Anticancer activity of Silver Nanoparticle by using Cassia auriculata Extract

Abstract

In this study, green synthesis of silver nanoparticles (Ag NPs) has done using traditional herbal namely Cassia auriculata extract by the simple Green synthesis method. The synthesized Ag nanoparticles were studied by the characterization techniques includes X-ray diffraction (XRD) crystallography for nature of crystalline with relevant parameters, Transmission electron microscopy (TEM) for particle size as well as the SAED patterns for amorphous, crystalline or polynanocrystalline and Photoluminescence analysis were carried out for the prepared NPs. Ag NPs were fabricated utilizing Phyto-aquatic extract of Cassia auriculata which act as a reducing agent, and it was converted into a precursor solution to coat on cotton fabrics for antibacterial applications. To further, its performance on anticancer application was studied for Michigan Cancer Foundation-7 (MCF-7) line breast cancer.

Keywords: Cassia auriculata, Silver nanoparticles, cotton substrate and MCF-7 cell line.

1. Introduction

Silver nanoparticles have attracted and demandable research of interest due to its distinct properties such as good catalytic activity, Surface Enhanced Raman Scattering (SERS) and antimicrobial activity [1]. Silver is widely used as catalyst for the oxidation of methanol to formaldehyde and ethylene oxide. Due to colloidal nature it use as substrate for surface enhanced spectroscopy, as it partly require electrical conducting surface. In this era silver is use as antimicrobial agent. Recent focuses towards silver nanoparticle synthesis for increasing the treat of antibiotic resistance, caused by the misuse of antibiotic [2].

AgNPs can be prepared by biosynthesis technique. The synthesis of AgNPs in two-phase aqueous organic systems is based on the initial spatial separation of reactants (metal precursor

and reducing agent) in two immiscible phases. The rate of subsequent interaction between the metal precursor and the reducing agent is controlled by the interface between the two liquids and by the intensity of interphase transport between the aqueous and organic phases, which is mediated by a quaternary alkylammonium salt. Metal clusters formed at the interface are stabilized, due to their surface being coated with stabilizer molecules occurring in the nonpolar aqueous medium, and transferred to the organic medium by the interphase transporter. This method allows preparation of uniform and size controllable nanoparticles. However, a highly deleterious organic solvent is employed in this method. Thus large amounts of surfactant and organic solvent, which are further added to the system, must be separated and removed from the final product. As a result, it is expensive to fabricate silver nanoparticles by this method. Thus the prepared silver solution need not be separated from the reaction solution and it can be directly used for antibacterial activities [3].

Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plants sources. This revival of interest in plant derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than t he costly synthetic drugs, many of which have adverse side effects. Infectious diseases are the leading cause of death worldwide. The clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens [4].

Bacterial pathogens have evolved numerous defense mechanisms against antimicrobial agents and resistance to old and newly produced drug is on the rise. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity [5].

The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial

substances in nature become inevitable. Plants are good sources for new safe, biodegradable and renewable drugs [6]. The use of plants as therapeutic agents in addition to being used as food is age long. This has led to intensified efforts on the documentation of medicinal plants. *Cassia auriculata* (family: Cesalpinaceae) is a common plant in Asia, profoundly used in Ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and opthalmia. It is one of the principle constituents of 'Avaarai panchaga chooranam'- an In dian herbal formulation used in the treatment of diabetes to control the blood sugar level [7].

2. Experimental

In first step, thoroughly well cleaned 100 grams of Cassia auriculata leaves were taken by immersing, rinsing and flushing 10 times by 5 litter of double deionized (DD) water at room temperature. Secondly, this wetted leafs were grinded in granite mortar with pestle for 1 hour and its greenish extracts were collected as well as filter by PTFE Filter Membrane. This filtered extract is collected in a sample holder and stored at refrigerator.

Meanwhile, precursor solution of 1 mM of silver nitrate ($AgNO_3$) was added in 20 ml of DD water in a beaker and vigorously stirring for 2 hours at magnetic stirrer. After that, this well dissolved solution was filtered by PTFE Filter Membrane to get pure extract without any foreign particles. Then 5 ml of leaf extract and 20 ml of $AgNo_3$ were well mixed by magnetic stirrer for 2 hours in an aluminum foil covered beaker. The beaker with makeup solution was carefully placed in microwave oven at the power of 750 watt to tune the aqua solution in to solid powder format. During the irradiation between the times periods 50 s to 90 s, the solution is switchover in to thick gel and lastly formed as solid powder. The resultant powder was grinded in a cleaned granite mortar with pestle for 2 hours and collected in the form of bright brownish fine powder in a sample cube. Furthermore, the certain amount of readymade powder in mg was directly taken

in water for antibacterial applications as well as rest of sample dispersed in ethanol solution for an anti-cancer application. The pure and cleaned pieces of cotton of 1 X 1.5 cm dimension were immersed in solution (fine powder + water) for 1 hour and dried under 60 watt incandescent lamp for 2 hours. This Ag NPs coated Cotton fabrics were employed for antibacterial activity which have discussed in the current work.

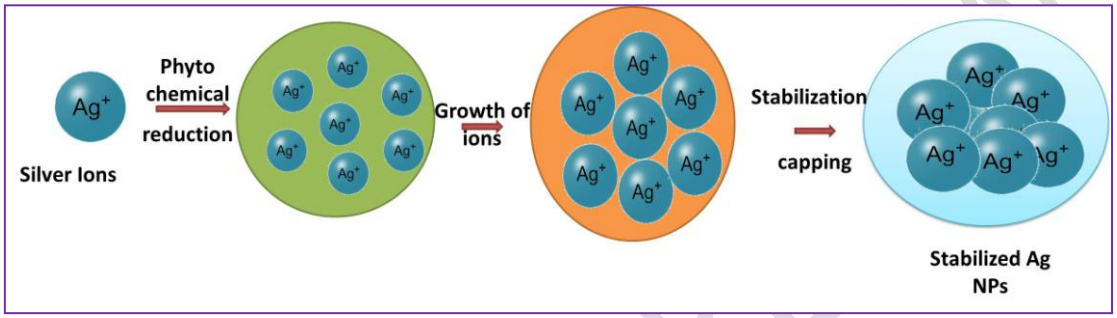

Fig 1. Mechanism for synthesis of nanoparticles under the capping and reducing action of phytochemicals of Cassia auriculata

As shown in the Fig 1, in the mechanism of synthesis of Cassia auriculata herbal extract assisted Ag^+ nanoparticles under the reducing action of phytochemicals, innumerable silver atoms/ions/molecules are combined to form of basic building block and then they are assembles themselves to form stabilized silver nanoparticles due to the capping action of extract components [8].

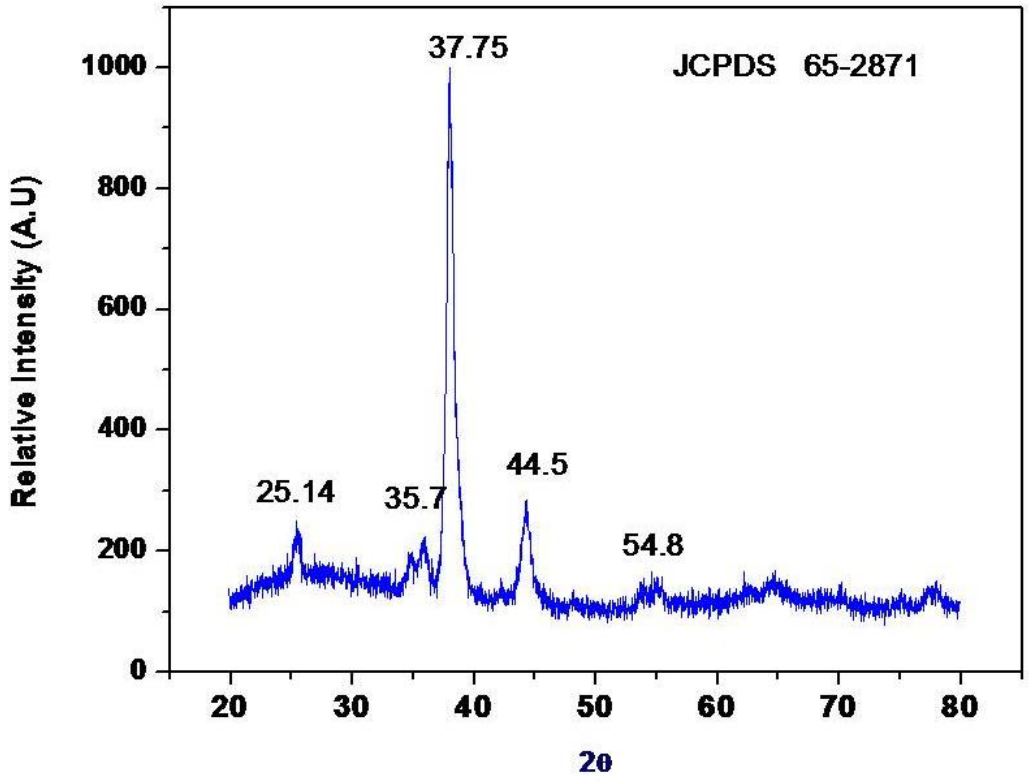

Fig 2 XRD Analysis of Ag Nanoparticle

To study the crystalline nature of the silver nanoparticles, the XRD analysis was performed. XRD pattern of derived AgNPs shows six intense peaks in the whole spectrum of 2θ values for *Cassia auriculata* extract shown in Fig 2. The full width at half maximum values was used to calculate the size of the nanoparticles. A few intense additional and yet unassigned peaks were also noticed in vicinity of the characteristic peaks of Silver. These sharp Bragg peaks might have resulted from some present in the *Cassia auriculata* extract. The XRD pattern revealed six peaks corresponding to 6 diffraction facets of silver. The presence of minor peaks suggests that the prepared silver nanoparticles are Cubic in nature [9]. The crystal size of Silver NPs are 54 nm and its lattice constants are 4.086 Aº by JCPDS but the calculated lattice constant are nearly 4.123 Aº with face centered cubic.

Results and Discussion

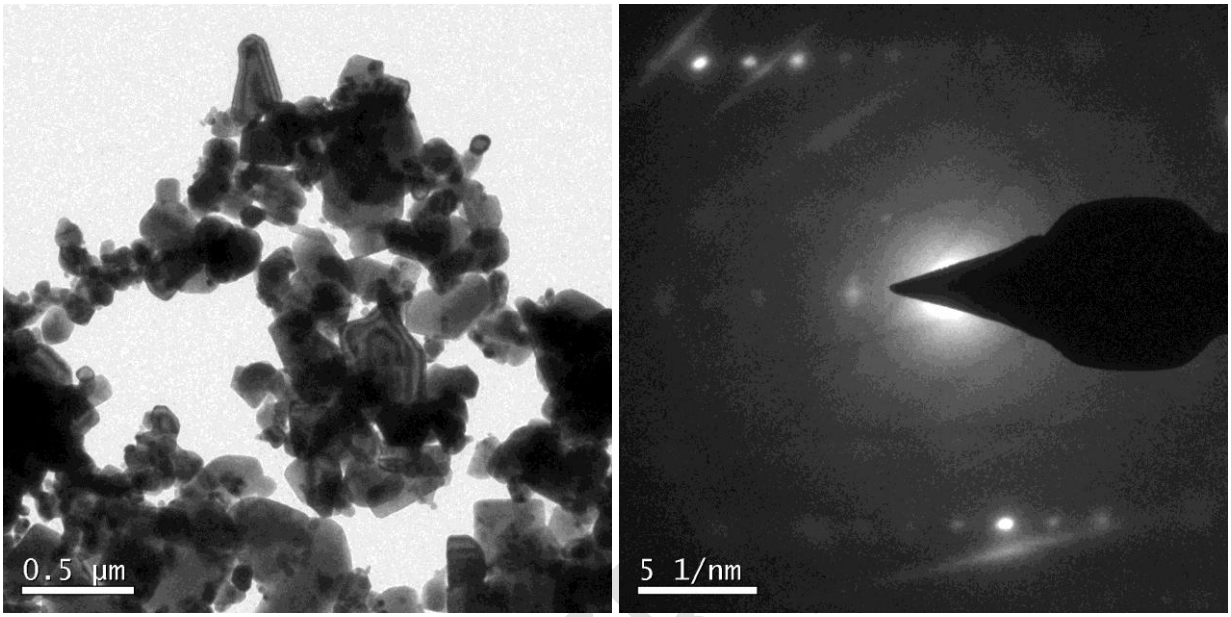

Fig 3(a-b) TEM with SAED pattern analysis of Ag Nanoparticle

The TEM images indicated equally spherical shaped orthorhombic crystals. Colloidal silver nanoparticles from *Cassia auriculata* were analysed using transmission electron microscopy (TEM). The size and shape of Ag nanoparticles synthesized using *C. auriculata* extract was visualized using 200 kV Ultra High Resolution TEM [10]. Formation and stability of silver nanoparticles using *Cassia auriculata* extract in aqueous colloidal solution shows that, by increasing concentration of silver nitrate and its particle size is nearly 70 nm which is shown in Fig 3(a-b).

Fig. 4 shows the PL analysis of Ag NPs by using Cassia auriculata. It is seen that Ag exhibits emission wavelengths of 361 nm and 421 nm, at the excitation wavelength of 320 nm which is attributed to the high ability for recovery of the Cubic network of Ag NPs atom by Cassia auriculata. It is also observed that Ag NPs show relatively higher intensity [11]

Silver is being promoted as an environmentally-friendly alternative to chemical-based disinfectants. Filling hydro gels with nanoparticles makes them better. Adhesives and increases their mechanical properties [12]. The adhesive material made from a hydro gel filled with nanoparticles, that could prevent wound dressings from falling off in contact with water or when we sweat, or even deliver drugs through the skin Nano-silver soap containing 99.99% purity silver nanoparticles solution, more than ordinary soap, antibacterial, the role of bactericidal, facial acne, sores and other common skin diseases at significantly improved, because contains natural plant extracts, at the same time cleaning the skin more delicate skin flexible as shown in Fig 5(a-b).

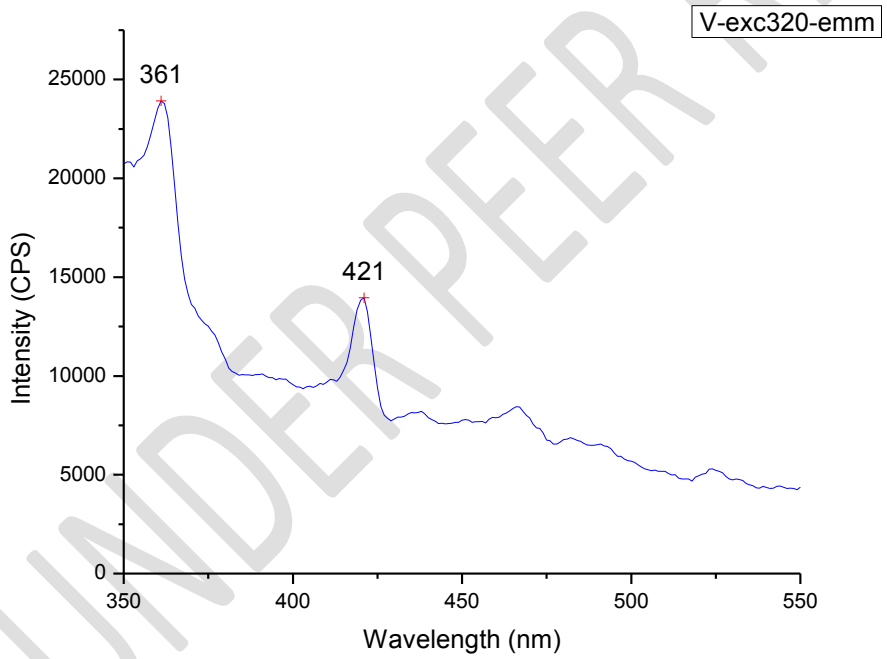

Fig 4 Photoluminescence analysis of Ag NPs by using *Cassia auriculata*

Antimicrobial mechanism of green synthesis of Ag NPs

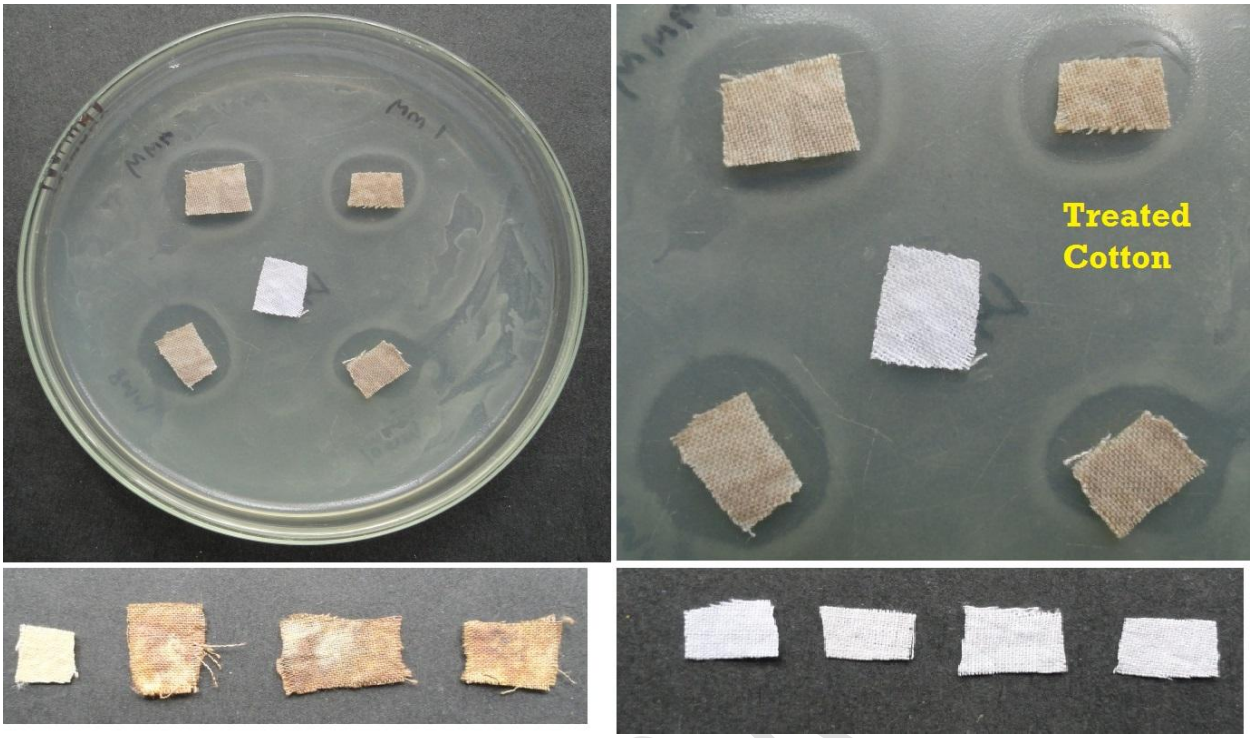

Fig 5 (a) Antibacterial activities by Ag NPs coated Cotton fabrics (b) Described image of (a)

In the present study Ag prepared by using synthesis of *Cassia auriculata* was tested for its antimicrobial activity against some human bacterial wound dressings [13]. Such resistance could be due to the permeability barrier provided by the cell wall or the membrane accumulation mechanism. The extract restricted the growth of pathogen on the media around the well. The maximum inhibition zone (21 mm) was observed in ethanol extract of *Cassia auriculata* against wound dressings.

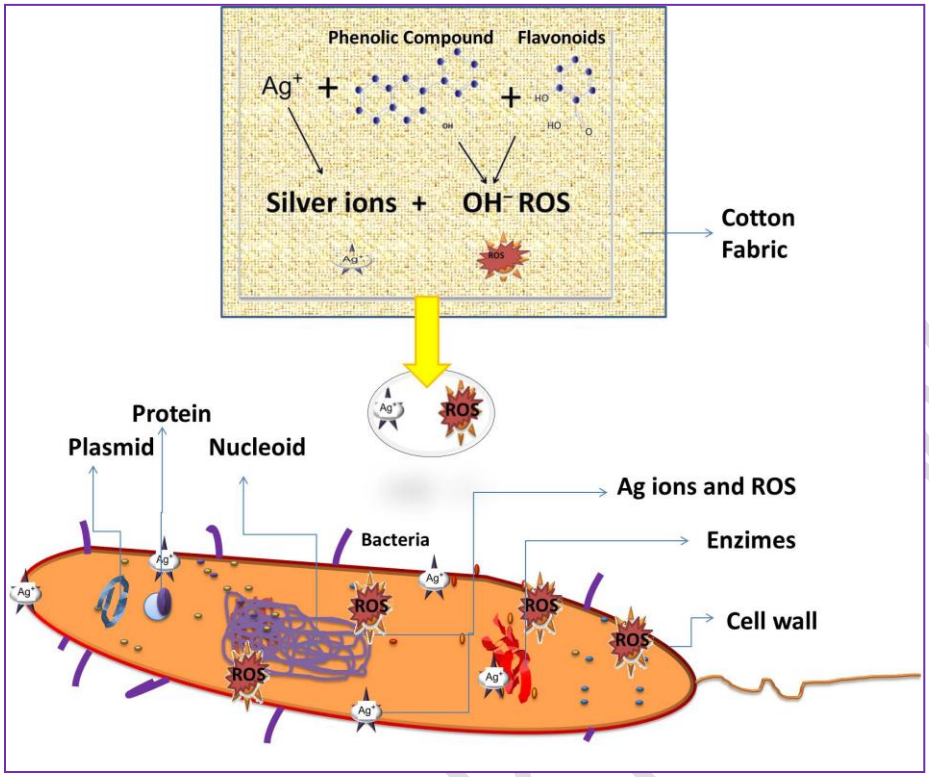

Fig 6. Mechanisms of antimicrobial activity of the Ag NPs coated cotton fabric

There are many researchers suggesting that the releasing of reactive oxygen species (ROS) and metal ions from Ag NPs coated Cotton fabrics in the antimicrobial activity. An increase of the surface to volume ratio normally by the reducing action of capping agent of entitled herbal is one of the important physical aspects in nanoparticles. In the Fig 6, Silver ions from the Ag NPs and emerged ROSs from major phyto chemical components such as phenolic and flavonoids groups having the vital role in the mechanism of bacterial inhibition are the important considerations at all. First, the cell wall of bacteria is damaged by electrostatic attraction between positive silver ions and negative charged component of bacteria meanwhile the ions are strongly interact with nuclic acids to perturb the cell divisions. In next stage, co action of both of these ions and species riding the entities of bacteria such as H-bonds in DNA,

respiratory chain enzymes, nucleic acids is damaging them effectively which is shown in the Fig 6 clearly [12].

Pathogenic bacterial contamination is at the root of many persistent and chronic bacterial infections. Synergistic superhydrophobic surfaces functionalized with a cationic antimicrobial agent of carbon adsorbent components show promising antimicrobial efficacies, also bactericidal activity due to the compromised low surface energy stemming from the introduced hydrophilic biocides. Synergistic antibacterial cotton bits with superhydrophobic bacterial repellency and photodynamic bactericidal activity for Escherichia coli (Gram-negative) and Staphlococcus aureus (Gram – positive) [15].

The modified cotton textile was constructed by integrating tunable micro/nanoscale roughness, and surface perfluorination. The triple-scale structured superhydrophobic surfaces exhibited ≲ 90% reductions in both waterborne and airborne bacterial adhesions. Subsequently, after being exposed to visible light for 45 min, the synergistic surface demonstrated complete inactivation (100% killing) against Escherichia coli (Gram-negative) and Staphlococcus aureus (Gram – positive) bacterial cells via photodynamic bactericidal capacities. This synergistically antibacterial surface not only improves the antibacterial efficiency but also leads to long-lasting antimicrobial performance, each of which is highly desirable in combating bacterial infections [16]

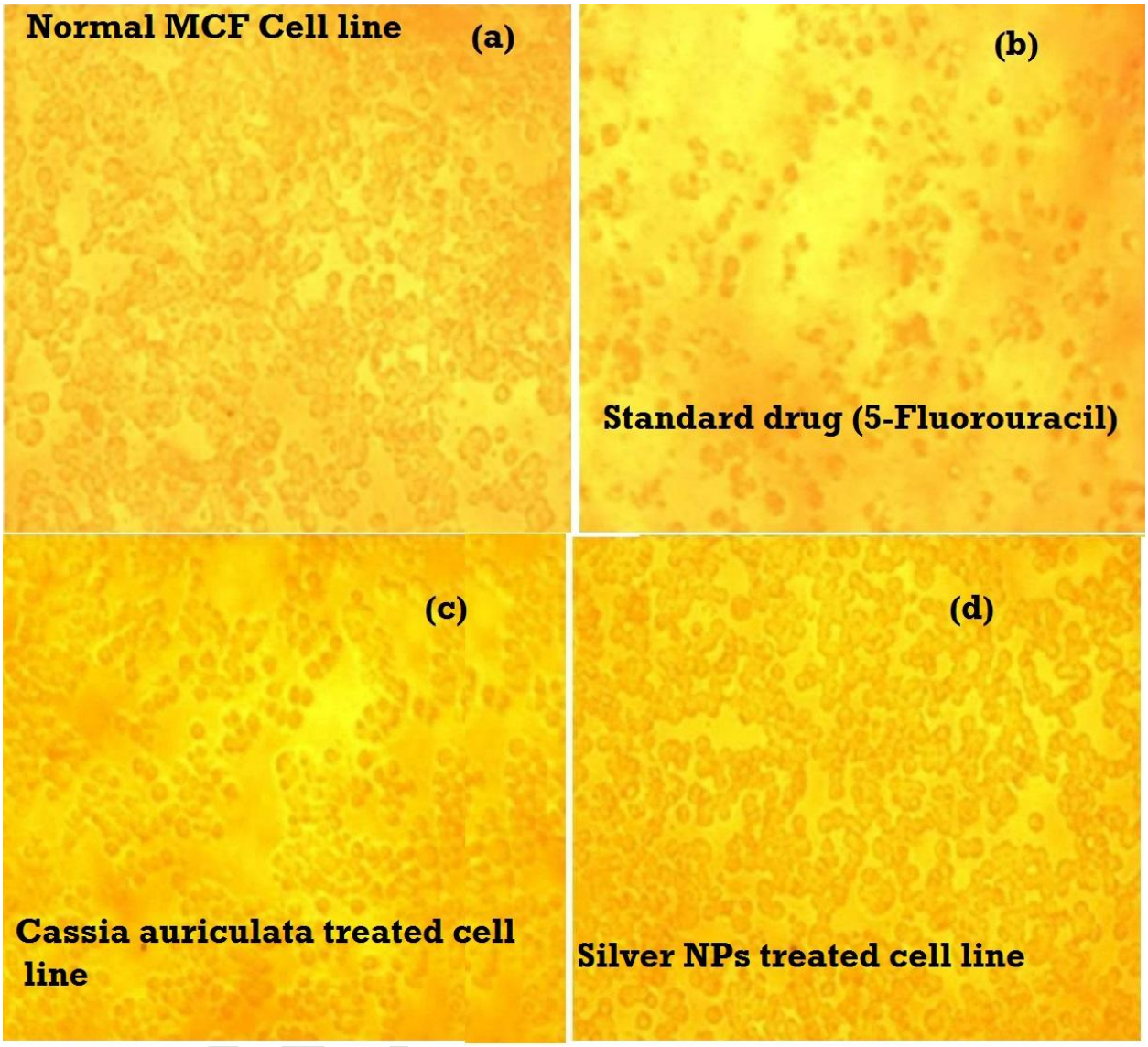

Figure 7 (a-d) Plate showing the anticancer activity of MCF-7 cell line of Silver nanoparticles *Cassia auriculata* leaf extracts

Natural derivatives play an important role to prevent the cancer incidences as synthetic drug formulations cause various harmful side effects to human beings. Cancer chemotherapeutic agents can often provide temporary relief from symptoms, prolongation of life and occasionally complete remission. A successful anticancer drug should kill or incapacitate cancer cells without causing excessive damages to normal cells. This ideal situation is achievable by inducing apoptosis in cancer cells. Chemopreventive agents comprise diverse groups of compounds with

different mechanisms of action with ultimate ability to induce apoptosis. Given that disruption of cell cycle plays a crucial role in cancer progression, its modulation by phytochemicals seems to be a logical approach in control of carcinogenesis [17]. The ability of a substance to affect specific phases of the cell cycle may provide clues as to its mechanism of action. A reduction in cell growth and an induction in cell death are two major means to inhibit tumor growth [18]. Apoptosis is one of the important pathways through which chemopreventive and chemotherapeutic agents inhibit the growth of cancer cells [19].

Table 1. Anticancer activity of ethanolic extract of *Cassia auriculata* on MCF-7 cell line

Concentrations	Cell viability	Cell inhibition	IC 50
(µg ml- ^{1})	(%)	(%)	(µg ml- ^{1})
7.8	61.52	38.48	
15.6	58.78	41.32	
31.2	51.29	48.71	
62.5	43.33	56.67	
125	37.55	62.45	84.56
250	28.82	71.18	
500	20.21	79.79	
1000	15.46	84.54	
2000	8.75	91.25	
Vehicle control	100	0	
(DMSO)			

In the present study, the ethanolic extract of *Cassia auriculata* was used to check whether it had the capability of inducing cytotoxic effects on the MCF-7 cell lines. In the MTT assay method both the cell lines were used by using the concentration range of 7.8-2000 µg/ml and the IC_{50} concentration were determined. The IC_{50} concentration of MCF-7 cell line was 84.56 µg/ml. It was noticed that the crude extract of *C. auriculata* had toxic effects on the above cell line, who

reported the IC_{50} value of ethyl acetate fraction of *H. pinifolia* collected from the Vellar Estuary on MCF-7 cell line of 66.68 µg/ml [20]. The *in vitro* cytotoxicity was meant to determine the IC_{50} of the crude sample towards to the cells shown in Fig 7(a-d).

It is evident that the acetone extracts of *H. pinifolia* exhibited less prominent antiproliferative activity on the *Vero* cell line. The extracts mediated antiproliferative activity is limited to the cancer cell lines rather than the normal cell lines. This indicates that the specific inhibitory effect may be due to the apoptosis-inducing ability of the acetone extracts of *H. pinifolia* in response to the defective gene expression in cancer cell lines rather than the normal cell line. With the significant antiproliferative activity of the extracts of plant against MCF-7 cancer cell lines, the mechanisms of action could, possibly, be due to the dose-dependent apoptosis-inducing ability, by necrosis of cancer cell lines, by enhanced neoplastic transformation followed by apoptosis or by any other mechanisms related to epigenetic and signal transduction pathways. These metabolites obstruct various hormone actions and metabolic pathways associated with the development of cancer [21] shown in Fig 7(a-d). In the present study suggested that the percentage of cell inhibition was noted in the different concentrations of ethanolic extract of *Cassia auriculata* ranges from 7.8 to 2000 µg/ml. The lowest cell inhibition (26.72 %) was recorded in the lowest concentration and highest cell inhibition (90.42 %) was noted in the higher concentration of ethanolic extract of *Cassia auriculata* shown in Table (1) and (2) [22].

Table 2. Anticancer activity of Silver nanoparticle on MCF-7 cell line

Concentrations	Cell viability	Cell inhibition	IC_{50}
(µg ml- ^{1})	(%)	(%)	(µg ml- ^{1})
0.78	73.28	26.72	
1.56	60.55	39.45	
3.13	51.46	48.54	
6.25	37.38	62.62	
12.5	31.65	68.35	17.25
25	22.25	77.75	
50	14.37	85.63	
100	09.58	90.42	
Vehicle control	100	0	
(DMSO)			

4. Conclusion

Silver was used as antiseptic cloth silver compounds have a similar mode of operation. All of these substances release silver ions to liquid phase. In the present study, the MCF-7 cell lines incubated with the test extract was stained with propidium iodide stain. It was observed that there was reduced cell size and nuclear condensation of cells by the methanolic extracts of *S. isoetifolium* than the control cells. Released silver ions interact strongly with nitrogen, phosphorus and sulfur containing compounds in bacteria, algae and fungi. We find hydrophobic coatings for cars, self-cleaning surfaces preventing dirt accumulation or T-shirts that remain fresh and odor free for an extended period of time by using this Silver NPs. The antimicrobial activity of *Cassia*

auriculata extracts were studied against two different bacterial species Escherichia coli (Gram-negative) and Staphlococcus aureus (Gram – positive) and the results were reported.

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