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RESEARCH ARTICLE

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Biogenic Synthesis of Silver Nanoparticles (AgNPs) using *Celosia cristata* L. Leaves Extract and Their Antimicrobial Activity against Otorhinolaryngological Isolated Pathogen

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ABSTRACT

This work focused on the biogenic synthesis of silver nanoparticles (AgNPs) by silver nitrate using *Celosia cristata* leaves extract in four different solvents namely petroleum ether, acetone, methanol, and water. Silver nitrate and leaves extract were used as a precursor and capping reducing agent respectively. Biogenic AgNPs were characterized and identified by UV-Vis spectrophotometer, X-ray diffraction (XRD), Field emission scanning electron microscope (FE-SEM), and energy dispersive spectroscopy (EDX). Disc diffusion method was used for antibacterial activity of AgNPs and effective antibacterial activity was shown against IS-3, IS-4, IS-6, and IS-7 as compared to a positive control (ciprofloxacin).

Keywords: Silver nanoparticles, FE-SEM, X-ray diffraction, antibacterial activity, *Celosia cristata*.

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INTRODUCTION

From the last few decades, the field of nanotechnology had gained much importance in several research and development working areas. Silver nanoparticles (AgNPs) are universally utilized metal nanoparticles [1], these nanoparticles were also used in personal care, household products and clothes. [2-3] Synthesis of AgNPs can be done by radiation, chemical, photochemical and electrochemical methods. However, these processes are very slow, costly and hazardous to humans. Biogenic synthesized AgNPs are good alternatives to the above methods. [4] Biogenic synthesized AgNPs are nontoxic to human and effectively works against bacteria at very low concentration. [5] Many bacteria developed antibiotic resistance to most of the antibiotics, so there is a requirement of alternate antimicrobial substances. AgNPs works against all pathogenic bacteria and no bacteria developed antimicrobial resistance against it. [6] *C. cristata* is a household decorative plant belongs to family *Amaranthaceae*. It is commonly known as Fire flame and Cockscomb in English and Chi Kuan in Chinese. This is a herbaceous plant with a height of up to 30 cm. Leaves are simple and alternate with green sometimes red or purple in color. Flowers are divided into three parts i.e. spikes, plumes, and crests. Flowers

also vary in color; red, yellow, orange, and pink are common. This plant showed the presence of flavonoids, alkaloids, carbohydrate, gum, protein, steroids, saponins, amino acids, vitamin B1, B2, C and E. *C. cristata* used as traditional medicine for the treatment of uterine bleeding, hemorrhoids, bloody stool, wound healing, burning, leucorrhoea, amenorrhea, hyperactivity of liver and dysentery. [7-9]

This research paper focused on the biosynthesis of AgNPs by different solvents (petroleum ether, acetone, methanol, and water) leaves extract of *C. cristata* which were characterized by UV-VIS spectrophotometer, XRD, EDX, FE-SEM, and their antimicrobial activity was also investigated.

MATERIALS AND METHODS

Experimental

Silver nitrate ($AgNO_3$) used as a precursor for AgNPs synthesis was purchased from Sigma-Aldrich. Sterile cotton swab, Muller Hilton agar medium, nutrient agar medium, nutrient broth, and antimicrobial susceptibility discs were purchased from HiMedia.

Plant Material

These plants were collected from Sitarganj, Uttarakhand and identified as *C. cristata* by the BSI Dehradun and herbarium sheet with the Acc. No. 118058 was submitted.

C. cristata leaves extract preparation

Young green leaves of the plants were taken and washed with running water and shade dried at room temperature for 15-16 days. 200 g of powdered leaves were taken for successive solvent extraction by the Soxhlet apparatus.

Isolation of otorhinolaryngological pathogen

All otorhinolaryngological specimens were collected by sterile cotton swab from the otorhinolaryngological infection suffering patients. All specimens were cultured on nutrient agar medium at 37°C for 24 hours and all isolated were stored on nutrient agar medium slants at 4°C for further use.

Silver nanoparticles (AgNPs) synthesis

For the AgNPs synthesis, 10^{-3} molar (10^{-3}M) and 10% (w/v) silver nitrate solution leave extract were prepared. 10ml of leaves extract was added in 90 ml of 10^{-3}M $AgNO_3$ solution. After adding the leaves extract in the solution, the color change in the solution occurred slowly. The formation of silver nanoparticles was confirmed by the brown color of the solution. This solution was left in dark for 24 hours. After 24 hours centrifugation of colloidal suspension was done for 20 minutes at 10000 rpm. Unbound particles were removed by centrifugation and supernatant was replaced by double distilled water. Settled nanoparticles were dried in the oven at 55°C for 24 hours.

Analysis of silver nanoparticles

UV-Visible spectra analysis

Brown color appearance of the colloidal solution confirmed the AgNPs formation. Further, UV-Vis absorbance spectra were tested between the wavelength range of 300-550 nm. Systronics double beam UV-Vis Spectrophotometer: 2201 was used for analysis and double distilled water was used in reference cuvette.

X-ray diffraction (XRD) analysis

The dried AgNPs powder was analyzed by XRD on Powder X-Ray Diffractometer (Bruker D8-Advance) at 40kv voltage with monochromic Cu-Kα radiation (λ = 1.5406 Å) at 2θ angle. The scanning region of XRD was 20°-80°. The domain size of nanoparticle crystal was calculated by Scherer formula.

$D = 0.94\lambda/\beta COS\Theta$

Field Emission Scanning Electron Microscope (FE-SEM) analysis

FE-SEM analysis was done to determine the size and shape of nanoparticles. Carl Zeiss Ultra Plus Field-Emission Scanning Electron Microscope was used at the voltage of 15kV in a high vacuum to reach the highest magnification.

Energy dispersive X-ray (EDX) Analysis

EDX analysis was done to analyze the abundance of silver element. Carl Zeiss Ultra Plus Field-Emission Scanning Electron Microscope with EDX attachment was used for EDX analysis.

Antimicrobial activity

Antimicrobial susceptibility of AgNPs was analyzed by disc diffusion method. 50µg/ml concentration of powdered AgNPs was prepared in double-distilled water. Muller Hinton agar (MHA) medium was used to prepare bacterial culture lawn containing AgNPs discs at 37°C for 24 hours. Ciprofloxacin (5 mcg) and distilled water discs were used as positive and negative control respectively.

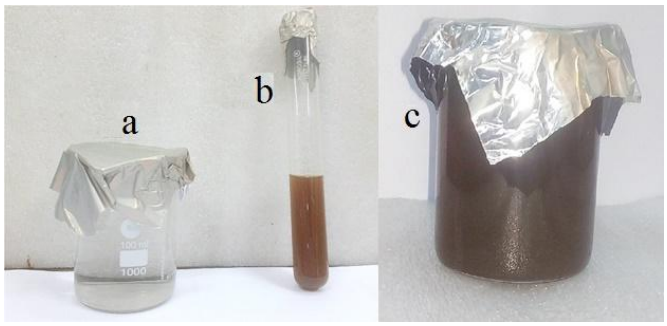

Fig. 1: Photographs of a: $AgNO_3$, b: Plant extract, and c: AgNPs Solution

RESULTS AND DISCUSSION

UV-Visible spectra analysis

The formation of the AgNPs indicated by the color change of the silver nitrate solution after adding the plant extract, during this reduction process colorless solution changed into brown color (Figure 1). UV-Visible absorption spectra of AgNPs solutions were analyzed between 300-550 nm and the surface plasmon resonance (SPR) bands were observed strongly between 401-415 nm (Figure 2). The range of SPR bands confirms the abundance of spherical AgNPs. The SPR peak of AgNPs was found between the 350-550 nm

wavelengths. [4] The SPR peak of biogenic (*Urtica dioica* Linn. leaves) synthesized silver nanoparticles at 414 nm, was similar to our observation. [10]

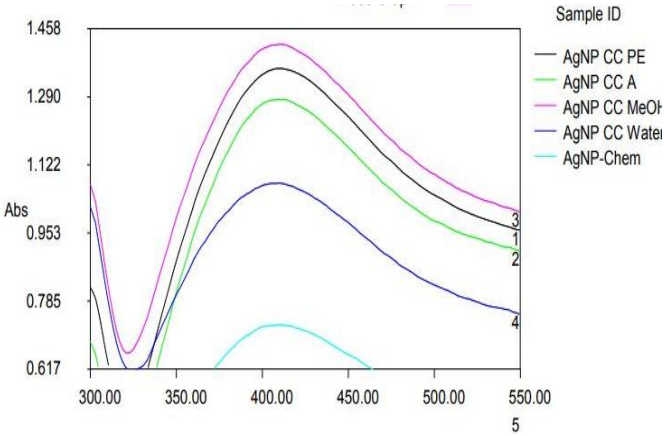

Fig. 2: UV–Vis spectra of synthesized AgNPs by *C. cristata* leaves extract of different solvent (PE: Petroleum ether, A: Acetone, MeOH: Methanol and Water) and chemically synthesized AgNPs (AgNPs-Chem)

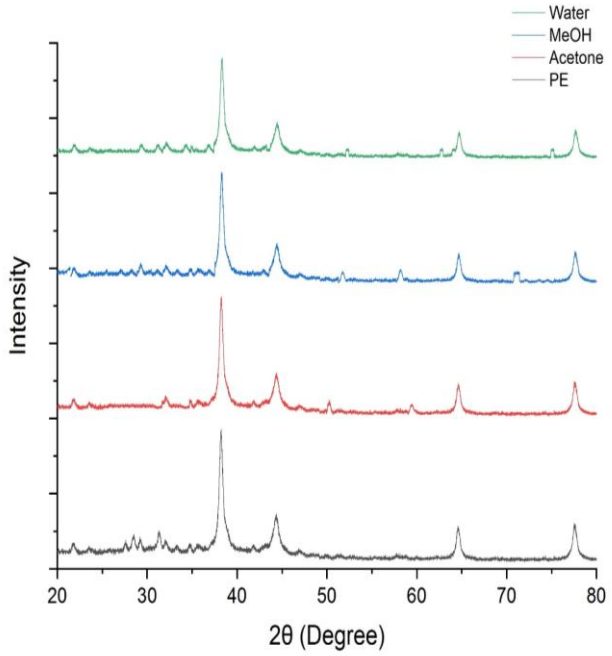

Fig. 3: XRD pattern of synthesized AgNPs by *C. cristata* leaves extract of different solvent (PE: Petroleum ether, Acetone, MeOH: Methanol and Water)

XRD Analysis

The crystalline properties of AgNPs were analyzed by XRD pattern (Figure 3). Four main peaks were observed in each XRD pattern of biogenic (*C. cristata* leaves extract of petroleum ether, acetone, methanol, and water) synthesized AgNPs. The diffraction peaks at 38.23° (111), 44.37° (200), 64.64° (220) and 77.65° (311) for petroleum ether; 38.25° (111), 44.39° (200), 64.66° (220), and 77.68° (311) for acetone; 38.24° (111), 44.36° (200), 64.67° (220) and 77.59° (311) for methanol; 38.28° (111), 44.40° (200), 64.69° (220) and 77.62° (311) for water were observed and suggested that all crystals had FCC structure which is further confirmed by JCPDS file no. 84-0713, 04-0783 and 87-0720. The mean crystal size was 13.08, 13.25, 13.25 and 13.00 nm respectively. Similar diffraction peaks were observed by Ghozali *et al* [4], Philip [11], and Shankar *et al.* [5]

FE-SEM analysis

Crystal shape, size, and morphology of synthesized AgNPs were analyzed by FE-SEM at 25000X. Individual and aggregates of AgNPs were observed in FE-SEM images (Figure 4). All particles were spherical in shape while aggregated AgNPs had no well-defined shape. The size of AgNPs in images was 10-60nm also observed by Vanaja & Annadurai. [12]

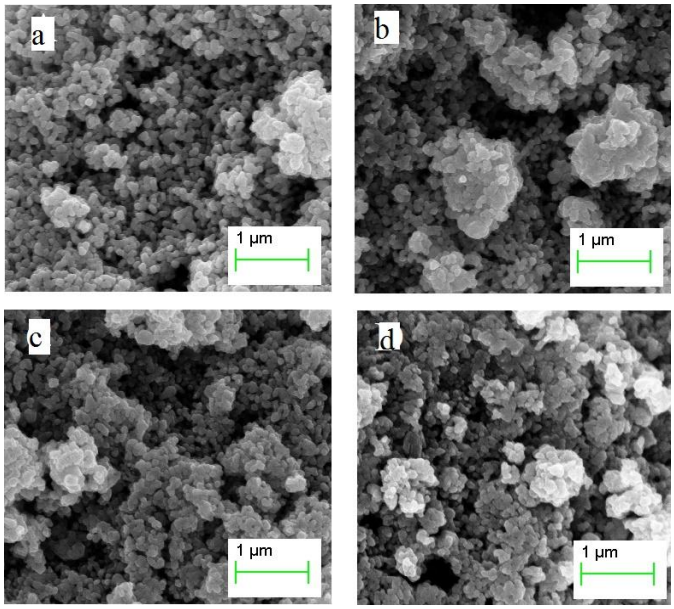

Fig. 4: FE-SEM images synthesized AgNPs by *C. cristata* leaves extract of different solvent (a: Petroleum ether, b: Acetone, c: Methanol and d: Water)

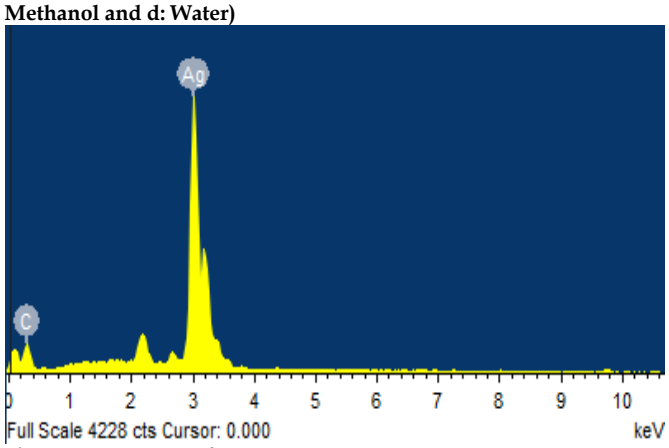

Fig. 5: EDX pattern of AgNPs

EDX Analysis

A strong peak of silver metal was observed at 3 keV which confirms the silver nanoparticle synthesis while a weak peak of oxygen was also observed on EDX (Figure 5). No other peaks were observed in EDX which confirm the highest purity of synthesized AgNPs.

Antimicrobial activity

Antimicrobial activity of biogenic synthesized nanoparticles was analyzed by the disc diffusion method; the inhibition zone was calculated in diameter against otorhinolaryngological isolates namely IS-1, IS-3 (*Alcaligens faecalis*), IS-4, IS-6, IS-7 (*Staphylococcus sciuri*), IS-8 and IS-10 (*Bacillus subtilis*). These 7 isolated

bacteria were tested against biogenic synthesized and chemically synthesized AgNPs; ciprofloxacin (5 mcg) and distilled water were used as positive and negative control respectively. Biogenic synthesized nanoparticles were more toxic against IS-3, IS-4, IS-6, and IS-7 as compared to ciprofloxacin while all isolates showed more zone of inhibition for biogenic synthesized nanoparticles as compares to chemically synthesized nanoparticles. The highest zone of inhibition (28.33 ± 0.88 mm) was shown by methanol leaves extract AgNPs in IS-4 while least zone of inhibition (10.67 ± 0.88 mm) was shown by petroleum ether extract AgNPs in IS-7 (Figure: 6). Almost similar observations were found by Anandalakshmi *et al* [13], they synthesized biogenic AgNPs of *Pedalium murex* leaves extract and found the highest antibacterial activity against *Escherichia coli* and *Bacillus subtilis*. While Mahitha *et al* [14] synthesized silver nanoparticles of *Bacopa monniera* whole plant extract and found antibacterial activity against *Staphylococcus aureas, B. subtillis*, *E. coli*, and *Klebsiella pneumoniae*.

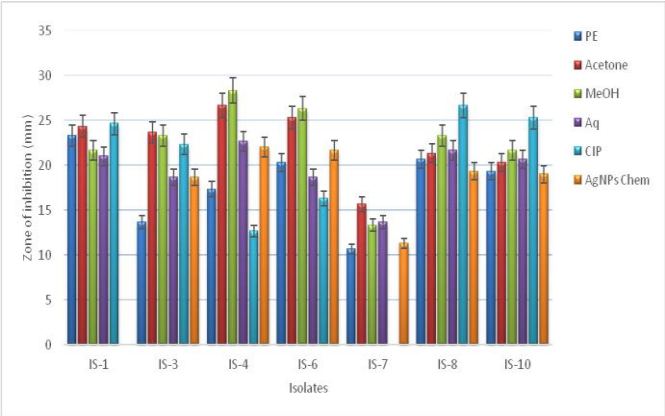

Fig. 6: Antimicrobial activity of synthesized AgNPs by *C. cristata* leaves extract of different solvents (PE: Petroleum ether, Acetone, MeOH: Methanol and Water), CIP: ciprofloxacin and chemically synthesized AgNPs (AgNPs-Chem)

The present work demonstrated that *C. cristata* leaves are capable to synthesize the biogenic AgNPs, *C. cristata* leaves extracts work like a reducing and capping agents. Characterization of AgNPs was performed by UV-Vis spectrophotometer, XRD, FE-SEM, and EDX. SPR in AgNPs was confirmed by the UV-Vis absorption spectra, the abundance of silver metal in AgNPs was confirmed by EDX, FE-SEM study suggested that AgNPs had uniformly spherical shape with 10-60 nm in size range and FCC crystal structure were confirmed by XRD pattern. The biogenic synthesized AgNPs showed excellent antimicrobial activity against IS-3 (*A. faecalis*), IS-4, IS-6, and IS-7 (*S. sciuri*).

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