



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2018; 7(5): 1308-1311

Received: 07-07-2018

Accepted: 09-08-2018

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Evaluation of antimicrobial activity of silver nanoparticle using *Eichhornia crassipes* leaves extract

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Abstract

This study highlights the synthesis of silver nanoparticles using *Eichhornia crassipes* leaves extract. Antibacterial activity of silver nanoparticles was assessed by using disc diffusion method against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, *Aspergillus flavus*, since *Bacillus* species and *S. aureus* strains may cause diarrhoea and an enteropathogenic form of *E. coli*. *Candida albicans* is the most common cause of fungal urinary tract infections, intense itching, swelling, and irritation. *Aspergillus flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation. The results of this study clearly indicate that silver nanoparticles synthesized from plant extract of *Eichhornia crassipes* has many pharmaceutical applications for the control of deadly pathogens.

Keywords: *Eichhornia crassipes*, antibacterial activity, silver nanoparticles

Introduction

A large number of plants are being used in medicine for therapeutic and prophylactic purposes. The beneficial medicinal effects of plant products typically result from the combinations of secondary metabolites present in the plants. The therapeutic properties of medicinal plants are attributed owing to the presence of active substances such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarins (Daniel, 2005) [4]. These affect the body of human beings, interact with the pathogens and interrupt their growth at different stages of development and make the body disease free. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process (Lok *et al.*, 2007) [9]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds (Ip *et al.*, 2006) [6]. Currently, there is a growing demand for the devising of environmentally agreeable protocols for the synthesis of nanomaterials that would avoid the hazardous by-products associated with current physicochemical processes (Kumar *et al.*, 2009) [8]. Biological methods of synthesis have smooth way for the “greener synthesis” of nanoparticles and they offer enhanced manipulation and control over crystal growth and their stabilization. This has aggravated an upsurge in research on the synthesis routes that allow superior control of shape and size for various nano technological applications. The use of environmentally benign materials like plant extract for the synthesis of silver nanoparticles offer numerous benefits of eco friendliness and compatibility for pharmaceutical and other biomedical applications including antimicrobial activity (Jain *et al.*, 2009) [7]. The aim of the study to synthesis of silver nanoparticles using *Eichhornia crassipes* leaves extract and evaluated the antibacterial activity of silver nanoparticles.

Materials and Methods

The *Eichhornia crassipes* leaves were collected in January 2015 from Koraiyaru River, Mannargudi, Thiruvarur district, Tamil Nadu. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rapiant Herbarium and centre for molecular systematics, St. Joseph’s college Trichy-Tamil Nadu. India. A Voucher specimen (SJCBO12335) has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India.

Preparation of flower extract

The dried leaves were pulverized well with mortar and pestle to make a powder. Twenty grams of powder sample was mixed into 100 ml of deionized water and the mixture was boiled for 10 min. After cooling the flowers extract was filtered with Whatman No. 1 filter paper. The filtrate was stored at 4 °C for further use.

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Synthesis of Ag nanoparticles using leaves extracts

For the Ag nanoparticles synthesis, 5 ml of *Eichhornia crassipes* leaves extract was added to 45 ml of 1 mM aqueous AgNO₃ solution in a 250 ml Erlenmeyer flask. The flask was then incubated in the dark at 5hrs (to minimize the photo activation of silver nitrate), at room temperature. A control setup was also maintained without flowers extract. The Ag nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 15 min followed by re-dispersion of the pellet in de-ionized water. Then the Ag nanoparticles were freeze dried using SEM analysis (Arunachalam *et al.*, 2012) [12].

Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method (NCCLS, 1993 and Awoyinka *et al.*, 2007) [11, 3]. Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mins. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing bacteria specie were spread on Nutrient agar plates and fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing each 30µl of plant extract, AgNO₃, AgNPs and Standard solution as Chloramphenicol and fluconazole were laid down on the surface of inoculated agar plate. The plates were incubated at 37 °C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hours for yeasts strains. Each sample was tested in triplicate.

Measurement of Zone of Inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

Statistical analysis

The results were presented as mean ± SD. Data was statistically analyzed using student “t” test. P. values set as lower than 0.05 was considered as statistically significant.

Results and Discussion

Synthesis of Silver nanoparticles

Phytosynthesis of Ag nanoparticles by the aqueous leaves extract of *Eichhornia crassipes* leaves was carried out in this work. During the visual observation, silver nitrate incubated with leaves extract showed a colour change from yellow to brown after 5 hrs whereas no colour change could be observed in silver nitrate without leaves extract. The appearance of brown colour in leaves extract treated flask is clear indication for the formation of Ag nanoparticles. The use of plant and plant extract in nanoparticle synthesis is considered advantageous over microbial based system because it reduces the elaborate process of maintaining cell cultures. The particle size growth can also be controlled by altering synthesis conditions like pH, reductant concentration, temperature, mixing ratio of the reactants etc. The plant based synthesis can be carried out either extracellular or intracellular. Intracellular synthesis takes place inside the plant whereas the extracellular synthesis occurs *in vitro*. Our earlier report indicates that UV-Vis spectral studies confirmed the surface plasmon resonance of green-synthesized silver nanoparticles. Biomolecules were responsible for reducing and capping of AgNPs, which were confirmed by FTIR measurements. SEM studies revealed spherical and uniform-shaped silver nanoparticles with size in the range 10-40 nm. (Prabakaran and Mani, 2017) [13]. Present finding agreement with Mohammed Rafi Shaik *et al* (2018) [10] who observed the brown colour in the reaction mixture indicates the synthesis of silver nanoparticles form *Origanum vulgare* extract with 1mM silver nitrate solution.

Antimicrobial activity of *Eichhornia crassipes* and Silver Nanoparticles

Silver nanoparticles biosynthesized from *Eichhornia crassipes* leaves extract was tested individually against test organisms for antimicrobial activity by agar disc diffusion method. For this study Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*), Gram negative (*Escherichia coli*) bacterial specie and *Candida albicans*, *Aspergillus flavus* of fungus strains were used. This was performed by determining ZoI (zone of inhibition) which is rapid and inexpensive to determine the susceptibility of a particular test organism as antimicrobial agent. This was executed by measuring the zone of inhibition using a vernier caliper (Table 1 and Fig 1).

Table 1: Antibacterial activity of AgNPs, AgNO₃ and *Eichhornia crassipes* leaves extract

Microorganisms	AgNO ₃ (30µl)	Plant extract (30µl)	AgNPs (30µl)	Standard (30µl)
Bacteria				
<i>Escherichia coli</i> (mm)	0.30±0.02	3.10±0.21	7.30±0.51	11.40±0.79
<i>Staphylococcus aureus</i> (mm)	0.30±0.02	2.80±0.19	6.50±0.45	10.50±0.73
<i>Bacillus subtilis</i> (mm)	0.20±0.01	1.10±0.07	5.40±0.37	10.90±0.76
Fungal				
<i>Candida albicans</i> (mm)	0.20±0.01	1.30±0.09	6.80±0.47	10.70±0.74
<i>Aspergillus flavus</i> (mm)	0.10±0.01	0.70±0.07	4.80±0.33	11.10±0.77

Values were expressed as Mean ± SD for triplicate

Bacterial standard : Chloramphenicol
Fungal standard : Fluconazole

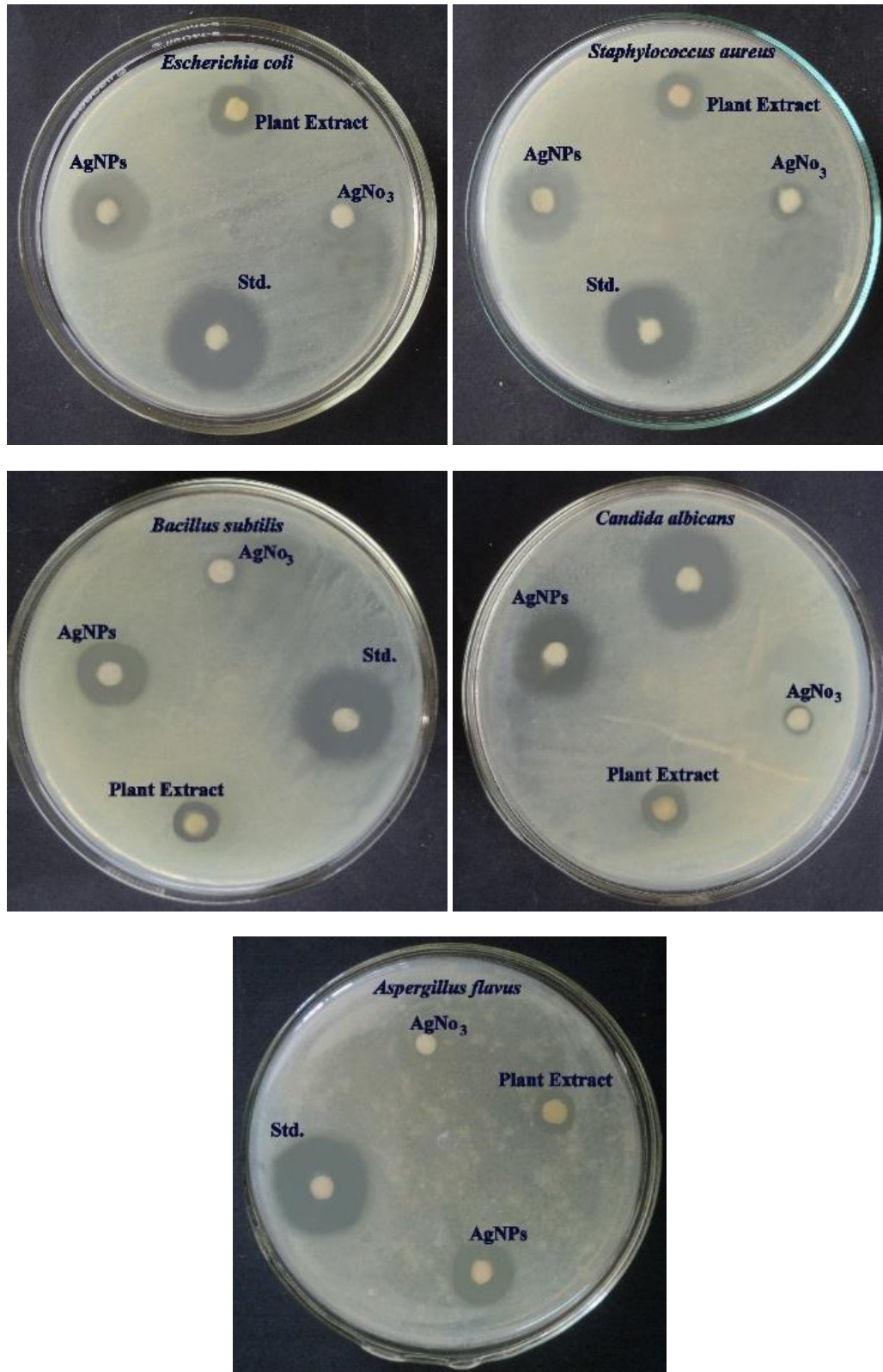


Fig 1: shows the antibacterial activity of AgNPs, AgNO₃ and *Eichhornia crassipes* leaves extract AgNO₃ = Silver Nitrate; AgNPs = Silver Nanoparticles

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects (Gardea-Torresdey *et al.*, 2003) [5]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria.

In this study selected gram positive and negative bacterial species and fungus were used. After 24 hours of incubation,

the inhibitory effect of AgNPs from *Eichhornia crassipes* leaf extract was significant as compared to *Eichhornia crassipes* leaf extract alone and standard chloramphenicol. Silver nanoparticles possess better antimicrobial activity than *Eichhornia crassipes* leaf extract.

Experimental evidence indicates that DNA loses its replication ability once the bacteria have been treated with silver ions (Pal *et al.*, 2007) [12]. Ahmad *et al.* (2011) [1] mentioned that the pathogenic effect of nanoparticles can be attributed to their stability in the medium as a colloid, which

modulates the phosphotyrosine profile of the pathogen proteins and arrests its growth. The growth of microorganisms was inhibited by the green synthesized SNPs showed variation in the inhibition of growth of microorganisms may be due to the presence of peptidoglycan, which is a complex structure and after contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria (Ahmad *et al.*, 2011) ^[1].

Conclusion

Biosynthesis of silver nanoparticles was carried out using by using the aqueous extracts of medicinal plants with the bio-reduction of silver ions in short period and tested for their antimicrobial activity. The AgNPs of *Eichhornia crassipes* leaf extract have shown good antimicrobial efficacy and hence has a potential to be used as antimicrobial agent against wide range of microbes over conventional antibiotics.

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