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Research article

**BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM
CASSIA AURICULATA LEAF EXTRACT AND *IN VITRO* EVALUATION OF
ANTIMICROBIAL ACTIVITY**

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ABSTRACT: The synthesis of metals and nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. Biosynthesis of silver nanoparticles was investigated by reducing silver nitrate with *Cassia auriculata* leaf extract at room temperature. The plant belongs to family Ceasalpinaceae and the plant is having promising medicinal properties for a wide range of human diseases. The synthesized nanoparticles characterized by the UV-Vis spectroscopy, revealed the formation of silver nanoparticles by exhibiting the typical surface plasmon absorption maxima at 420-435 nm. The peaks in the X ray Diffraction pattern are in good agreement with the standard values of the face-centered-cubic form of metallic silver. Fourier transform infrared spectroscopy indicates that the compounds attached with silver nanoparticles could be polyphenols with aromatic ring and bound amide region and transmission electron microscope reveals that the particles are spherical and polydispersed. The antimicrobial activity of synthesized nanoparticles were evaluated against *E.coli*, *Serratiamarcenscense*, *Bacillus subtilis*, *Aspergillusniger* and *Aspergillusflavus*. Fungi were most susceptible to silver nanoparticles followed by bacteria.

Keywords: Biosynthesis, Silver nanoparticles, *Cassia auriculata*, Ceasalpinaceae, antimicrobial.

INTRODUCTION

Bio-nanotechnology has emerged as integration among biotechnology and nanotechnology for developing biological synthesis and environmental-benign technology for synthesis of nanomaterials. The use of nanoparticles is gaining impetus in the present century as they possess defined chemical, optical and mechanical properties. Among them, the metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against antibiotics, and the development of resistant strains (Rai et al., 2009; Gong et al., 2007). A list of some of the applications of nanomaterials to biology or medicine or agriculture is fluorescent biological labels, drug and gene delivery, antimicrobials, and anti-insect molecules. Bio-Nanotechnology combines biological principles with physical and chemical approaches to produce nano-sized particles with specific functions. It also represents an economic substitute for chemical and physical methods of nanoparticles formation. Nanoparticles exhibit completely new or improved properties based on specific characters such as size, distribution and morphology. Specific surface is relevant for catalytic reactivity and other related properties such as antimicrobial activity in silver nanoparticles. These method of synthesis can be divided into intracellular and extracellular (Ahmad et al., 2005) with three main steps, which must be evaluated based on green chemistry perspectives, including (a) selection of solvent medium, (b) selection of environmentally benign reducing agent, and (c) selection of nontoxic substance for the Ag NPs stability. Among the various biosynthetic approaches, the use of plant extracts has advantage such as easily available, safe to handle and possess a broad viability of metabolites. It has been reported that medicinally valuable angiosperms have the greatest potential for the synthesis of metallic nanoparticles with respect to quality and quantity (Kumar and Yadav, 2009; Mohanpuria et al 2008; Song and Kim 2009).

The main phytochemicals responsible for the synthesis of nanoparticles are terpenoids, flavones, ketones, aldehyde amides etc. Every plant contains thousands of various alkaloids, steroids, flavonoids, terpenoids and other molecules with bioactivity.

The most effectively studied nanoparticles today are those made from noble metals, in particular Silver(Ag),Platinum(Pt),Gold(Au) and Palladium(Pd). Among the above four, silver nanoparticles play a significant role in the field of biology and medicine. Silver has been known to exhibit a countable toxicity to a wide range of microorganisms and because of this reason silver based compounds have been used in many bactericidal applications. Ag compound have also been used in the clinical field to treat burns and number of infections. Several salts of Ag and their derivatives are commercially used as antimicrobial agents. Primarily, silver nanoparticles are considered as an alternative to silver ions (obtained from silver nitrate) which were used as antimicrobial agents. Cassia sps have been used as traditional medicine for centuries. The whole plants have been employed in herbal medicine around the world. (Burkill 1995). Cassia auriculata is a common plant, profoundly used as antipyretic (Wealth of India), hepatoprotective (Rao and Vedavathy 1991), antidiabetic, antiperoxidative and antihyperglycemic (Manickam, et al 2002), conjunctivitis and ophthalmia (Joshi 2000), ulcers, leprosy, skin and liver diseases. Although there are several reports on synthesis of silver nanoparticles from plants and its antimicrobial activity (Ankannaand Savithramma 2011; Nasrollahi et al. 2011;Geethalakshmi and Sarada 2010). Here we report the synthesis of silver nanoparticles of varying sizes using aqueous leaf extract of *Cassiaauriculata*at different concentrations. The synthesized nanoparticles were evaluated for its antimicrobial activity against human pathogens bacteria as well as fungi.

MATERIALS AND METHODS

Plant collection and Extract preparation

The healthy leaves of *Cassia auriculata* were collected from the botanical garden Gulbarga University, Gulbarga. The leaves were gently washed with soap solution and bavistine to remove the dust and any other contaminants then shade dried at room temperature for about ten days. Dried leaves were powdered and 1%, 3% and 5% of aqueous extract was prepared by boiling the powder in distilled water for 5-10 minutes, filtered and used as reducing agent.

Synthesis of Silver Nanoparticles

10ml of aqueous leaf extract was added to 100ml of 10^{-3} M AgNO_3 solution in a 250 ml conical flask at room temperature. The color of the solution started changing within 5-10mins from yellow to dark brown (Fig.1). Further characterization was done by UV-Vis spectrophotometer, Fourier transform infrared (FTIR), X-ray diffraction (XRD) and Transmission electron microscope (TEM).

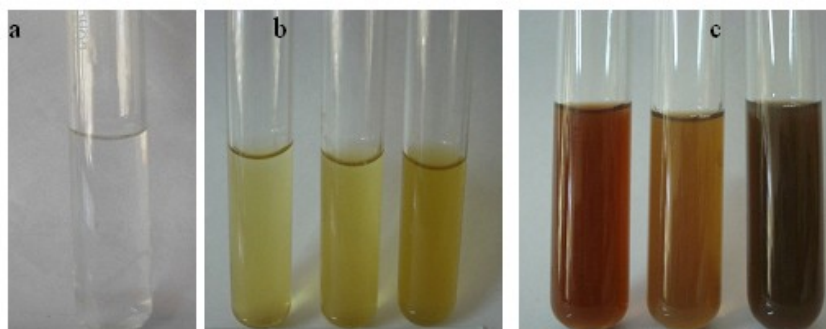


Figure 1: (a) 1mM Silver nitrate (b) Leaf extracts 1%, 3% & 5% (c) Leaf extracts + Silver nitrate after reduction

Antimicrobial assay of synthesized nanoparticles was done on *Escherichia coli*, *Bacillus subtilis* and *Serratiamarcescens* and fungi *Aspergillusniger*, *Aspergillusflavus* by disc diffusion method. Sterile 6mm of diameter of whatman no.1 filter paper disks were prepared by applying 30 μ g/ml 1%, 3% and 5% of synthesized Ag NP's and Cefotaxime as standard for bacteria and 50 μ g/ml of Ag NP's and Clotrimazole as standard for fungi. The test organisms were subculture in nutrient broth media (bacteria) and Czapekdox broth media (fungi) for 12hrs (bacteria) and 48 hrs (fungi). These cultures were used for antimicrobial assay. The nutrient agar media/Czapekdox agar media was poured in sterile petriplates under sterile conditions and left to solidify. About 0.5ml of bacterial/fungal suspension was uniformly spread on media and disks were placed with standard antibiotic disc. The culture plates were incubated at 38° C and zone of inhibition was measured after 24hrs and 48hrs

RESULTS AND DISCUSSION

UV-Vis spectra Analysis

The reduction of silver ions into silver particles during exposure to the plant extract was followed by color change from yellow to dark brown. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the plant extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from yellowish to brown due to reduction of silver ion, which may be the indication of formation silver nanoparticles (Jain et al, 2009). Almost all the herbal mediated silver nano solutions after incubation time, were showed the color change from light to dark color. The UV visible spectroscopy of the synthesized nano particles were in the range of 435nm (1%), 430nm (3%) and 420nm (5%) (Fig.2).

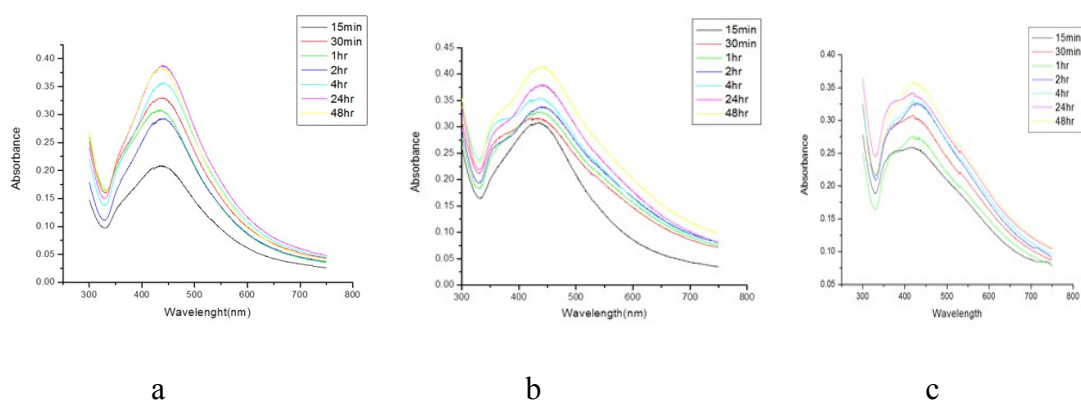


Figure 2: UV-Vis spectra of Ag nanoparticles at the following concentrations of leaf extract (a) 1% (b) 3% (c) 5%

FTIR analysis

The FTIR spectrum indicates various functional groups present at different positions. IR spectroscopy study has confirmed that the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles (i.e. capping of AgNP) to prevent the agglomeration of the particles, and thus, the nanoparticles are stabilized in the medium. The peaks in the region between 3412 to 2849 were assigned to O-H stretching of alcohol and phenol compounds and aldehyde -C-H- stretching of alkanes. The peaks in the region 1597 to 1508 and 1385 to 827 corresponds to N-H(bond) of primary and secondary amides and -C-N- stretching vibration of amines or -C-O- stretching of alcohols, ethers, carboxylic acids and anhydrides(Fig.3). FTIR analysis reveals the dual function of biological molecules possibly responsible for the reduction and stabilization of silver nanoparticles in the aqueous medium.

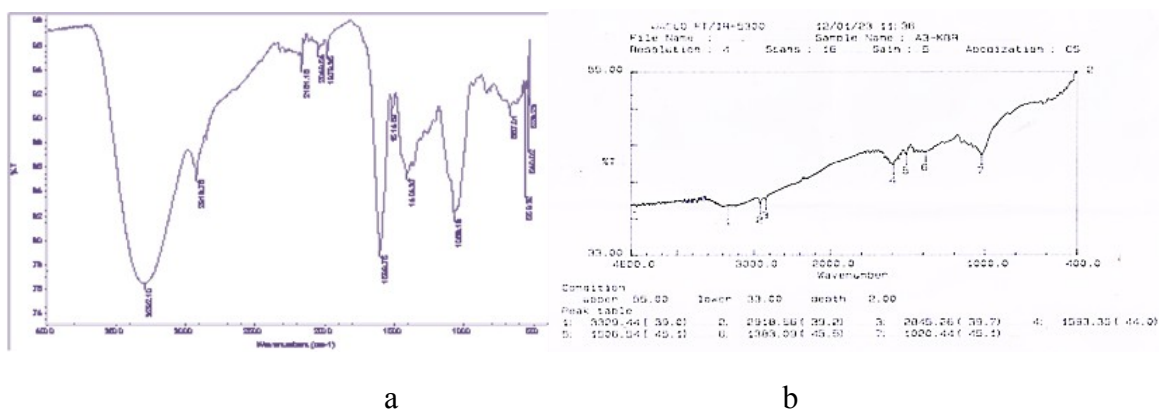


Figure 3: FTIR spectra of dried powder of *Cassia auriculata* (a) Leaf extract (b) Ag nanoparticles

XRD analysis

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of silver nanoparticles into 5ml of deionized water. After freeze drying of the purified silver particles, the structure and composition were analyzed by XRD. The crystallite domain size was calculated from the width of the XRD peaks using the Scherrer formula. $D = 0.94 \lambda / \beta \cos \theta$, where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. The average particle size obtained as 35nm (1%), 25 nm (3%) and 11nm (5%).

An XRD pattern obtained for the silver nanoparticles shows a number of Bragg reflections corresponding to (111), (200) and (210) sets of lattice planes are observed which may be indexed based on the structure of silver (Fig.4). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature.

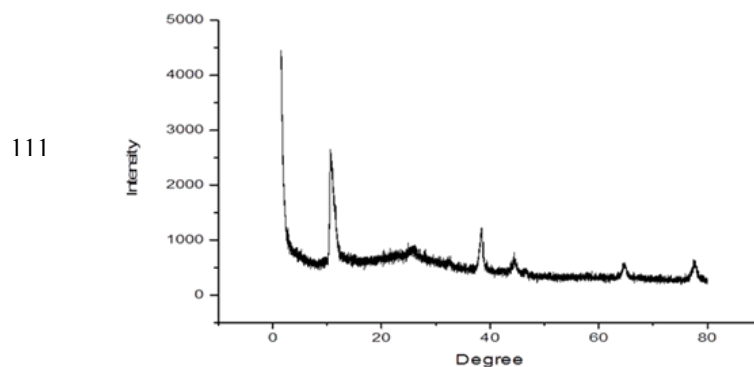


Figure 4: XRD spectra of synthesized Ag nanoparticles

TEM analysis of Silver nanoparticles

The TEM image of the silver nanoparticles produced at different concentrations were roughly circular, spherical in shape and polydispersed (Fig.5). There is no marked difference in the shape at various initial biomaterial dosages as reported by Huang et al (2007).

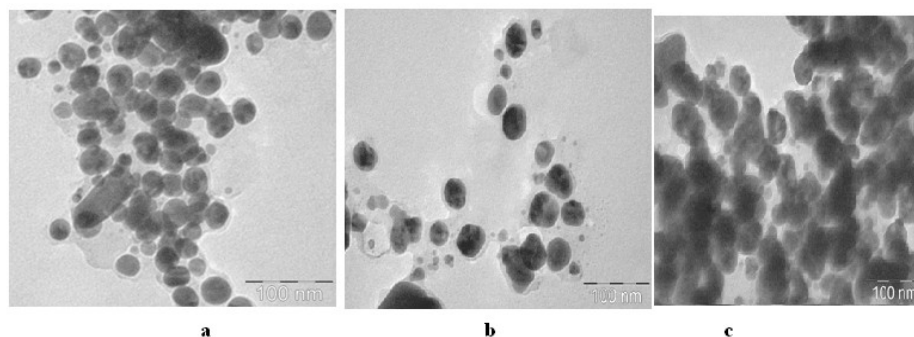


Figure 5: TEM images of Ag nanoparticles (a) 1 % (b) 3% (c) 5%

Antimicrobial assay

Antimicrobial assay of biosynthesized silver nanoparticles was examined against a gram negative *E.coli*, *Serratiamarcescens* and gram positive bacteria *Bacillus subtilis* and fungal strains like *Aspergillusniger* and *Aspergillusflavus*. AgNPs exerted highest toxicity against *Aspergillusniger* and intermediary effects on *E.coli*, *B.subtilis* and *A. flavus* and exhibited lowest effect on *Serratiamarcescens* (Fig.6). AgNPs synthesized at 5 % concentration were found to be significantly toxic to the fungi whereas standard antibiotic Clotrimazole did not show any zone of inhibition at 50 μ g/ml on *A.niger*. AgNPssynthesized at 3 % concentration were found better on all the bacteria tested followed by 1%. The maximum toxicity was observed in silver nanoparticles synthesized from 3% and 5% of leaf extract. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Our results are in agreement with those of found in *Boswelliaovalifoliata* (Savithamma et al 2011).

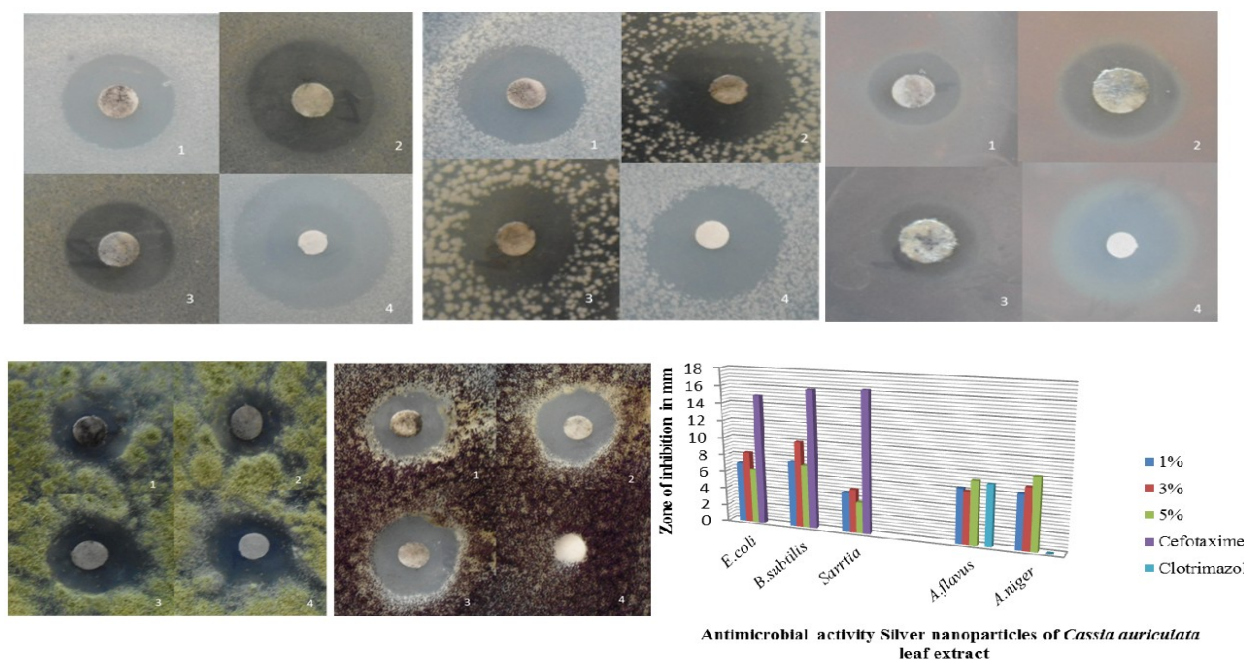


Figure 6: Antimicrobial activity of 30 μ g/ml & 50 μ g/ml (bacteria & fungi) Ag nanoparticles (1)1%, (2)3%, (3)5%, (4) Antibiotic.(a) *E.coli* (b) *B.subtilis*(c) *Sarratia* (d)*A.flavus* (e) *A.niger* (f) Comparative graphical representation of inhibition zones.

The mechanisms behind the activity of nano silver on bacteria are not yet fully elucidated, the three most common mechanisms of toxicity proposed up to now are: 1) uptake of free silver ions followed by disruption of ATP production and DNA replication (Lok et al., 2006) formation of Reactive Oxygen Species (ROS) (Park et al., 2009) direct damage to cell membranes (Raffi et al., 2008). The bactericidal activity of nanoparticles depends on the stability in the cultured medium too. The main problem is that bacteria have developed resistance towards many antibacterial agents. Hence to use Ag in various fields against microorganisms, it is needed to prepare the Ag NPs with cost effective methods and to find out the mechanism of antimicrobial activity. The Ag NPs produced here shows significant inhibitory activity against *E.coli* and *B.subtilis* and least inhibition against *Sarratiamarcescens*.

There are alarming reports of opportunistic fungal infections. The infections caused by opportunistic fungi are included under new spectrum of fungal pathogens. The results suggest AgNPs may have exerted antifungal activity by disrupting the structure of cell membrane and inhibiting the normal budding process due to the destruction of membrane integrity. The present study indicates AgNPs have considerable antimicrobial activity, deserves further investigation for chemical applications.

CONCLUSION

The present study demonstrated the extracellular synthesis of silver nanoparticles from leaf extract of *Cassia auriculata* at different concentrations and resulted in spherical and polydispersed nanoparticles. Increase in concentration of extract was found to decrease in size of silver nanoparticles. The results showed that Ag nanoparticles presented good antimicrobial activity against common pathogens. It can be concluded that the silver nanoparticles constitute an effective antimicrobial agent against common pathogenic microorganisms.

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