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Removal of water contamination using silver Nanoparticles synthesized from *Eichornia crassipes* (Root extract)

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Abstract

The present study was aimed to water contamination using silver nanoparticles synthesized from *Eichornia crassipes* (Root extract) and analyses anti-microbial activity. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 1mM $AgNo_3$ solution through the extract of *Eichornia crassipes* as reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy and SEM analysis. The SEM analysis showed the cubic structure of silver nanaparticle. Further these biologically synthesized nanoparticles were found to be highly toxic against different human pathogens like bacteria and fungi. These nanoparticle are pathogenic against *Pseudomonas aeruginosa*, *Escherichia coli* and fungi *Aspergillus flavus*.

Keywords: silver nanoparticles, *Eichornia crassipes,* SEM

Introduction

Nanotechnology is the manipulation of matter on an atomic and molecular scale. Nanomaterials involve structures having dimensions of nanometres (nm), that is, one billionth (or 10^{-9}) of a metre, typically between 1 and 100 nanometres in size. At such dimensions, materials can show significantly different physical, biological and/or chemical properties from materials at bigger dimensions, which opens up a range of new possibilities for technology.

A number of approaches are available for the synthesis of silver nanoparticles. for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route. Among these the use of environmentally benign materials like plant extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol (*Geethalakshmi et al., 2010*).

Silver has long been recognized as having inhibitory effect on microbes present on polluted water (*Geethalakshmi et al., 2010*). Silver nanoparticles used as antimicrobial agent is well known and has already found applications in antimicrobial paint coatings, textiles, water treatment, medical devices and greenhouse gas emission, remediating environmental damage. It has also great impact in managing and preventing diseases and offering new safety, enhancing materials that are stronger self –repairing and able to provide protection.

Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogens. The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries (*Hasan, et al*., 2009). Majority of rural water sources for drinking are still the traditional ones that are dams, wells, rivers, streams and ponds which might harbour waterborne and vector-borne diseases. Among these diseases there is cholera, guinea worm, schistosomiasis, lymphatic filariasis, onchocerciasis, fungal, shigellosis, salmonellosis, yersiniosis, campylobacteriosis, parasitic and viral infections (*Zvidzai et al., 2007*).

Water is said to be bacteriologically contaminated or polluted either due to presence of certain pathogens or due to high increase of total viable count. Microbiological parameters such as viable count at 37co are used not for disease risk estimation but rather as indictor for the treatment water process. In polluted water, a major type of bacteria found is coliform bacteria. The most important species of the group include *Escherishia coli*, *Klebsiella spp* and *Enterobacter spp*. At the same time, non coliform bacteria were also found in polluted water such as *Streptococcus*, *Proteus* and *Pseudomonas* species (*El Emam et al., 2010*).

Bacteria produce pigments for various reasons and it plays an important role (*Ahmed et al, 2012*). These pigment production helps to identify the bacteria. The roots of *Eichhornia crassipes* is feathery, blue-black to dark purple and are dense near the root crown and heavily branched. These are naturally absorbing pollutants including lead, mercury and strontium, as well as some organic compounds believed to be carcinogenic. In the present study demonstrate the antimicrobial activities of nanoparticles synthesized from *Eichornia crassipes.*

Materials and Methods

Preparation of root extract

25g of *Eichornia crassipes* roots were thoroughly washed with distilled water, dried, cut into tiny pieces and were crushed with 100ml sterile distilled water and filtered through filter paper.

Synthesis of silver Nanoparticles

10ml of root extract was added into 90ml of aqueous solution of 1mM silver nitrate ($AgNo_3$) and kept at room temperature for 5 hours. Then the reaction mixture is centrifuged and UV-vis analysis was performed.

Preparation of nutrient agar

Weigh out 5.6 grams of nutrient agar powder and is added to 200ml of deionized water with gentle stirring. Sterilize the media by autoclaving at 121^0c for 15 minutes. Allow the mixture to cool to 50^0c. Then pour the Agar into the petriplate and keep this undisturbed to solidify.

Inoculation of the culture

A reusable metal spreader should be flame sterilized and is then allowed to cool. The spreader is placed in contact with the inoculum on the surface of the plate and positioned to allow the inoculums to run evenly along the length of the spreader. Evenly distribute the inoculums and to allow it to be absorbed into the agar. Avoid disturbing plates for 10 to 20 minutes after spreading. After that the spread plates have been permitted to absorb the inocula for 10 to 20 minutes they may be inverted and incubated as desired. After appropriate incubation, plates are inspected and the cultured colonies were again sub cultured for isolation.

Isolation of the culture

Sterilize the inoculating needle by flame until the metal turns red and cool it. Touch the inoculating needle to an individual colony growing on the plate. Carefully lift up the lid of the plate inoculating onto. Touch the inoculating needle to the very center of the surface of

the nutrient agar. Place the lid on the plate, and flame the inoculating needle to kill any remaining bacteria. Seal the plate with a layer of paraffin around the edges. The isolated cultures were again sub cultured for the analysis of antimicrobial activities.

Antibacterial Activity

The antibacterial activities of the crude extracts were determined in accordance with the agar-well diffusion method. Wells were bored into the agar using a sterile well cutter. These wells were filled with the solution of the extract and is allowed to stand at room temperature for about 2 h and then incubated in the microbial incubator. The plates were observed for zones of inhibition after 24 h. The effects were compared with those of streptomycin and tetracycline (purchased from HIMEDIA, Mumbai) respectively.

Antifungal Activity

The fungal isolates were allowed to grow on a microbial incubator until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores were evenly spread on the Agar plate using a glass spreader. Wells were then bored into the agar media using a sterile well cutter and the wells filled with the solution of the extract. The plates were allowed to stand on the laboratory bench for 1 h to allow for proper diffusion of the extract into the media. Plates were incubated in the microbial incubator for 24h and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with ***Bavistin*** (systemic fungicide, purchased from BASF, Mumbai.) at a concentration of 0.1g / 100 ml.

Result

The silver nanoparticles exhibit yellowish brown colour in aqueous solution due to excitat on of surface plasmon vibrations in silver nanoparticles. As the extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from watery to yellowish brown due to reduction of silver ion which indicated formation of silver nanoparticles. Figure.1 shows the UV-Vis spectra recorded from the reaction medium (root extract) after 5 hours. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 335nm, broadening of peak indicated that the particles are polydispersed. The biosynthesised silver nanostructures were further demonstrated and confirmed by the scanning electron microscope. Fig.2 showing the image of nanoparticles synthesized from root extract looks like the granules. Normally if the concentration of the sample increases the

Absorbance value also increases. In the present study, various concentrations of silver nanoparticles from root extract were plotted in a graph. (Fig.3)

Further the nanoparticles syntheses by green route are found highly toxic against multi drug resistant human pathogenic bacteria. Silver nanoparticles exhibited antimicrobial activity against *E. coli, Pseudomonas aeruginosa and Aspergillus flavus* as it showed a clear inhibition zone. The Silver nanoparticles synthesized from root extract shows higher activity against *Aspergillus flavus.*

Fig. 1. UV-Vis absorption spectrum of silver nanoparticles synthesized from *Eichornia crassipes* root extract.

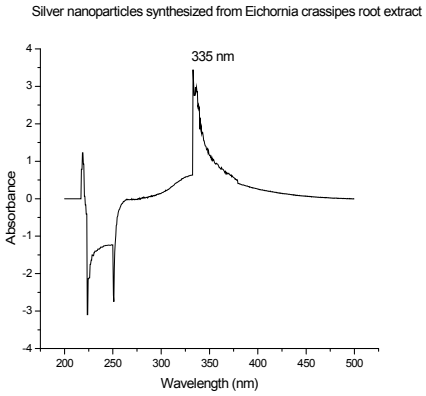
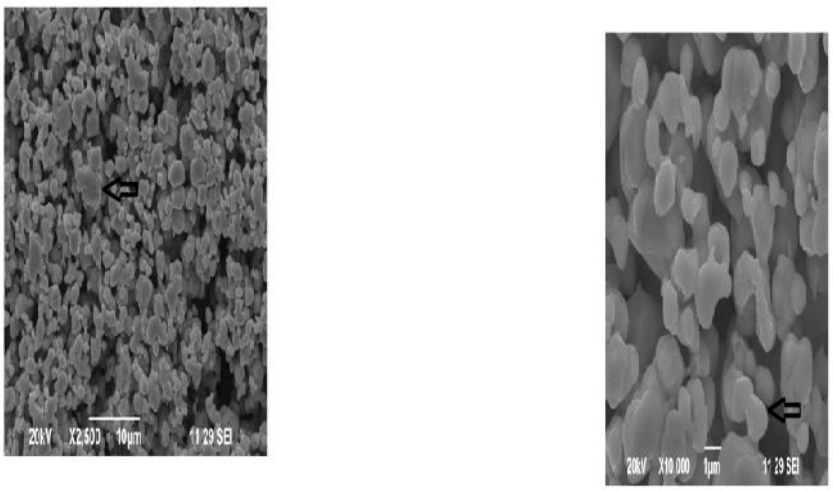
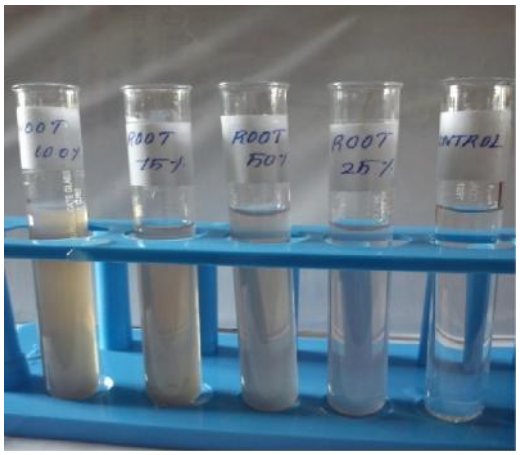


Fig.2. SEM image of silver nanoparticles from root extract



Variation in concentration of silver from root extract



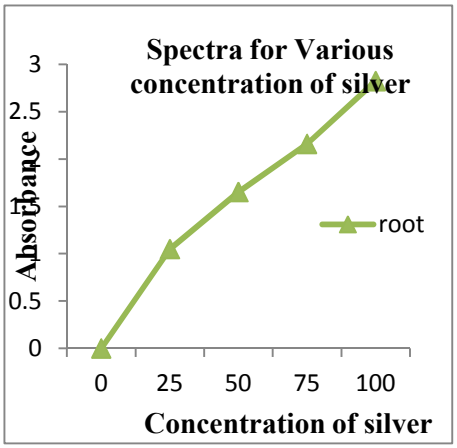
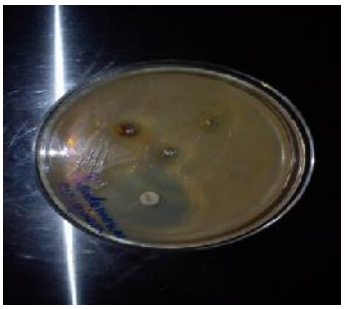


Table.1: Antimicrobial activities of Silver nanoparticles synthesized from *Eichornia crassipes*

Test organisms	Zone of inhibition in cm			
	Strep	Tet	Bavis	Root
Pseudomonas aeruginosa	3	4	-	1
Escherichia coli	2.4	2	-	1
Aspergillus flavus	-	-	2	1.8

Note: Strep – Streptomycin, Tet – Tetracycline (antibiotics),

Bavis – Bavistin (systemic fungicide)



Discussion

The silver nanoparticles are widely applicable in disease diagnosis, therapeutics, environmental remediation, etc. (*Abhilash, M., 2010*). Silver metal nanoparticles are formed in plants from silver salts. Therefore, the process necessarily requires a reduction of the salt to metal. A reducing agent must be present or formed in the plant in order to effect this reduction. Plants contain a complex mix of organic compounds capable of reducing metal ions. This includes a variety of sugars. The fluid within a plant root has a reduction potential. This reduction potential, dictates the electrochemical reactions that are possible, or rather what state an electrochemical equilibrium will occupy (*Haverkamp, 2011*).

Reduction of the silver ions were observed immediately when the silver nitrate solution was contacted with *Eichornia crassipes* (root) extract, and the colour was changed from water colour to the formation of silver nanoparticles of fairly well defined dimensions. *(Sivashankar, et al., 2012). It was* seen by the UV-Vis spectroscopy by using a quartz cuvette with water as reference. Maximum absorbance was seen at 335nm, indicating that the formation of silver nanoparticles. SEM analysis showed the structure of the nanoparticles. The mechanism of the antimicrobial action of silver ions is closely related to their interaction with the microorganisms. The present findings revealed that, the silver nanoparticles from *Eichornia* (root) extract showed antimicrobial activity against all the tested pathogens. Among these it showed maximum inhibition against *Aspergillus flavus*. The possible mechanism of action is, the metal nanoparticles are carrying the positive charges and the microbes are having the negative charges which create the electromagnetic attraction between the nanoparticles and the microbes. When the attraction is made, the microbes get oxidized and die instantly. Moreover, the silver nanoparticles showed antimicrobial activity against *E. coli, Pseudomonas aeruginosa* and *Aspergillus flavus.* The oxidative stress in the cell wall which increases the production of lactate dehydrogenase, which is an indicator of cell membrane damage *(Ravikumar et al., 2012).*

Silver ion release over a period of several weeks from nanoparticle sources of various sizes and doses was evaluated in vitro against *Pseudomonas aeruginosa* strain. Nano-Ag may exert an antifungal activity by disrupting the structure of the cell membrane and inhibiting the normal budding process due to the destruction of the membrane integrity (*Thirumalaiarasu et al., 2010*). Plant mediated nanoparticles are evaluated as potent antimicrobial agents against various human and phytopathogenic microorganisms (*Baker et al., 2013*).

AgNPs are attractive because they are non-toxic to the human body at low concentrations and have broad spectrum antibacterial actions. In fact, it is well known that Ag+ ions and Ag-based compounds are toxic to microorganisms. The protected silver and silver compounds showed high antimicrobial activity *against S. aureus, P. aeruginosa* and *E. coli* without the loss of solubility (*Tyagi et al. 2012*).

Antimicrobial silver nanoparticles (AgNPs) are the most widely used engineered nanomaterials. They are commonly incorporated into a wide variety of commercial goods (e.g., personal care products, food containers, laundry additives, clothing, paintings, and home appliances) and are also used for water treatment, drug and gene delivery, bone prostheses, implantable materials, biosensors, and bioimaging devices. The widespread use of AgNP-containing products raises the likelihood of accidental or incidental environmental releases that could impact microbial ecosystem services (e.g., biogeochemical cycling) (*yu yang,et al.,2013*).

Conclusion

In this study, *Eichornia crassipes* (root) extract have been used as a reducing agent for the synthesis of silver nitrate into silver nanoparticles. Here, the synthesized silver nanoparticles are confirmed from the yellowish brown colour formation and monitored quantitatively by UV-Vis spectroscopy. The SEM result showed the granular shape of the nanoparticles. This is the first report of synthesizing silver nanoparticles using *Eichornia crassipes* plant extract. The present investigation was concluded that the silver nanoprticles synthesized from *Eichornia crassipes* (root) extract demonstrated a strong activity against both bacteria and fungi. This investigation can be used in the folk medicine, source of antibacterial substances for possible treatment of many diseases including bacterial and fungal infections and cleanup microbial contamination in water bodies.

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