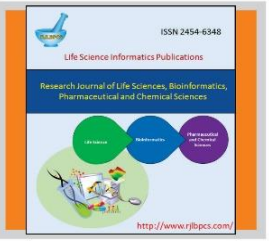

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BIOREDUCTION OF GOLD NANOPARTICLES FOR PHARMACOLOGICAL APPLICATIONS

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ABSTRACT: Nanotechnology pacts with the Nanoparticles having a size of 1-100 nm used knowingly regarding therapeutic interaction, nuclear dynamics, and all other areas. The effect of nanoparticles on the natural environment is of rising concern among environmental experts and the wider community. Gold nanoparticles has usually manufactured from chemicals, which are somewhat toxic and combustible in nature. This survey deals with an environmentally responsive and biosynthesis procedure for pharmacological uses of gold nanoparticles, derived from *Breynia retusa* leaves aqueous extract. The formation and characterization of AuNPs has confirmed by UV-Vis spectrometry, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM) with Energy-Dispersive Spectroscopy (EDX), Dynamic Light Scattering analyses (Particle size and Zeta potential) and X-ray Diffraction (XRD). The antimicrobial and anticancer deeds had run out against some human pathogenic strains and HeLa cancerous cells with different concentrations respectively. The Au nanoparticles inhibited the increase of the microbial pathogens and cancer cells expressively, in a back breaker and a duration dependent manner.

KEYWORDS: *Breynia retusa*, gold nanoparticles, antimicrobial activity, anticancer activity.

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1.INTRODUCTION

Nanotechnology is a tremendously powerful technology; the subject of nanotechnology is one of the upcoming areas of inquiry in the modern study of fabric science. Nanoparticles show completely

new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly in various subjects [1]. Which contains a vast promise for the invention and evolution of many types of novel products with its potential medical applications in early disease detection, treatment and prevention. Nanotechnology applications are extremely suited for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticles synthesis, which has found to be reliable and eco-friendly [2]. Metal nanoparticles have gained a great deal of attention due to their unique chemical, optical, magnetic, mechanical and electric magnetic properties. The synthesis of metal and semiconductor nanoparticles is a huge region of research due to its potential applications, which has implemented in the development of novel technologies [3]. Gold nanoparticles represent a novel class of biocompatible vectors capable of living up to this promise by selective cell and nuclear targeting of which will provide new means for the situation- specific diagnosis and handling of medical conditions [4]. This study outlines the methodology for conjugation of AuNps with target specific biomolecules and details the results of studies evaluating the target specificity and Cytotoxicity effects of thus conjugated gold nanoparticles.

2. MATERIALS AND METHODS

Sample collection

The *Breynia retusa* plant has collected from Tiruchirappalli district, Tamil Nadu, India during November to December 2018.

Aqueous Extraction

The plant materials have 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 has sterilized at 121°C for 15 min and weighed. Sterilized fine powder, 20 g has taken, mixed with 200 ml of Milli Q water and held in boiling water bath at 100°C for 10 minute. The extracts have filtered with Whatman 1 filter paper and the filtered extracts were stored in a refrigerator at 4°C for further works to avoid microbial contamination.

Manufacturing of gold nanoparticles

The gold chloride prepared at the concentration of 10^{-3} M with pre-sterilized Milli Q water. A quantity of 10 ml plant extract has 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 have tightly covered with aluminum foil in order to avoid photo reduction of gold ions, incubated at room temperature under dark condition and observations has recorded.

Characterization of nanoparticles

After AuNPs. formation, it has characterized by UV-vis spectroscopy (UV spec), Fourier transform-infra red spectroscopy (FTIR), Scanning electron microscope (SEM), energy dispersive spectroscopy (EDS), dynamic light scattering (DLS - particles analyzer/ 'Z' potential) methods and X-ray diffraction (XRD) [5].

UV-VIS spectroscopy

The Au nanoparticles has 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 mm/min. Baseline correction of the spectrophotometer has carried away by using a white character.

Fourier transforms-infra red (FT-IR) spectrometry

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

Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

In this research work, Joel JSM-6480 LV SEM machine has used to characterize the mean particle size and morphology of nanoparticles. Compositional analysis on the sample was carried away by the energy dispersive X-ray spectrometry (EDS) attached with the SEM. The EDS analysis of Ag sample has done by the SEM (JEOLJSM 5800) machine. The EDS normally reveals the presence of phases.

Dynamic Light Scattering analyses (Particle size and Zeta potential)

In parliamentary law to get out the particle size distribution the Au powder has spread in water by horn type ultrasonic processor [Vibronics, model VPLP1]. Dynamic light scattering (DLS) which has grounded on the laser diffraction method with multiple scattering techniques has employed to analyze the average particle size of gold nanoparticles. The prepared sample has dispersed in deionized water followed by ultra-sonication. Then solution has filtered and centrifuged for 15 minute at 25^0C with 5000 rpm and the supernatant has collected. The supernatant was diluted in 4 to 5 times and then the atom distribution in liquid was studied on a computer controlled particle size analyzer (ZETA Sizer Nano series, Malvern instrument Nano Zs) to get out the particle size dispersion. Zeta potential describes the electrical voltage in the double layer of ions surrounding a molecule at the limit of the particle surface and the adsorbed ions in the diffuse layer [6]. Zeta potentials has found with a Zetaphorementer IV (CAD, France).

X-ray diffraction method

The phase evolution of calcined powder as good every bit that of sintered samples has studied by X-ray diffraction technique (Philips PAN analytical, The Netherlands) using Cu radiation. The generator voltage and current was set at 40 KV and 30 mA respectively. The Au sample has scanned in the range 10.0000 - 90.0000^{o} in continuous scan mode. The scan rate was 0.60/Sec.

Antimicrobial screening AuNPs

The gold nanoparticles has challenged against certain microbial strains (procured from MTCC and NCIM, India) for antimicrobial sensitivity using the disc diffusion method [7]. The test strains: *Aeromonas liliqueciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumoniae* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporum canis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). A sterile cotton swab has used to inoculate the bacterial and fungal suspension on the surface of MHA and PDA agar plates. The 15 and 30 µL of sample-coated disc has placed on agar plates, one by one. For the negative control study, the sterile triple distilled water has used. The plates has incubated at 37±1°C for 24–48 h (for bacteria) and 25±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition has measured with a ruler. All the trial has performed thrice and mean values have shown.

Determination of Anticancer activity

The AuNPs was dissolved in DMSO, diluted in culture medium and used to handle the chosen cell line (Hela) (obtained from NCCS) over a sample concentration (5 different concentrations – 0.63, 1.25, 2.5, 5, 10 %) range of 0.63 - 10 % for a period of 24 h. The DMSO solution has 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) has carried out according to the method described by standard procedure [8]. To each well, 20 µl of 5 mg/ml MTT in phosphate-buffer (PBS) has added and wrapped with aluminum foil, and incubated for 4 h at 37 ^{0}C. The purple formazan product has dissolved by the increase of 100 µl of 100 % DMSO to each good. The absorbance has monitored at 570 nm (measurement) and 630 nm (reference) using a 96 well plate reader (Bio-Rad, Hercules, CA, USA). The information has gathered for four replicates. Each and used to calculate the respective way. The part of inhibition has calculated, from this information, using the formula:

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

The IC50 value has seen as the complex concentration that is needed to reduce the absorbance to half that of the dominance.

3. RESULTS AND DISCUSSION

Biosynthesis of Au nanoparticles

The plant aqueous solution and gold chloride solutions have developed separately. The plant extract has mixed with gold chloride for the synthesis of gold nanoparticles. During this operation, the colour has varied from pale greenish to pink colour, suggested that formation of gold nanoparticles [9].

UV-VIS Spectral analysis

The UV-VIS spectroscopy studies revealed the presence of beard peaks at 540 nm (Figure 1). The absorption spectra of Au nanoparticles formed in the reaction media have absorbance maxima at 540 nm [10]. A remarkable broadening of peak at around 480 nm to 680 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 increments. The strength of the absorption peak increases with increasing time. This characteristic colour variation is due to the inflammation of the SPR in the metal nanoparticles [11]. The diminution of the metal ions occurs rapidly; more than 90% of reduction of Au+ ions are complete within two Hrs. After addition of the metal ions to the plant selection. The metal particles have observed to be stable in solution even 4 weeks after their synthesis. By stability, we imply that on that point has no observed variation in the optical properties of the nanoparticles solutions with time. On the behalf of UV-vis data, it has cleared that reduces metal ions [12].

Fourier Transform Infra-Red (FTIR) Spectroscopy

The synthesized gold nanoparticles has subjected to FT-IR analysis to discover out the bioactive compounds synthesized by the plant and associated with the nanoparticles. The FTIR images of the Plant samples show a bit of functional bonds associated with them, which provide them with stability by capping them. From figure 2, 676 cm-1, 1651 cm-1, 2091 cm-1, 2368 cm-1, 3448 cm-1. The 676 cm-1 corresponds to the C-OH out-of-plane bending, 1651 corresponds to the C=O bond, 2091 cm-1 and 2368 cm-1 corresponds to the C-N bond, 3448 corresponds to the N-H bond. Therefore, the synthesized nanoparticles has surrounded by proteins and metabolites having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the potent ability to bend metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; Capping of gold nanoparticles) to prevent Agglomeration and thereby stabilize the medium. This indicates that the biological particles could possibly do the dual purposes of organization and stabilization of gold nanoparticles in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles. Flavanones or terpenoids could adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating Agents in

sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles [13]. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehyde groups in the molecules to carboxylic acids. These issues can addressed once the various fractions of the plant extract has separated, identified and individually assayed for reduction of the metal ions [14]. This rather detailed study is presently underway.

Scanning Electron Microscope (SEM) and Energy Dispersive Spectroscopy (EDS)

The SEM image of gold nanoparticles synthesized by green synthesis process by using 5 % leaves extract and 1mM HAuCl4 concentration it gave a clean image of highly dense gold nanoparticles. The SEM image showing gold nanoparticles synthesized using plant extract confirmed the development of gold nanostructures (Figure 3). The EDS reading proved that the mandatory phase of gold (Au) and potassium (K) is present in the sample. It has discovered the presence of pure gold nanoparticles in higher parts than other agents have. This is probably due to the presence of substrate over which the NP sample has held during SEM microscopy (Figure 4). As EDS equipment works at low vacuum (1-270 pa) it allows to keep non-conducting samples without the need to handle them with a thin conductive film, and consequently no evidence of interference by the coating material [15].

Dynamic Light Scattering of Particle Size analyzer

The Figure 5 shows the particle size of the Au nanoparticles samples. After analysing data, it was found that the graphical representation of the average particle size distribution of Au nanoparticles. They were in an orbit of 20-80 nm. However, beyond 100 nm range the percentage of nanoparticles present is high. The highest fraction of Au-NPs present in the solution was 30 nm. From the plot, it was apparent that the solution has consist of nanoparticles having various sizes, which are indeed in agreement of the result obtained by SEM analysis [16].

Dynamic Light Scattering of Zeta Potential Measurement

The Figure 6 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and has related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nanogold. The overall absorbance of Zeta Potential revealed the energetically moderate stable. Therefore, the particles undergo agglomeration/ aggregation to stabilize themselves. Therefore, 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.

XRD analysis

The XRD image of the sample after the addition of the gold chloride hydrate has depicted (Figure 7). Its represents the XRD pattern of the produced gold nanoparticles. The position at 38.1, 44.2, 64.4,

and 77.6 in peak pattern represents presence of gold and the value is consistent. Although the Figure.7 is in agreement with Bragg's reflection values at 2Θ, the produced gold nanoparticles exhibit irregular morphology. The XRD patterns clearly show that the nanoparticles are crystalline in nature.

Antimicrobial studies

The antimicrobial activity assay is the AuNPs has challenged against various NCIM and MTCC microbes using the disc diffusion method. The test concentrations (15 and 30 µL/disc) produce zone on MHA and PDA plates for bacteria and fungi, respectively. The sample has most effective against *Salmonella typhimurium* NCIM 2501 (B5) while smaller effect has noticed from *Micrococcus luteus* NCIM 2871 (B4) in the bacterial division. Nevertheless, in fungi, which has effective against *Trichophyton rubrum* MTCC 3272 (F4) whereas smaller effect has not observed. The higher (30 µL/disc) concentration got larger zone effect than the small (15 µL/disc) concentration against certain microorganisms. All the microbial strains depict higher sensitivity to the higher concentration (30 µL) for the test sample when compared to the positive control except B3, B4 and B6. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity has directly related to the sample (Table 1). The gold nanoparticles not only interact at the surface of cell membrane, but also enter inside the bacteria and cause damage of the cells by interacting with phosphorus/ sulphur containing DNA and its replication [17]. In bacteria, the test sample has most effective against B5 while smaller effect has noticed from B4. In fungi, this has effective against F4 whereas smaller effect has observed in F2. All the microbial strains depict higher sensitivity to the higher concentration (30 µL) and the concluded that the silver materials are an efficient alternative to antibiotics for the treatment. This nanoparticles release gold ions in the bacterial cells, which enhance their bactericidal activity [18],[19],[20]. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (sterile triple distilled water), reflecting that antimicrobial activity has directly related to the sample.

Anticancer studies

The cytotoxic effect of the AuNPs has examined on HeLa cell lines. (Sample conc. = 0.63– 10%). The cytotoxicity effect is very high in biosynthesized AuNPs in all concentrations against HeLa cell lines. 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 10 % sample has control cancerous cell. 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 alter hypoxia status in the cancer cell. **(**Figure 8). 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 alter hypoxia status in the cancer cell. The cytotoxicity effect was compared with the standard anticancer drug 5-FU against HeLa cells and their LC_{50} value was observed [21], [22], [23]. Similarly, cytotoxicity of chemically synthesized AuNPs against HeLa cells [24], [25], [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 cell]s [27],[28],[29], [30]. Indeed, the results of present study provide conclusive evidence for cytotoxic effect of AuNPs on cancer cell lines rather than normal cell lines.

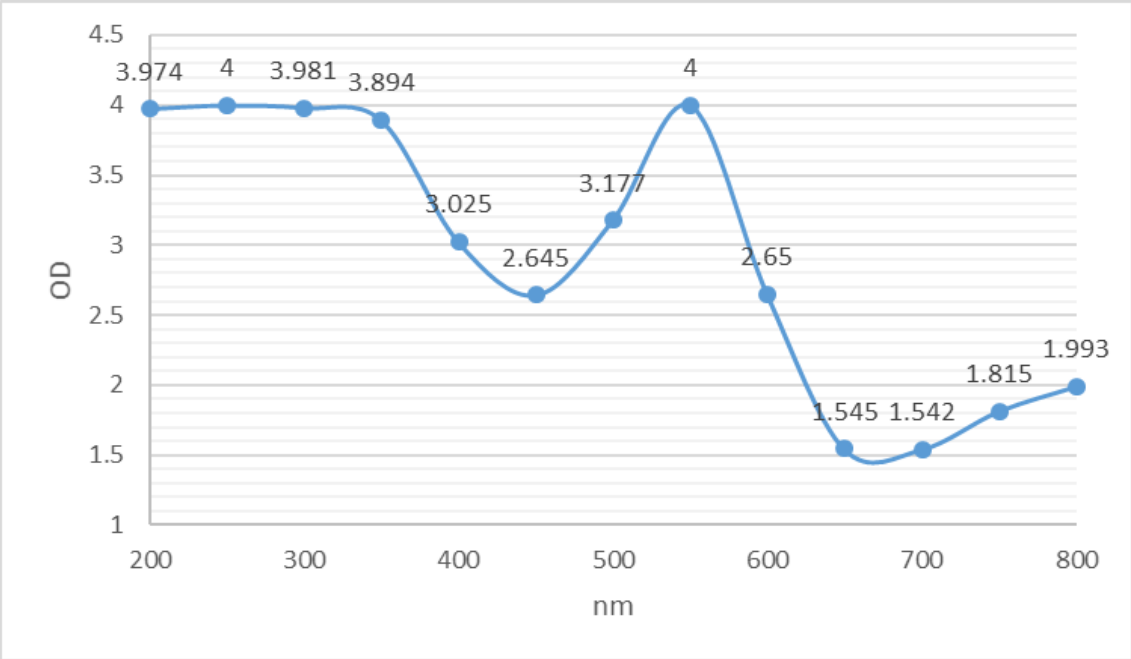


Figure 1: UV-Spectrum of AuNPs

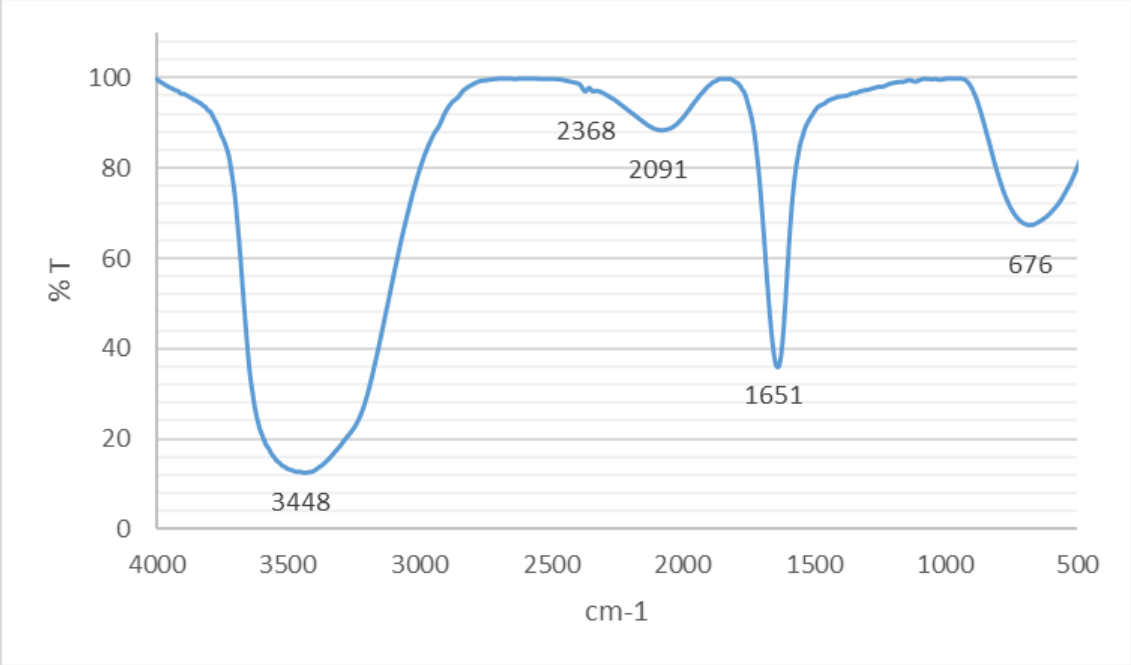


Figure 2: FTIR spectrum of AuNPs

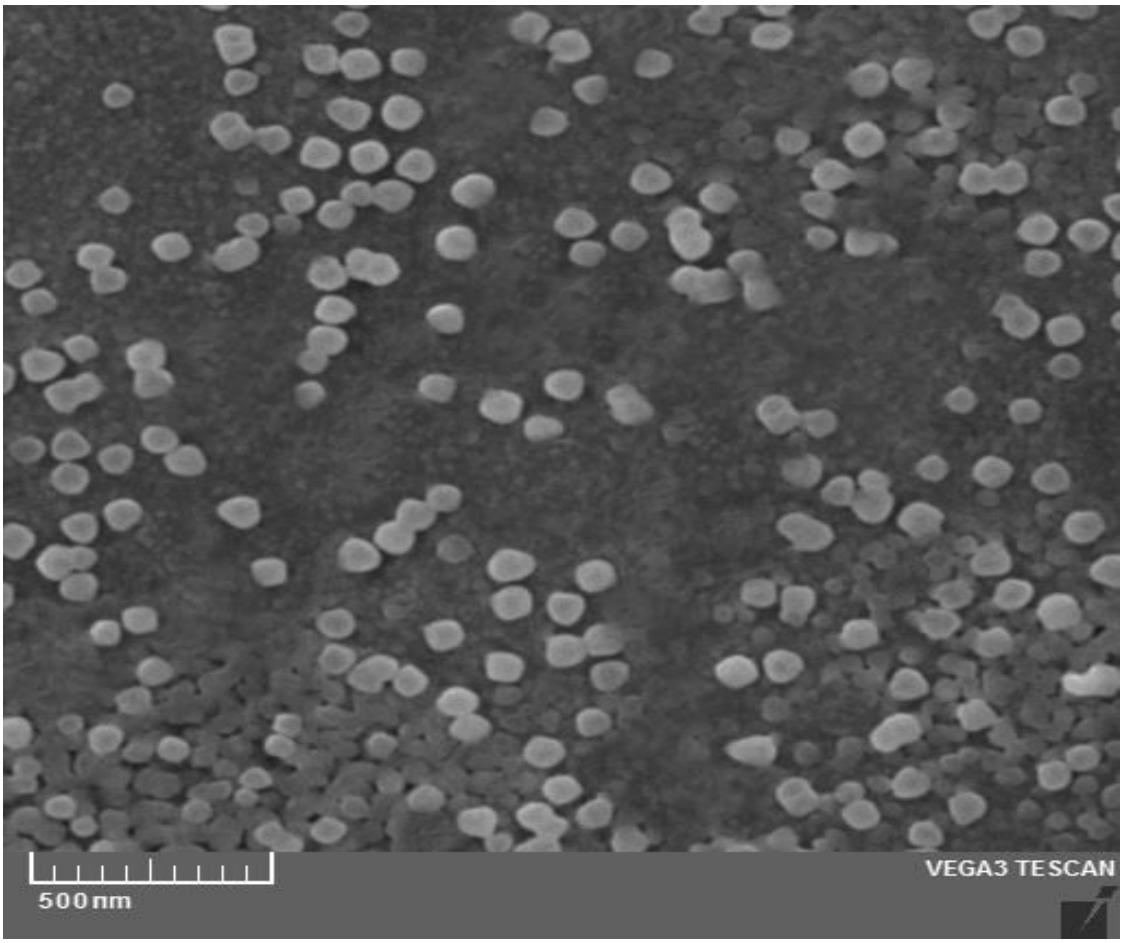


Figure 3: SEM Image of AuNPs

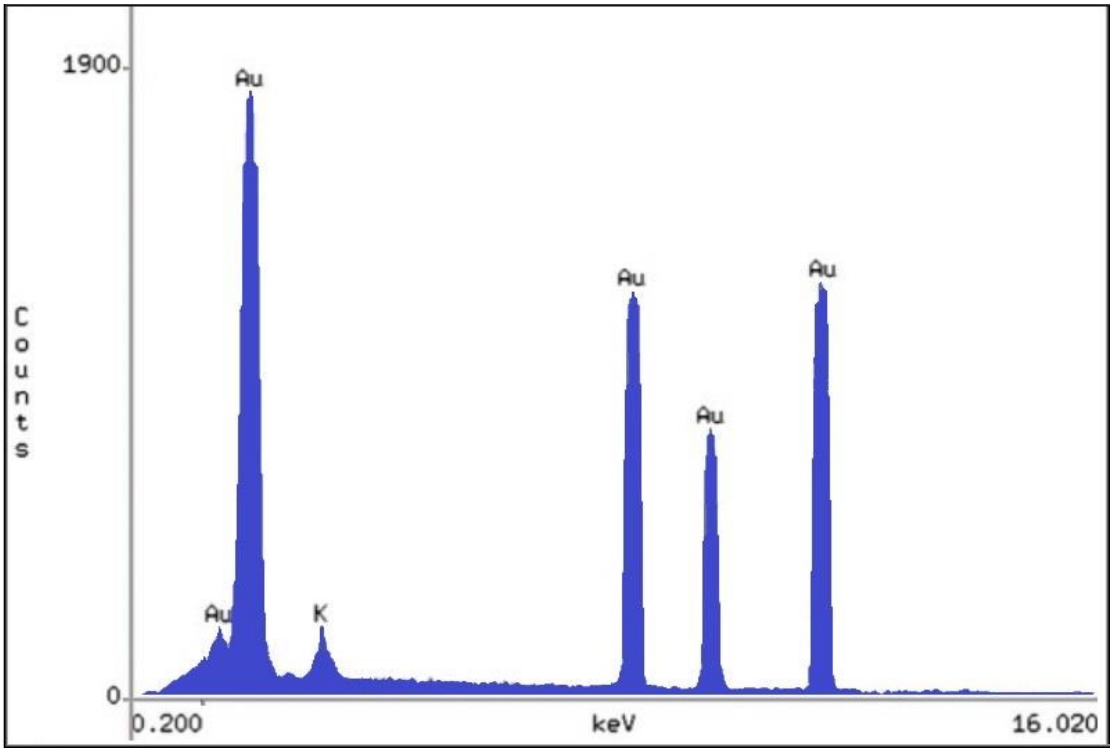


Figure 4: EDAX Spectrum of AuNPs

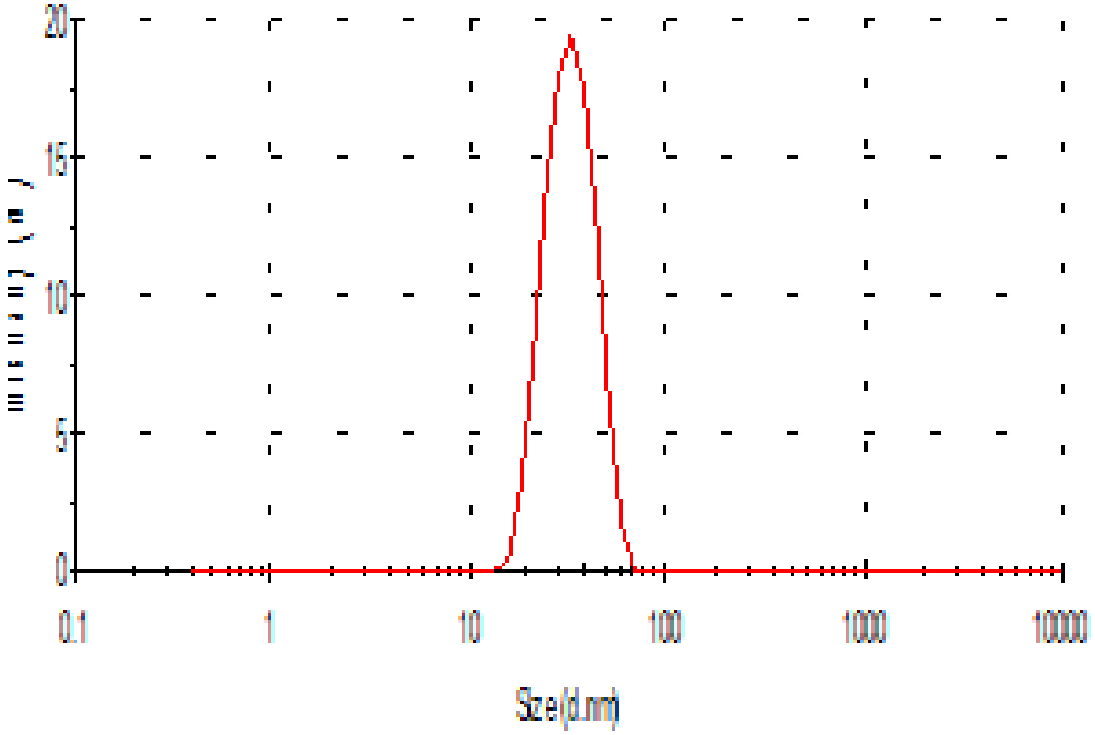


Figure 5: DLS-Size distribution of AuNPs

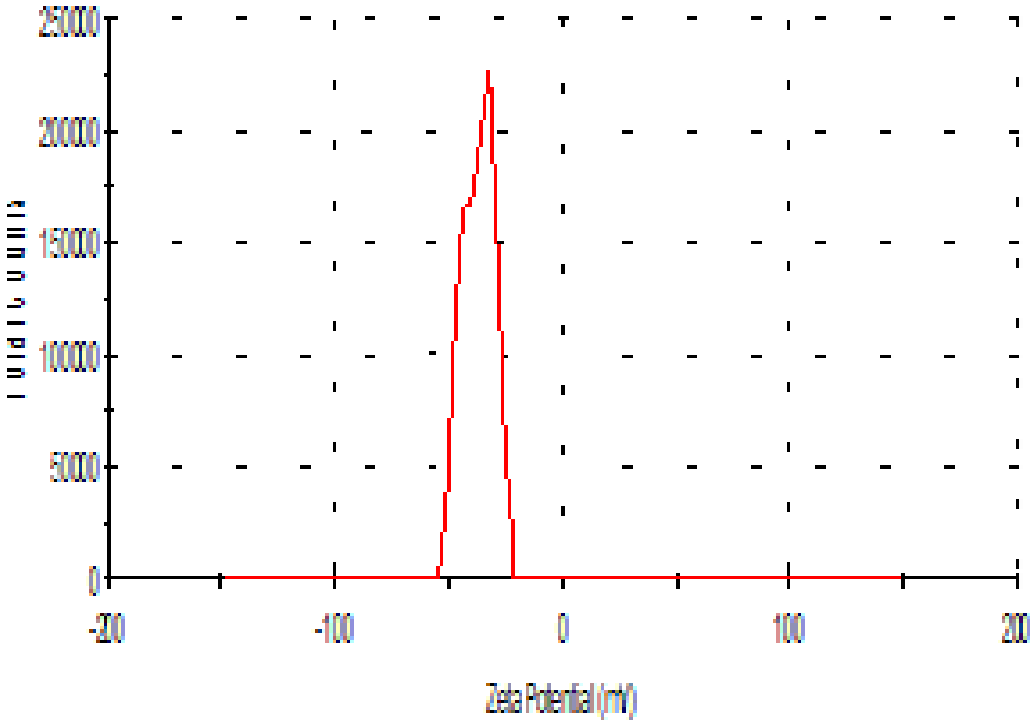


Figure 6: DLS-Zeta Potential of AuNPs

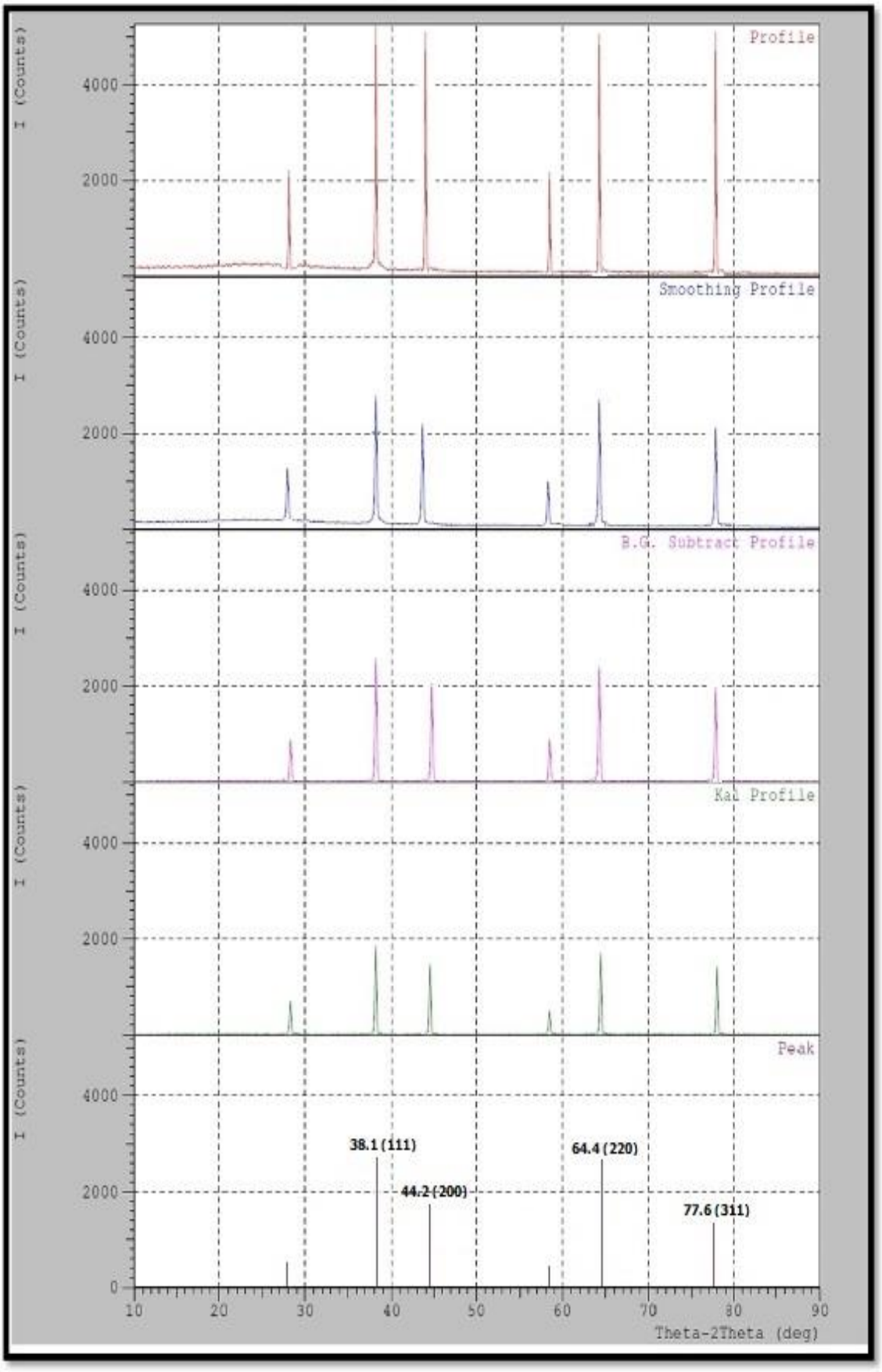


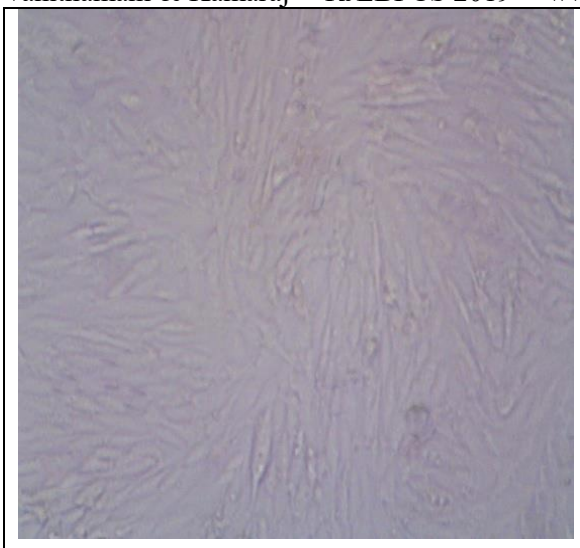
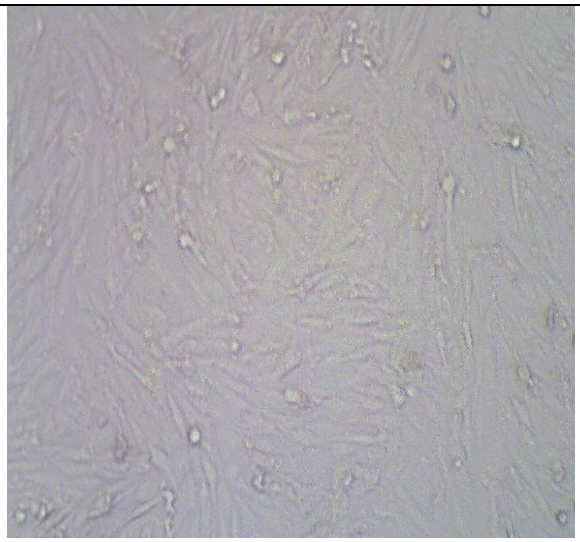
Figure 7: XRD charaterization of AuNPs

Table 1: Antimicrobial activity of AuNPs

S.No	Test Microorganisms	AuNPs µL/disc		PC	Diseases	Route of Transmission
Bacteria		15	30	10 mcg		
1.	*Aeromonas liquefaciens* (B1)	12	13	14	Wound Infections / Gastroenteritis	Water / Food
2.	*Enterococcus faecalis* (B2)	11	13	8	Endocarditis / Epididymal Infections	Water / Food
3.	*Klebsiella pneumoniae* (B3)	16	20	28	Acute diarrhoea / Dysentery	Water / Food
4.	*Micrococcus luteus* (B4)	14	16	38	Skin & Pulmonary infections	Soil / Water / Air / Food
5.	*Salmonella typhimurium* (B5)	13	15	0	Typhoid	Water / Food
6.	*Vibrio cholerae* (B6)	10	12	16	Cholera	Water / Food
Fungi						
7.	*Candida albicans* (F1)	12	14	10	Skin infection / Gastrointestinal tract Infection	Air / Wound / Soil / Water
8.	*Cryptococcus* sp. (F2)	10	12	9	Bronchiectasis / Endophthalmitis.	Air / Wound / Soil / Water
9.	*Microsporum canis* (F3)	13	15	9	Tinea capitis /Ringworm	Air / Wound / Soil / Water
10.	*Trichophyton rubrum* (F4)	11	14	7	Tinea corporis / Tinea pedis	Air / Wound / Soil / Water

PC - Positive Control (Using antibiotic disc; Bacteria – Methicillin (10mcg/disc); Fungi – Itraconazole (10mcg/disc); Samples – 15, 30 mg/ml (well)

Graph 1: Anticancer activity (MTT Assay)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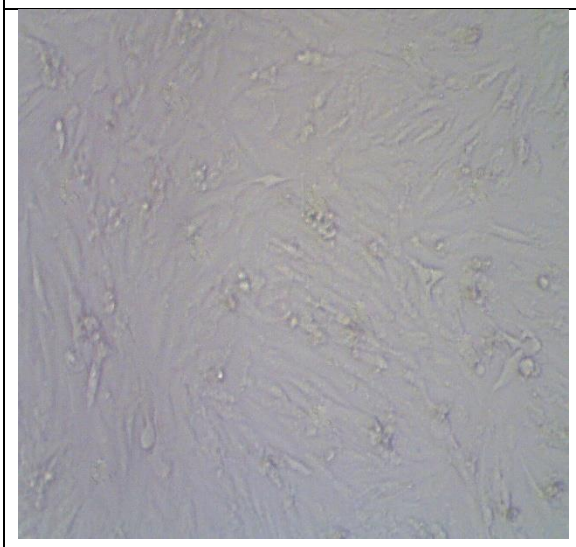	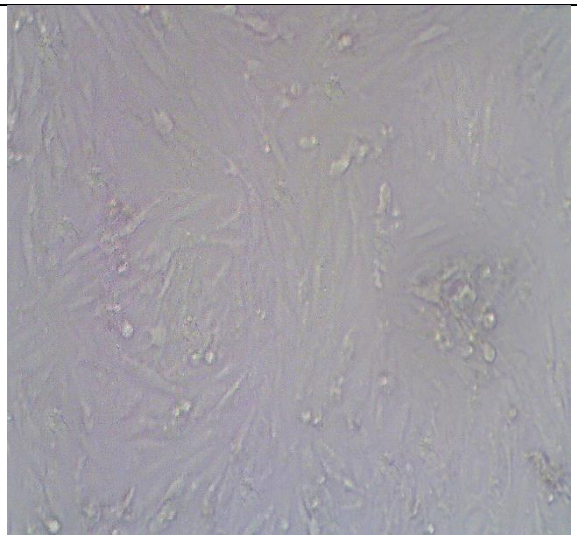
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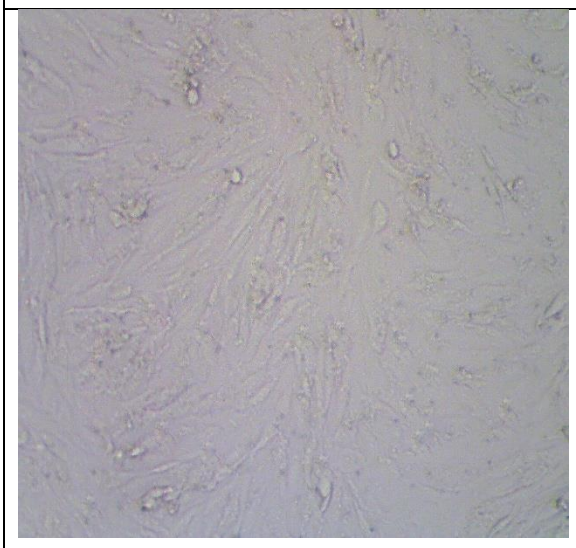	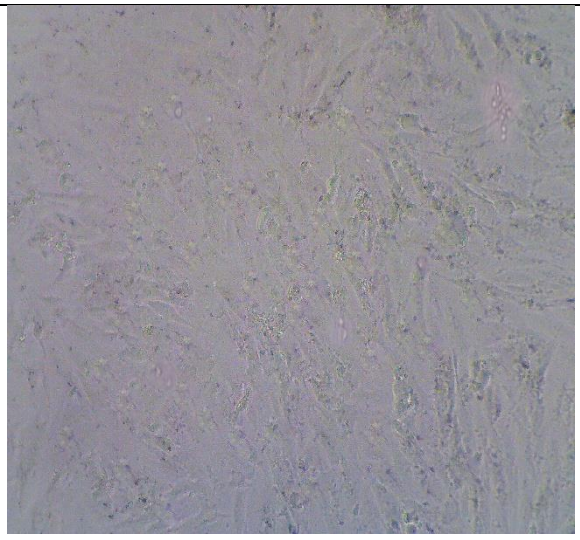
5 %	10 %

Figure 8: Anticancer activities of AuNPs

4. CONCLUSION

In our present investigation, we carried an in-depth survey on the synthesis and characterization of gold nanoparticles and their application on biological systems. At nanoscale, gold exhibits remarkably unusual physical, chemical and biological attributes. Effective green synthesis of nanoparticles will have greater import and application in antimicrobial and anticancer research. From this work, we found that the *Breynia retusa* will be promising modern medicine in the near future.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

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