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Research Article

PHYTOSYNTHESIS OF SILVER NANOPARTICLES USING *CERIOPS TAGAL* AND ITS ANTIMICROBIAL POTENTIAL AGAINST HUMAN PATHOGENS

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ABSTRACT

Synthesis of silver nanoparticles using mangrove aqueous extract of *Ceriops tagal* and to evaluate the antibacterial efficacy against pathogenic bacteria and fungi. The synthesized silver nanoparticles were characterized by UV-visible spectrophotometer, transmission electron microscopy (TEM) and atomic force microscopy (AFM) techniques. The physical property of nanoparticles in suspension was determined by measuring the zeta potential (ζ-potential) of the colloidal suspension. The standard disk diffusion method was employed to evaluate the antimicrobial activity of silver nanoparticles. The characteristic surface plasmon absorption band of silver nanoparticles (SNPs) was observed around 424 nm. The microscopic images of TEM and AFM showed the presence of spherical shaped nanoparticles with an average size of 30 nm. The zeta potential of the colloidal suspension was found to be -34.18 mV. The SNPs exhibited antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Proteus mirabilis*, *Candida albicans*, *Candida tropicalis* and *Aspergillus niger*. The research provides an important direction in the development of bio-based approach for synthesis of antimicrobial silver nanoparticles.

Keywords: *Ceriops tagal*, Mangrove, Silver nanoparticles, Zeta potential, Antimicrobial activity

INTRODUCTION

Nanotechnology is a developing interdisciplinary field of research interspersing physical, chemical, biological and engineering sciences. Top-down and bottom-up approaches are commonly used for the synthesis of nanoparticles. In top-down approach, the nanosized materials are obtained by breaking down the larger materials. The bottom-up approach involves the arrangement of atoms or molecules in precise control to molecular structures in the nanometer range. Bottom-up approach is commonly employed for biological and chemical synthesis of nanoparticles [1]. The chemical synthesis usually requires the use of toxic reducing agents such as sodium borohydride, hydrazine and additionally involve a volatile organic solvent such as toluene or chloroform [2]. Hence the development of eco-friendly and sustainable methods for synthesis of nanoparticles from biological resources is gaining momentum. Bacteria, fungi and plants are the potential sources utilized for the synthesis of nanoparticles [3-6]. The synthesis of nanoparticles from plants is more advantageous over other biological processes as they are safe to handle and does not require any elaborative processes of purification or maintenance of culture [7].

Silver is known for its antimicrobial activity from ancient times. It is been used in different forms like silver nitrate, silver sulfadiazine or metallic silver for the treatment of burns, wound, and severe bacterial infections [8]. The high surface area to volume ratio of silver nanoparticles (SNPs) prompts higher antimicrobial activity compared to the bulk silver [9]. The use of silver nanoparticles as antimicrobial agent is significant, as several pathogenic bacteria and fungi have developed resistance against various antibiotics due to their overuse [10]. Silver nanoparticles at non-cytotoxic concentrations have proven to exert antiviral activity against HIV-1 [11].

Ceriops tagal, a mangrove plant, is a rich source of biologically active compounds like triterpenoids, diterpenoids, tannins and polysaccharides [12]. The decoction of the bark of *C. tagal* was used to treat haemorrhages and malignant ulcers [13]. He *et al* isolated a pentacyclic triterpens from *C. tagal* which exhibited antiviral property [14]. The current study is aimed at the synthesis of silver nanoparticles using the unexplored mangrove plant *Ceriops tagal* and to assess the antimicrobial activity of SNPs against human pathogens.

MATERIALS AND METHODS

Chemicals

Silver nitrate ($AgNO_3$) and all other chemicals were purchased from SD Fine Chemicals, India. Muller Hinton agar and Potato Dextrose agar were purchased from Himedia, India.

Microorganisms

The microbial strains used for the experiments were *Pseudomonas aeruginosa* (NCIM-2036), *Bacillus cereus* (NCIM-2458), *Staphylococuss aureus* (NCIM-2672), *Escherichia coli* (NCIM-2809), *Proteus mirabilis* (NCIM-2388), *Candida albicans* (NCIM-3557), *Candida tropicalis* (NCIM-3110) and *Aspergillus niger* (NCIM-1207). The strains were purchased from National Chemical Laboratory (Pune, India).

Preparation of extract

Ceriops tagal leaves were collected from the Pichavaram mangrove forest,Tamil Nadu, India. The leaves were thoroughly washed with distilled water, shade-dried and powdered. The leaves were macerated and were placed in a rotary shaker at 130 rpm at room temperature (25 °C) for three days. The extract was centrifuged at 10,000 rpm for 20 min. The supernatant was filtered using whatmann filter paper 1 and further used for experiments.

Synthesis of silver nanoparticles

The aqueous extract was interacted with 0.008 M $AgNO_3$ solution at room temperature in the rotary shaker at 130 rpm and was observed for color change.

UV–Visible spectroscopy

The preliminary characterization of the synthesized silver nanoparticles was carried out using Systronic double beam spectrophotometer (UV-Vis Systronic-2201) by diluting the reaction mixture in Millipore water 10 times. The spectra were recorded from 300-600 nm at a resolution of 0.1 nm.

Microscopic characterization

Transmission electron microscopy

The shape and size of the AgNPs were determined using TEM. The transmission electron micrographs of the silver nanoparticles were taken using the Technai G 10 (Philips 80 kV). The colloidal solution was diluted, and a drop of it was placed on copper grid. The copper grid was allowed to dry.

Atomic force microscopy

AFM studies were carried out by drop coating the colloidal suspension onto a glass slide and scanning at a rate of 100 mV/s in the range 50_m×50_m using Nanosurf easysurf 2 (Switzerland).

Zeta potential measurement

The zeta potential of the synthesized nanoparticles was determined by means of zeta potential analyzer (90 Plus Particle Size Analyzer,

Brookhaven Instruments Corporation, using Zeta Plus software). The zeta potential measures the direction and velocity of particles under the influence of known electric field. The magnitude of the zeta potential gives an indication of the colloidal stability.-

Antibacterial assay

Antimicrobial assays were carried out using standard disk diffusion method. The bacterial and fungal lawns were prepared on Muller–Hinton agar (MHA) and Potato dextrose agar (PDA) by using sterile cotton swabs at a concentration of 10^8 CFU/mL for bacteria and 10^6 colony forming unit (CFU) per ml for fungi [15]. The sterile paper disks (6 mm in diameter; Himedia) were impregnated with 20 µL test solution (SNPs, $AgNO_3$ and plant extract) and were placed on MHA and PDA plates using a sterile pair of forceps. The MHA plates were maintained at 37°C for 18 h and PDA plates were incubated for 2-3 days. Standard antibiotics, Gentamicin (30 µg/disk) and Amphotericin–B (20 µg/disk) were used as positive control.

RESULTS AND DISCUSSIONS

UV-Vis spectroscopy

The formation of silver nanoparticles using aqueous leaf extract was monitored by UV–visible spectroscopy. On addition of silver nitrate to the aqueous extract, the color changed from pale yellow to brown. The appearance of brown color was an indication for the formation of silver nanoparticles. The color change was due to the excitation of surface plasmon vibrations which provides a distinctive spectroscopic signature for SNPs. The unique optical properties of nanosized metallic particles originate from the collective oscillations of conduction electrons in resonance with the incident light termed as Surface Plasmon resonance (SPR) [16]. From figure 1 (inset) it is evident that the solution color changed from pale yellow to brown. The appearance of brown color was an indication of the formation of silver nanoparticle formation in the reaction mixture. The absorption maxima of SNPs peak around 424 nm was observed at the end of the fifth hour of the reaction (Fig. 1). The absorption maxima of silver nanoparticles were reported in the range of 400–500 nm [17].

Microscopic characterization

Spherical nanoparticles ranging from 10 nm to 40 nm were observed in TEM micrographs (Figure 2a). The AFM results (Figure 2b) also well corroborated with the TEM measurements.

Zeta potential (ζ) measurement

The ζ-potential value of the colloidal solution was was determined to be −34.18 mV. The negative surface charge could be due to the adsorption of bioactive components present in the aqueous extract onto the nanoparticles surface. The stability of the colloidal system is determined by the magnitude of the zeta potential (ζ). If the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be fewer tendencies for the particles to come together and aggregate. The particles in suspension with ζ-potentials more positive than +30 mV or more negative than -30 mV are considered stable [18]. However, if the particles in suspension have low zeta potential values then there will be no force to prevent the particles coming together and aggregating. From Figure 3 it is clear that the colloidal suspension was highly stable with a ζ-potential of -34.18 mV.

Antimicrobial activity

The silver nanoparticles exhibited antimicrobial activity against pathogenic fungi and bacteria**.** Among the bacterial strains *Pseudomonas aeruginosa* showed the maximum zone of inhibition (ZOI) of 21 mm for SNPs, followed by *Staphylococcus aureus* and *Bacillus cereus* with ZOI of 14 mm and 13 mm respectively. *Escherichia coli* and *Proteus mirabilis* exhibited similar inhibitory activity against SNPs. *Candida tropicalis* and *Aspergillus niger* was found to be more sensitive to SNPs as compared to *Candida albicans* (Table 1). Silver nanoparticles demonstrated substantial antimicrobial activity as compared to $AgNO_3$ for both bacteria and fungi (Figure 1). The leaf extract did not show any activity against the test organisms. Gentamicin and Amphotericin–B exhibited comparable activity for *Pseudomonas aeruginosa* and *Aspergillus niger* (Table 1).

Silver nanoparticles exhibited higher antimicrobial activity than that Ag^+ (Figure 4); this is attributed to the large surface area of nanoparticles, which allows them to interact with microbial membrane. Li *et al* propounded a mechanistic mode of SNPs on *E. coli*, they found out that the nanoparticles attach to the surface of the cell membrane and alter its permeability, thus leading to the leakage of ions and other materials. Secondly, SNPs invade the inner membrane and inactivate respiratory chain dehydrogenases, thus leading to inhibition of respiration and growth of cells. Silver nanoparticles could also affect some proteins and phosphate lipids and damage the membrane, eventually resulting in cell death [19]. There are many reports on the antimicrobial effects of silver nanoparticles but the antifungal effects of silver nanoparticles are still unexplored. The mechanism regarding the antifungal activity is not explored much, however it is speculated that silver nanoparticles inhibit the normal budding process, probably through the destruction of membrane integrity [20].

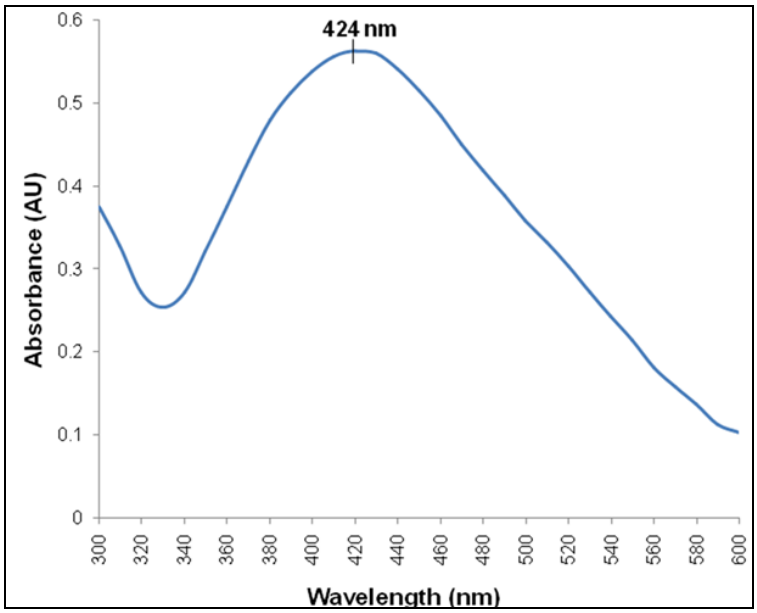

Fig. 1: UV-vis spectra of silver nanoparticles synthesized from *Ceriops tagal*

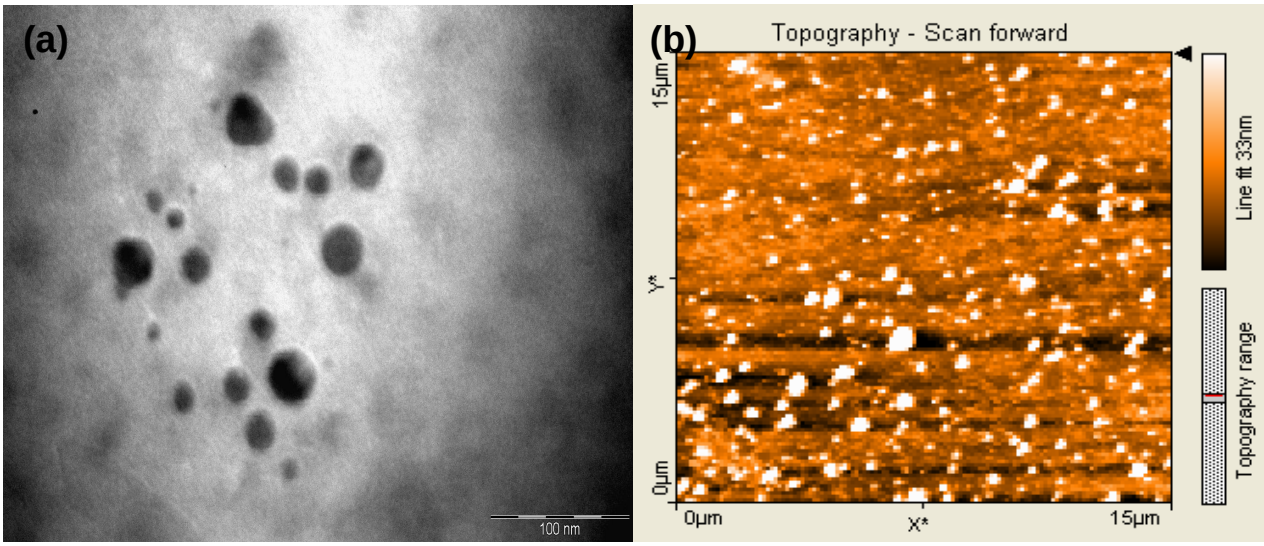

Fig. 2: Micrographs of (a) TEM and (b) AFM images

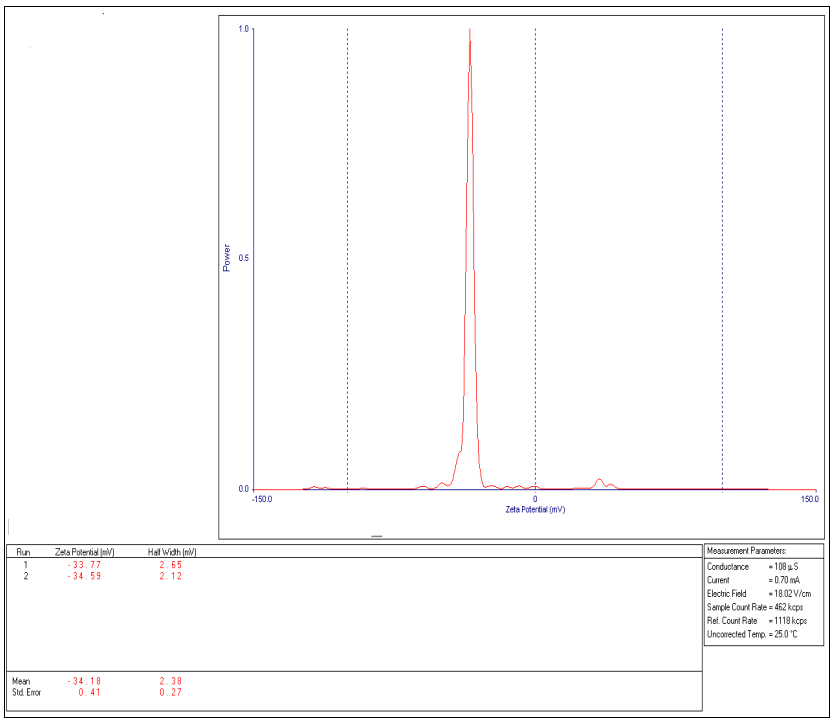

Fig. 3: Zeta potential of nano silver colloidal solution

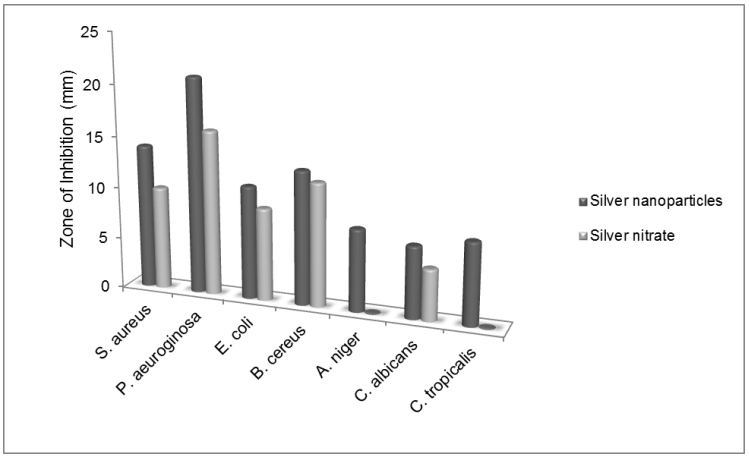

Fig. 4: Antimicrobial activity of SNPs and $AgNO_3$

Table 1: Antimicrobial activity of SNPs, $AgNO_3$, Aqueos extract and reference drugs against pathogenic microorganisms

Microorganism	Zone of inhibition (mm)			
	SNPs	$AgNO_3$	Aqueous extract	Reference drugs
Bacteria				Gentamycin (30 µg/disk)
Staphylococcus aureus	14	10	-	18
Pseudomonas aeuroginosa	21	16	-	20
Escherichia coli	11	9	-	19
Bacillus cereus	13	12	-	20
Proteus mirabilis	11	8	-	21
Fungi				Amphotericin–B (20 µg/disk)
Aspergillus niger	8	-	-	9
Candida albicans	7	5	-	20
Candida tropicalis	8	-	-	18

CONCLUSION

We have demonstrated a simple and eco-friendly process for the synthesis of silver nanoparticles from *Ceriops tagal*. Within five hours of interaction time, spherical shaped nanoparticles below 40 nm size were formed. The higher negative zeta potential represented the stability of colloidal stability of nanoparticle suspension. The silver nnanoparticles exhibited substantial antimicrobial activity against pathogenic bacteria and fungi. In the present scenario, SNPs have appeared as a promising antimicrobial candidate in the medical field. Hence it could be potentially applied in the fabrication of silver impregnated antimicrobial materials for biomedical applications. The method described is highly efficient and cost-effective to produce stable silver nanoparticles.

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