Synthesis and characterization of gold nano particles using barleria cristata for antimicrobial and anticancer applications

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Abstract. A nano revolution is necessary for the integration of nanoscience and medicine. Drug delivery, electronics, biological sciences, optics, and catalysis are among the fields in which metal nanoparticles find application. The objective of this work is to extract leaves from Barleria cristata in order to produce ecologically friendly gold nanoparticles (Au NP) for pharmacological studies. Several human pathogens were used to assess the biological mechanism and antibacterial properties of gold nanoparticles (AuNPs) in relation to their potential as a cancer treatment. An aqueous extract with a natural pH of 7.4 was obtained by reacting with 1 mM chloroauric acid ($HAuCl_4.3H_2O$), and it was then kept at room temperature. The colour changed from pale yellow to pink right away, indicating the reduction of Au3+ ions to Au 0. Using a UV-Visible spectrophotometer, the synthesised AuNPs were seen. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), field emission transmission electron microscopy (FE-TEM), and particle size distribution and zeta potential analysis are a few examples of analytical techniques. The disc diffusion method was used to confirm the extract's antibacterial properties in vitro on a few human illnesses. The MTT test was used to confirm the extract's anticancer properties on Hela carcinoma cell lines, revealing IC50 values of 50 µg/mL.

1. Introduction

Metal nano particles are very significant because of their massive surface area and high fraction of surface atoms. Researchers are particularly interested in producing anoparticles and analysing their size for a range of applications because of their distinct physicochemical properties (Beevi et al., 2012). Because of their unique chemical and physical features, metal nanoparticles have garnered significant attention in study because of their importance in science and technology. Green synthesis methods, advances in the

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new nanotechnological process, and all therapeutic applications of medicine are included in modern nanotechnology. Among the many applications for gold nanoparticles are biosensing, electronics, biocatalysis, enzyme electrodes, super conductors, and cancer treatment. Recently, a variety of methods have been discovered to produce noble metal nanoparticles with the ideal size and shape. Because more environmentally friendly material synthesis techniques are needed, one of the main areas of study at the intersection of biotechnology and nanotechnology is the biosynthesis of nanoparticles. Biological molecules are shown to have a significant advantage over chemical reducing agents because they are not biodegradable. Plant-based nanoparticle synthesis is becoming more and more popular due to its ease of use and lack of negative environmental effects (Huangetal.,2007a). Because of the rise in drug-resistant bacteria and resistant strains, scientists are intrigued by the remarkable antibacterial properties of metallic nanoparticles. Cancer is eventually lethal as a result of aberrant cells growing and spreading unchecked. The synthesis of nanoparticles using an ethno-pharmacological method is a remarkable technological development that highlights the mutually beneficial interaction between medical sciences and nanoscience. In this connection, synthesising pharmacologically relevant plant components have been considered as a common way to functionalize gold nanoparticles for antidiabetic nanomaterials. Sub-nanotechnology advancements have demonstrated prospects for novel therapeutic agents, biological and biomedical programmed in pharmaceutics, and diagnostics. The nanoparticle drug delivery technology provides the advantage of increasing therapeutic medication concentrations in cancer tissues by employing both passive and active targeting mechanisms (Anitha et al., 2011).These days, nanoparticles can be used in water treatment and antifouling compounds (Muthukumar et al.,2015). Hela carcinoma cells were used to test the in vitro anticancer activity in gold nanoparticles of Barleria cristata that had undergone green production, characterization, and biofunctionalization. Our recent research has clearly proven that it is possible to synthesise Au-NPs in a much greener manner without sacrificing their therapeutic characteristics, therefore plant extracts a good option to generate Au-NPs with enhanced antibacterial and anticancer activity.

2. Materials and Methods

2.1. Plant Collection and Leaf Extractions

The plant material, leaves of B. cristata, was collected in January 2016 from the Tiruchirappalli area in Tamil Nadu. After allowing the leaves to dry in the shade for up to five days and giving them three thorough washing in distilled water, the leaves were ground into a fine powder. After the plant material was sterilised for 15 minutes at 121°C, the fine powder was weighed. 200 ml of Milli Q water and 20 g of sterilised fine powder were combined, and the mixture was heated to 60°C in a boiling water bath for 10 minutes. The extracts were filtered through Whatman 1 filter paper to avoid microbiological contamination, and they were then chilled at 4°C for further analysis.

2.2. Biosynthesis of Nanoparticles

For the purpose of biosynthesising gold nanoparticles, pre-sterilized Milli Q water is used at a concentration of 10-3 M to generate gold chloride. Ten millilitres of plant extract and ninety millilitres of 10-3 M gold chloride were mixed to make gold nanoparticles. Comparable doses of gold chloride have been consumed without the principal matching controls containing any plant extracts. Aluminium foil was firmly wrapped around the

saline bottles to stop photoreduction of the gold ions. After that, they were kept in a dark, room temperature incubator while observations were conducted.

2.3 Anticancer Activity

To perform an anticancer investigation, in vitro samples and AuNPs were dissolved in DMSO, diluted in culture media, and treated over a range of 1 - 50 µg/mL f or a period of 24 and 48 hours to the chosen cell line (Hela) (obtained from NCCS). The DMSO solution served as the solvent control. A miniaturised viability assay was performed using 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in compliance with standard protocol (Sinthiya and Koperuncholan, 2015). After adding twenty-one millilitres of MTT (5 mg/mL) in phosphate-buffered saline (PBS) to each well, covering them with aluminium foil, and heating them to 37°C for four hours, the experiment was conducted. Each well was filled with 100 µl of 100% DMSO in order to dissolve the purple formazan product. Using a 96-well plate reader (Bio-Rad, Hercules, CA, USA) and two wavelengths—570 nm for measurement and 630 nm for reference—the absorbance was determined. Four sets of identical data were collected. For each, the associated means are found. These data were used to calculate the percentage of inhibition using the following formula: the mean absorption of untreated cells, or measure the mean absorbance of treated cells (test) The typical uptake of untreated cells, or reference.

2.4 Biosynthesis of Gold Nanoparticles by *B. Cristata* Leaves

The colour of the gold nanoparticles shifted from pale yellow to pink after incubation, suggesting that biosynthesis was taking place. To characterise gold nanoparticles, spectroscopic data analysis was used of Myristica dactyloides, which showed the greatest resistance against Shigella dysenteriae and Salmonella typhi. Rauvolfia tetraphylla ethanol extract has the best antibacterial activity and shows the maximum efficacy against E. coli and Enterobacter aerogenes, according to Suresh et al. (2008). It was discovered that some examined fungus, such as Penicillium spp. and A. niger, were more susceptible to crude extract than others. Many phytochemical constituents are effective antibacterials against a wide range of microorganisms, such as tannins (Fazal Mohamed et al., 2011), flavonoids (Ahmed John and Koperuncholan, 2012a), and terpenoids (Ahmed John and Koperuncholan, 2012). The results of this investigation clearly demonstrate the antibacterial and antifungal qualities of the leaf extracts that were extracted in ethanol, methanol, acetone, chloroform, and petroleum ether.

3 Characterizations of Gold Nanoparticles

3.1 UV-Vis Spectroscopy

Research employing UV-VIS spectroscopy revealed the presence of 545 nm beard peaks. The plasmon resonance of the gold nanoparticles was noted. When the precursor chloroauric acid solutions were mixed with the plant extracts, gold (Au) nanoparticles were created. When B. cristata leaf extract was applied at a 0.1% concentration, the associated chloroauric acid (HAuCl4) aqueous solutions' colour changed from pale yellow to pink for gold nanoparticles. The change in colour is due to the excitation of surface Plasmon vibration, which is indicated by the formation of gold nanoparticles (Figure 1).

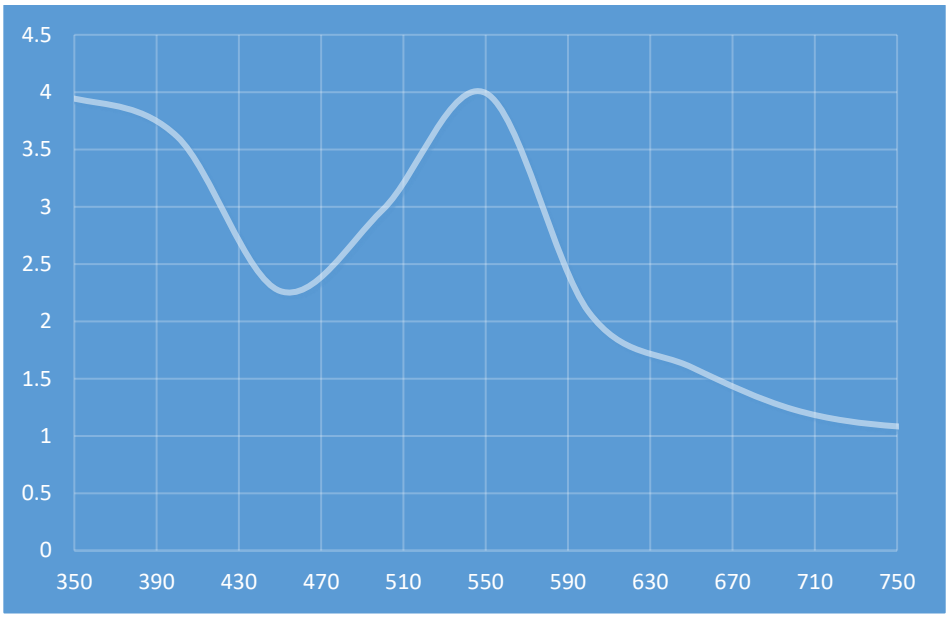

Fig. 1. UV-Spectrum of AuNPs

3.2. Fourier Transform Infra-Red Spectroscopy

The FTIR spectrum of AuNps derived from leaf extract is shown here. It demonstrates that there was a discernible absorption in the 400–4000 cm^1 range. These include β-dikeone, enolic form, C=O, 2729.17 (alkyl ethers for C-H stretching), and 3356 (secondary amine, free, N-H asymmetric stretching). (Figure 2).

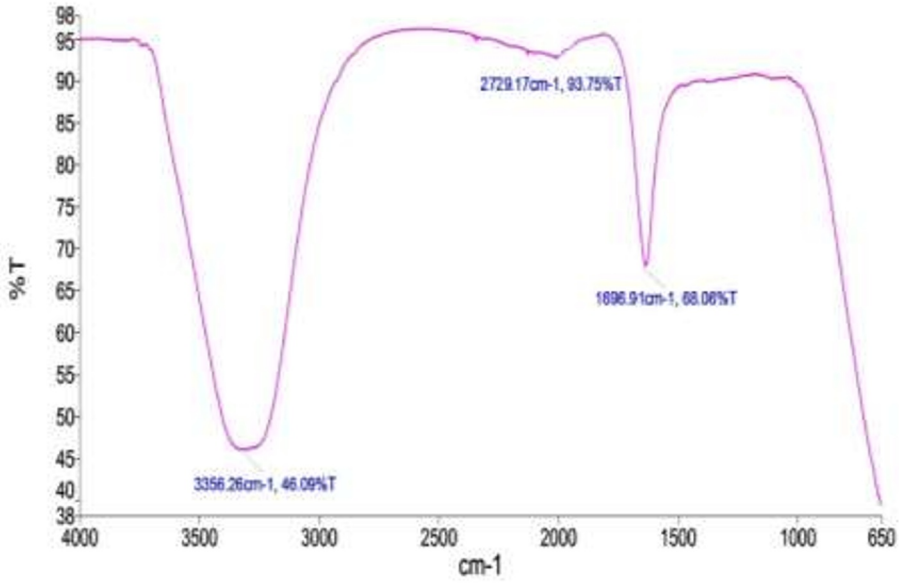

Fig. 2. FTIR-Spectrum of Au NPs

3.3. Scanning Electron Microscope with Energy Spectroscopy

The production of nanoparticles with spherical rod, triangular, and circular structures—all with a diameter of around 40 nm—was shown by the products' SEM absorbance (Figure 3a).Energy dispersive spectroscopy is used for sample evaluation or chemical characterization. The leaf extract of B. cristata exhibits potential in the production of gold nanoparticles. SEM analyses revealed higher concentrations of 40 nm-sized gold nanoparticles with rod, circular, and triangle shapes. EDS revealed higher proportions of pure gold nanoparticles (Figure 3b).

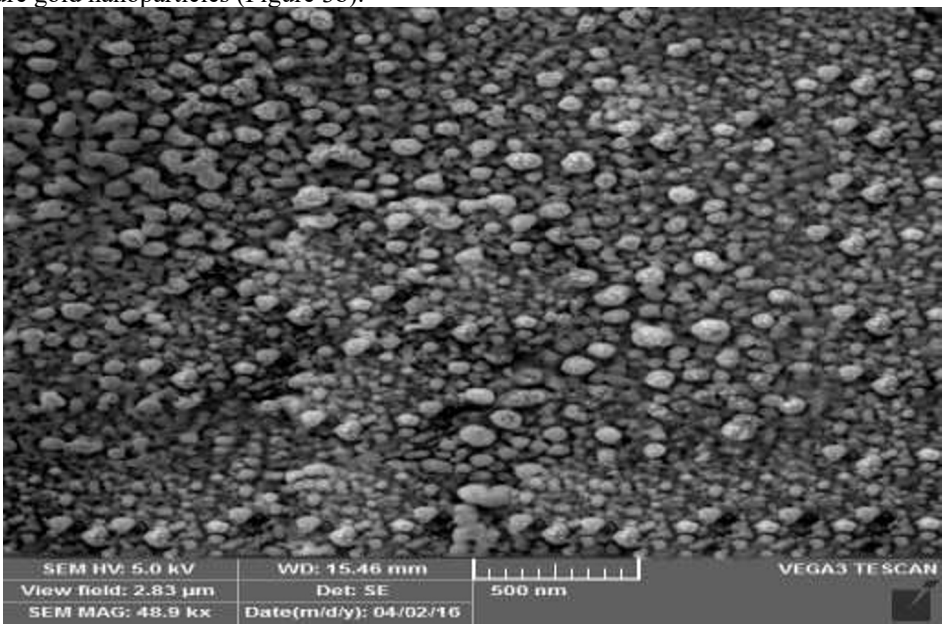

Fig. 3(a): SEM Image of Au NPs

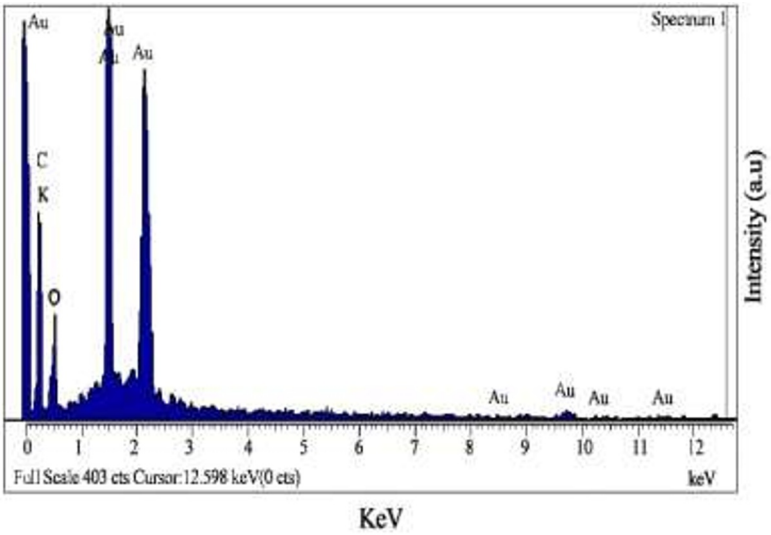

Fig. 3(b). EDAX spectrum of AuNPs

3.4. X-Ray Diffraction Pattern for Biosynthesized Au Nanoparticles

The synthesis of gold nanoparticles was confirmed by the UV-VIS spectrum and an evident colour change in the reaction mixture, both of which were produced using B. cristata. XRD analysis was then used to ascertain the phase distribution, crystallinity, and purity of the generated nanoparticles. An analysis of the synthesised particle's X-ray diffraction (XRD) pattern allowed for the identification of peak profiles of relevant particles. This finding showed peaks at 2θ of 38, 44, 65, and 77; these correspond to Bragg's reflections, which are (220), (111), (200), and (311). There have been sightings of more peaks in addition to the known ones. This might be explained by the extracts' raw material makeup, which contains salts and other metabolites. These variables would have responded with the ionic gold during the synthesis process. These compounds could be the cause of the other peaks' arrangement. (Figure 4)

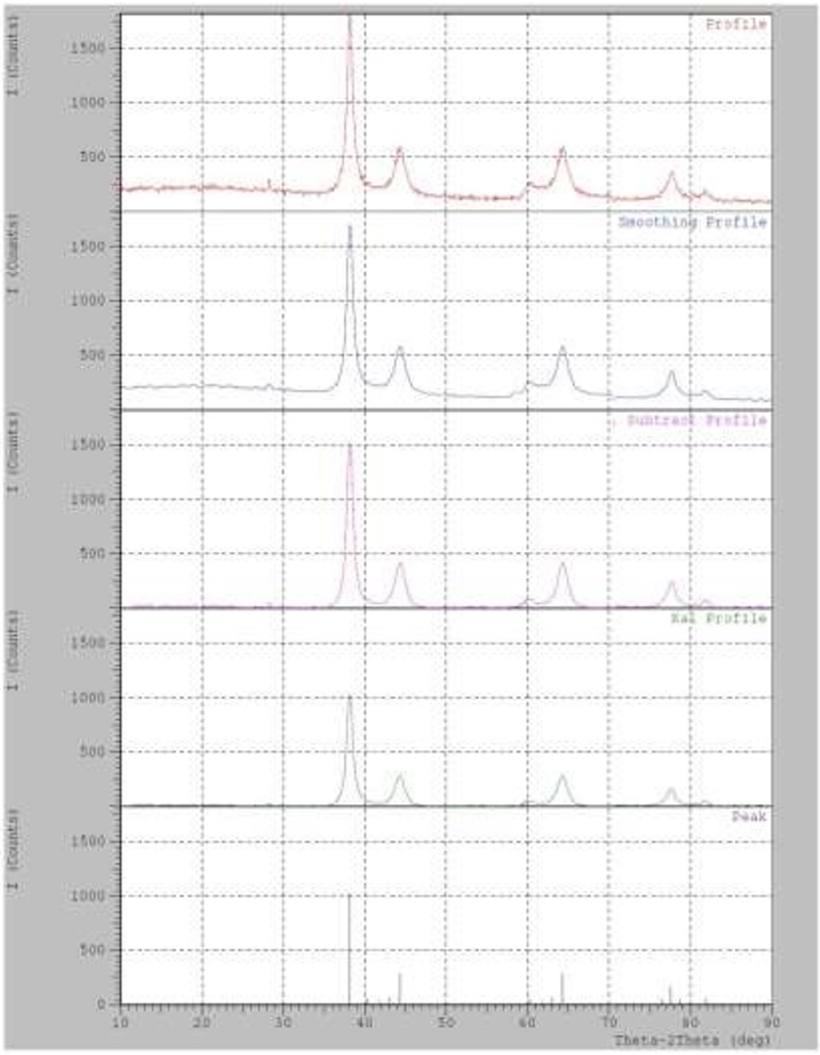

Fig. 4. XRD Pattern of Au NPs

3.5 Field Emission Transmission Electron Microscopy (Fe-Tem) Analyses

The size, shape, and surface morphology of the synthesised Au nanoparticles were examined by FE-TEM analysis. The different magnifications of uniformly distributed, spherical Au nanoparticles with sizes ranging from 10 to 30 nm are displayed in Figure 5 (a–c). The average diameter of the spherical AuNps was measured to be 25.261 nm, with a standard deviation of 9.107 nm. The spherical AuNps were discovered to have a standard deviation of 436.985 nm and an average surface area of 563.742 nm, respectively. P. Vijay Kumar, S. Mary Jelastin Kala (2019), and Maurizio Celentano Anshuman Jakhmola (2018) state that the main cause of the nanostructures' spherical shape is the interfacial surface tension phenomenon. Fig. 5(d), which depicts the selected area diffraction pattern (SAED), shows the four Bragg's reflection planes, which are, respectively, (111), (200), (220), and (311). This is in excellent accord with the reported X-ray diffraction analysis [N. Basavegowda, A. Idhayadhulla (2014)] and validates the creation of a face-cantered cubic lattice structure.

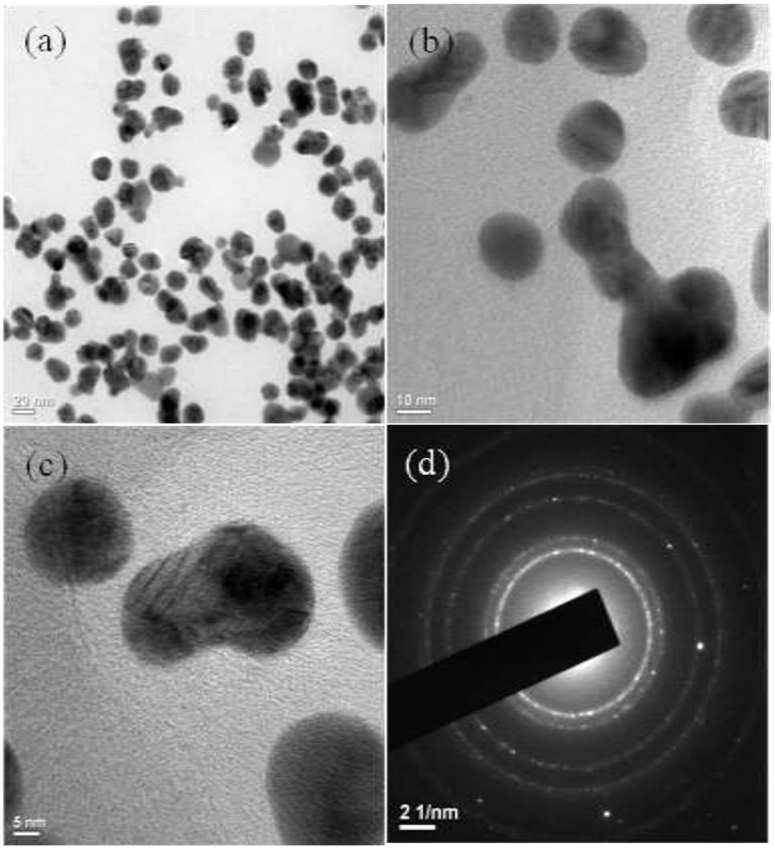

Fig. 5. FE-TEM images at magnification of (a) 20 nm (b) 10 nm (c) 5 nm and (d) SAED pattern of Au nanoparticles.

3.6. Particle Size Distribution and Zeta Potential Analysis

Figure. 6(a) shows the size of Au nanoparticles that were investigated using the Dynamic Light Scattering (DLS) method. The samples had a narrow range of particle sizes, and the Z-average diameter (dz) of the colloidal Au nanoparticles was found to be 93.11 nm. The surface charge, which is influenced by the Au nanoparticle composition, was measured using zeta-potential analysis, as shown in Figure. 6(b). It is utilised to evaluate the electric potential at the boundary of the Au NP double layer. According to an examination of the Au nanoparticles' Zeta potential values, the potential range for these particles was -16.23 to 6.09 mV. This suggests that free negatively charged carboxyl ate groups (COO−) may be present on the surface of the nanoparticles. The positive charge around the Au nanoparticles was found to be caused by quaternary ammonium groups present in the plant extract. The high negative potentials imply that Au nanoparticles are stable in water. [Jennifer Gubitosa Sakai Laksee (2018)].

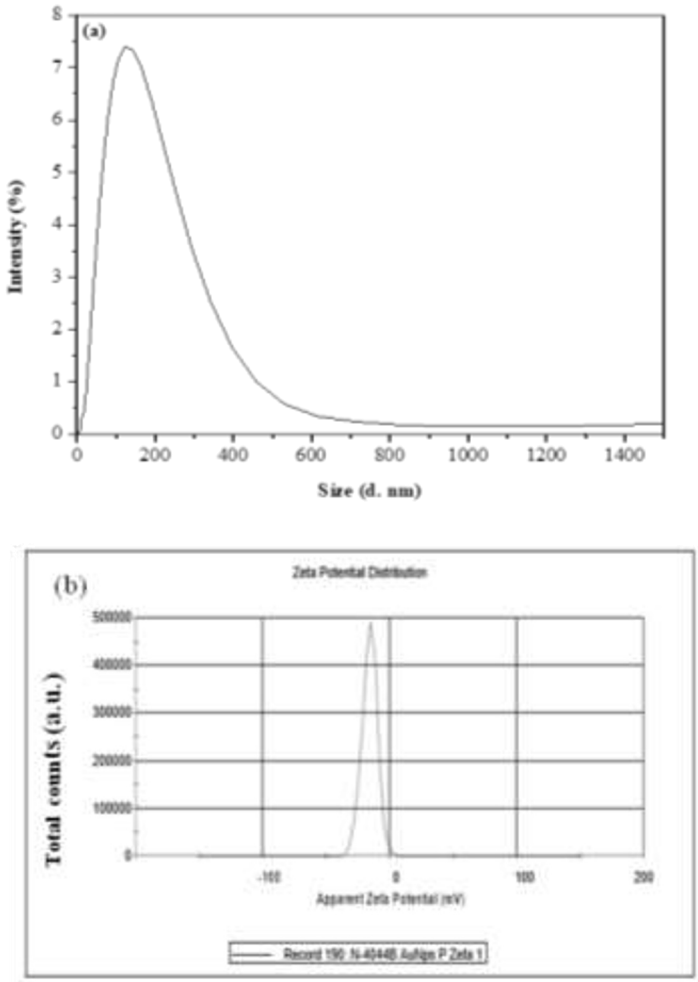

Fig. 6. Au NPs Dynamic Light Scattering (DLS) (a), Zeta-potential analysis of Au NPs Stability (b)

3.7. Anticancer Activity

Au NPs' Cytotoxic effect was tested on human cell lines (HeLa cells) for 48 hours (sample concentration = 0.1 – 50 µL). HeLa cell lines are highly susceptible to the Cytotoxic effects of biosynthesized Au NPs (Graph 7). The Au NPs significantly inhibited the growth of the cancer cells, and this effect varied in terms of dose and duration. By comparing treated and untreated cells, the Cytotoxic activity of the complex was ascertained, and the dosage values of exposure required to reduce survival to 50% (IC50) were established. Au NPs can be used to regulate a cancer cell with a 50 µL sample. The sample's amphiphilic nature, which facilitates entry into cell membranes, lowers energy status in tumours, and alters the hypoxic state of cancer cells, may account for some of its fatal effects. The Cytotoxicity effect on HeLa cells was compared using the traditional anticancer drug 5-FU, and the LC50 value was recorded. Several in vitro studies demonstrate that Au NPs are detrimental to mammalian cells. It's interesting to note that some studies have shown Au NPs can disrupt genes related to the progression of the cell cycle, damage DNA, and induce apoptosis in cancer cells. Indeed, the results of this investigation clearly demonstrate that AuNPs are more lethal to cancer cell lines than to normal cell lines. The anti-angiogenic properties of GNP have been observed both in vitro and in vivo (Koperuncholan and Ahmed John, 2011). GNP was found to interact with fibroblasts, vascular permeability factors (galectins that bind heparin), and cardiac endothelium growth factors. Because these substances mediate angiogenesis, including that which takes place in cancer tissues, GNPs inhibit their function. Given that high angiogenesis—the process of generating new blood vessels in organs or tissues—is regarded to be one of the key causes driving the growth of tumours, the presence of antiangiogenic capabilities in GNPs may make them attractive for use in tumour therapy. Additionally, the same researchers demonstrated that gold nanoparticles promote the apoptosis of chronic lymphocytic leukaemia cells that are stable to programmed death and prevent the proliferation of multiple myeloma cells (Koperuncholan and Ahmed John, 2011a; Koperuncholan and Manogaran, 2015).

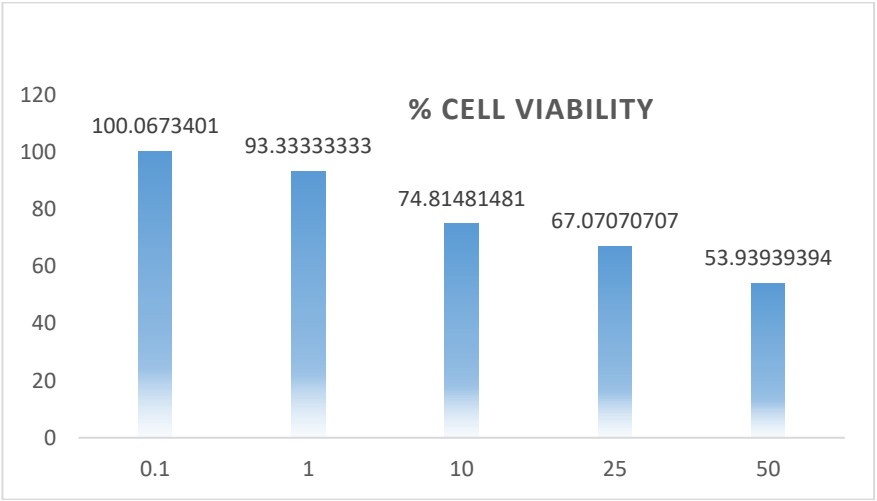

Fig. 7. Anticancer activity of Au NPs

4. Conclusion

Lastly, we describe a rapid, simple, and inexpensive biological method for producing gold nanoparticles from Barleria cristata leaf extract. Plant extracts' quick colour change

provided proof that AuNPs had been produced, and spectroscopic methods including Zeta Potential analysis, UV-visible, FTIR, FE-TEM, SAED, particle size distribution, and SEM were used to describe these nanoparticles. The AuNPs that are biosynthesised from B.cristata leaves exhibit potent antibacterial and anticancer effects when tested against a range of pathogens and human cancer cell cultures, respectively. These biosynthesized gold nanoparticles have a wide range of potential medical applications.

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