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GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLES FROM *ALPENIA PURPURATA*

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Abstract

Nanotechnology is a broad interdisciplinary area of research, development and industrial activity which has grown very rapidly all over the World for the past decade. The development of an eco-friendly process for the synthesis of nanoparticles is an important and emerging area in the field of nanotechnology. In the present study, silver nanoparticles (AgNPs) were synthesized by using aqueous leaf extract of the medicinal plant *Alpenia purpurata* (Vieill). The synthesized AgNPs were initially noticed through visual color change from yellow to reddish brown. This is confirmed by UV–visible spectroscopy. The spectra of the reaction medium containing silver nanoparticles showed maximum absorbance at 437 nm. Morphology and size of AgNPs were determined by transmission electron microscopy (TEM). The prolonged stability of AgNPs was due to capping of oxidized polyphenols and carboxyl protein which was established by fourier transform infrared spectroscopy (FTIR) study. The silver nanoparticles have shown anticancer activity against PA 1 ovarian cancer cell lines. The synthesized AgNPs show good cytotoxic activity. The out comes of this study indicate that these nanoparticles could be effectively utilized in pharmaceutical, biotechnological and biomedical applications.

Keywords: *Alpenia purpurata, MTT, TEM, SEM, Silver nano particles, Cytotoxic activity*.

Introduction

Nanotechnology is the engineering of functional systems at the molecular scale. The synthesis of silver nanomaterials or nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions [1]. Medicinal plants play a very important role in clinical applications, hence they are assumed to be effective and safe. Research on medicinal plants has shown that nanotechnology could offer new solutions in the quality control and delivery aspects of herbal compounds. Nanotechnology is partly built on the principles of solubility of chemicals which can be manipulated to yield a product from a variety of solvents, containing the desirable concentration or quantity of bioactive ingredients. Such chemical engineering technique is going to overcome difficulties in obtaining uniform bioactive extractions from medicinal plants. In medicines, silver and silver nanoparticles have an ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds [2]. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for green nanotechnology [3]. The family Zingiberaceae comprises more than 1200 species that are native to tropical regions and many of these are valued as ornamentals or employed as raw materials in the production of fibre, paper, dyes, foods, spices and perfumes. *Alpinia* is the largest genus of the family with more than 200 species [4]. Plants belonging to Zingiberaceae (Ginger family) are known for a number of medicinal properties [5-7]. Spectrums of essential oils are present in the members of Zingiberacae [8]. Rhizome exract of some members of the medicinal Zingiberales are widely used in dietary intake as well as in traditional systems of medicine [9]. *Alpinia* is the largest genus in ginger family in which *A.purpurata* (Vieill.) K .Schum. is a very popular garden plant in India [10]. Rhizome has sharp odour, improves appetite, taste and voice. It is also used for headache, rheumatism, sore throat and renal disease [11]. Phytochemical studies on *A.purpurata* revealed that it possess flavonoids, rutin, kaempferol-3-rutinoside and kaempferol-3-oliucronide [12]. The phytochemical constituents of *A.purpurata* promote antimicrobial activity against certain microorganisms [13]. In addition to the purported anti-inflammatory activity, its phytomedicinal potential to treat tuberculosis is also described [14]. *A.purpurata* may serve as potential antioxidant and anticancer agents against ovarian cancer cell lines [15]. In the concept of using plants for the synthesis of nanoparticles to increase medical and pharmaceutical applications the present study was undertaken in producing silver nanoparticles using *A. Purpurata*.

Materials and Methods

Preparation of extract

The whole plant of *Alpenia purpurata* were collected from Palakad, Kerala. Washed in sterile water to remove any dirt or other unwanted objects, shade dried and preserved. The plants were authenticated by Dr. G.V.S. Murthy, Botanical Survey of India, Coimbatore. A voucher specimen has been deposited in the laboratory for future reference respectively. 1 g of plant powder was mixed with 100 ml of water and kept on orbital shaker at 120 rpm for 12 h. After that, the extracts were filtered with Whatman No. 1 filter paper and stored at 4°C in refrigerator until further use.

Biosynthesis of silver nanoparticles (AgNPs)

AgNPs were synthesized following the procedure of [16] with slight modification. AgNPs were synthesized by mixing aqueous $AgNO_3$ solution (1 mM) and plant extracts in the ratio of 1:1 and incubating the mixture at room temperature for 6 h. Following incubation, the AgNPs formed were collected by centrifugation at 18,000 rpm for 20 min. The collected pellet was washed three times with double distilled water, transferred to a Petri plate and dried at room temperature.

Characterization of AgNPs

UV–Vis spectra analysis

The bioreduction of Ag^+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring UV– Vis spectra of the resulting diluents. UV–Vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on Shimadzu 1700 pharm spec UV spectrophotometers at a resolution of 1 nm.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using instrument LEO 1420 VP Scanning Electron Microscopic. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5min.

TEM analysis of silver nanoparticles

Transmission Electron Microscopic (TEM) analysis was performed with 200 keV Transmission Electron Microscope (JEOL, Ultra high resolution), PP resolution: 0.19 nm. Thin film of the sample were prepared on a carbon coated grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper. Later on, film on the TEM grid was allowed to dry by placing it under a mercury lamp for 5 minutes for the characterization of size and shape of synthesized silver nanoparticles [17].

FT-IR analysis

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by Shimadzu 8400S, FT-IR spectrophotometer. The sample is gently mixed with 100 mg of micronized Potassium bromide powder and compressed into a disc shaped holder. For each spectrum a 256-scan interferogram was collected with a 4 cm^{-1} resolution in the mid-infrared region at room temperature [18].

EDX analysis of silver nanoparticles

To gain further insight in to the features of the silver nanoparticles, analysis of the sample was performed using Energy Dispersive microanalysis techniques. EDX analysis was carried to confirm the presence of silver in the particles as well as to detect other elementary compositions of the particles.

Determination of anticancer activity by MTT assay

Cell growth inhibition was determined by MTT assay [19]. The human lung cancer cell line (A549) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium (EMEM) containing 10% fetal bovine serum (FBS). The monolayer cells were detached with trypsin-EDTA to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with a medium containing 5% FBS to give a final density of $1x10^5$ cells/ml. 100µl/well of cell suspension were seeded into 96-well plates at a plating density of 10,000 cells/well and incubated for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours, the cells were treated with serial concentrations (10.0, 5.0, 1.0 and 0.1 µg/ml) of AgNPs. The

nanoparticles were initially dissolved in dimethylsulfoxide (DMSO) and diluted to twice the desired final maximum test concentration with serum free medium. Additional four dilutions were made to provide a total of five sample concentrations. Aliquots of 10 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulted the required final sample concentrations. The absorbance was read at a wave length 595nm using a microtitre ELISA plate reader. Following sample addition, the plates were incubated for an additional 48 hours. Experiments for extract were carried out in triplicate including untreated cell control and blank cell – free control. Cell viability was expressed as percentage over the control.

Statistical analysis

All experiments were done in duplicate, and the results were presented as Mean ± Standard deviation. The experimental data were analyzed by using SPSS.

Results and Discussions

UV–Vis spectra analysis

It is generally recognized that UV-VIS spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspension [20] which proved to be a very use full technique for the analysis of nanoparticles in figure 1 shows the UV-Vis spectra. It is observed that the maximum absorbance of Ag nanoparticles occurs at 437nm. The presence peak at 425nm confirms silver in the nanoscale range [21] . The absorption peaks are found to increase with the time which is in accordance with the color change observed in visual examination [22].

FTIR analysis

FTIR analysis was carried out using Shimadzu. Figure 2 shows the FTIR analysis of the Silver nanoparticles. The aqueous extract of *Alpenia purpurata* a very strong absorption band observed around 3400.50 cm^{-1} and 3383.14 cm^{-1} due to the presence of bonded N-H and O-H group. Absorption bands observed around 1618.28 cm^{-1}, 1384.89 cm^{-1}, 1087.85 cm^{-1} due to the presence of N-H, -C-H- and C respectively. It is used to probe the chemical composition of the surface of the AgNPs and the local molecular environment of the capping agents on the nanoparticles. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared and placed in fourier transform infrared (FTIR) for the analysis of the nanoparticles [23]. FT-IR spectral measurement was carried out to identify the potential biomolecules in *Alpenia purpurata* extract that handles reducing and capping the bioreduced silver nanoparticles. IR spectroscopic study confirmed that the plant extract has the ability to perform dual functions of reduction and stabilization of AgNPs.

SEM analysis

Figure 3 shows the scanning electron micrographs of AgNPs obtained from the proposed green synthesis (bioreduction) method. Scanning electron microscopy provided further insight into the morphology and size details of the AgNPs. SEM image shows high-density Ag nanoparticles synthesized by *Alpenia purpurata.* It was shown that relatively spherical and uniform Ag nanoparticles were formed with diameter of 13–51 nm. In the SEM images, the assembling of AgNPs is shown on the surface. Similarly monodispersed silver nanoparticle was reported by using the extract of *Coccinia grandis* leaf extract [24].

TEM analysis

Scanning under TEM (Philips CM-10) revealed that the size of silver nanoparticles was 18-20 nm and the tiny particles were seemed to be spherical in morphology as shown in the following images Figure 4 (a). Synthesized silver nanoparticles were mostly spherical and their dimensional ranges were from 2 to 10 nm. Thus TEM characterization studies confirm that the synthesized silver nanoparticles were in nanometer size range. Figure 4 (b) showed the selected area of electron diffraction pattern (SAED) of the synthesized AuNPs showing the rings. Figure 4

(c) shows the energy dispersive X-ray (EDX) spectrometers confirmed the presence of elemental silver signal of the silver nanoparticles. The EDX spectrum revealed that the nanostructures formed were solely of silver.

Anticancer activity

Cell viability was measured using MTT assay. *In vitro* cytotoxic activity against PA 1 at different concentration was evaluated. The invitro screening of AgNPs showed potential cytotoxic activity against PA 1 (human ovarian cancer cells). Figure 5 showed that PA 1 cells proliferation were significantly inhibited by AgNPs with an IC_{50} value of 78.2 µg/ml and plant extract alone showed 93.5 µg/ml. The plates were observed under an inverted microscope to detect the morphological changes is expressed in figure 6. Cell growth was inhibited and eventually the cell death occurred and aggregated to form round dead cells at the highest concentration. The results of this study suggest that the cytotoxicity of biologically synthesized AgNPs increased with the increasing concentration of nanoparticles. AgNPs of certain non-irregular shapes can be adsorbed readily to the surface of the biomolecules, which show higher surface plasmon resonance and will have a greater contrast effect than those of photothermal dyes that are used regularly in the detection of cancer cell lines [28, 29]. These results demonstrate that silver nano-particles mediate a dose and time dependent increase in toxicity. Compounds possessing antiangiogenic properties are known for their potential ability to block the activity of abnormally expressed signaling proteins [30].The cytotoxic effect of SNP on cell viability has a major role in antitumor activity, thereby reducing disease progression. The cytotoxic effects of silver are the result of active physiochemical interaction of silver atoms with functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA [31].

Conclusion

In this study, *Alpenia purpurata* plant extract has the ability to synthesize the nanoparticles. Nanoparticles synthesis initiated within 10 min and gradually increased up to 16 h. The size of the silver nanoparticles ranges from 50 to 70 nm, predominantly spherical shapes with crystalline nature. Elemental analysis by EDX shows that strong peak at 3 keV confirms the presence of silver nanoparticles. Carboxyl and amine groups from plant extract may be involved in the bioreduction process of silver ions to nanoparticles and stabilizing mechanism confirmed by FT-IR analysis. The silver nanoparticles show high anticancer against ovarian cancer cell lines PA 1. This green synthesized nanoparticle could be used in the medical field against human diseases. The use of plant extracts for making silver nano particles is an inexpensive, easily scaled up and environmentally benign. It is especially suited for making nanoparticles that must be free of toxic contaminants as required in therapeutic applications. The plant extract based synthesis can provide nanoparticles of a controlled size and morphology.

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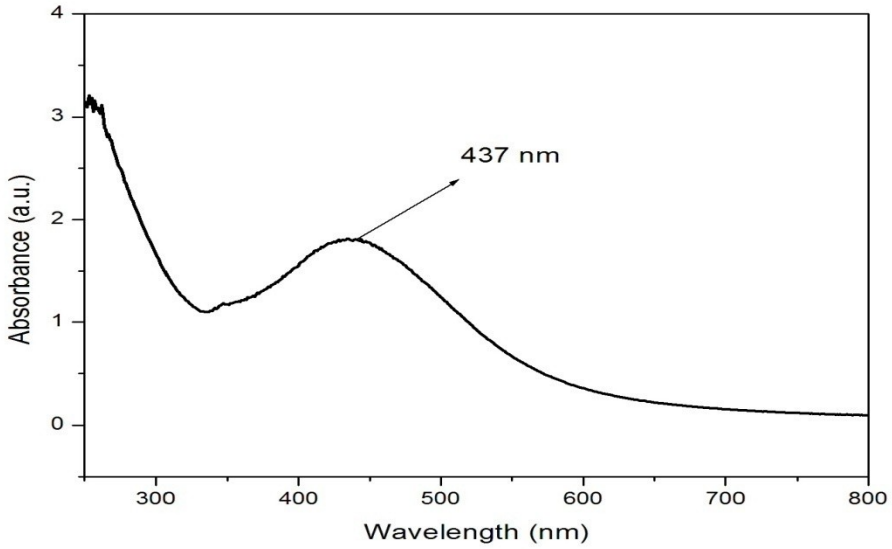

Figure 1. UV – Vis absorption spectrum of synthesized AgNPs

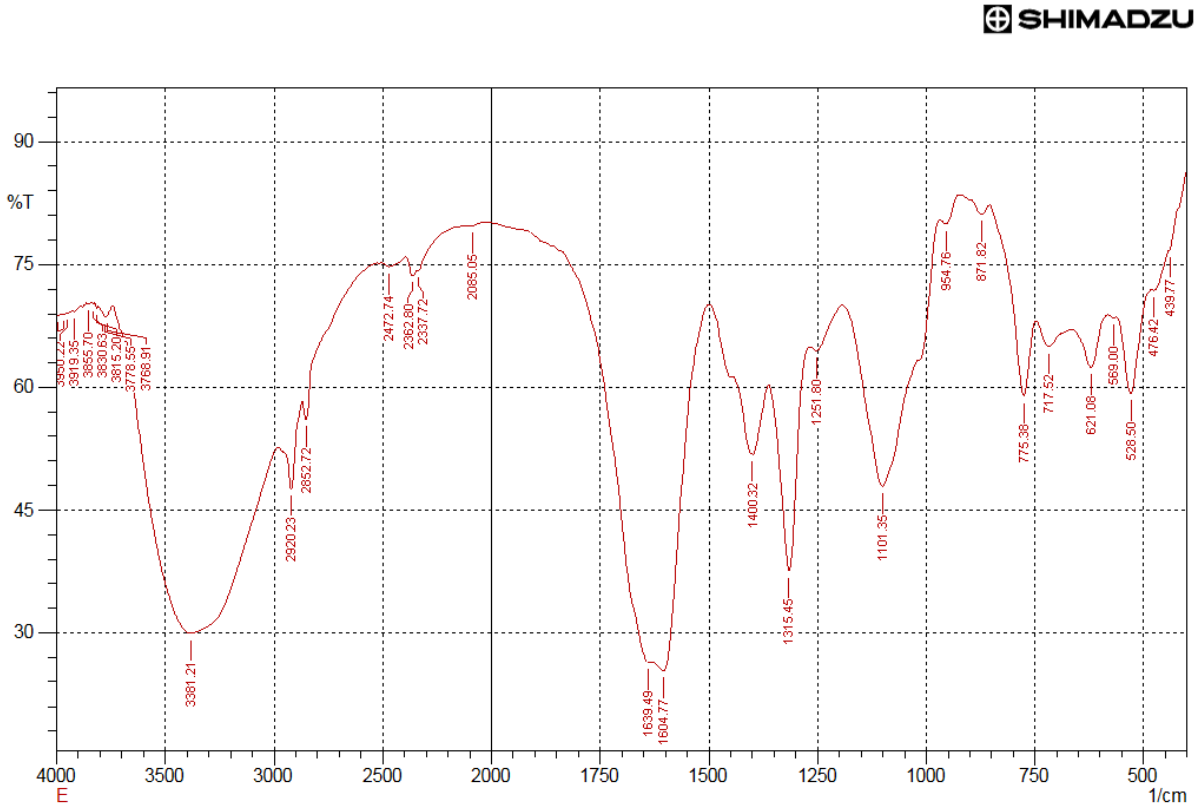

Figure 2 Fourier transform infrared spectroscopy (FTIR) analysis of synthesized AgNPs

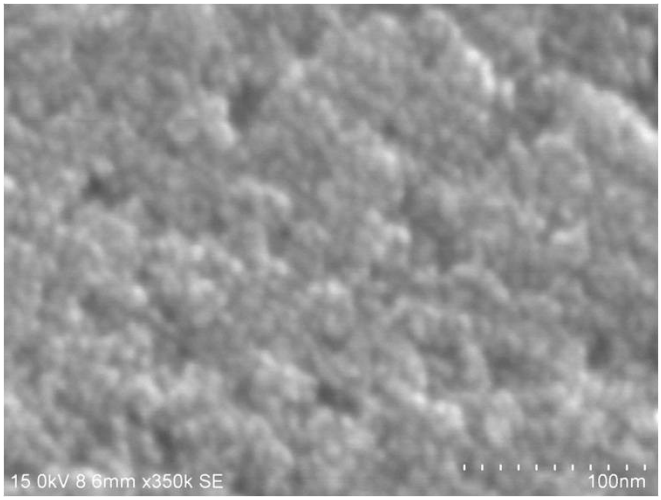

Figure 3. Scanning electron microscope (SEM) micrograph showed the synthesized of AgNPs using aqueous extract *Alpenia purpurata*

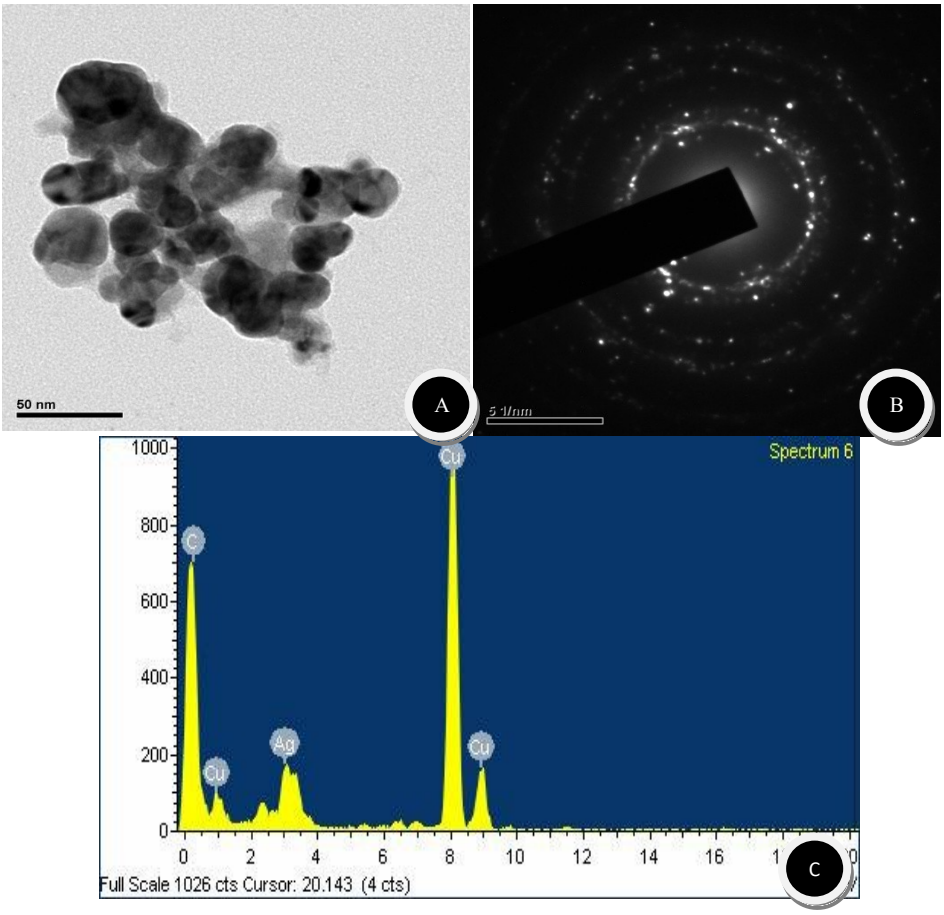

Figure 4 (a) High-resolution transmission electron microscopy (HRTEM) micrograph of synthesized AgNPs; (b) selected area of electron diffraction pattern (SAED) of the synthesized AgNPs showing the rings (c) EDX analysis showing the chemical composition of synthesized AgNPs.

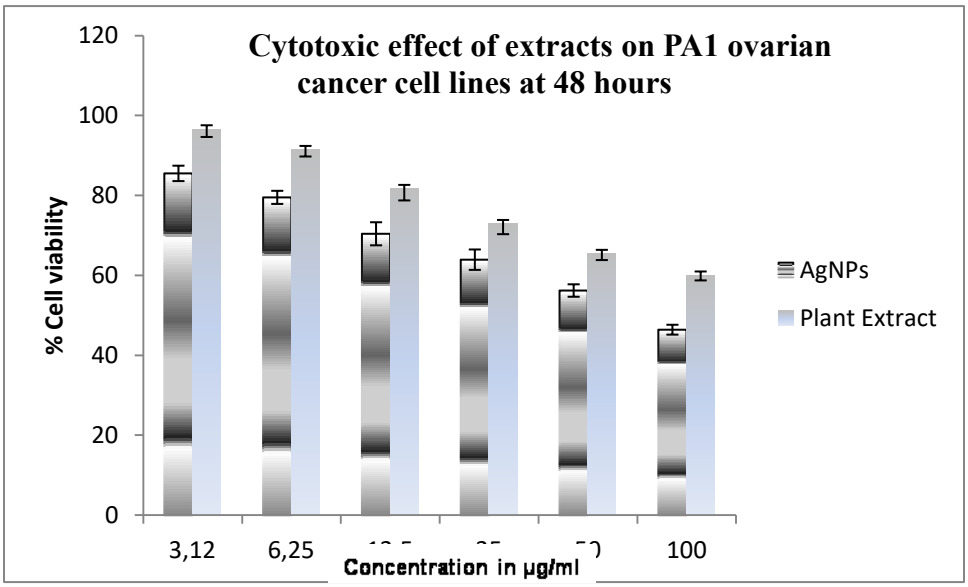

Figure 5. Cytotoxic effect of plant extracts on PA 1 ovarian cancer cell lines at 48 hours. The AgNPs and plant extract showed the IC 50 value of 78.2 µg/ml and 93.5 µg/ml respectively.

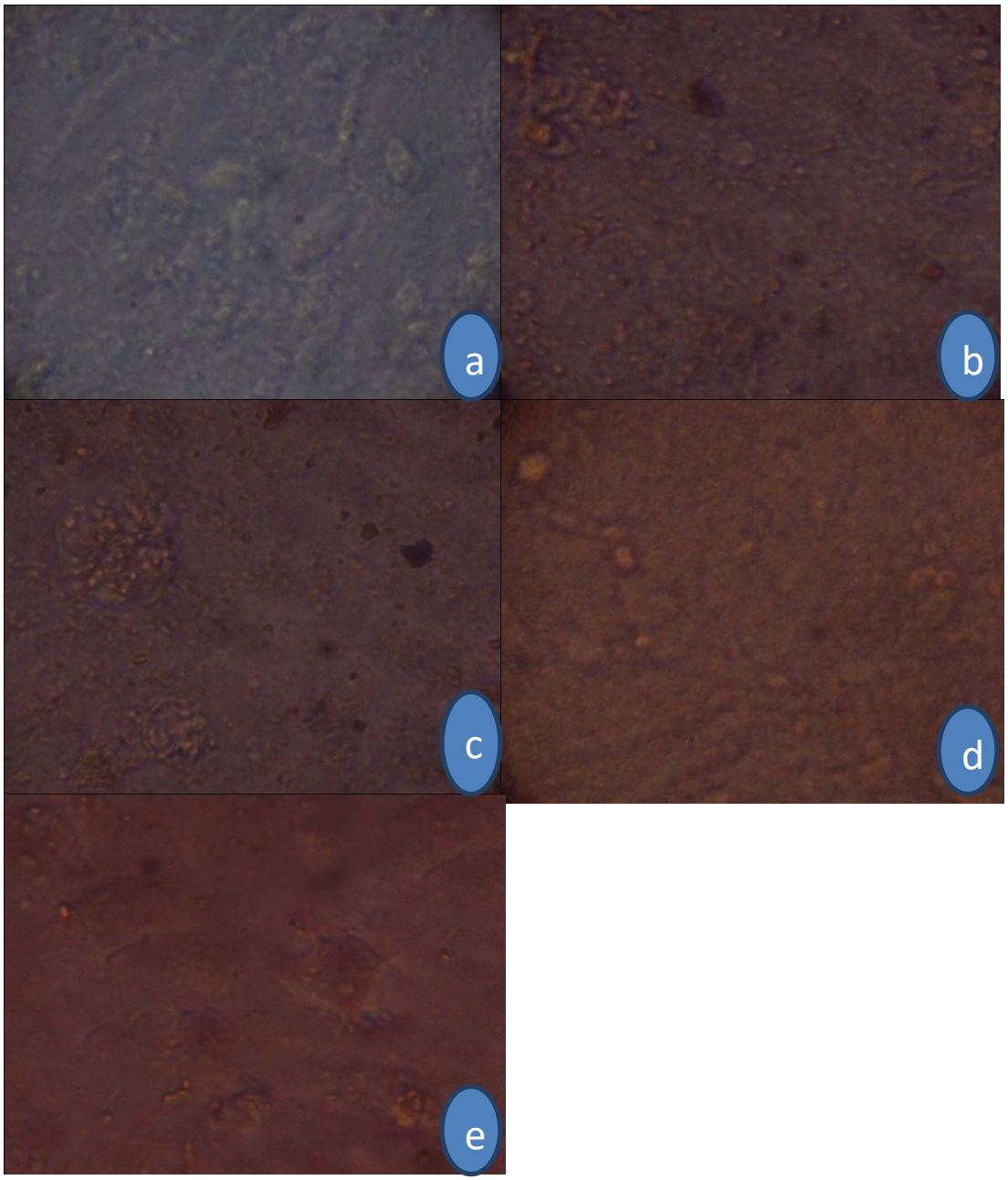

Figure 6. Cytotoxic effect of synthesised AgNPs against PA 1 cancer cell line showed cell toxicity (% at different concentration) (a) Control, (b) 6.5 µg/, ml, (c)12.25 µg/ml, (d) 25 µg/ml, (e) 50 µg/ml