

Green synthesized of silver nanoparticles using *Canna indica* leaf extract and its characterization

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Abstract: In recent years a number of physical and chemical techniques were applied for the development of metal nanoparticles (MNPs). In present study, we synthesized silver nanoparticles from leaf extract of *Canna indica*. The resulting silver nanoparticles were characterized by using UV –visible absorption spectra of silver nanoparticles formed in the reaction media has absorbance maxima at 454.5nm, X-ray diffraction (XRD) intensities were recorded from 10 ° to 80 ° at 2theta angles and EDX analysis to study the crystalline nature and presence of the silver nanoparticles and Fourier transform infrared spectroscopy (FTIR) spectra revealed the presence of different functional groups. Further, the SEM and TEM analysis of silver nanoparticles were analyzed for determination of particle size and its shape.

Keywords: *Canna indica*, silver nanoparticles, UV–Visible Absorption spectra, Fourier transform infrared spectroscopy.

Introduction:

Nanotechnology provides the tools and technology platform for the investigation and transformation of biological systems, and biology offers inspiration models and bio-assembled components to nanotechnology. Nano biotechnology is defined as a field that applies the Nano scale principle and techniques to understand and transform bio systems (living and non -living) and which uses biological principles and materials to create new devices and systems integrated from the nanoscale¹. Key advances have been made in the ability to make measurements at the sub-cellular level and in understanding the cell as highly organized, self-repairing, self-replicating, information rich molecular machines^{2,3}. Smalley, classified nanotechnologies into wet and dry nanotechnology, the first one describes the living bio systems and second one deals with man -made objects at nano scale structures⁴

Among the different living organisms used for nanoparticles synthesis, plants are of particular interest in metal nanoparticles synthesis because of its advantage over other environmentally benign biological process as it eliminates the elaborate process of maintaining cell cultures. Plant mediated synthesis of nanoparticles is gaining importance due to its simplicity and ecofriendly. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver nanoparticles in medical industry is topical ointments to prevent infection against burn and open wounds⁵. The reduction of Ag⁺ ions by combinations of bio molecules found in the extracts such as vitamins, enzymes/proteins, organic acids such as citrates, amino acids, and polysaccharides⁶. Currently, the investigation of this fact has regained importance due to the increased bacterial resistance to antibiotics, caused by their over usage. Many evidence based studies of the past shows that there is virtually no bacterial strain resistant to silver's powerful antibacterial effects.

Canna indica L., is commonly known as Indian shot or Canna lily. Several varieties are common all over India and are grown in gardens. It is extensively used in constructed wetland for removal of organic pollutants, nitrogen, phosphorus and heavy metals^{7,8}. The flowers are red or yellow and showy. It encloses a variable number of rounds, shiny black seeds. In folkloric medicine, root decoction is used for the treatment of fever, dropsy, and dyspepsia. Seed juice is used to relieve earaches. The flowers are said to cure eye diseases^{9,10}. The leaves of *C. indica* showed antimicrobial activity¹¹, analgesic activity, and the rhizomes showed a good anthelmintic activity against *Pheritima posthuma*¹². The flower are said to cure eye diseases¹³ shows antibacterial activity¹⁴. Flowers contain lutein, β -carotene, violaxanthin. Its leaves have chemical constituents like lignin, furfural, hemicelluloses. While rhizomes has 5,8-Henicosdine, Tetracosane, Tricosane^{15,16}. The water extract of rhizomes of *C. indica* has been reported to have HIV-1 reverse transcriptase inhibitory activity¹⁷ while its essential oil shows antibacterial activity¹⁸. Methanolic extract of Aerial Parts of *Canna indica* shows Antioxidant Activity¹⁹. Anthocyanins and methylated anthocyanidin glycosides were also isolated from *canna indica* flowers^{20, 21}.

In view of the importance of *Canna indica* and silver nanoparticles, the present work has been planned to synthesize and characterize the silver nanoparticles synthesized from the leaf extracts of *Canna indica* using AgNO_3 using greener techniques.

Material and methods:

Preparation of *Canna indica* leaf Extract:

The AR grade silver nitrate (AgNO_3) was purchased from Sigma-Aldrich chemicals and fresh *Canna indica* plant were collected from surroundings of Botanical garden, University College of Science, Osmania University, Hyderabad, India (Fig 1).

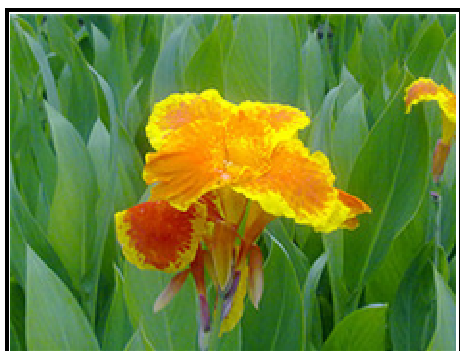


Fig.1: *Canna indica* (Kardali plant)

The *Canna indica* fresh leaves were used for the reduction of Ag^+ ions to Ag^0 was prepared by taking 25g of thoroughly washed finely cut leaves in 500 ml Erlenmeyer flask along with 150 ml of distilled water and then boiling the mixture on sand bath for 30 min at 50°C . Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4°C and used for further experiments.

Synthesis of silver nanoparticles:

In experiment, the *Canna indica* leaf extract (2 ml) was added to 10 ml of 1 mM AgNO_3 aqueous solution and heated the mixture for 20 min at 40°C on magnetic stirrer. The bio reduced component was used to measuring UV-Vis spectra of the mixture.

UV-Vis spectral analysis:

The color change in reaction mixture was recorded through visual observation. Synthesized silver nanoparticles was confirmed by sampling the aqueous component after reaction and the absorption maxima was scanned by UV-Vis spectrophotometer at the wavelength of 300-700 nm on FLICO SL-159 Spectrophotometer.

X-ray diffraction studies:

The formation and quality of compounds were checked by X-ray diffraction (XRD) spectrum. The mixture was centrifuged at 10000 rpm for 15 minutes in centrifuge, followed by redispersion of the pellet with

Millipore water. The dispersed pellets were dried in an incubator at 37°C. The size of the purified Ag nanoparticles was analyzed by X-ray powder diffraction crystallography Phillips, Holland model diffractometer with Cu-K α radiation ($\lambda=1.540\text{nm}$).

FTIR analysis of silver nanoparticles:

To remove any free biomass residue or compound that is not the capping ligand of the nanoparticles, the residual solution of 100 ml after reaction was centrifuged at 10000 rpm for 15 min. The supernatant was again centrifuged at 10000 rpm for 30 min and the pellet was obtained. This is followed by redispersion of the pellet of Ag-NPs into 1 ml of deionized water. Thereafter, the purified suspension was dried to obtain dried stable powder. Finally, the dried nanoparticles were analyzed by FT-IR Spectrometer.

SEM –EDX and TEM analysis of silver nanoparticles:

The dried and stable powder was analyzed with Transmission electron microscope (TEM) (Philips CM-10) and Scanning electron microscope (SEM) (Hitachi S - 4500) to investigate morphology and size of the particles.

Table.1: The composition (EDX) of silver nanoparticles synthesized from *Canna indica* leaf extract

Element	Weight%	Atomic%
C K	5.96	19.30
O K	17.99	43.73
Cl K	9.69	10.63
K K	3.81	3.79
Ag L	62.56	22.56
Totals	100.00	

Results and Discussion:

It is well known that silver nanoparticles exhibit Reddish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles. The leaf extracts was used for the synthesis of silver nanoparticles. The reaction started within ten minutes of the incubation with silver nitrate. This was confirmed by the appearance of Reddish brown color in the reaction mixture (Fig 2).

Fig 2

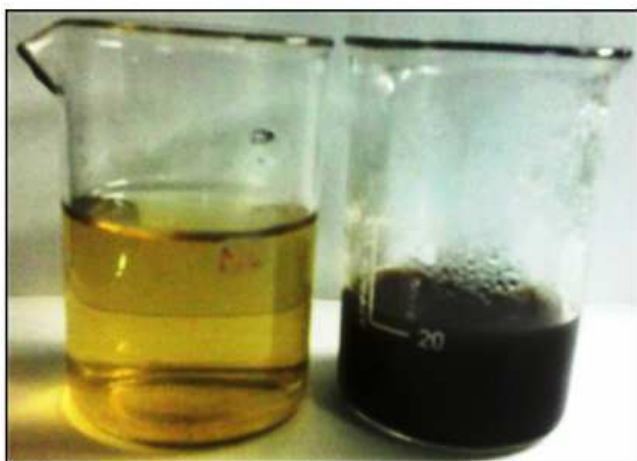


Fig.2: *Canna indicaleaves* extract and Silver nanoparticles (dark reddish color).

Figure 3 shows the UV-Vis spectra which are recorded after the completion of the reaction. In order to validate the synthesis of silver nanoparticles after 20min incubation, peak specific for the synthesis of silver nanoparticles was obtained at 454.5 nm. The frequency and width of the surface Plasmon absorption depend on the size and shape of the metal nanoparticles.

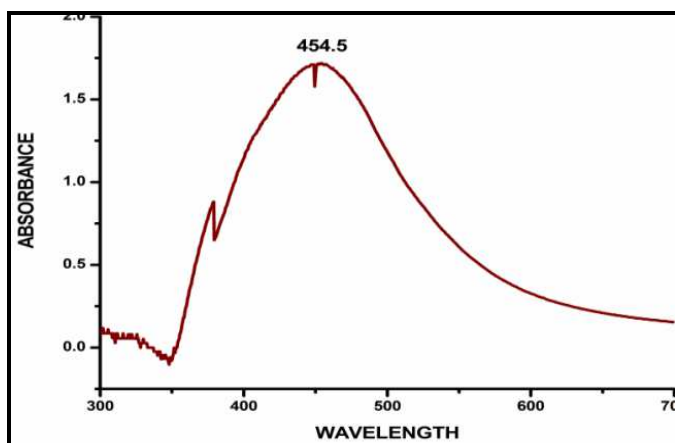


Figure 3: UV-Vis Spectroscopic analysis of Silver Nanoparticles

The FT-IR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the silver nanoparticles synthesized by *Canna indica* leaf extract. Figure 4 represents the FTIR spectrum of the leaf extract and shows peaks situated at about 3851.84cm^{-1} , 1558.48cm^{-1} , 1384.88cm^{-1} , 858.48cm^{-1} , 433.98cm^{-1} , in the region of 4000 cm^{-1} to 500 cm^{-1} .

The FTIR spectra showed the presence of different functional groups like Alcohol (O-H stretching, H-bonded), Alkane (C-H stretching), Alkene (C=C stretching), Aromatic (C=C stretching), Amine (C-N stretching) respectively. The absorption at about 1384 cm^{-1} is notably enhanced indicating residual amount of NO_3 in the solution. The peak at 1558 cm^{-1} may be assigned to symmetric stretching vibrations of $-\text{COO}^-$ (carboxylate ion) groups of amino acid residues with free carboxylate groups in the protein. The peak at 3851 cm^{-1} indicates polyphenolic OH group along with the peak of 858 cm^{-1} which represents the aromatic ring C-H vibrations, indicate the involvement of free catechin.

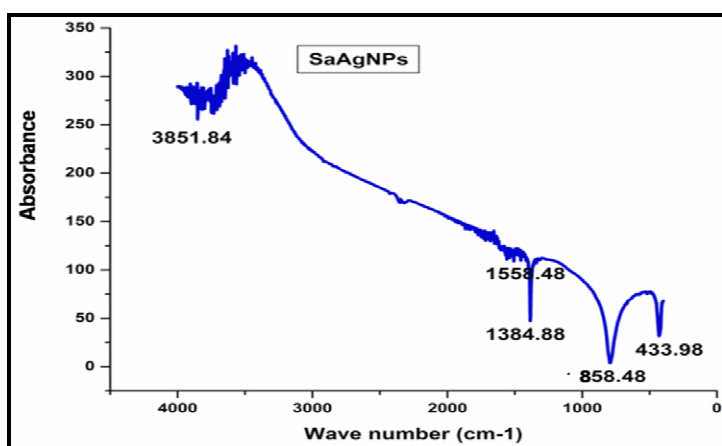


Figure 4: FTIR analysis of synthesized silver nanoparticles

To study the crystalline nature of the silver nanoparticles of *Canna indica*, the XRD analysis was undertaken, Figure 5 revealing peaks at degree (2θ) 38.12, 44.26 and 64.42 corresponding to diffraction components of silver. The extra peaks are formed due to the unreacted molecules of leaf extract. The broadening of X-ray peaks observed is primarily due to the small particle size. The spectra were recorded in Seifert -Jso-Debye flex 2002 X-ray diffractometer. The mean size of silver nanoparticles was calculated using the Debye-Scherrer's equation. An average size of the AgNPs synthesized by *Canna indica* was 30.0nm.

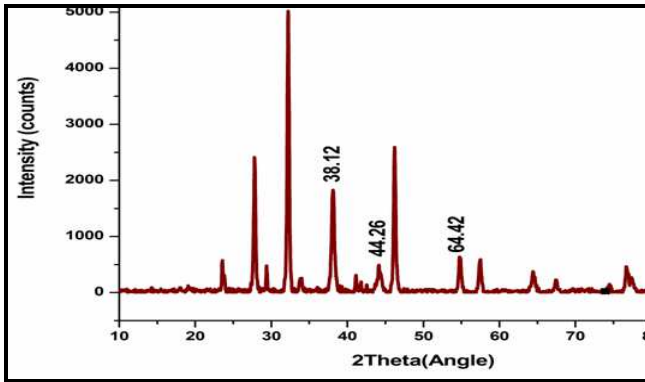


Figure 5: XRD analysis of *Canna indica* silver nanoparticles

Scanning and Transmission Electron Microscopic studies were carried out to study the morphology and shape of the silver nanoparticles. The SEM pictures indicated that the formed particles were from spherical to oval in shape as shown in Figure 6A. EDX analysis confirmed the presence of elemental silver as the major constituent (Fig 6B and Table 1).

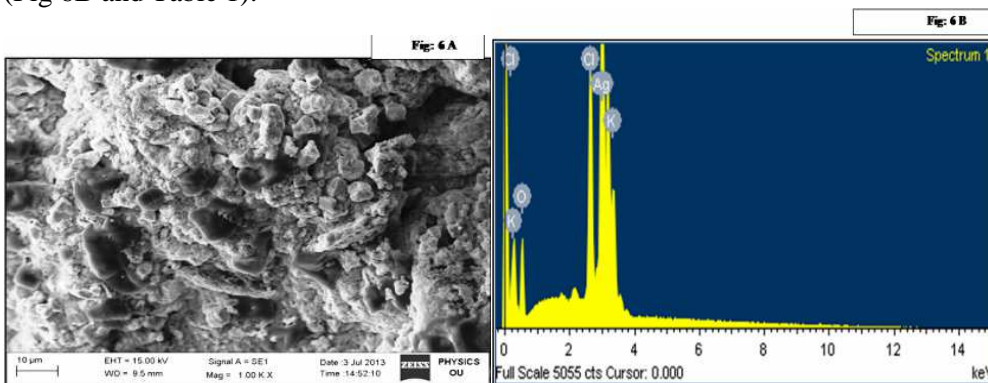


Figure 6: SEM analysis and EDX spectrum of synthesized silver nanoparticles

From the figure 7, the size of the formed silver nanoparticles was found to be within the range 25-30 nm.

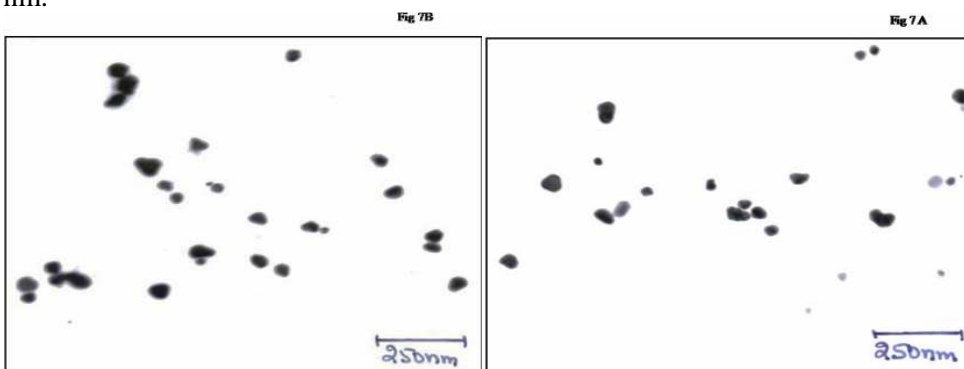


Figure 7: TEM images of biosynthesized silver nanoparticles using *Canna indica* leaf extracts at 250 nm scales

Conclusion:

In conclusion, there has been an exponentially increasing interest in biological synthesis of AgNPs. In this study, AgNPs were synthesized by an ecofriendly and rapid method using *Canna indica* leaf extract. *Canna indica* leaf extract has been used as a reducing agent for the synthesis of silver nitrate into silver nanoparticles. Green synthesized silver nanoparticles are confirmed by color change which was characterized by UV-Vis spectroscopy at 454.5nm. Further characterization with SEM and TEM analysis shows the spherical AgNPs of particle size ranging from 25 to 30 nm. FTIR showed the structure, the respective bands of the synthesized nanoparticles, and the stretch of bonds. EDX showed the elemental composition of synthesized silver nanoparticles.

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