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An investigation on cytotoxic effect of bioactive AgNPs synthesized using *Cassia fistula* flower extract on breast cancer cell MCF-7

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ABSTRACT

A single step protocol to produce biofunctionalized silver nanoparticles (AgNPs) using the aqueous extract of *Cassia fistula* flower as "natural factory" was investigated. The reaction between silver ions and aqueous flower extract after the bioreduction process has resulted in the formation of reddish brown color colloidal solution. XRD pattern showed the face centered cubic crystalline structure of AgNPs and exhibited spherical morphology as characterized by FE-SEM. FTIR studies identified different functional groups involved in effective capping of AgNPs. The *zeta potential* affirmed the phytoreduced AgNPs possess good stability and the size of the particle was measured by DLS. The synthesized AgNPs displayed effective cytotoxic potential against MCF7 and the inhibitory concentration (IC_{50}) was recorded at 7.19 μg/mL. The apoptotic effects of the AgNPs were also confirmed by AO/EB staining. The investigation presents preliminary evidence that biosynthesized AgNPs can be used in the development of novel anticancer drugs.

1. Introduction

Nanoparticles have gained interest in recent years and are successfully employed in delivering therapeutic agents [1]. For the past two decades, numerous nanoparticles based diagnostic and therapeutic agents have been developed to treat several diseases such as diabetes, asthma, allergy etc. [2]. Green nanotechnology has received much attention due to its numerous advantages such as cost effective, eco-friendly and easily scaling-up nature. Among the various sources available, plants have been considered as the preferred choice of materials owing to its bioreducing and stabilizing potential [3].

In current scenario, silver nanoparticles (AgNPs) have gained increasing interest due to enormous applications such as in nonlinear optics, coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions and as antibacterial capacities [4]. AgNPs have been reported to possess anti-fungal [5,6], anti-inflammatory [7], anti-viral [8] anti-angiogenesis [9]. Different biological sources such as bacteria, fungi, algae and plants are exploited for the green route synthesis of nanoparticles. There are different types of nanomaterials such as copper, zinc, magnesium, gold, selenium and silver have been used nowadays, but silver have been proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms [10]. However, plant mediated nanoparticle synthesis is a cost effective, faster and preferred approach. Plants serve as readily available sources of bioactive compounds such as alkaloids, amino acids, flavanoids, terpenoids and other phenolic intermediates that could act as effective reducing agents for the bioreduction of metals into nanoparticles which have a wide range of biological applications. This process may be associated with the phytoremediation concept [11,12].

Cassia fistula (*C. fistula*) commonly known as Indian labrum and Golden Shower in English, is a native plant of India. Traditionally, *C. fistula*flower is used to treat fever, skin diseases, abdominal pain and leprosy [13] and the flower extract is used to treat stomach troubles [14]. The flower extract is known to exhibit antibacterial, antifungal [15], antioxidant [16] and antidiabetic properties [17]. The present study deals with the biosynthesis of AgNPs using*C. fistula* flower extract and to assess its cytotoxic effect against breast cancer cell line MCF-7.

2. Materials and methods

2.1. Phytosyntheis of AgNPs

C. fistula flowers were collected and finely powdered prior to the experiment. The dried flower powder was mixed with deionized water, boiled, filtered and the extracts were collected.

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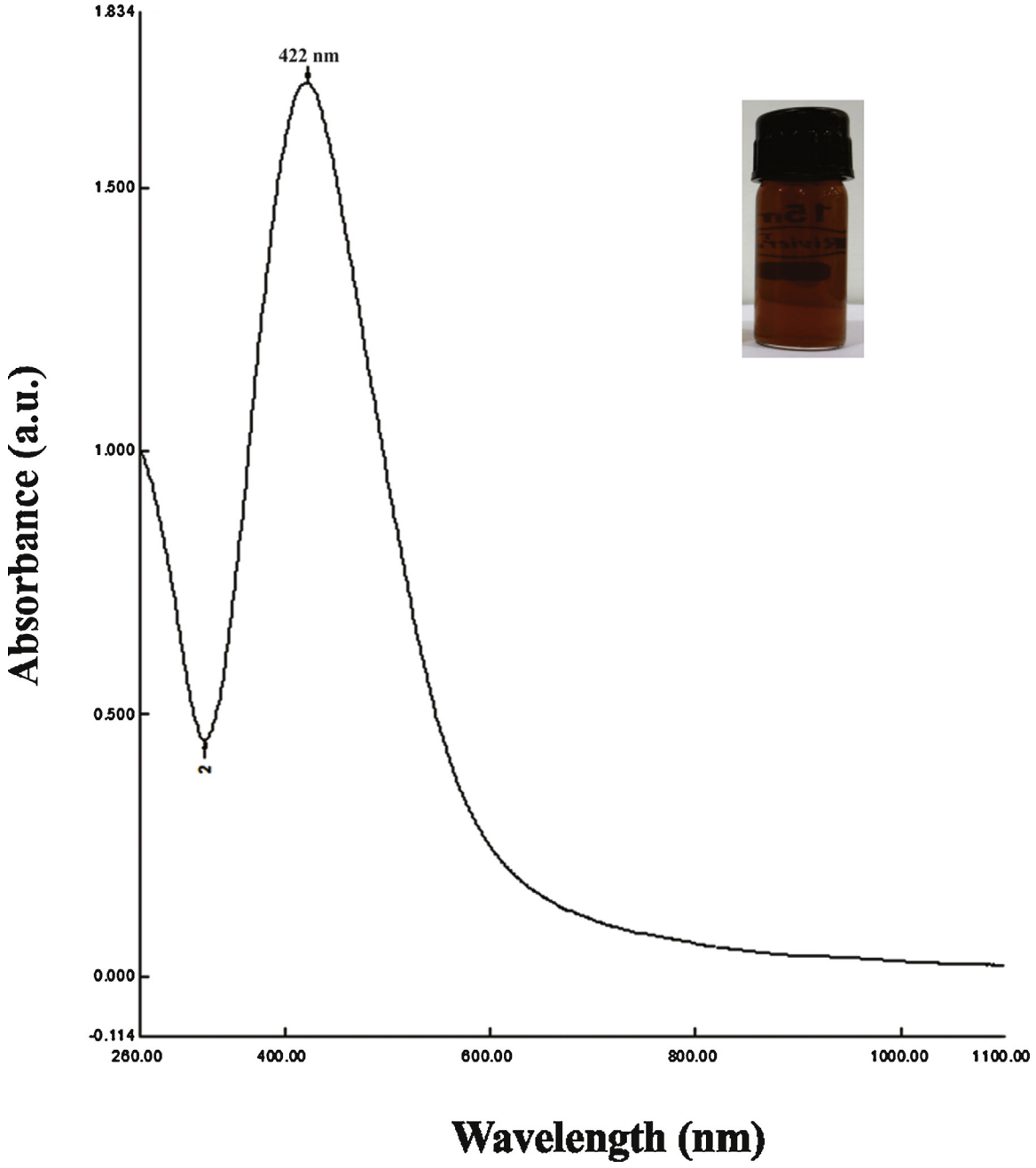

Fig. 1. UV spectra of AgNPs synthesized with 1.5 mL of flower extract. The absorption spectra of AgNPs at 422 nm.

About 1.5 mL of the extract was added to 30 mL of 1 mM $AgNO_3$ solution and the reaction was left to take place at ambient conditions.

2.2. Characterization of AgNPs

The biosynthesized AgNPs were characterized by Ultraviolet–visible spectrophotometer (UV–vis, Schimadzu 1800), X-Ray Diffraction (XRD, Rigaku smart lab), Fourier Transform Infrared Spectroscopy (FTIR, 4000–400 cm^{-1}-PerkinElmer), Dynamic Light Scattering (DLS, Malvern Zetasizer Nano Series) and Field Emission-Scanning Electron Microscopy (FE-SEM, FESEM-SUPRA 55-CARL ZEISS).

2.3. Cell culture

The breast cancer cell line MCF7 and Vero cell line were purchased from NCCS, Pune, India. The cells were grown in Minimal Essential media supplemented with 10% Fetal Bovine Serum (FBS), 100 μg/mL penicillin, 100 μg/mL streptomycin and grown at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. The cells were allowed to grow to 70–80% confluence and were seeded at a density of 1×10^6 cells per well and incubated for 24 h in 95% air and 5% CO_2 incubator.

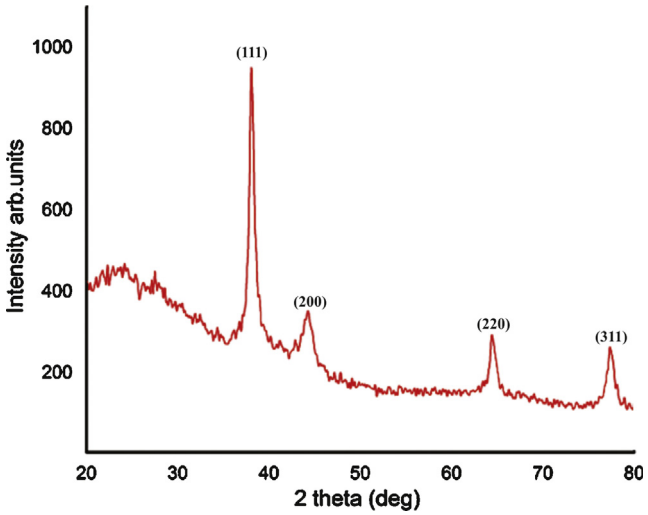

Fig. 2. XRD patterns of biosynthesized AgNPs.

2.3.1. In vitro cytotoxicity by MTT assay

The cytotoxic activity of AgNPs against MCF7 and Vero cell lines was determined by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay [18]. Various concentrations of the AgNPs in 0.1% DMSO were added and incubated for 24 h at 95% air and 5% CO_2 incubator. After incubation, 10 μL (5 mg/mL in PBS) of MTT was added to each well and incubated for 4 h at 37 °C. The resulting formazan was dissolved in 100 μL of DMSO and the viable cells were determined by measuring the absorbance at 570 nm. The concentration of AgNPs showing 50% inhibition of viability (IC_{50} values) was calculated.

$$\%\text{Cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100\%$$

2.4. Fluorescence microscopic study by Acridine Orange (AO)/Ethidium Bromide (EB) staining method

Approximately, 1×10^6 cells/mL in 100 μL medium was suspended in 96 well titer plates and treated with IC_{50} concentration of AgNPs. The cells were incubated for 24 h and 100 μg/mL of AO/EB dye mixed solution was added. The cells were incubated at 37 °C at 95% air and 5% CO_2 for 30 min and washed with 200 μL of warm phosphate buffer. Finally, the live and apoptotic cells were visualized under an epifluorescence microscope (NIKON) at 40× magnification with an excitation filter at 510–550 nm.

3. Results and discussion

The color change from yellow to reddish brown after 15 min of incubation under stirring conditions was observed. UV–vis spectroscopy showed absorption peak (λ_{max}) at 422 nm for AgNPs (Fig. 1) which is in accordance with the earlier reports of green synthesis of silver nanoparticles using the *Syzygium cumini* fruit extract [19] and *Mangifera indica* leaf extract [20].

The XRD spectrum of the biosynthesized AgNPs showed four intense peaks 38°, 44.27°, 64.47° and 77.3° that could be indexed to

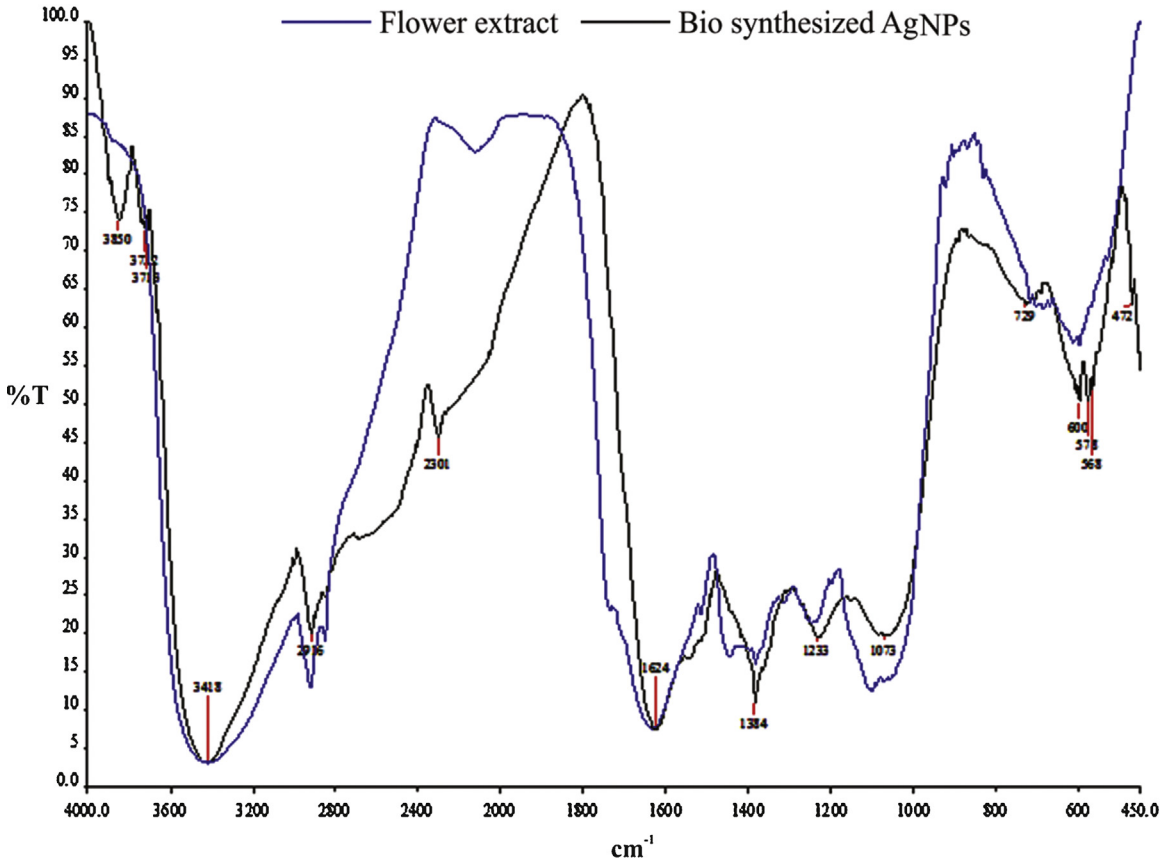

Fig. 3. FTIR spectra of *C. fistula*flower extract.

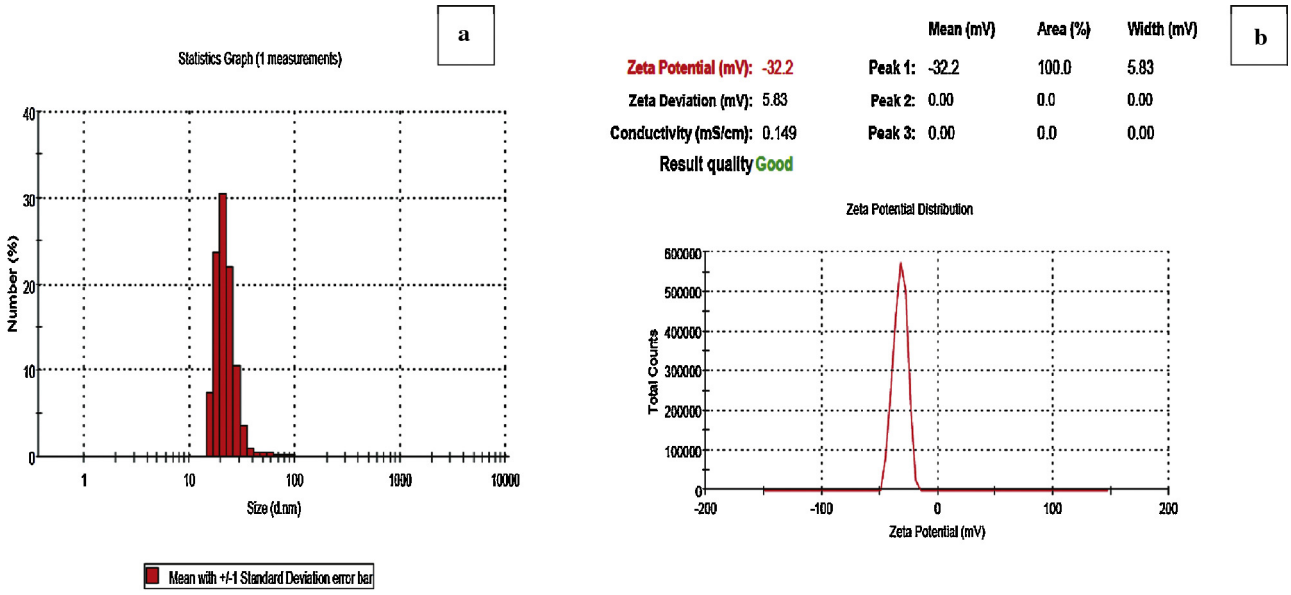

Fig. 4. (a) Particle size measurement of AgNPs. (b) Size distribution analysis of synthesized AgNPs.

(1 1 1), (2 0 0), (2 2 0) and (3 1 1), respectively (Fig. 2). The obtained XRD pattern was consistent with the early reported documentation of AgNPs synthesized using *Bacillus licheniformis* [21].

FTIR data of the extract and biogenic AgNPs was recorded to reveal the interaction of nanoparticles with active biomolecules involved in capping and stabilizing process (Fig. 3). A first peak shift from 3417 to 3418 cm^{-1} denotes stretching vibration of the O—H group of phenol [22]. Second peak shift 2921–2916 cm^{-1} represent C—H stretching vibration [23]. A third peak shift from 1633 to 1624 cm^{-1} affirmed stretching mode of carboxyl group coupled to amide I band [24]. A peak located at 1384 cm^{-1} signifies C—N stretching vibrations of aromatic amines [25]. The peak shift from 1248 to 1233 cm^{-1} indicates presence of an amide III band [26]. A peak located at 1073 cm^{-1} in biogenic AgNPs arises due to the C—OH vibration [27]. Thus, these FTIR data confirmed the presence of proteins that could be responsible for the bioreduction of metal ions and formation of nanoparticles [28].

Zeta potential analysis showed a sharp peak at −32.2 mV that indicated the biogenic nanoparticles are negatively charged on their surface and the particles are polydisperse with size ranging from 21 to 30 nm (Fig. 4a and b). This result is an agreement with the particle size of silver nanoparticle (27 to 32 nm) synthesized using *Catharanthus roseus*leaf extract [29]. FE-SEM results showed the nanoparticles are spherically shaped with size measured between 33 and 51 nm (Fig. 5). This size is larger than DLS measurement which is due to the hydrodynamic size of nanomaterials. The sizes and shapes of metal nanoparticles are influenced by a number of factors, including pH, precursor concentration, reductant concentration, time of incubation, temperature, and the method of preparation [30].

The size and dose concentration of nanoparticles play an important role in inducing cytotoxicity [31]. Fig. 6a illustrates the cytotoxicity of biosynthesized AgNPs against MCF-7 and Vero cells. The cytotoxicity of AgNPs against MCF-7 was observed in a dose dependent manner. After the incubation period, 90.5% and 89.7% cell death was noticed against MCF-7 and Vero cell lines at 1000 μg/mL. The inhibitory concentration 50% (IC_{50}) against MCF-7 and Vero cell lines were observed at 7.19 μg/mL and 66.34 μg/mL, respectively. AgNPs synthesized from various biological sources and the cytotoxicity induced against MCF-7 with their respective IC_{50} values were listed in Table 1. Interestingly the dose required to induce cytotoxicity against MCF-7 in the present study was much lower when compared to AgNPs synthesized from other sources. The high cytotoxic effect of AgNPs may also be attributed to its size and capping of biomolecules such as protein or phenol on the surface of nanoparticles.

The AgNPs treated and untreated cells were stained by AO/EB staining. The control/untreated cells showed green fluorescence due to the permeabilization of the AO that specifically stains live cells and the AgNPs treated cancer cells showed red fluorescence due to loss of membrane integrity (Fig. 6b). This result is consistent

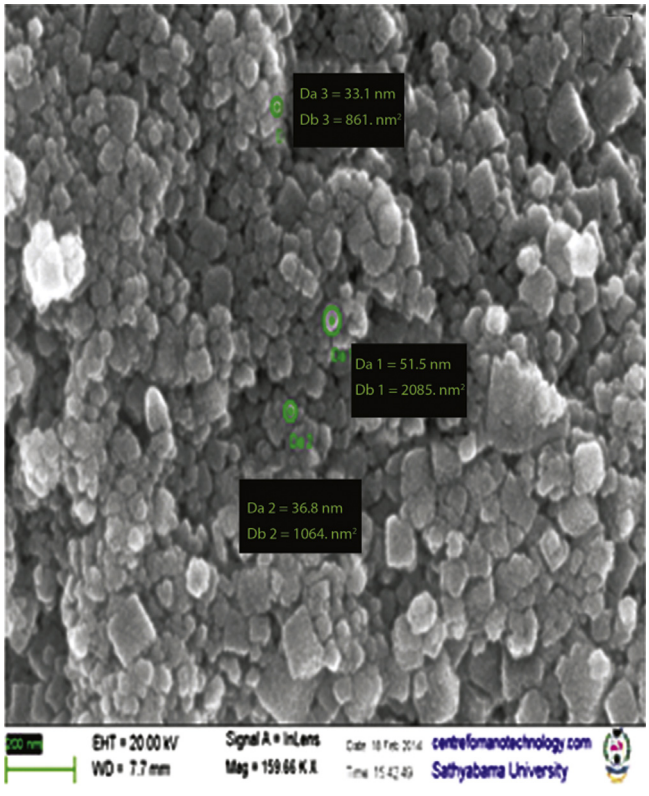

Fig. 5. FE-SEM micrographic images of synthesized AgNPs.

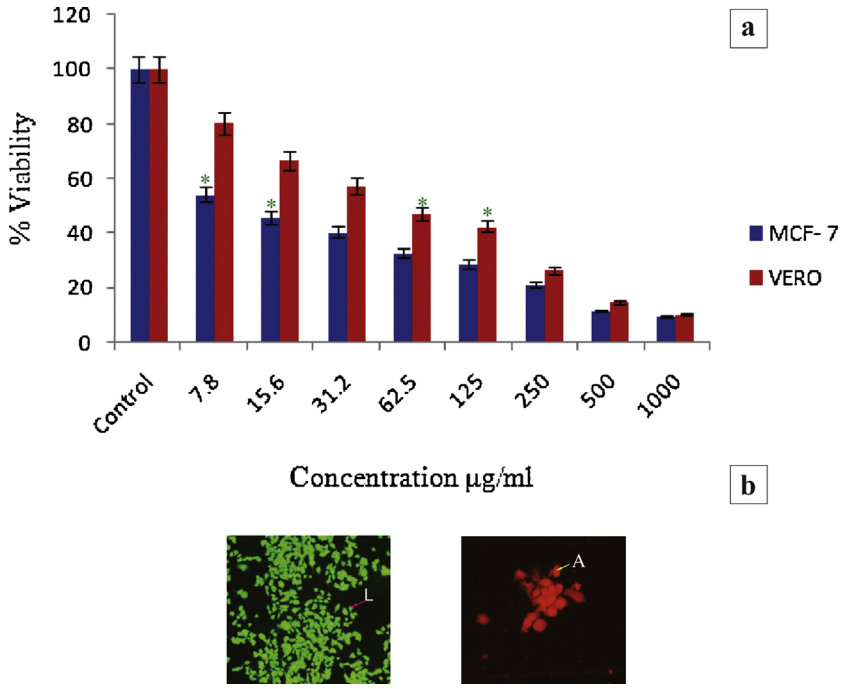

Fig. 6. (a) Cytotoxicity effect of AgNPs against MCF-7 and VERO cell lines at 24 h. (b) AO/EB staining of control live cells (L) showing green fluorescence and apoptotic cells (A) showing red fluorescence.

Table 1
IC_{50} values of AgNPs from various sources on MCF-7 cell lines.

Biosynthesis of AgNPs from various sources	IC_{50} on MCF-7	Reference
Datura iinnoixa(leaf extract)	20 µg/mL	[32]
Annona squamosa (leaf extract)	50 µg/mL in 24 h and 30 µg/mL in 48 h	[33]
Malus domestica(fruit extract)	10 µg/mL	[34]
Escherichia fergusoni(bacterial extract)	17.41 µg/mL	[35]

with the earlier reports of silver nanoparticles mediated apoptosis evaluated by fluorescence staining [36]. To the best of our knowledge, the potential anticancer effect of AgNPs of the flower extract of *C. fistula* has been investigated for the first time in the present study and there will be a wide scope for detailed investigation in the future of the application of AgNPs in cancer therapy.

4. Conclusion

Our findings indicated that the green synthesized nanoparticles using flower extract of *C.fistula* could provide an efficient application in medicine. The phytofabricated AgNPs were well characterized by FESEM, *zeta potential*, FTIR and the crystalline nature was confirmed by XRD. AgNPs showed good antiproliferative activity against MCF7 cells and further research has to be carried out for application in pharmaceutical industry.

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