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Cicer arietinum Leaf Extract Mediated Synthesis of Silver Nanoparticles and Screening of Their Antimicrobial Activity

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Silver nanoparticles have received substantial attention for various reasons—like magnetic and optical property, electrical conductivity, catalysis, antimicrobial activities. The use of silver and other metal ions for their sustained antifungal, antibacterial and antiviral effects has also been evaluated. Silver nanoparticles synthesized from wet chemical method are quite often toxic and flammable, while green chemical approach for the synthesis of silver nanoparticles provides an opportunity for large scale production of nanoparticles without any conflicting effect. This process is simple, rapid, economic and environment friendly, so in this study we have synthesized Ag NPs from *Cicer arietinum* leaf extract, characterized using UV-Vis spectrophotometer, FTIR, XRD, SEM, EDS, AFM and evaluated its antimicrobial activity.

Keywords: Silver Nanoparticles (Ag NPs), PL, FTIR, XRD, AFM, SEM, EDS, *Cicer arietinum* Leaf Extract, Surface Plasmon Resonance.

1. INTRODUCTION

Nanobiotechnology has emerged as an integration of biotechnology and nanotechnology for developing biosynthetic and environmental friendly technology for the synthesis of nanoparticles and their applications. Nanobiotechnogy is likely to make impact on human life to a great extent. Synthesis of nanoparticles via green route opened up a new area of research.[1,2] Nanoparticles show novel and improved catalytical,[3] optical,[4] electronical[5] properties and high reactivity compared to bulk material owing to their large surface area.[6,7] Nano sized metal particles exhibit remarkable physical, chemical and biological properties.[8]

Metallic nanoparticles are traditionally synthesized by wet chemical synthesis where the chemicals used are often toxic and flammable. Since noble metal nanoparticles are widely used in biological applications. So, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis that do not use toxic chemicals. Biological methods of nanoparticle synthesis using micro-organisms, leafs including algae, fungi,[9–11] bryophytes, pteridophytes etc. have been suggested as possible eco-friendly alternatives to chemical and physical methods.[12] Among the various inorganic metal nanoparticles, silver nanoparticles have received substantial attention for various reasons—like magnetic and optical property, electrical conductivity, catalysis, antimicrobial activities.[13] The use of silver metal ions for their sustained antifungal, antibacterial and antiviral effects has been practiced.[14]

In the present research work, we report the synthesis of silver nanoparticles using *Cicer arietinum* leaf extract as a reducing agent. Ag NPs are formed by reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *Cicer arietinum* and characterized using UV-Vis Spectroscopy, PL, FTIR, XRD, SEM, EDAX and AFM. To the best of our knowledge this is the first time, synthesis of silver nanoparticles, using *Cicer arietinum* leaf extract.

2. EXPERIMENTAL DETAILS

2.1. Preparation of Leaf Extract

The leaf material were collected from university campus and washed with sterile distilled water. The leaf extract was prepared by taking 20 g of thoroughly washed leaf material in a 250 mL Erlenmeyer flask with 100 mL of distilled water, and then boiling the mixture for 10 min in

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a water bath. The leaf broth was then cooled and filtered through Whatman No. 1 filter paper (pore size 25 μm).

2.2. Synthesis of Silver Nanoparticles

Aqueous solution of 1 mM silver nitrate ($AgNO_3$, Merck, India) was prepared and used for the synthesis of Ag NPs. 10 ml of Leaf extract was added into 90 ml of aqueous solution of 1 mM $AgNO_3$ solution, drop by drop for bioreduction process, at room temperature. The resulting Ag NPs solution was purified by repeated washing with DW and centrifugation at 7000 rpm for 20 min. The colour of the solution changed from light yellow to brown indicating the formation of silver nanoparticles.

2.3. UV-Visible Spectroscopy

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 2 hours after diluting a small aliquot of the sample into distilled water. UV Vis spectral analysis was done by using UV-Vis spectrophotometer (Shimadzu).

2.4. Photoluminescence (PL) Spectroscopy

The PL emission spectrum of Ag NPs was recorded by (RF5301 PL Shimadzu) Spectrofluorimeter using a four side polished quartz cuvette of path length 10 mm. For PL analysis sample were dispersed in distilled water.

2.5. Fourier Transform Infrared Spectroscopy (FTIR)

After the complete reduction of Ag^+ ions by the leaf extract, it was analysed by FTIR spectrophotometer (IR Affinity-1 Shimadzu) in the range of 4000–500 cm^{-1} for knowing the possible functional groups responsible for the formation of silver nanoparticles.

2.6. X-Ray Diffraction (XRD) Measurements

The dried mixture of silver nanoparticles was analysed by an X'Pert Pro X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ–2θ configurations.

2.7. Scanning Electron Microscopy

SEM analysis was done using scanning electron microscope (Carl ZEISS EVO^R-18) operated at 20 kV. For the SEM analysis sample was first sonicated for 15 min. A drop of this solution was loaded on carbon coated copper grids and solvent was allowed to evaporate to leave a thin film on the substrate.

2.8. EDS Measurements

EDS measurement of the *Cicer arietinum* reduced Ag NPs drop coated onto Al substrate were performed on a SEM instrument equipped with Oxford instrument nano analysis X-act EDAX attachment.

2.9. Atomic Force Microscopy

Purified Ag NPs in suspension were also characterized for their morphology using Atomic Force Microscope (Nanosurf). A small volume of sample was spread on a well cleaned glass substrate and allowed to dry. AFM image was obtained in contact mode using a silicon probe cantilever of 125 μm length, resonance frequency 13 kHz, force constant 0.2 N/m.

2.10. Antibacterial Assay

The Ag NPs synthesized from *Cicer arietinum* leaf extract were tested for bactericidal activity against pathogenic *E. coli* bacteria by standard disc diffusion method. Luria Bertani (LB) broth was used to cultivate bacteria.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Absorbance Studies

There was a visible colour change from green to brownish as the leaf extract was mixed in the aqueous solution of the silver ion complex which indicated formation of silver nanoparticles shown in Figure 1. This was further confirmed by UV-Visible spectrophotometer by obtaining a spectrum in visible range of 300 nm to 800 nm. A typical absorbance peak at 418 nm of silver nanoparticles was obtained due to the surface Plasmon vibrations of silver nanoparticles, indicating reduction of $AgNO_3$ into silver nanoparticles. Figure 2 shows the UV-Visible spectrum recorded from the sample after 2 hours of the reaction. Broadening of the peak shows that particles were polydispersed.

3.2. PL Analysis

The synthesized Ag NPs were found to be Photoluminescent. Figure 3 shows the luminescence spectrum of freshly prepared Ag NPs, which exhibited a sharp and strong peak near 365 nm and a broadened band between 500–600 nm.

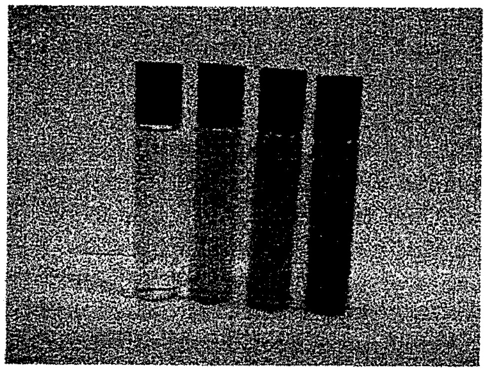

Figure 1. A visible observation of change in colour during silver nanoparticles formation (a) $AgNO_3$ solution (1 mM) (b) *Cicer arietinum* leaf extract (c) synthesized Ag NPs in brown colour solution after 1/2 hrs of incubation (d) after 2 hrs of incubation.

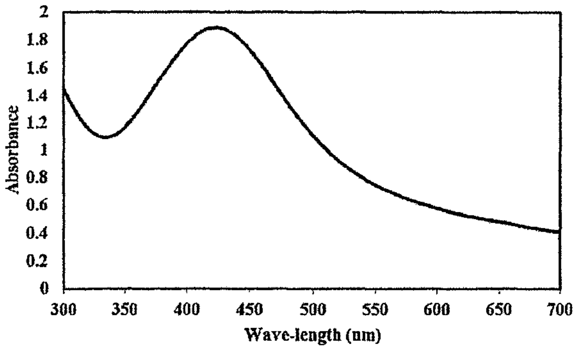

Figure 2. UV-Vis absorption spectrum of silver nanoparticles synthesized by reacting 10^{-3} M aqueous $AgNO_3$ solution with the *Cicer arietinum* leaf extract after 2 hrs.

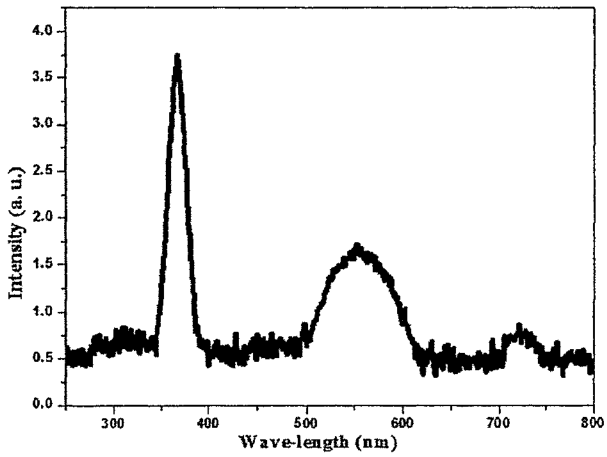

Figure 3. Photoluminescence spectrum of Ag NPs.

3.3. FTIR Analysis

FTIR measurement carried out to identify the possible interaction between biomolecule and SNPs. The FTIR measurements of biosynthesized silver nanoparticles show bands at around 763, 1605, 1722, 3165 and 3679 cm^{-1}. Absorption peak at 1605 cm^{-1} may be assigned to the

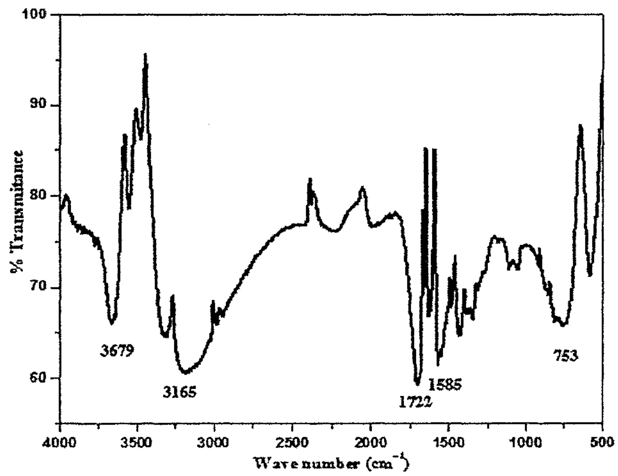

Figure 4. FTIR spectra of vacuum dried powder of silver nanoparticles.

Table I. Result obtained from XRD analysis.

S. no.	2θ value	Plane	Element	Phase
1	38.04	111	Ag	Cubic
2	45.97	103	Ag	Hexagonal
3	54.73	006	Ag	Hexagonal
4	76.61	201	Ag	Hexagonal

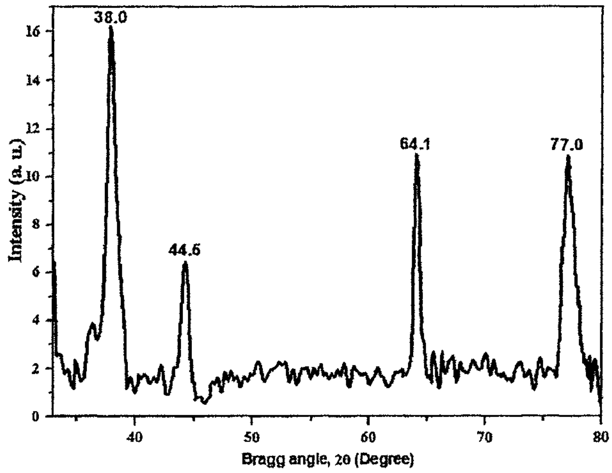

Figure 5. XRD pattern of silver nanoparticles.

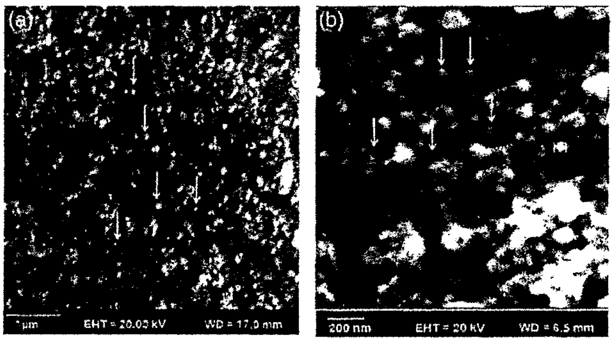

Figure 6. SEM images of silver nanoparticles (a) the spherical shaped nanoparticles in the range of 90 nm in 1 μm scale (b) in 200 nm scale.

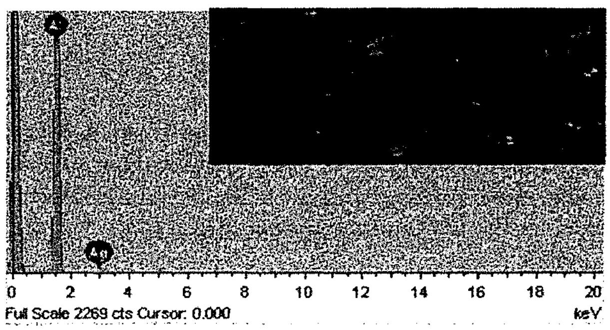

Figure 7. EDS spectra of synthesized silver nanoparticles.

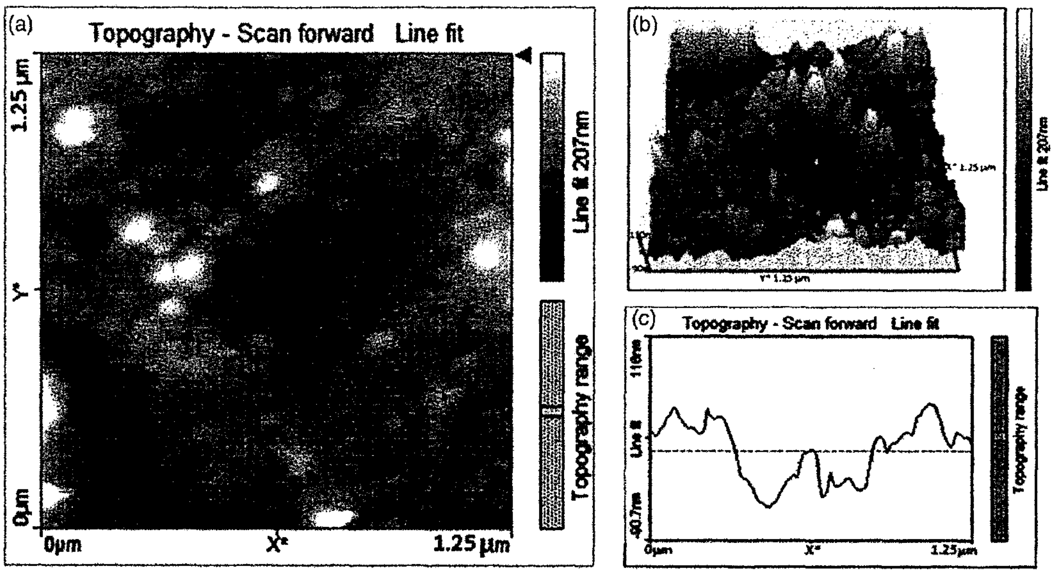

Figure 8. AFM images of silver nanoparticles synthesized by *Cicer arietinum* leaf extract (a) two dimensional (b) three dimensional (c) line graph.

primary amines (N–H) and peak at 1722 cm^{-1} assigned to carbonyl functional group in ketone, aldehydes and carboxylic acids.

The band at 3156 cm^{-1} is characteristic to hydroxyl functional group in carboxylic acid compound present in high concentration while the band at 3679 cm^{-1} show hydroxyl functional groups present in alcohol and phenols in low concentration. The absorption peak at 763 cm^{-1} is due to the aromatic C–H group (Fig. 4).

3.4. XRD Analysis

Structural characterization of biologically synthesized Ag NPs has been performed using XRD analysis. A typical XRD pattern of Ag NPs exhibited 4 XRD peaks appear at 38.0, 44.6, 64.1, 77.0 (Table I) which is due to the interaction of primary X-ray with the plans of silver nanoparticles (JCPDF card file no. 00-041-1402). The XRD analysis revealed that the sample contained a mixed phase (cubic and hexagonal) structures of silver nanoparticles (Fig. 5).

3.5. SEM Analysis

Figure 6 illustrates the SEM micrographs of Ag NPs being formed using *Cicer arietinum* leaf extract. It was observed that Ag nanoparticles were spherical in shape and particles were found to be round 40–60 nm.

3.6. EDS Analysis

Spot profile spectra recorded from the silver nanoparticles showed a strong silver signal along with an Al signal which is due to the thin film made on the Al substrate taken for the EDAX measurement (Fig. 7).

3.7. AFM Analysis

The Ag NPs were characterized by AFM for its topography and morphology. Figures 8(a) and (b) show two dimensional and three dimensional AFM images of Ag NPs synthesized by *Cicer arietinum* leaf extract. The particle size of Ag NPs was measured and found in the range of 40–60 nm.

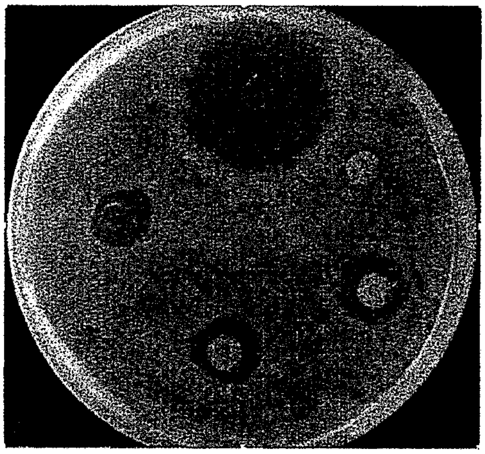

Figure 9. Image of antibacterial activity of discs containing 50 ppm Ag NPs and Standard antibiotics against pathogenic *E. coli.* bacteria (N = Ag NPs, R = Rifampicin, C = Cefimex, A = Ampicilin, T = Tetracycline).

3.8. Antibacterial Assay

The results indicated that silver nanoparticles synthesized from *Cicer arietinum* leaf extract showed a clear inhibition zone against *E. coli* bacteria and possess stronger antibacterial activity compared to standard antibiotic Ampicilin (Fig. 9).

4. CONCLUSIONS

The present work shows the ecofriendly use of a *Cicer arietinum* leaf extract first time for the synthesis of silver nanoparticles. The structural characterization was done using SEM, AFM, and XRD analysis. UV-Vis spectroscopy revealed the surface Plasmon property, while FTIR analysis confirmed that the bioreduction of Ag^+ ion into silver NPs was due to the reduction by capping material of leaf extract. In addition visible photoluminescence emissions were observed from the synthesized silver nanoparticles. The Ag NPs synthesized via green route exhibit antibacterial activity against pathogenic *E. coli* bacteria. Antibacterial property of Ag NPs on pathogenic bacteria opens a door for a new range of potential antibacterial agents.

In summary, the present study signifies a simple, rapid, ecofriendly, cost effective biogenic process for the large scale production of Ag NPs.

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