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Poisonous weed leaf extract mediated biosynthesis of silver nanoparticles and evaluation of their antibacterial activity

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Silver nanoparticles were synthesized by using leaf extract of a poisonous weed *Ipomoea carnea* Jacq. Leaf extract reduces silver ions to silver nanoparticles. Synthesized nanoparticles were confirmed by UV-Visible spectrophotometer and UV-Visible absorption spectra of the reaction mixture showed λ max at 475 nm. The average particles size was confirmed by XRD peaks was 20 nm. SEM image showed aggregates of spherical silver nanoparticles. Antibacterial efficiency of silver nanoparticles was evaluated by disc diffusion assay method. Silver nanoparticles exhibited antibacterial activity against *Staphylococcus aureus* NCIM-2079 and *Pseudomonas aeruginosa* NCIM 2200. This cost-effective, eco-friendly and easily scald up biosynthesis method of silver nanoparticles synthesis using leaf extract of a poisonous weed *Ipomoea cornea* Jacq. will be compatible for pharmaceutical and medical applications.

Keywords: Silver nanoparticles, *Ipomoea carnea* Jacq. Scanning Electron Microscopy, X-Ray Diffraction

Introduction

There are many approaches available for the biosynthesis of silver nanoparticles. Silver nanoparticles can be synthesized by the methods like reduction in solutions (Goia and Matijevie, 1998), chemical and photochemical reactions in reverse mycelles (Taleb *et al.* 1997), thermal decomposition of silver compounds (Esumi *et al.* 1990), radiation assisted (Henglein, 2001), electrochemical (Rodriquez-Sanchez *et al.* 2000), sonochemical (Zhu *et al.* 2000), microwave assisted (Pastoriza-Santos and Liz-Marzan 2000). In recent years, silver nanoparticles are synthesized via green chemistry route (Begum *et al*. 2009; Bar *et al*. 2009; Song and Kim 2009; Malabadi *et al*. 2012; Nalwade *et al.* 2013). Biological synthetic methods of nanoparticles have many advantages as they are cost effective, eco-friendly and compatible for pharmaceutical and other biomedical applications. There is no need to use pressure, energy, temperature and toxic chemicals.

Among the noble metals, silver is the metal of choice in the field of biological system, living organisms and medicine (Parashar *et al*. 2009). Silver has been recognized as having inhibitory effect on microbes present in medical and industrial processes (Jose *et al*. 2005; Lok *et al*. 2007).

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Herein we report synthesis of silver nanoparticles in the aqueous solution of silver nitrate by the leaf extract of a weed *Ipomoea cornea* Jacq. This weed is toxic to cattle. It is reported to have stimulatory allelopathic effects. Roots are boiled to use as laxative and to provoke menstruation. Traditional healers for treatment of skin diseases have used it. The milky juice of plant has been used for the treatment of leucoderma and other related skin diseases. Only external applications have been recommended due to poisonous nature of the plant. It has depressant effect on central nervous system, also shows muscle relaxant property.Silver nanoparticles synthesizedusingthis obnoxious weed was evaluated for their antibacterial activity against *Staphylococcus aureus* NCIM-2079 and*Pseudomonas aeruginosa* NCIM 2200.

Materials and Methods

Plant material and preparation of extract

Fresh leaves of *Ipomoea carnea* Jacq. were collected from the college campus. Leaves were washed with tap water, then with distilled water and dried with blotting paper and cut into small pieces. Leaf pieces were dispersed in 100 ml sterile distilled water and boiled for 30 min at 100 ^{0}C. It was filtered through Whatman No. 1 filter paper and volume of the filtrate was adjusted to 100 ml by adding sterile distilled water.

Synthesis of silver nanoparticles

1 mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 10 ml of *Ipomoea carnea* Jacq. leaf extract was added into 90 ml of 1 mM silver nitrate solution. It was kept for 4 h. The colour change of reaction mixture from yellow to dark brown was checked periodically. This indicated the synthesis of silver nanoparticles.

UV-Vis Spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 4 h after diluting 100 µl of the sample with 1 ml sterile distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-2450 (Simatzu).

XRD measurement

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of sterile distilled water. After freeze drying of purified silver nanoparticles, the structure and composition were analyzed by XRD (RIGAKU-D Machine). The data was collected in the 2Θ range. The crystalline domain size was calculated from the width of XRD peaks using Scherrer'sequation.

Dabye- Scherrer's equation

$$D = K\lambda / \beta \cos \Theta$$

Where, D = average crystalline domain size; β is the Full Width at Half Maximum (FWHM), K= 0.94, λ = 1.540598 A^o and Θ is the diffraction angle.

SEM analysis of silver nanoparticles

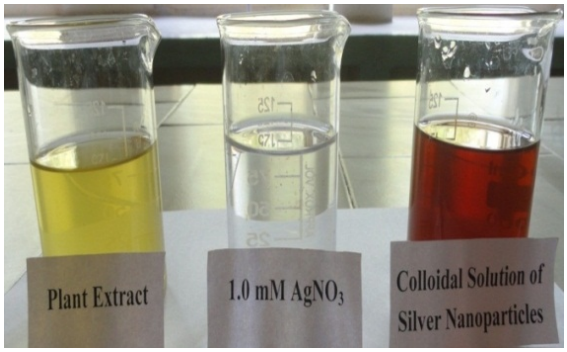
Scanning Electron Microscopic (SEM) analysis was done using PHILIPS-XL-30 SEM machine. Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting under a mercury lamp for 5 min.

Antibacterial assays

The antibacterial assays were done on *Staphylococcus aureus* NCIM-2079 and *Pseudomonas aeruginosa* NCIM 2200by disc diffusion method. Bacterial cultures were procured from National Chemical Laboratory, Pune, India. Nutrient agar medium was used to cultivate bacteria. 20 ml molten and cooled media (Nutrient agar) was poured in sterilized petridishes. The plates were left overnight at room temperature to check for any contamination to appear. Bacteria were grown in the nutrient broth for 24 h. A 100 ml nutrient broth culture of bacterial organism (1 x 10^5cfu/ml) was used to prepare bacterial lawn. Sterile paper discs of 6 mm diameter were prepared.Two discswere loaded with 30 µl of silver nanoparticles suspended 'hydrosol' and others with 30 µl of each antibiotic. These plates were incubated at 37 ^{0}C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc. The diameter of each zone of inhibition was measured.

Results and Discussion

Plant extracts are effective against various plant pathogens. Plants contain compounds such as barberine, emetine, quinone and sanguinarine still find specialized uses. The use of plant extracts has opened awareness for the control of pathogenic microorganisms. As the *Ipomoea carnea* Jacq. leaf extract was mixed in the aqueous solution of silver nitrate, it started to change colour from yellow to dark brown due to reduction of silver ions (Figure 1), which indicated formation of silver nanoparticles. Silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles (Mulavney, 1996).



A B C

Figure 1. Photograph of (A) *Ipomoea carnea* Jacq. leaf extract, (B) 1.0 mM $AgNO_3$ solution without leaf extract, (C) Colloidal solution of silver nanoparticles

UV-Vis spectra recorded from the reaction medium after 4 h is shown in Figure 2. Absorption spectra of silver nanoparticles formed in the reaction media has absorption peak at 475 nm, broadening of peak indicated that the particles are polydispersed. UV-Vis spectroscopy is commonly used to examine size and shape controlled nanoparticles in aqueous suspensions (Wiley *et al*. 2006).

XRD studies were carried out to confirm the crystalline and structural information. Three intense peaks were observed between 10 to 70 range of 2Θ. Bragg reflections were obtained at (111), (200) and (220) lattice planes. This reveals that particles are crystalline in nature. The particle size ranges between 15 to 28 nm with an average of 20 nm. Silver nanoparticles were spherical in shape. XRD pattern displayed is consistent with reports on microstructures (Fu *et al*. 2003).

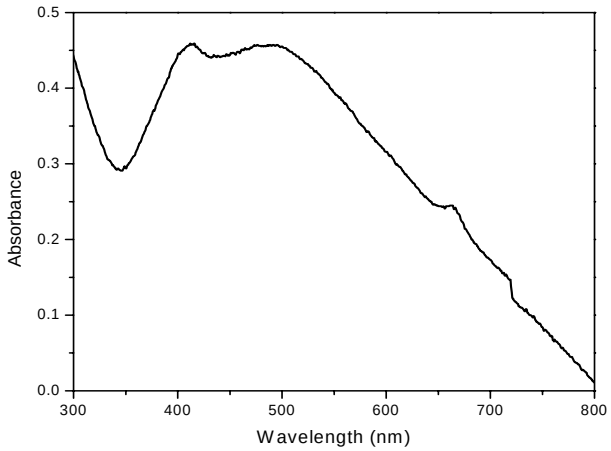


Figure 2. UV-Visible spectra of Ag nanoparticles

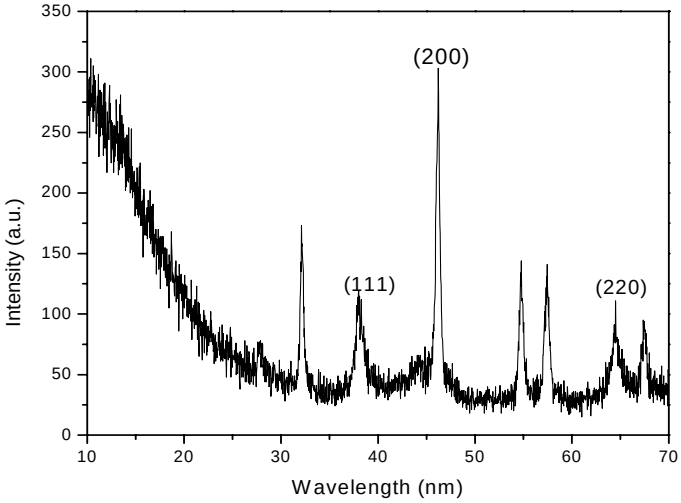


Figure 3. XRD pattern recorded for the silver nanaoparticles

The SEM image (Figure4) showed the high density silver nanoparticles synthesized by the *Ipomoea carnea* Jacq. leaf extract. There wereaggregates of silver nanoparticles. The particles were spherical.

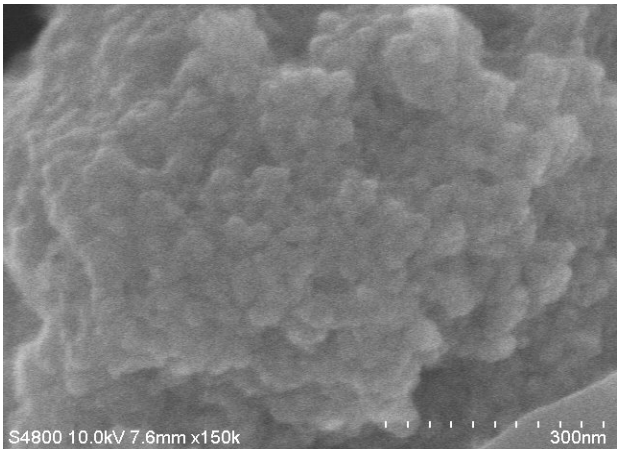
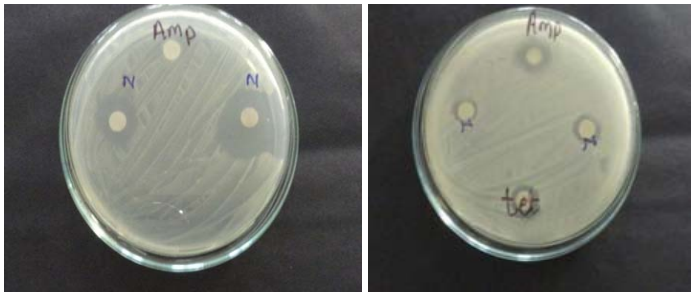


Figure 4. SEM image of silver nanoparticles synthesized using leaf extract of *Ipomoea carnea* Jacq.

Molecular basis for the biosynthesis of silver nanoparticles is not known, but it is speculated that the organic matrix contains silver binding proteins that provide amino acid moieties that serves as the nucleation sites. Proteins / enzymes that have been found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles (Balaji *et al.* 2008). According to Geethalakshmi and Sarada (2010), polyols are mainly responsible for the reduction of silver ions. Polyol compounds and the water-soluble heterocyclic compounds are mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles, respectively (Huang *et al*. 2007).

The inhibitory activities of silver nanoparticles in culture media are reported in Figure5comparable with the standard antibacterials. The inhibitory zone of silver nanoparticles was 20 mm in diameter for *Staphylococcus aureus* NCIM-2079 and 10 mm in diameter for *Pseudomonas aeruginosa* NCIM 2200. Similar antibacterial activity of silver nanoparticles was reported against *E. coli* and *Pseudomonas aeruginosa* (Jain *et al.* 2009); *Bacillus cereus* and *Pseudomonas aeruginosa* (Elumalai *et al.* 2010); *Proteus vulgaris, Vibrio cholera* (Prabhu *et al.* 2010); *Bacillus subtilis*, *Staphylococcus aureus, E. coli* (Malabadi *et al.* 2012); *Klebsiella pneumoniae* (Nalwade *et al*. 2013).



A **B**

Figure 5. (A) *Staphylococcus aureus* NCIM-2079, (B) *Pseudomonas aeruginosa* NCIM 2200

The mode of action of both silver nanoparticles and silver ions was reported to be similar, although the nanoparticles were reported to be effective at significantly lower concentration than that of the ions. However,it was proposed that the bactericidal mechanism of silver nanoparticles and silver ions are distinctly different. For treatment with silver nitrate, a low molecular weight central region was formed within the cell, as a defense mechanism, whereas for treatment with nanoparticles, no such phenomenon was observed (Morones *et al.* 2005). With the detail study of DNA / Protein migration profiles it was demonstrated that silver nanoparticles have no direct effect on either cellular DNA or protein (Gogoi *et al.* 2006), although the silver nanoparticles were more efficient bactericidal agent compared to the silver ions. For *E. coli* (ATCC 10536) and *Staphylococcus aureus* (ML 422), silver nanoparticles demonstrated greater bactericidal efficiency compared to penicillin (Sarkar *et al*. 2007).

Conclusions

The study concluded that leaf extract of the *Ipomoea carnea* Jacq. is capable of synthesizing silver nanoparticles in aqueous solution. These silver nanoparticles revealed to possess an antibacterial activity against *Staphylococcus aureus* NCIM-2079 and *Pseudomonas aeruginosa* NCIM 2200. The poisonous weed can be utilized for the synthesis of silver nanoparticles by "green route" which have applications in many fields.

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