Research Article

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SYNTHESIS OF ENVIRONMENTAL STABLE SILVER NANO PARTICLES FROM THE LEAF EXTRACT OF *POLYALTHIA LONGIFOLIA* AND TO STUDY ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

The synthesis of stable nanoparticles from biological sources is evolving a new era of research interests in nanotechnology. To develop a novel approach for the improvement of aquaculture by introducing the green synthesis of silver nanoparticle and this can be achieved by inhibiting the pathogenic microorganisms which are harmful to the aquaculture environment. The present work leads to the synthesis of nanoparticles from the aqueous leaf extract of *Polyalthia longifolia* using 1 mM Silver nitrate. The synthesis and characterization of silver nanoparticles were confirmed by UV-vis spectroscopy, FTIR, TEM, XRD and XPS. The antibacterial activities were carried out against fish infecting organisms such as *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Edwardsiella tarda* and *Vibrio cholerae* by using Agar well-Diffusion method.

Keywords: Aqueous leaf extract, UV-Vis spectroscopy, FTIR, TEM, XRD, XPS and Agar well diffusion method.

INTRODUCTION

The field of nanotechnology is one of the most active researches nowadays in Modern material science and technology. Nanoparticles are fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is their exhibits larger surface area to volume ratio[1]. *Azadirachta indica* leaf extract has also been used for the synthesis of silver, gold and bimetallic (silver and gold) nanoparticles. The major advantage of using the neem leaves is that it is a commonly available medicinal plant and the antibacterial activity of the biosynthesized silver nanoparticle might have been enhanced as it was capped with the neem leaf extract[2]. Silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganisms at low concentrations and without any side effects[3]. Increasing common application is the use of silver nanoparticles for antimicrobial coatings and many textiles, keyboards, wound dressings and biomedical devices now contain silver nanoparticles that provide protection against bacteria. Large amount of nanoparticles can be easily synthesized from plant and the majority of these are non-toxic. These have been used for inhibiting the bacteria and fungi and preventing burns and wound infection. In the recent years, plant-mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. There are some reports on synthesis of nanoparticles from stem and leaf extracts[4-7] and studies on the biological activities like antimicrobial activity of these silver nanoparticles[8-11]. Nanoparticles are mostly prepared from novel metals such as Gold, Silver, Platinum and Lead. Among the Nobel metals, silver (Ag) is the metal of choice in the field of biological systems, living organisms and medicine[12]. The size dependent use of silver nano particles as carrier molecules in applications, such as drug delivery, diagnostics, nano biosensors, etc. are increasing with the advancement in technology[13,14]. The synthesis of silver nanoparticles by using the leaf extract of *O. bacillicum* is evaluated for its antimicrobial activity against on selected bacterial species[15]. The use of medicinal plants in the treatment of diseases has long been established as traditional treatment by traditional healers. Most of the plants have bioactive compounds as their secondary metabolites that possess antibacterial activity *in vitro*[16]. *Polyalthia longifolia* is evergreen tree native to India to grow over 30 feet height. The traditional healers have been using this plant preparation to treat fever, skin diseases, helminthiasis etc.[17] The methanol extract of *Polyalthia longifolia* exhibited noncytotoxic and antibacterial property[18]. *Polyalthia longifolia* (*P. longifolia*) (Annonaceae) is a tall handsome evergreen tree and it is cultivated all over India. The plant has been used as traditional systems of medicine for treatment of various diseases. The plant extracts and isolated compounds were studied for various biological activities like cytotoxicity, antibacterial and antiulcer activitities[19,20] Aquaculture fish production increased significantly over the past few decades necessitating intensive fish culture practices. Due to this practice a number of associated stressors like overcrowding, transport, handling, grading and poor water quality tends to adversely affect the health of cultured fish. These practices are the major factors that make the fish susceptible to disease. *Aeromonas hydrophila* is the most widespread pathogen and it can be

easily spread through accidental abrasions. This bacterium causes hemorrhagic septicemia, characterized by presence of small superficial lesion, focal hemorrhages, particularly in the gills and opercula, ulcers, abscesses, exophthalmia, and abdominal distension[21]. Thus, the present study investigates the role of silver nanoparticles synthesized from *Polyalthia longifolia* for antibacterial activity against selected fish infecting pathogenic organisms.

MATERIALS AND METHODS

Collection of Plant material and synthesis of silver nanoparticles

Polyalthia longifolia leaves were collected from VELS University campus, Pallavaram, Chennai, India. The taxonomic identification and voucher specimen was numbered (PARC|2015|3032) by Prof. P. Jayaraman, Ph.D. Institute of Herbal Botany, Plant Anatomy Research centre, Tambaram, Chennai, India. Leaf extract of the plant was prepared by mixing 10 g of dried Powder with 100 mL deionized water and boiled it for 20 minutes. 10 mL of leaf extract was mixed with 90 mL of 1 mM aqueous of $AgNO_3$ and kept it for sunlight for 20 minutes. A colour change was obtained from yellow to reddish brown and it confirmed the reduction of Ag^+ ions in the aqueous medium.

Collection of Culture

The bacterial test organisms used for the present study were *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Edwardsiella tarda* and *Vibrio cholerae*. The bacterial cultures were collected from Fish Immunology Laboratory, VELS University, Pallavaram, Chennai, India. The isolates were identified and sub cultured in slants on nutrient agar medium and maintained in the Department of Microbiology.

UV-vis spectra analysis

The reduction of Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using DU 800 Spectrophotometer.

FTIR

FTIR spectra was obtained using a Bruker Spectrum 100 spectrophotometer, operated at the resolution of 4 cm^{-1}. The sample was drop cased on a silicon wafer and the material was analyzed and the spectra was recorded in diffuse reflectance mode.

TEM

Sample for transmission electron microscopy (TEM) was made by drop casting the silver nanoparticles solution onto a carbon coated copper grid and performed using a JEOL 1010 TEM instrument operated at an accelerating voltage of 100 kV.

XRD

XRD measurement was carried out on a Bruker AXS X-ray diffraction system operating at a voltage of 40 kV and current of 40 mA with CuKa radiation.

XPS

XPS measurement was done with a Thermo K-Alpha XPS instrument at a pressure better than 1 _ 10_9 torr with core levels aligned with C 1s binding energy of 285 eV.

Antibacterial test

The Muller-Hinton Agar was used for the antibacterial activity. The medium was sterilized and poured into the sterile petri plates. After solidification plates were seeded with appropriate microorganisms (*Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Edwardsiella tarda* and *Vibrio cholerae*) by streaking eventually on the surface of the medium using sterile cotton swab. The wells were prepared by using sterile steel borer. Synthesized silver nanoparticles and the crude plant extract were poured in 25 µl concentrations in respective wells. Tetracycline (25 µl) used as control. After incubation the zone of inhibition were measured.

RESULT AND DISCUSSION

UV-vis spectra analysis

The bio-reduction of Ag^+ in aqueous solution was monitored by UV-vis spectroscopy. The absorbance spectra of synthesised nanoparticles were detected at various absorbance 400 nm to 800 nm. The synthesised silver nanoparticles from the aqueous solution showed the absorption peak around 550 nm that indicates the particles are completely dispersed in the aqueous solution. (Figure 1)

Fourier Transform Infrared spectroscopy (FTIR)

FTIR technique was used to detect free biomass residue that is not the capping ligand of the synthesized silver nanoparticles. The dried powder was subjected to FTIR spectroscopy measurement. The peak at 673.63 cm^{-1} corresponds to C-N stretching of the amine. The peak at 1640.15 cm^{-1} observed for the silver nanoparticles which has C=C and this indicates the aromatic rings, suggest the presence of the metal nanoparticles. The peak at 2367.76 cm^{-1} corresponds to O-H groups of carboxylic acids. The peak near 3423.04 cm^{-1} was assigned to O-H stretching in phenols. (Figure 2)

Transmission electron microscopy (TEM)

Transmission electron microscopy was prepared by placing a small amount of sample placed on Carbon coated grid and allowing the water to evaporate. The

resulting nanoparticle was obtained at the range of 100 nm in size of nanoparticles. (Figure 3)

XPS

Experiment\X-Ray 015 400 um - FG ON\Point #004

XPS spectra were obtained by irradiating a material with a beam of X-ray and measuring its kinetic energy. It showed number of electrons that escapes from the top of the materials and allowed to settle are acquired through this high resolution XPS spectra. (Figure 4)

XRD

The nanostructure was confirmed by the characteristic peaks observed in the XRD image at 2Θ. The different lines observed at this angle were 41.5, 47, 65 and 77 which have been indexed as 111, 200, 220 and 311 respectively. (Figure 5)

Antibacterial test

The aqueous crude extract of the plant showed maximum activity in *V. cholerae* as compared to other species. Synthesized silver nanoparticles showed maximum antibacterial activity in *P. aeruginosa*, *V. cholerae* and minimum activity was observed in *A. hydrophila*, *Edwardsiella tarda*. Tetracycilne was used as positive control and this was showed maximum activity in *A. hydrophila*, *P. aeruginosa* and minimum activity in *V. cholerae*, *Edwardsiella tarda*. (Figure 6 & 7)

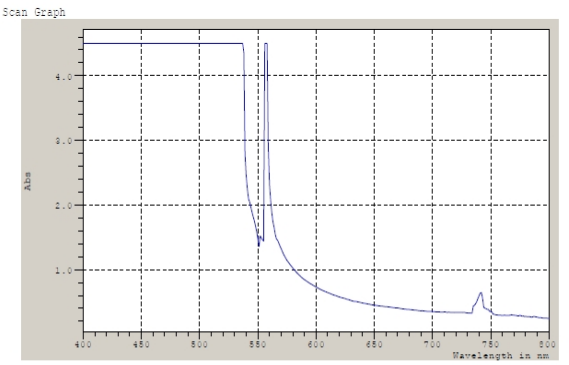

Figure 1: UV-VIS absorption spectrum of silver nanoparticles

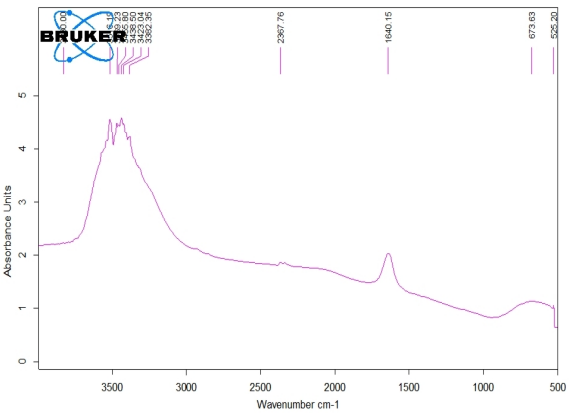

Figure 2: FTIR spectra of silver nanoparticles

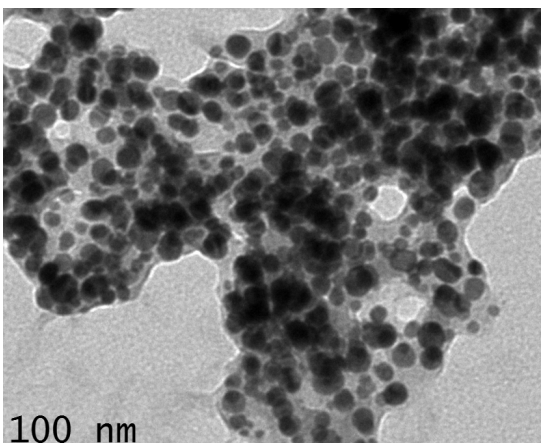

Figure 3: TEM image of silver nanoparticles

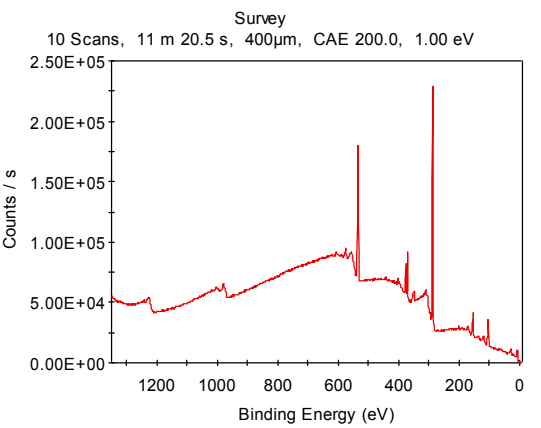

Figure 4: XPS spectra of silver nanoparticles

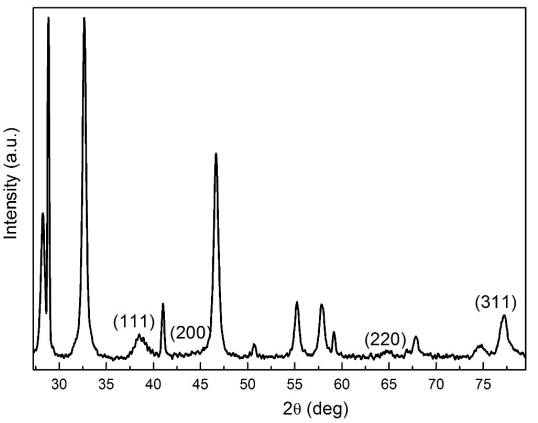

Figure 5: XRD image of silver nanoparticles

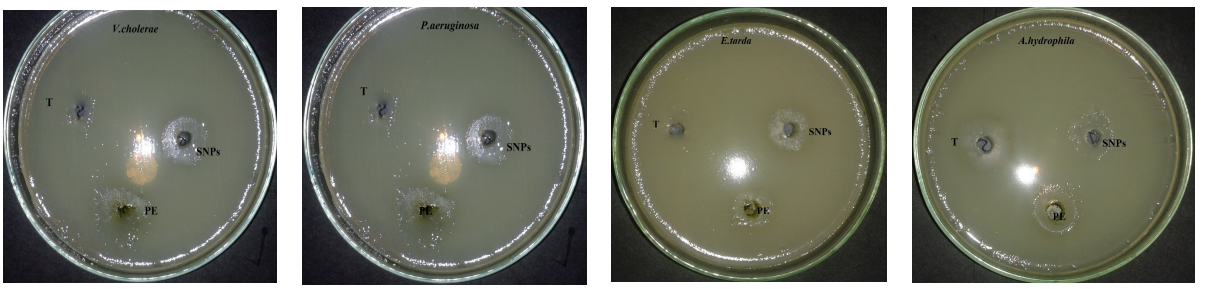

Figure 6: Antibacterial Activity

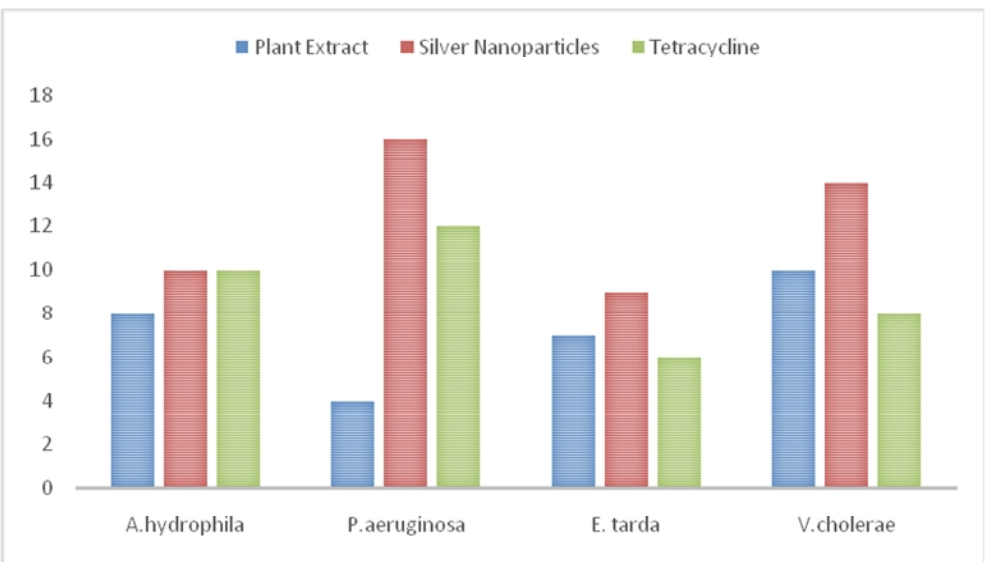

Figure 7: Antibacterial activity (Zone of inibition)

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