles of particular shape and size depending on specific requirements [10][11]. 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 [12]-[14]. Especially, gold nanoparticles (AuNps) have aroused great interest in the field of medical applications due to the inert nature and the biocompatibility of gold as well as the well-known chemistry of nano-gold [15][16]. One example in the field of therapeutics is the nano enabled hypothermia treatment, in which the nanoparticles are bound to specific target cells or proteins and irradiated with laser light. The gold nanoparticles convert the light to highly localized heat, which leads to damage of the tagged cells exclusively, thus enabling the destruction of specific cells with high selectivity and efficiency. Many diseases are considered treatable by this technique, including cancer which is one of the leading causes of mortality in the world [15][17]. Inorganic nonmaterial have been widely used for cellular delivery due to their versatile features like wide availability, rich

functionality, good compatibility, and capability of targeted drug delivery and controlled release of drugs [18]. The main object of the present study is to synthesize the Au nanoparticles by using green plants (*Couroupita guianensis*) with low cost. And also to understand the anticancer efficacy of biosynthesized AuNPs and raw plant extraction.

2. Materials and Methods

2.1 Plant material

The plant materials were collected from Pudukkottai district of Tamil Nadu in India during the period of January to February 2016.

2.2 Aqueous extraction

The plant material was collected individually, washed thoroughly thrice with distilled water, shade-dried up to 5 days and prepared fine powder by grinding. 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, mixed with 200 ml of Milli Q water and kept in boiling water bath at 60°C for 10 min. The extracts were filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C for further studies to avoid microbial contamination.

2.3 Development of colloidal bio-nano drugs

Development 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.

2.4 Dynamic light scattering particle size analyser for AuNPs characterization

In order to find out the particle size distribution the AU powder was dispersed in water by horn type ultrasonic processor (Vibronics, model: VPLP1). Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of gold nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 25^0C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particles distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries, Malvern instrument Nano Zs) to find out the particle size distribution.

2.5 Dynamic light scattering zeta potential measurement for AuNPs characterization

Zeta potential describes the electrical potential in the double layer of ions surrounding a particle at the boundary of the particle surface and the adsorbed ions in the diffuse layer (Ives, 1956; Henderson, 2008). Zeta potentials were determined with a Zetaphorementer IV (CAD, France).

2.6 Anticancer screening of solvent extracted plant and AuNPs

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 µg/mL) range of 1 - 50 µg/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[19][20]. 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 ^{0}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:

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

The IC_{50} value was determined as the complex concentration that is required to reduce the absorbance to half that of the control. The morphological changes/ apoptosis analysis of the control and treated cells were assessed by acridine orange/ethidium bromide (AO/EtBr) staining method [21]. The cell suspension of each sample (Hep G2 - 10^6/well cells) was treated with 25 µl of AO and EB solution (3.8 µM of AO and 2.5 µM of EtBr in PBS) and incubated for 5 min. The excessive unbinding dye was removed by washing with PBS twice. Both control and treated cells were visualised under fluorescent microscope with UV filter range between 450 to 490 nm (CarlZeiss, Germany). Three hundred cells per sample were counted in triplicate for each dose point and digitised images were captured. The apoptotic (shrunken, fragmented

nuclei) and necrotic (EtBr positive) cells were judged by the staining, nuclear morphology, membrane integrity and shape of the cells and the percentages of apoptotic and necrotic cells were calculated.

3. Results and discussion

3.1 DLS analysis

The particle size distribution (PSD) of synthesized gold nanoparticles, it was found that Au nanoparticles size were in the range of 20-100nm. However, beyond 100 nm range the percentage of nanoparticles present is high. The highest fraction of AuNPs present in the solution was of 31nm is very appropriate since it gives lowest average size of nanoparticles (Figure 1).

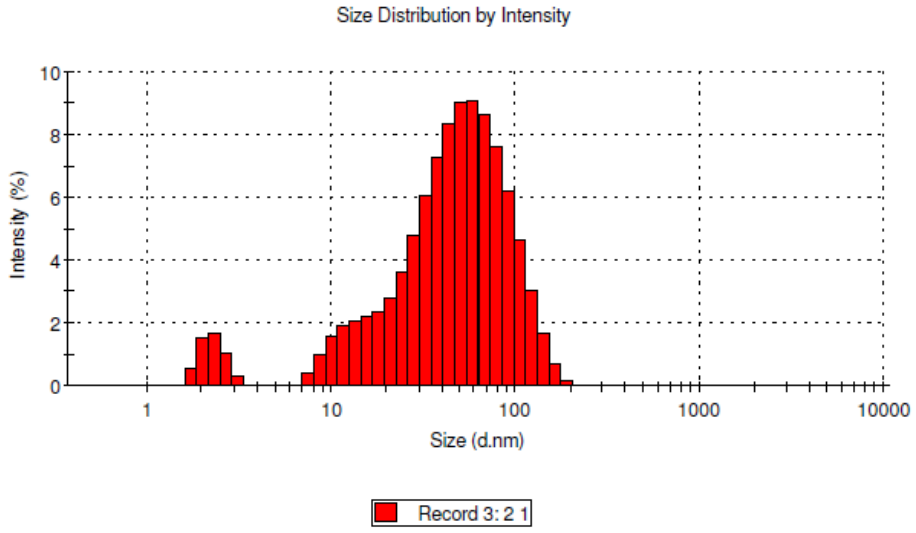
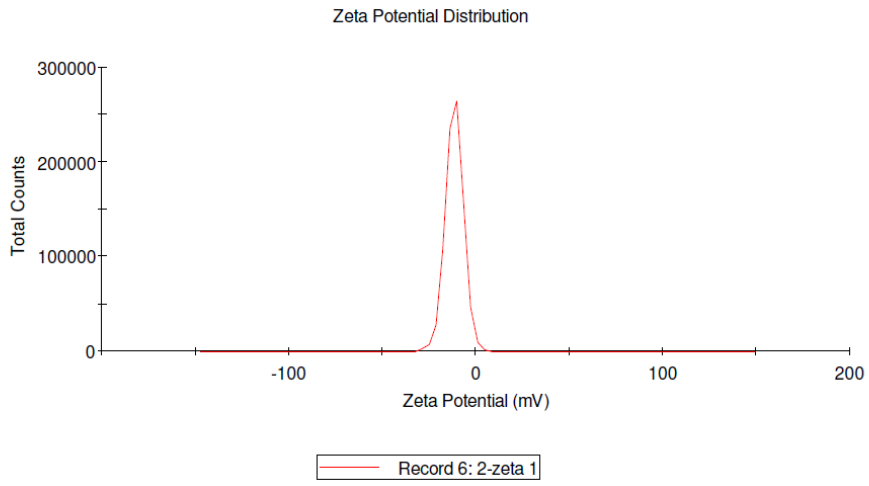


Figure 1: Dynamic light scattering of particle size analyser of Au Nanoparticles

3.2 Zeta potential analysis

The Figure 2 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nanogold. The overall absorbance of Zeta Potential revealed the energetically very unstable. Therefore, the particles undergo agglomeration/ aggregation to stabilize them. So there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis. Zeta potential (surface potential) has direct relation with the stability of a form/structure as mentioned below (Figure 2).



Zeta Potential (mV): from ±10 to ±30 = Incipient instability

Figure 2: Zeta Potential Measurement of Au Nanoparticles

3.3 Anticancer activity

The cytotoxic effect of the AuNPs and raw plant extractions were examined on human cell lines (Hep G2 cells) for 24 h and 48 h (Sample conc. = 0.1 – 50 µL). The cytotoxicity effect is very high in biosynthesized AuNPs than raw plant extraction against Hep G2 cells (Figure 3, Figure 4, Figure 5 and Figure 6). The AuNPs and raw plant 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% (IC_{50}), compared to untreated cells [22][23][24]. In AuNPs, the 50 µL sample is enough to control cancerous cell (Figure 3, Graph 1 & Figure 4 Graph 2). 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 tumours and also alter hypoxia status in the cancer cell. The cytotoxicity effect was compared with the standard anticancer drug 5-FU against Hep G2 cells and their LC_{50} value was observed [26]. 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.

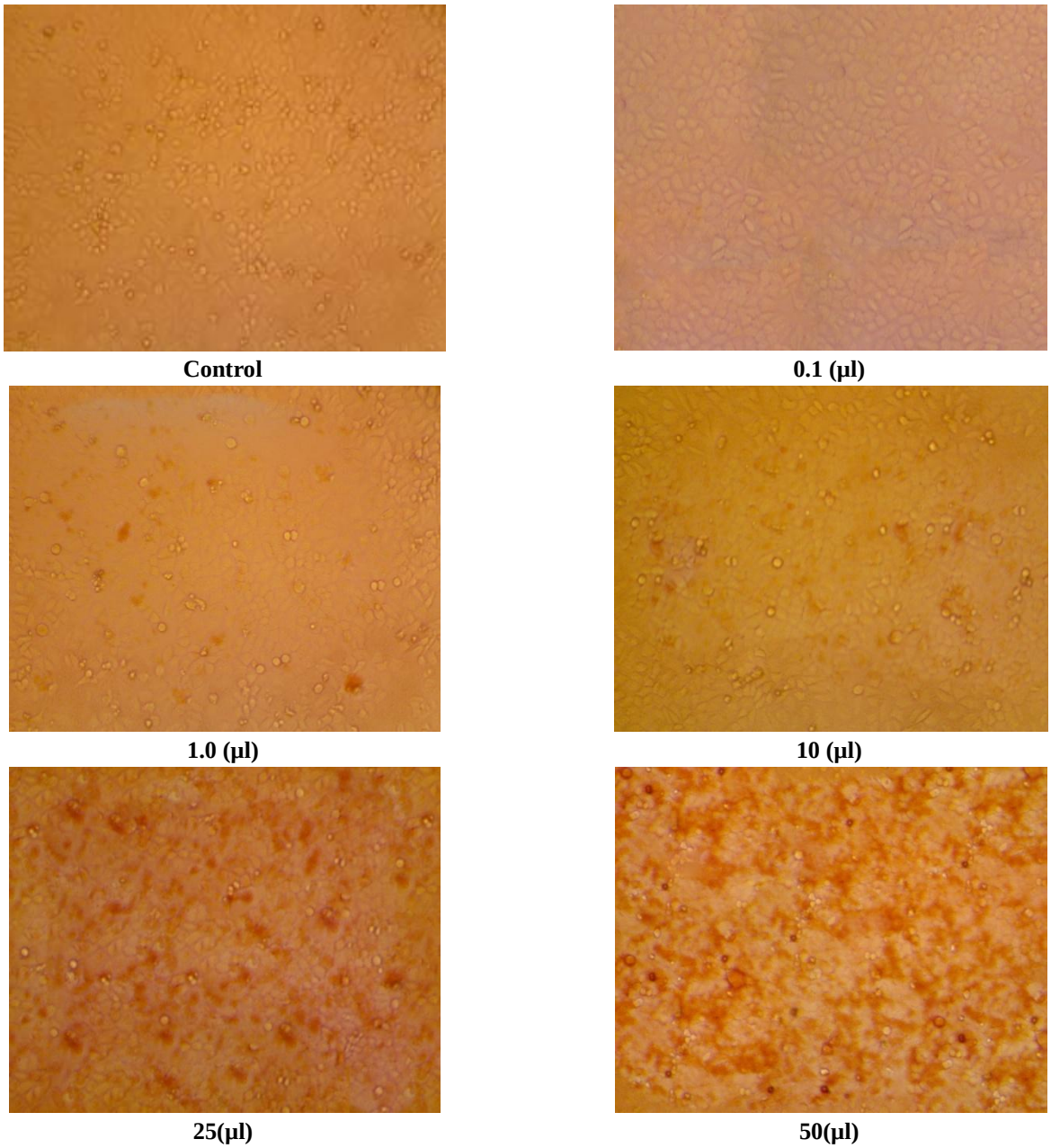


Figure 3: Anticancer activity of raw plant extraction against Hep G2 cancerous cells

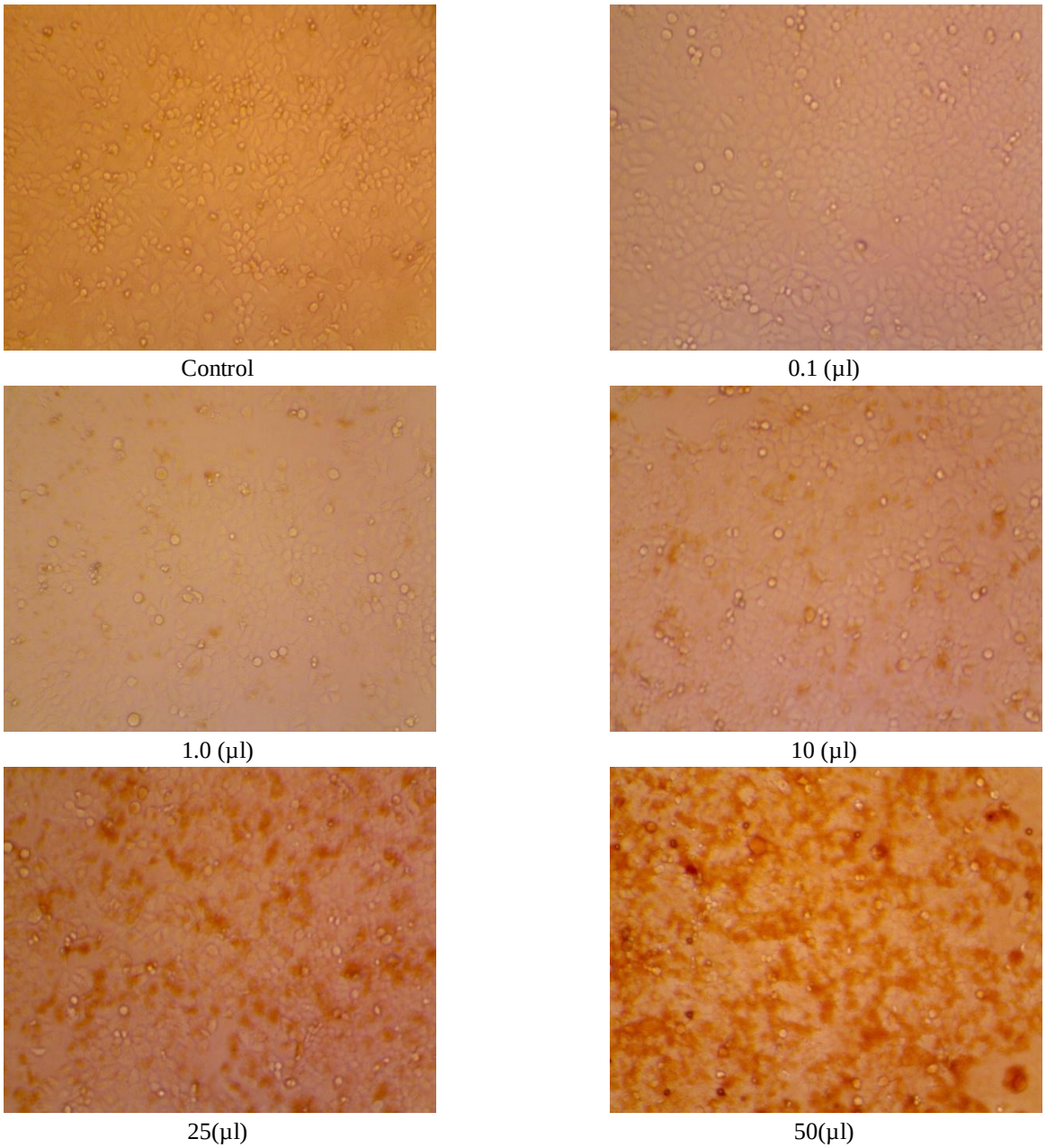


Figure 4: Anticancer activity of AuNPs against Hep G2 cancerous cells

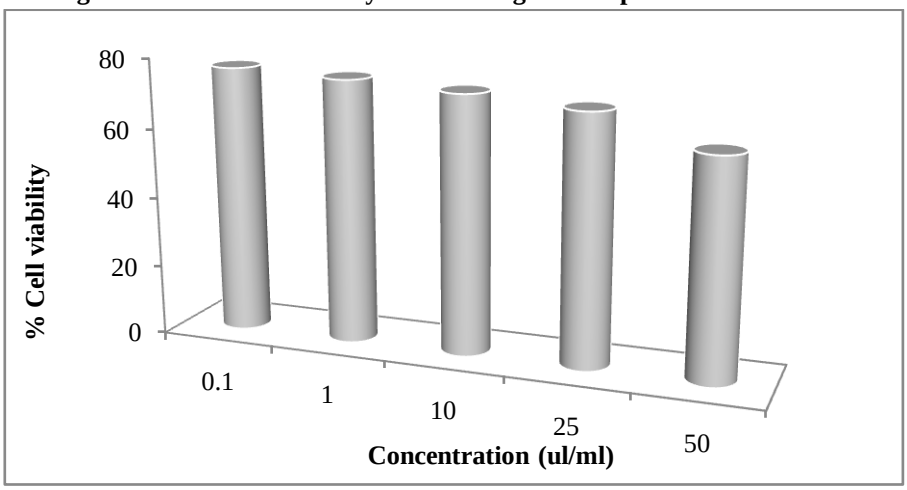


Figure 5: Anticancer activity of raw plant extraction against Hep G2 cancerous cells

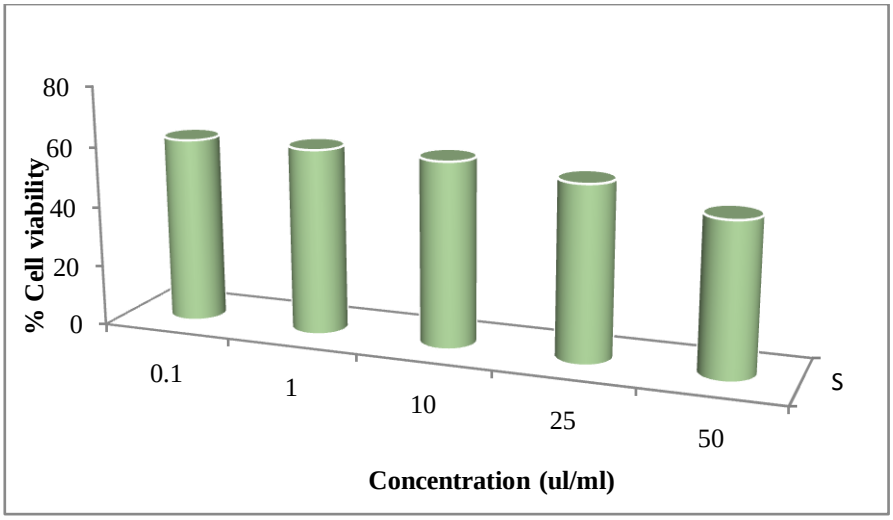


Figure 6: Anticancer activity of AuNPs against Hep G2 cancerous cells

4. Conclusion

The rapid biological synthesis of gold nanoparticles using *C. guianensis* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 20-100 nm and other techniques were confirmed the reduction of gold nanoparticles. The cytotoxicity effect is very high in biosynthesized AuNPs against Hep G2 cell lines than raw plant extraction. In AuNPs, the 50 µL sample is enough to control cancerous cell.

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