Anticancer activity of Silver Nanoparticle by using Cassia auriculata

Abstract

Silver Nanoparticle was prepared by using *Cassia auriculata extract by the method, the prepared powdered particle was characterized by using XRD and TEM to confirm the elements presented in it with its particle size in nm.* 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.* The adhesive material made from a hydro gel filled with nanoparticles, that could prevent wound dressings from falling off in contact with water.

Keywords; Cassia auriculata, Silver nanoparticles, wound dressings and MCF (full expression???)-7 cell line

1. Introduction

Silver nanoparticles (AgNPs) 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 its use as substrate for surface enhanced spectroscopy, it partly requires 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 alkyl ammonium 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 other ophthalmic diseases. It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar level [7].

2. Experimental

The *Cassia auriculata* leaves were washed several times with deionised water. 100 gm of finely cut *Cassia auriculata* leaves were taken and boiled in 300 ml of distilled water for 3mins and filtered. After filtration it was centrifuged at 10,000 rpm for 15mins, the supernatant was collected and stored at 4°C. A particular concentrations of leaf extracts (5ml) were taken separately and added 10 mL of 1mM silver nitrate solution with constant stirring and exposed to short burst of microwave irradiation at a frequency of 2.45 GHz in a domestic microwave oven (National Model N N-GD 576M), at a power output of about 100W in a cyclic mode (on 15s, off 15s) to prevent overheating as well as aggregation of metals at room temperature and Direct boiling. The colour change of the solution was checked periodically. The colour change of the leaf extract from yellow to dark brown indicated the silver nanoparticles were synthesized from the leaves. The same was performed for both conventional and homogenization method of extracts. The dried silver nanoparticles were subjected to Fourier Transform Infrared spectroscopy (FTIR) analysis by Potassium Bromide pellet (FTIR grade) method in 1:100 ratios and spectrum was recorded in Nicolet Impact 400 FT-IR Spectrophotometer using diffuse reflectance mode [8].

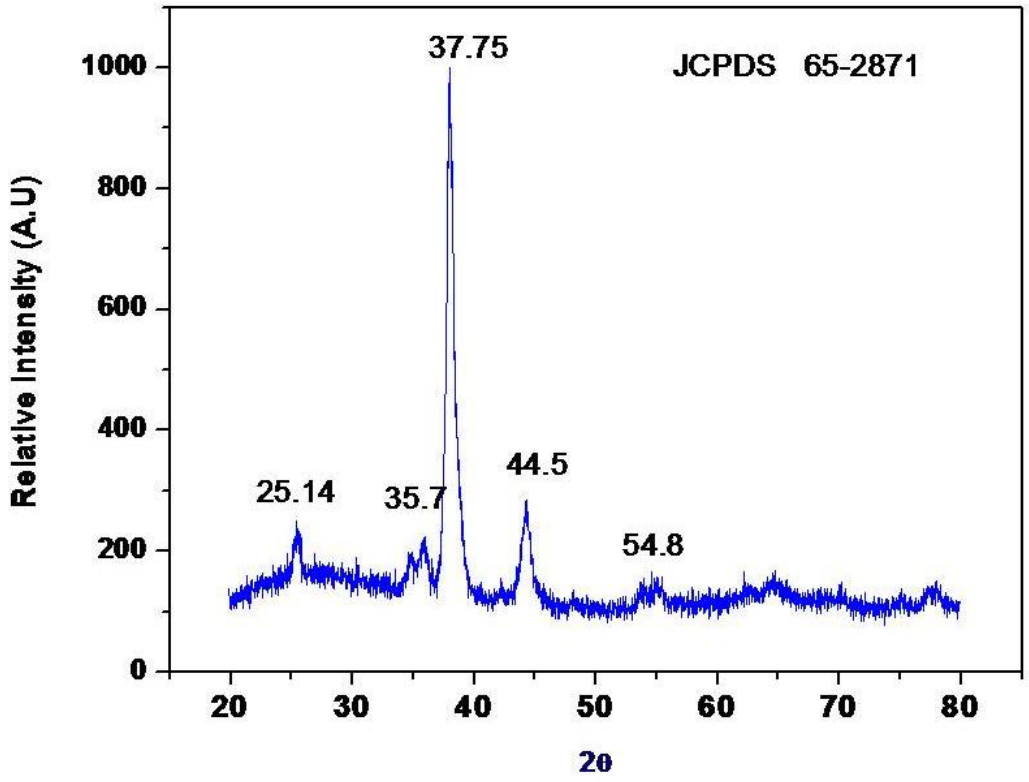

Figure 1 XRD Analysis of Ag Nanoparticle

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. 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.

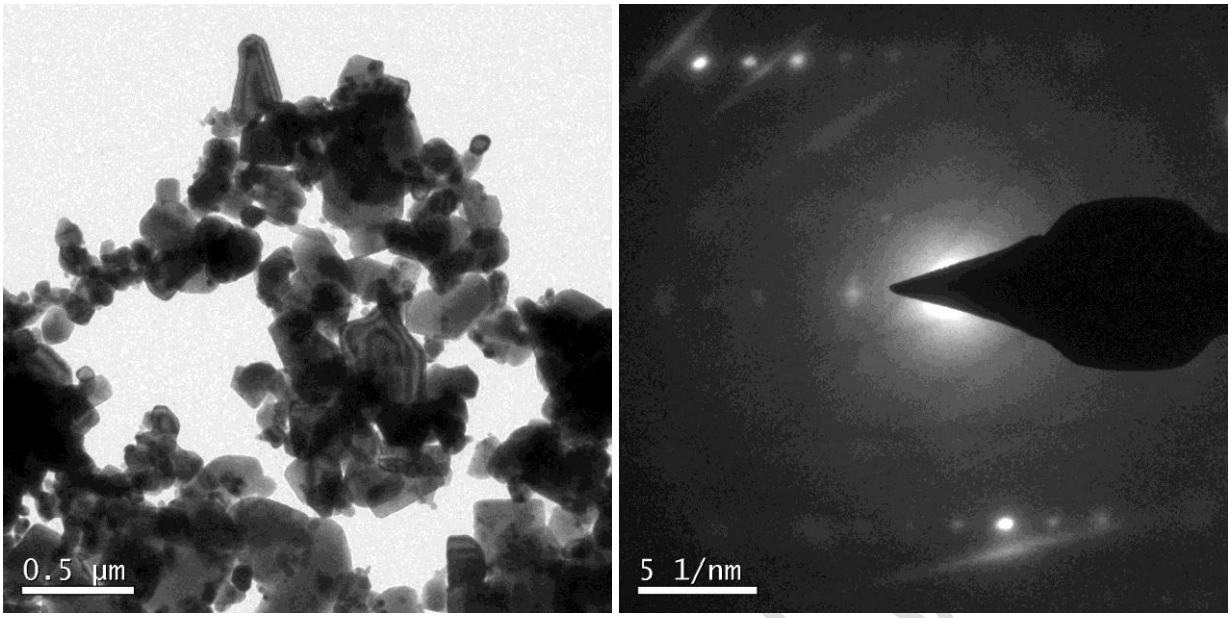

Figure 2 TEM with SAED pattern analysis of Ag Nanoparticle

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. 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 Figure 2.

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. 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 Figure 3(a-b).

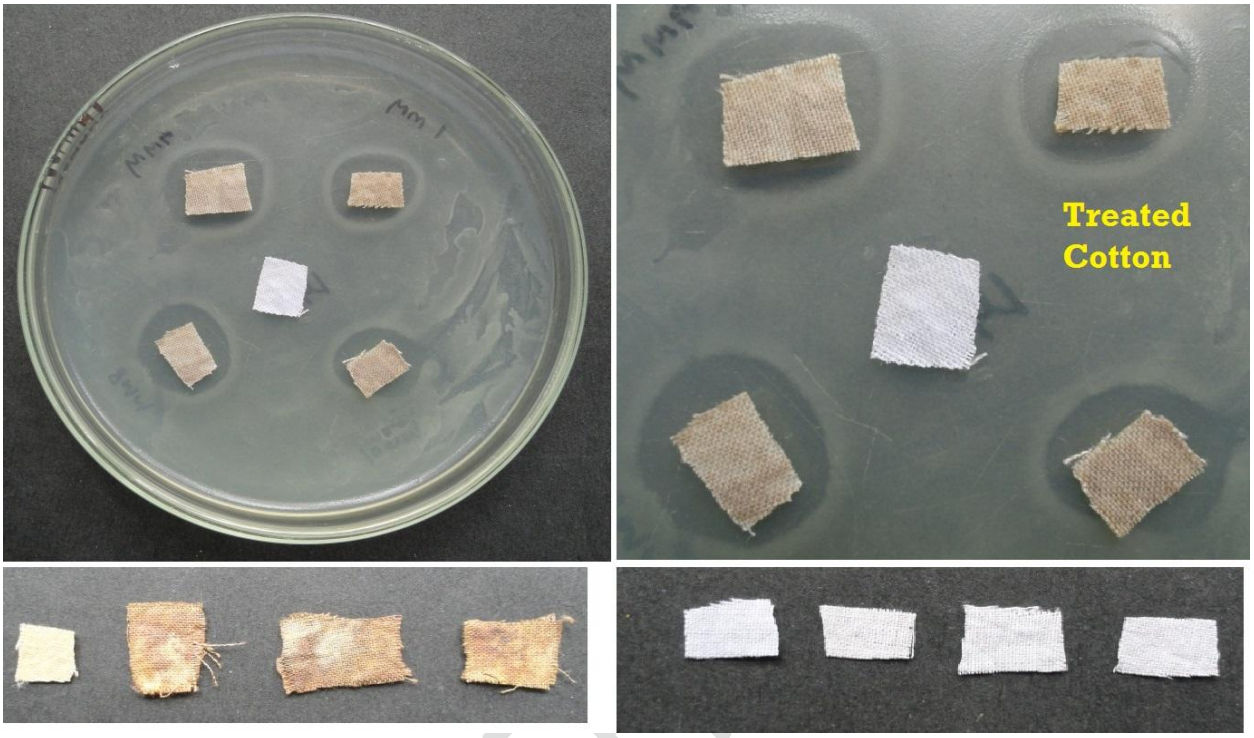

Figure 3(a-b) Silver nanoparticles decorated Cotton fabrics for wound healing

In the present study ethanol extract was tested for its antimicrobial activity against some human bacterial wound dressings [11]. 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.

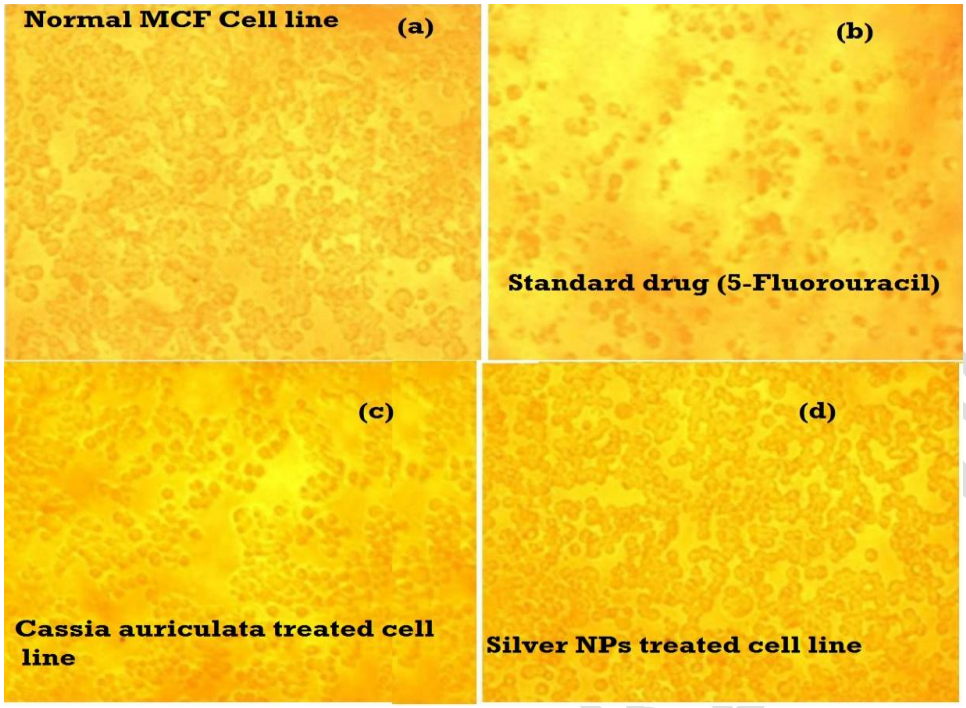

Figure 4 (a-d) Plate showing the anticancer activity of MCF-7 cell line of Silver nanoparticles *Cassia auriculata* leaf extracts

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 [12]. 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 [13].

Apoptosis is one of the important pathways through which chemopreventive and chemotherapeutic agents inhibit the growth of cancer cells [14].

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. The *in vitro* cytotoxicity was meant to determine the IC_{50} of the crude sample towards to the cells.

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 [16]. 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).

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)			

3. Heading where it is?????

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.

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