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Research Article

Green Synthesis of Silver Nanoparticle Using *Eichhornia crassipes* and Study of *in-vitro* Antimicrobial Activity

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Abstract: Different biological methods are gaining recognition for the production of silvernanoparticles (Ag-NPs) due to their multiple applications. One of the most important applications of Ag-NPs is their use as an anti-bacterial agent. The use of plants in thesynthesis of nanoparticles emerges as a cost effective and eco-friendly approach. In this study preparation of silver nanoparticles by using leave extract of *Eichhornia crassipes* has been investigated. Characterization of different properties of the prepared nanoparticles by techniques like, UV-VIS spectroscopy, Zetasizer, TEM, and FT-IR was carried out. In-vitro antibacterial study of the prepared nanoparticles on different microorganisms like *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia hermanii*, and *Pseudomonas aeruginoss* was carried out. The results showed the formation of silver nanoparticles in the range of 18–30 nm and average of 21.71+/- 4.42 nm in size. The silver nanoparticles showed the antimicrobial activity against Gram positive and Gram negative bacteria. *Eichhornia crassipes* was found to display strong potential for the synthesis of silver nanoparticles that can be used as antimicrobial agents by rapid reduction of silver ions.
Keywords: Silver, *Eichhornia crassipes*, nanoparticles, Antibacterial

INTRODUCTION

Due to swift industrialization and urbanization, our environment is undergo huge smash up and a large amount of perilous and superfluous chemical, gases or substances are released. Therefore now it is our need to learn about the secrets that are present in the Nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticle synthesis which was found to be reliable and ecofriendly [1]. The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [2] they are gaining the interest of scientist for their novel methods of synthesis. Over the past few years, the synthesis of metal nanoparticles is an important topic of research in modern material science. Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. However, there is still need for economic commercially viable as well as environmentally clean synthesis route to synthesize the silver nanoparticles. Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes [3]. In medicines, silver and silver nanoparticles have an ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds [4], medical devices and implants prepared with silver-impregnated polymers [5]. In textile industry, silver-embedded fabrics are now used in sporting equipment [6]. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of nanoparticles, this method requires capping agents for size stabilization of the nanoparticles. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for green nanotechnology [7]. Many biological approaches for both extracellular and intracellular nanoparticles synthesis have been reported till date using microorganisms including bacteria, fungi and plants [8, 9]. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, use of plant extracts also reduces the cost of microorganism's isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms [7].

MATERIALS AND METHODS

Chemicals used:

The chemicals used like ethanol, agar, peptone, beef extract, Sodium chloride, Sodium hydroxide, Silver nitrate, etc were of analytical grade.

Microbial strains used:

Following four microbial strains were used for the antimicrobial activity determination.

Gram + ve: *Staphyloccous aureus* (MTCC 9886) and *Bacillus subtilis* (MTCC 1789)

Gram – ve: *Escherichia heranii* (MTCC 9144) and *Pseudomona aeruginoss* (MTCC 10070)

[MTCC: Microbial type culture collection] that were obtained from the culture collection of the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India and cultured and preserved in Institute of Pharmacy and Technology, Salipur microbiology laboratory.

Preparation of leave extract:

The leaves of *Eichhornia crassipes* were collected from the nearby village of Salipur, Odisha during early summer, washed in sterile water to remove any dirt or other unwanted objects, shade dried and preserved.The leave were authenticated by the taxonomist of Department of Botany, College of basic science and humanities, OUAT, Bhubaneswar and a specimen sample was preserved in the museum of Institute of Pharmacy and Technology, Salipur, Cuttack[10] .

Biosynthesis of silver nanoparticles:

In a typical synthesis for silver nanoparticles using aqueous-methanolic extract of *Eichhornia crassipes* leaves, the carefully weight biomass was added to 50 ml of 1 mM aqueous Silver nitrate ($AgNO_3$) solution, in conical flasks of 250ml content at room temperature [11].

UV–Vis spectra analysis:

The bioreduction of Ag+ in aqueous solution was monitored by periodic sampling of aliquots (0.2 ml) of the suspension, then diluting the samples with 2 ml deionized water and subsequently measuring UV–vis spectra of the resulting diluents. UV–vis spectroscopy analyses of silver nanoparticles produced were carried out as a function of bioreduction time at room temperature on Shimadzu 1700 pharm spec UV spectrophotometers at a resolution of 1 nm.

Size analysis:

Particle sizes of the prepared silver nanoparticles were determined by dynamic light scattering using a Malvern Zetasizer Nano-ZS. The nanoparticles suspended in purified water to a final concentration of 100µg/ml. The measurement was done in disposable polystyrene cuvettes at 25ºC with a detection angle of 90º and by adjusting the viscosity and refractive index that of water as 0.8872cP and 1.33 respectively. The particle size and the polydispersity index (PDI) of three batches of each sample were determined [12].

TEM Analysis:

Transmission Electron Microscopic (TEM) Analysis was performed with 200 keV Transmission Electron Microscope (JEOL, Ultra high resolution), PP resolution: 0.19 nm. Thin film of the sample were prepared on a carbon coated grid by dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper. Later on, film on the TEM grid was allowed to dry by placing it under a mercury lamp for 5 minutes for the characterization of size and shape of synthesized silver nanoparticles [12].

FT-IR analysis:

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 5000 rpm for 10 min and the resulting suspension was redispersed in 10 ml sterile distilled water. The centrifuging and redispersing process was repeated three times. Thereafter, the purified suspension was freeze dried to obtain dried powder. Finally, the dried nanoparticles were analyzed by Shimadzu 8400S, FT-IR spectrophotometer.The sample is gently mixed with 100 mg of micronized Potassium bromide powder and compressed into a disc shaped holder. For each spectrum a 256-scan interferogram was collected with a 4 cm^{-1} resolution in the mid-infrared region at room temperature[13].

Determination of Minimum Inhibitory Concentration:

An agar dilution assay was used for determining the minimum inhibitory concentration (MIC) of the three extracts. This method was used as a modification of Parish and Davidson [19]. Tubes of 15 ml molten agar were prepared and maintained at 50ºC. A single concentration of antimicrobial was added to the agar in each test tube to obtain a range of final concentrations of 10, 20, 30, 40 and 50µg/ml. After addition of the different concentration of extract to the agar, plates were poured and agar allowed solidifying. The test microorganisms were diluted in 0.1% (w/v) peptone water to 10^6 CFU/ml. The test microorganism was then added to the plates and spreaded by a spreader. A control plate, without added antimicrobial, was prepared and inoculated to ensure adequate growth of the test microorganism. The plates were incubated at 37ºC for overnight. The MIC was defined as the lowest concentration that completely inhibited growth up to 24 hr.

Antibacterial study:

For antibacterial activities, the samples were evaluated following a modified filter paper disc method. The samples were diluted to 50μg/ml with sterilized distilled water. The sterile filter paper (Whatman filter paper) discs of 6mm diameter were soaked with 50 µl of the solution and placed on bacteria seeded plate (10^6 CFU/ml) of solid nutrient agar. For positive control, Cefexime in same concentration was used as similar manner. The plates were first incubated at 4^{o}C for 12 hours to allow proper diffusion of the extract into the medium and then re-incubated at 37^{o}C for 24 hours. After incubation period, the inhibition zone was observed and measured after marking at six different positions. The mean zone of inhibition and standard deviation was calculated by using SSP software [14].

RESULT AND DISCUSSION:

Biosynthesis of silver nanoparticles:

Green synthesis of silver nanoparticles using 10^{-3} mM Silver nitrate is shown in Figure 1. The fresh leave extract of *Eichhornia crassipes* was yellowish-green in colour. However, after addition of Silver nitrate the colour was changed to dark brown [15].

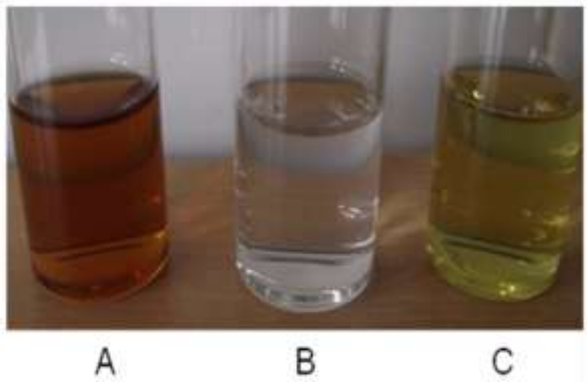

Figure 1: Preparation of silver nanoparticles
[A: Silver nanoparticles B: Only silver nitrate solution C: Leave extract]

UV-Vis Spectroscopy:

After addition of fresh leaves extract of *Eichhornia crassipes* to the aqueous solution of silver nanoparticles of different concentrations, the mixture showed a gradual change in color at room temperature with time from yellowish to wine-red and the colour intensified after 48 hours. The color developed was characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. The control sets showed no change in color under the same experimental conditions. The reduction of silver ion to silver nanoparticle was reflected in spectral data obtained by using a UV-Vis spectrophotometer. It was shown an absorbance peak around 430 nm for all four samples (Figure 2), which is specific for silver nanoparticles [16].

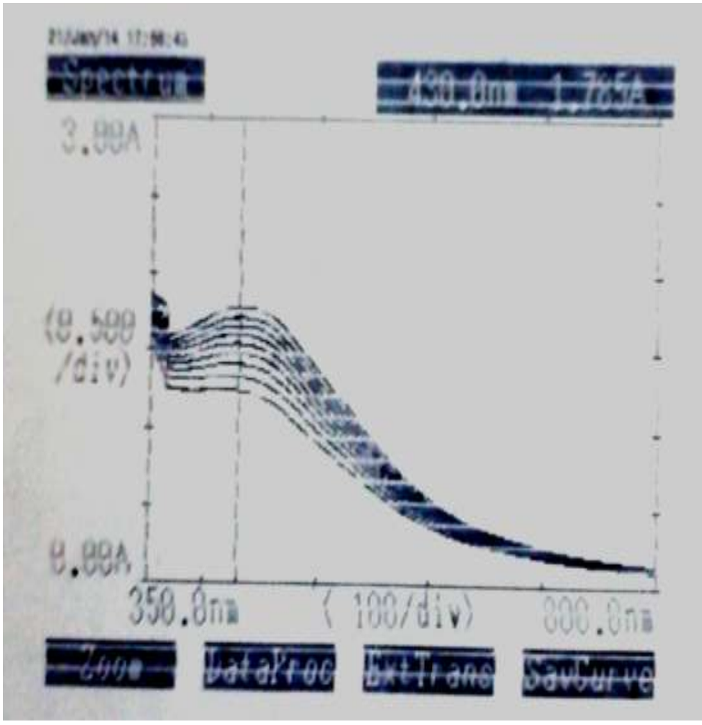

Figure 2: The absorbance spectrum of silver nanoparticles showing maximum absorbance at 430nm.

Size analysis:

Dynamic light scattering or Photon Correlation Spectroscopy is a technique used in material physics for determining the size distribution profile of nanoparticles in suspension or polymers in solution. Light scattering technique is used here to determine the size distribution profile of nanoparticles present in the final solution after ultracentrifugation. The study revealed that the average particle size of Silver nanoparticles range within 18-30 nm with average size of 21.71+/- 4.42 nm [16].

TEM Analysis:

The grid for the TEM analysis of Silver nanoparticles was prepared by placing a drop of the nanoparticles suspension on the carbon-coated copper grid and allowing the water to evaporate inside a vacuum dryer. Scanning under TEM (Philips CM-10) revealed that the size of silver nanoparticles was 18-20 nm and the tiny particles were seemed to be spherical in morphology as shown in the following images (Fig 3) [17].

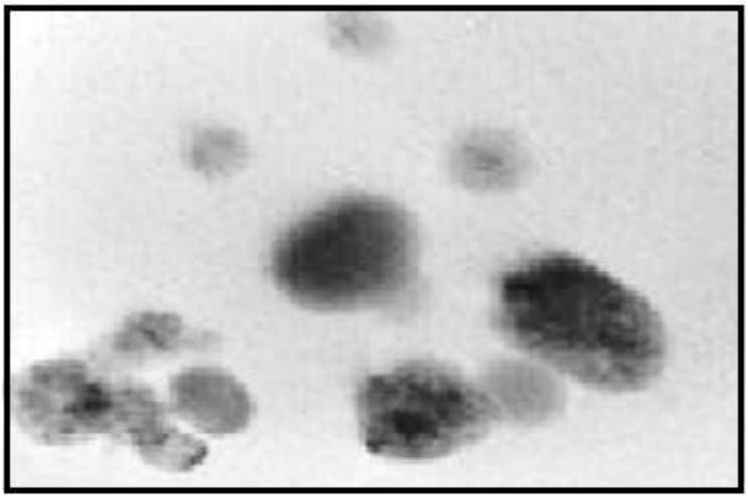

Figure 3: TEM images showing average particle size as 18-20 nm

FT-IR analysis:

Figure 4 shows the FTIR analysis results of the Silvernanoparticles. The prominent peaks were observed at 1054, 1242, 1400, 1506 cm^{-1} and a peaks in the wave number 3375cm^{-1}, that is, amide region. That was indicating binding as well as stabilization takes place by free amide groups present in proteinaceous substance used for synthesis of silver nanoparticles. The peaks found at 1400 and 1506cm^{-1} can be attributed to the C–C in alkene rings and C=C stretch of aromatic rings, respectively, whereas peaks at 1054 and 1242 cm^{-1} can be attributed to the ether linkages. It was confirmed from the observation that aliphatic amine, and aliphatic alkenes of alkaloids and terpenoids bound on the surface of silver nanoparticles. Depending on above observation, it can be assumed that the stabilization is achieved by the proteinaceous as well as aromatic compounds present in the extract [18].

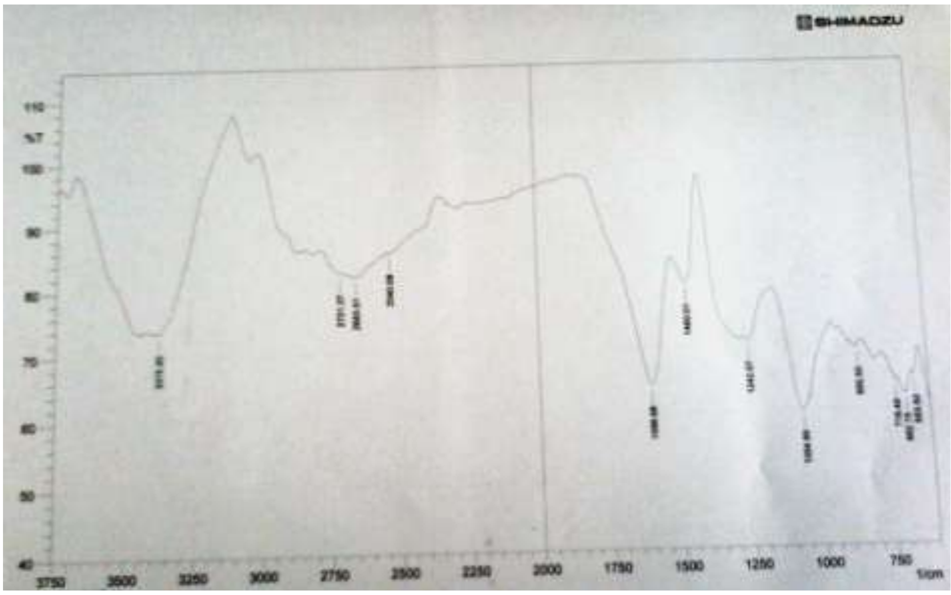

Figure 4: FTIR spectrum of silvernanoparticles.

Determination of Minimum Inhibitory Concentration:

The minimum inhibitory concentration of silvernanoparticles against different microorganism was shown in Table 1. *Staphylococcus aureus* had shown MIC of 20 µg /ml, *Bacillus subtilis* had shown MIC of 30 µg/ml. *Escherichia heranii and Pseudomonas aeruginoss* had shown MIC of 40 µg /ml.

Table 1: Minimum inhibition concentration (MIC) of silvernanoparticles.

Microorganisms	Concentration of silvernanoparticles				
	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml
Staphylococus aureus	+	-	-	-	-
Bacillus subtilis	+	+	-	-	-
Escherichia heranii	+	+	+	-	-
Pseudomonas aeruginoss	+	+	+	-	-

Antimicrobial study

Antibacterial activity of green synthesized silver nanoparticles against Gram negative *Escherichia heranii, Pseudomonas aeruginoss* and Gram positive *Staphylococcus aureus*, *Bacillus subtilis* bacteria at different concentrations showed that they revealed a strong dose-dependent antibacterial activity against the test microorganisms. It was seen that, as the concentration of green synthesized nanoparticles were increased, bacterial growth decreases in both the cases. The zone of inhibition of silver nanoparticles against the microorganisms *Staphylococcus aureus, Bacillus subtilis, Escherichia heranii and Pseudomonas aeruginoss* was shown in table 2. The results indicated that silvernanoparticles synthesized from *Eichhornia crassipes* leave extract showed antibacterial activity more in Gram positive than in Gram negative bacteria. As Gram negative bacteria were having high concentration of a lipid layer covering, that prevents penetration of silvernanoparticles inside the cell that may be a reason that gram negative microorganism were less sensitive towards silvernanoparticles as compared to gram positive microorganisms.

Table 2: Antimicrobial activity of silver nanoparticles and antibiotic against

Microorganisms	**Mean zone of inhibition in cm +/- SD**			
	Silver nanoparticle			**Antibiotic**
	1mg/ml	**500µg/ml**	**200µg/ml**	**100µg/ml**
Staphyloc속usaureus	1.42+/-0.12	0.92+/-0.12	0.67+/-0.05	1.18+/-0.07
Bacillus subtilis	1.3 +/-0.09	0.83+/- 0.05	0.65+/- 0.05	1.15 +/-0.05
Escherichia heranii	0.85+/-0.05	0.7+/-0.06	0.63+/-0.05	0.83+/-0.05
Pseudomonas aeruginoss	0.93+/-0.08	0.80+/-0.06	0.62+/-0.04	0.92+/-0.07

CONCLUSION

The rapid biological synthesis of silver nanoparticles using *Eichhornia crassipes* leave extract provides environmental friendly, simple and an efficient method for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical shape and the estimated sizes were in the range of 18-35 nm. The silver nanoparticles had shown antimicrobial activity against all the four tested microorganisms but more activity against gram positive microorganisms.

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