Green Synthesis and Characterization of Silver Nanoparticles using *Cassia auriculata* Leaves Extract and Its Efficacy as A Potential Antibacterial and Cytotoxic Effect

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Abstract

Silver nanoparticles (Ag NPs) were prepared using *Cassia auriculata* leaves extract as a reducing agent via green synthesis method. From the PXRD, UV-Visible, FTIR, studies the synthesized NPs were characterized. The morphologies of the prepared NPs were studied by SEM and TEM analysis. The synthesized NPs were tested for antibacterial and anticancer studies. The PXRD data indicated that the synthesized nanoparticles belong to cubic phase structure. Presence of strong silver peaks was confirmed by EDAX studies. The SEM and TEM data revealed that spherical like structure were obtained. Antibacterial (MIC from 75 to 150 µl) activities were noticed for green synthesized Ag NPs. Furthermore, *in vitro* studies revealed dose-dependent cytotoxic effects of Ag NPs treated PC-3 cell line. This is the first report on the green synthesis of Ag NPs using leaves extract of *C. auriculata*. Results of present study could contribute to synthesize new and cost-effective drugs from *C. auriculata* by using green approach. Copyright © VBRI Press.

Keywords: Green synthesis, *Cassia auriculata*, silver nanoparticles, antibacterial, anticancer.

Introduction

Nanoparticles are usually categorized as materials having structured components with at least one dimension less than 100 nm. Nowadays much attention has been focused on metal nanoparticles such as silver, gold, platinum, and copper as their properties may significantly differ from their respective bulk metals [**1**]. Silver nanoparticles have received special attention due to their chemical, physical, and biological properties that attributed to the catalytic activity and bactericidal effects [**2**]. They are used as antimicrobial agents [**3**], as topical creams to prevent wound infections [**4**], as anticancer agents [**5, 6**], catalysts [**7**] and anti-bacterial agents [**8**].

NPs can be synthesized by physical, biological and chemical techniques. Nanoparticles blend by substance strategies has different drawbacks which includes the utilization of unsafe dangerous synthetic substances, and high vitality utilization [**9, 10**]. Hence, with a specific end goal to conquer these constraints green combination techniques utilized either by utilizing biological microorganisms or plant extracts. Numerous research articles revealed the synthesis of Ag NPs utilizing plant extracts such as *Aloe Vera*, *Chenopodium album*, *Murraya koenigii*, *Cycas*, *Allium sativum*, *Ixora coccinea*, and *Sida cordifolia* [**11-17**].

Cancer is one of the leading diseases which will cause global death rates up to 15 million by 2020 and is characterized by proliferation of abnormal cells [**18**]. Cancer (uncontrolled cell growth) is one of the major diseases that affect humans and more than 20 % of the world's population. There are 200 different types of cancers found across the world which was served by the World Health Organization (WHO) [**19**]. Cancer is uncontrolled cell growth. Mutation in genes can causes cancer by accelerating cell division rates or inhibiting normal controls or programmed cell death. Prostate cancer was the second most commonly diagnosed cancer in men. Thus; many studies have focused on the developing anticancer agents to treat Prostate cancer. Prostate cancer affects the Prostate gland that produces some of the fluid in semen and plays a role in urine control.

In addition, the consumption of contaminated food causes serious illnesses throughout the world, where the contamination of food with pathogenic bacteria such as *E. coli, S. aureus* is a serious public health problem [**20-22**]. Food borne pathogens have recently become the most common global public health problem [**23, 24**]. Therefore, in order to overcome this problem, it is necessary to develop novel inorganic antibacterial agents

to combat *E. coli and S. aureus*. Metal NPs have good antibacterial activities but they are not highly effective against food borne pathogens.

Cassia auriculata (**Fig. 1**) is a legume tree in the subfamily *Caesalpinioideae*. It is ordinarily known by its nearby names matura tea tree, ranawara or avaram. The leaves are unpredictable, indiscriminate, splendid green and vast in estimate [**25**]. *Cassia auriculata* is appropriate for finishing roadways and home gardens as its blooms (flowers) are appealing. The plant has been appeared to have antibacterial activity in the research center.

Ag NPs material could provide opportunities to further explore its physicochemical properties and potential biological applications [**26**]. In the present work the green synthesis of Ag NPs using aqueous extract of *Cassia auriculata* leaves is an alternative to chemical methods and studied their antibacterial (foodborn) and anticancer activity against prostate cancer cell line.

Fig. 1. Photographic image of *Cassia auriculata* plant (insert leaf).

Experimental

Materials used

$AgNO_3$ (Silver nitrate) was purchased from Merck. *Cassia auriculata* leaves were collected from college of Devarayanadurga reserve forest, Tumkur, Karnataka, India.

Collection and preparation of leaves extract

The naturally gathered leaves of *Cassia auriculata* L. Rox (20 g) were altogether washed with tap water took after by twofold refined water to expel the tidy and earth particles. The cleaned leaves were taken in a 250 mL Erlenmeyer flask; 100 mL of twofold refined water was included and bubbled at 60 ℃ for 20 minutes on warming mantle. The leaves extract was filtered through Whatman's No.1 filter paper. The water extract (10 mL) was subjected to drying on petri plates in hot air oven and got the final weight of 60 mg/10 mL. The gotten product (60 mg) was used for the synthesis of Ag NPs (**Scheme 1**). In the present study, the leaves extract goes about as a decreasing and capping operator for the green union of Ag NPs.

Scheme 1. Schematic representation for the preparation of *Cassia auriculata* leaves extract.

Characterization techniques

Synthesized silver nanoparticles were characterized by Agilent Technologies-Cary 60 UV-Vis spectro-photometer. X-ray diffraction (Rigaku Smart Lab) measurement was used to study the phase and purity of the sample. Morphological distribution of the Nanoparticles was studied using TEM (Jeol/JEM 2100) and SEM (JEOL Model JSM- 6390LV) analysis.

Antibacterial assay

Antibacterial activity was screened against Gram –ve and Gram +ve bacteria by disc-diffusion method. Nutrient agar plates were prepared and swabbed using Sterile L- shaped glass rod with 100 µL of 24 h mature broth culture of individual bacterial strains. The discs were made by paper (5 mm) into each petri-plates. Ag NPs (1000 µg/well) was used to assess the activity of the NPs. The prepared NPs was dispersed in distilled water and it was used as a negative control and ciprofloxacin (5 µg/50 µL) was used positive control and incubated these petri-plates at 37 ℃ for 36 h. These petri-plates the developed zone of inhibition of every disc was measured in millimetre (mm) and the values were tabulated [**27**].

Anticancer activity assay

The cells were trypsinized and aspirated into a 15 mL centrifuge tube. Cell pellet was obtained by centrifugation at 300xg. The cell count was adjusted, using DMEM HG medium, such that 200µL of suspension contained approximately 10,000 cells. To each well of the 96 well microtitre plate, 200µL of the cell suspension was added and the plate was incubated at 37 ℃ and maintained the 5% CO_2 atmosphere for 24 h.

After incubation the spent medium was aspirated. 200 µL of different test concentrations (20, 40, 60, 80 and 100 µg/mL) from stock test drugs were added to the respective wells. The plate was again incubated at 37 ℃ with 5 % CO_2 atmosphere for 24 h. Then the plate was removed from the incubator and the drug containing media was aspirated. 200 µL of medium containing 10 % MTT reagent was then added to each well to get a final concentration of 0.5 mg/mL and the plate was incubated at 37 ℃ and 5 % CO_2 atmosphere for 3 h. The culture medium was removed completely without disturbing the crystals formed. Then 100 µL of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank. The inhibition concentration 50 % (IC_{50}) value was calculated from the dose response curve for the cell line [**28**].

Synthesis of silver nanoparticles using leaves extract

The aqueous $AgNO_3$ solution (5 mM) were equipped and used for the Ag NPs synthesis. The synthesis of silver nanoparticles was carried out at ambient-temperature for twenty-four hours and kept it in dark condition to prevent agglomeration. 10 mL of *Cassia auriculata* leaves extract was added to the $AgNO_3$ (90 mL) solution and located on a magnetic stirrer for ten minutes. After 24 h to get complete bioreduced Ag ions. The obtained silver nanoparticles were cleaned by frequent centrifugation at 8,000 rpm for fifteen minutes [**29**]. Additionally, the final material was dried and kept for additional inspection (**Fig. 2**).

Fig. 2. Green synthesis of Ag NPs using *Cassia auriculata* leaves extract.

Results and discussion

X-ray diffraction

Fig. 3 shows the XRD pattern, which is primary tool for the characterization of silver nanoparticles. The diffraction peaks at 2θ = 38.09°, 44.28°, 64.29° and 77.54° were indexed with the planes (111), (200), (220) and (311) for the resultant particles with cubic phase. The structure of obtained data well matches with the JCPDS card no. 4-783 [**30**]. Plotted XRD pattern indicates the formation high purity of the silver nanoparticles and there are no contamination peaks were detected. This calculation exposes that the average crystallite size (D) of Ag nanopowder was found to be ~9 nm using Debye Scherrer's equation.

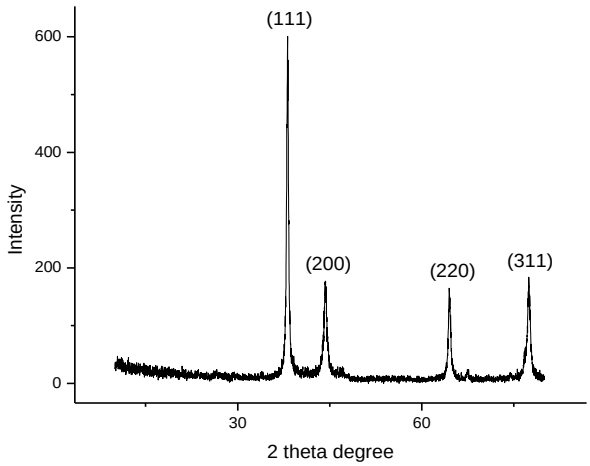


Fig. 3. PXRD pattern of the Ag NPs.

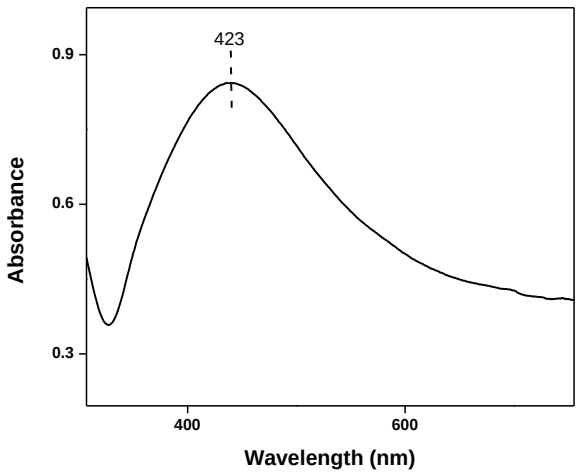


Fig. 4. UV-vis spectrum of Ag NPs.

UV-Vis spectroscopy

UV– visible absorbance unearthly incentive at 423 nm has affirmed the lessening of silver ions to metallic Ag nanoparticles (**Fig. 4**) by utilizing the leaves extract. The surface Plasmon resonance band at 423 nm affirmed the green union of Ag NPs [**31**]. The arrangement of Ag NPs was checked by UV– visible spectra at various time intervals; after 24 h the most extreme lessening and development of Ag NPs was watched which is set up in the absorbance force.

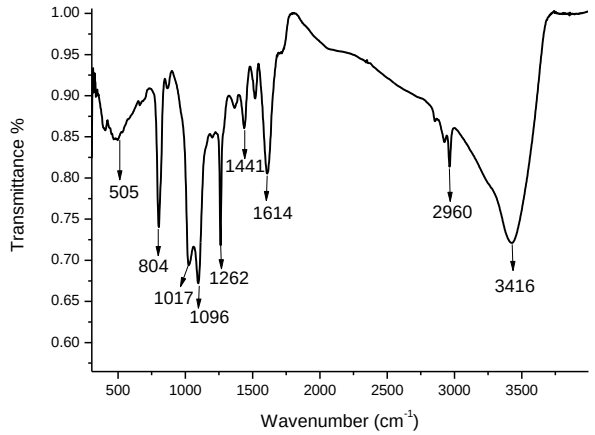


Fig. 5. FTIR spectrum of Ag NPs.

Fourier-transform infrared spectroscopy and phytochemical studies

The biogenic Ag NPs were characterized by FTIR spectrum (**Fig. 5**). The IR bands was observed at 3416, 2960, 1614, 1441, 1262, 1096, 1017, 804 and 505 cm^{-1}. The strong broad band which appeared at 3416 assigned to O-H Alcohol stretching, the medium bands at 2960 Alkane C-H, 1614 conjugated Alkene C=C, 1441 Carboxylic acid O-H, the strong bands at 1262 Aromatic ester C-O, 1096 Aliphatic ester C-O, 1017 Amine C-N, the medium bands at 804 Alkene C=C and 505 corresponds to Halo compound C-I [**32**].

The obtained FTIR spectrum comes about showed that the leaves extract phytochemical compounds for example, flavonoids, terpenoids, phenols, tannins, anthraquinones, carbohydrates, alkaloids, saponins and cardiac glycosides (**Table 1**), may take an interest during the time spent nanoparticles union.

Table 1. Phytochemical analysis *Cassia auriculata* leaves extract.

Sl. No.	Phytochemicals	Results
1.	Phenols	++
2	Flavonoids	+++
3	Terpenoids	++
4	Saponins	+
5	Alkaloids	+++
6	Carbohydrates	+
7	Cardiac glycosides	+++
8	Oxalate	—
9	Amino acids	—
10	Tannins	+
11	Anthraquinones	+

+: Confirms, —: Absent.

Scanning Electron Microscopy with Energy-dispersive X-ray (EDAX) studies

SEM images of Ag NPs are shown in **Fig. 6a** and **6b**). From the SEM images it is clearly confirmed that the material synthesized by green way is almost uniform spherical in nature. The particles are agglomerated crystals and looks like a spherical like structures [**33**].

The Energy-dispersive X-ray spectroscopy describes elemental analysis of the Ag nanoparticles. An obtained spectrum show characteristics of silver signals was given at the energy of three keV for silver (**Fig. 6 c**). [**34**].

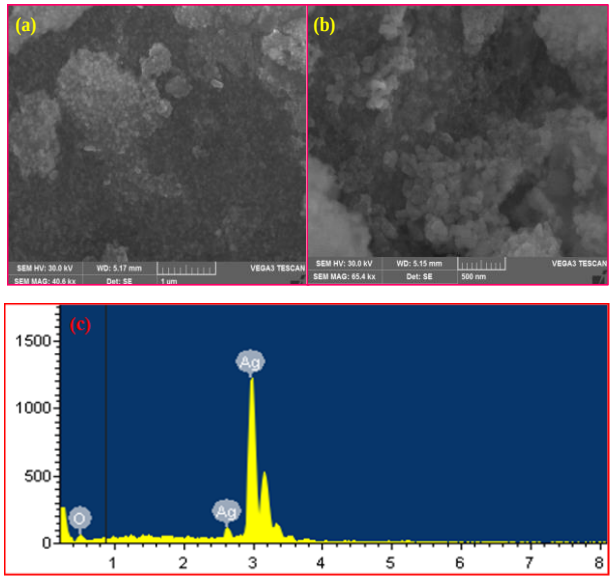


Fig. 6. (a), and (b) SEM images of Ag NPs (c) EDAX spectrum of Ag NPs.

Transmission electron microscopy

Fig. 7 demonstrates a very much scattered Ag NPs has recognized in the sizes run 30–50 nm with an average size of 35 nm. The particles are unmistakably distinguished by their spherical shapes [**35, 36**].

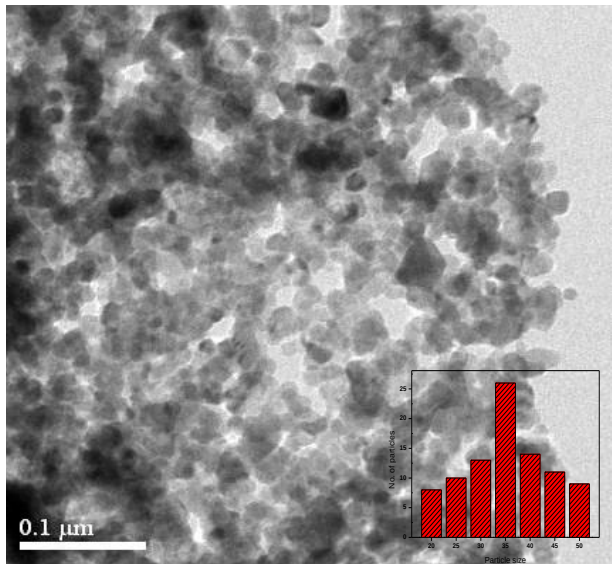


Fig. 7. TEM image of Ag NPs.

From the **Table 2**, green synthesis method has more advantages such as (shorter reaction time and enhanced reaction rate, improve the yields and high energy of efficiency) compared with some previously published data.

Table 2. Comparison of present work with some other previously published work.

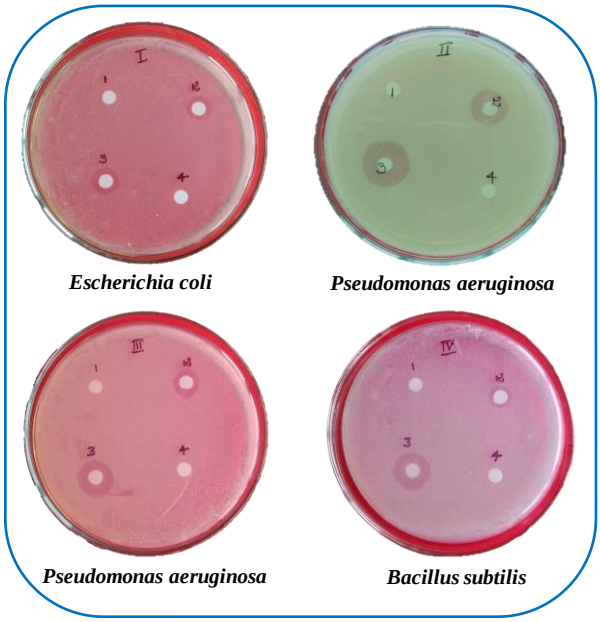
Method	Silver precursor	Reducing agent	Size (nm)	Reference
Green synthesis	$AgNO_3$	*Passiflora*	30-50	[37]
Green synthesis	$AgNO_3$	*Enicostemma axillare*	15-20	[38]
Green synthesis	$AgNO_3$	*Rhodiola rosea*	5-10	[39]
Green synthesis	$AgNO_3$	*Abutilon indicum*	20-100	[40]
Green synthesis	$AgNO_3$	*Cassia auriculata*	30-50	Present work

Antibacterial studies

The synthesized silver nanoparticles by the leaves extract of *Cassia auriculata* has a significant antibacterial activity against *Pseudomonas aeruginosa* followed by *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition was observed in the range of 10 to 17 mm (**Fig. 8; Table 3**) [**41-45**].

Table 3. Antibacterial activity of Ag NPs.

Strains	Control	Standard	Ag NPs	Leaves extract
Escherichia coli	–	10.01 ± 0.21	8.83 ± 0.33	–
Pseudomonas aeruginosa	–	17.50 ± 0.29	13.10 ± 0.21	–
Staphylococcus aureus	–	14.83 ± 0.17	10.83 ± 0.17	–
Bacillus subtilis	–	14.83 ± 0.33	7.50 ± 0.29	–



Notes: 1. Control, 2. Ag NPs, 3. Standard, 4. Plant extract.

Fig. 8. Antibacterial activity of Ag NPs.

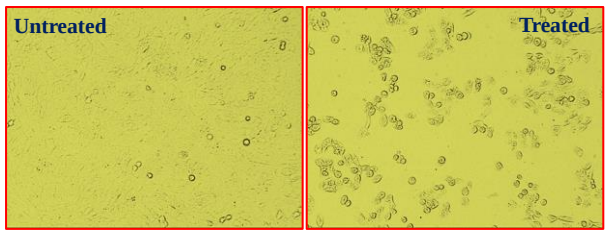


Fig. 9. Cytotoxicity of green synthesized Ag NPs against human prostate cancer cell line PC-3.

Anticancer studies

The present work agreement with the desired application for cancer treatment. Anticancer activity in terms of the cell viability against PC-3 cell line is presented in **Fig. 9**. MTT assay was performed to assess the effect of Ag NPs concentration (20-100 µg/mL) on cell viability of PC-3 cell line. The percentage of cell death in PC-3 cells gradually increases with increasing the concentration of Ag NPs concentration. The PC-3 cells are exposed to Ag NPs at the concentration of 20, 40, 60, 80 and 100 µg/mL. Curve software has been utilized to evaluate the IC_{50} values [**46**]. 103 µg/mL is selected as the IC_{50} of Ag NPs (**Fig. 10**). The results clearly illustrate that the Ag notably inhibits the cancer cells with a moderate concentration. The percentage of live cells were reduced as the concentration of Ag NPs against PC-3 cell line. A significant decrease in the cell viability showed a dose-dependent toxicity exhibited by Ag NPs. At present, Ag NPs have been used in biomedical applications including chemotherapy and drug delivery etc. [**47**].

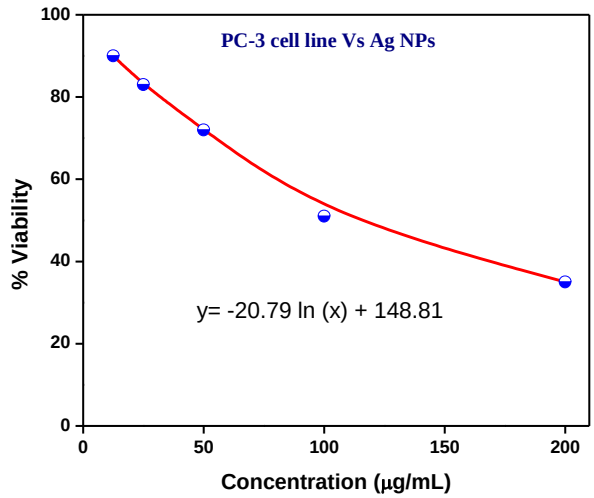


Fig. 10. Percentage of cell inhibition.

Conclusion

In the present work, green synthesis method was employed to obtain spherical Ag NPs from the assistance of *Cassia auriculata* leaves extract as a green reducing agent and stabilising agent. XRD, FTIR, SEM, TEM, and UV-Vis techniques were utilized to characterize the as synthesized NPs. SEM and TEM images reveal that nanoparticles possess spherical like shapes. The average

crystallite size was found to be 35 nm. Ag NPs exhibited significant antibacterial activity against the gram positive and negative bacterias. Furthermore, the cytotoxic activity of the Ag NPs was high against the prostate cancer cell line (PC-3) with IC_{50} value of 103.24 µg/mL. Overall, the green synthesized Ag nanoparticles will be useful in biomedical applications (food packaging and pharmaceutical industries).

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Conflicts of interest

The authors declare that they have no conflicts of interest in this work.

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