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Research Article

SYNERGISTIC EFFECT OF BIOGENIC SILVER NANOCOLLOID IN COMBINATION WITH ANTIBIOTICS: A POTENT THERAPEUTIC AGENT

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ABSTRACT

We have reported an eco-friendly process for the synthesis of stable silver nanoparticles from *Rhizophora apiculata*. The absorption maximum of silver nanoparticles as characterized by UV–Visible spectrophotometer was found to be around 422 nm. The transmission electron microscopy study revealed the presence of spherical shaped nanoparticles with an average size of 15 nm. The antibacterial activity of gentamicin and chloramphenicol increased in the presence of AgNPs against the test strains. The research provides an important direction in the development of new antimicrobial agents.

Keywords: Antibacterial activity, Antibiotics, *Rhizophora apiculata*, Silver nanocolloids, stability

INTRODUCTION

Nanotechnology emerges from the physical, chemical, biological and engineering sciences where novel techniques for the synthesis of nanoparticles are employed for the development of eco-friendly and sustainable methods. Recently, there has been renewed interest in applying fundamental principles of green chemistry to produce environmentally benign nanoparticles. The bio-based approaches for the synthesis of nanoparticles are rapidly gaining importance due to its easy synthesis, eco-friendly and formation of stable and biocompatible nanoparticles. Bacteria, fungi, plants and seaweeds are the potential sources utilized for the synthesis of nanoparticles [1-7]. The synthesis of nanoparticles from plants is more advantageous over other biological processes as they are safe to handle [5].

Rhizophora apiculata, a mangrove tree, have been used as a folkloric medicine in Asian countries like India, China as an astringent for vomiting, diarrhea and nausea. The extracts of *Rhizophora apiculata* have shown to exhibit larvicidal, anti-ulcer, antimicrobial, antiviral and antitumor activities [8-10]. The presence of reductants like phenolic compounds, polysaccharides and steroids, directs the formation of nanoparticles in the solution [8].

Since time immemorial, silver is been used in different forms like metallic silver, silver nitrate, or silver sulfadiazine for the treatment of burns, wound, and severe bacterial infections [11]. The use of silver nanocolloids is significant, as several pathogenic bacteria and fungi have developed resistance against various antibiotics. For example, *S. aureus* has exhibited resistance to methicillin, *E. coli* showed resistance to numerous antibiotics like, kanamycin, streptomycin, tetracycline and ampicillin [12,13]. According to Fayaz et al., the silver nanocolloid combined with antibiotics has shown good antibacterial activity [14]. The current study is aimed at the synthesis of silver nanoparticles using aqueous extracts of *Rhizophora apiculata* and to assess the enhanced antibacterial activity of Ag nanocolloid in combination with gentamicin and chlroamphenicol against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus mirabilis*.

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$) was purchased from SD Fine Chemicals, antibiotic disks, Muller-Hinton agar from Himedia.

Preparation of the aqueous extract

Rhizophora apiculata leaves were collected from the Pichavaram mangrove forest, Tamil Nadu, India. The leaves were thoroughly washed, shade-dried and powdered. Ten grams of the powdered leaves were macerated in 100 ml millipore water and kept in a rotary shaker at 130 rpm at room temperature (25 °C) for one day. The extract was centrifuged at 10,000 rpm for 10 min. The supernatant was filtered using 0.45 micron filter paper and further used for experiments.

Synthesis and characterization of silver nanocolloids

The aqueous extract (20 ml) of *R. apiculata* was interacted with 80 ml of 0.004 M $AgNO_3$ solution at room temperature in the rotary shaker at 130 rpm and was observed for color change. To remove the non-AgNPs components and maximum recovery silver nanoparticles from the colloidal solution, centrifugation was carried out at 10,000 rpm for 20 min. The pellet obtained by centrifugation was dispersed in millipore water and centrifuged again twice. The purified colloidal suspension was subsequently used for characterization.

The preliminary characterization of the synthesized silver nanoparticles was carried out using systronic double beam spectrophotometer (UV-vis Systronic-2201) by diluting the colloidal suspension in millipore water 10 times. The spectra were recorded from 300-600 nm at a resolution of 0.1 nm. The shape and size of were determined using transmission electron microscopy. The electron micrographs of the silver nanoparticles were taken using Technai G 10 (Philips 80 kV). The morphology of AgNPs was also determined using atomic force microscopy (AFM). The AFM studies were carried out by drop coating the purified colloidal suspension onto a glass slide using Nanosurf Easy Scan 2 (Switzerland) instrument. To check the stability of the colloidal suspension the zeta potential (ζ-potential) was checked periodically by using a Brookhaven Zeta 90 Plus analyzer. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. The measurement of zeta potential is based on the direction and velocity of particles under the influence of electric field.

Antimicrobial activity

Antimicrobial activity was evaluated against four human pathogenic bacteria, *Bacillus cereus* (NCIM-2458), *Staphylococuss aureus* (NCIM-2672), *Escherichia coli* (NCIM-2809) and *Proteus mirabilis* (NCIM-2388) in triplicates. The antibacterial activities of AgNPs were investigated by standard disk diffusion method [15]. The bacterial lawns were prepared on Muller–Hinton agar by using sterile cotton swabs at a concentration of 10^8 colony forming units (CFUs)/mL. To determine the combined effects, standard antibiotics- gentamicin (30 µg/disk) and chlroamphenicol (30 µg/disk) disks were impregnated with 20 µL of silver nanocolloids. The disks were placed onto the agar plate inoculated with the test pathogenic bacteria and then incubated at 37°C. After 18 hrs, the zones of inhibition were measured.

RESULTS AND DISCUSSIONS

The bioreduction of silver ions with concomitant formation of silver nanoparticles using aqueous leaf extract was monitored by UV–visible spectroscopy. The UV–vis spectrum in Fig. 1 represents the formation of silver nanoparticles as a function of time. The solution color changed from colorless to brown. The appearance of brown color was an indication of the formation of AgNPs in the reaction mixture. A sharp peak around 422 nm was observed at the end of the 4th hour of the reaction (Fig. 1). The absorption maxima of silver nanoparticles were reported in the range of 400–500 nm [16].

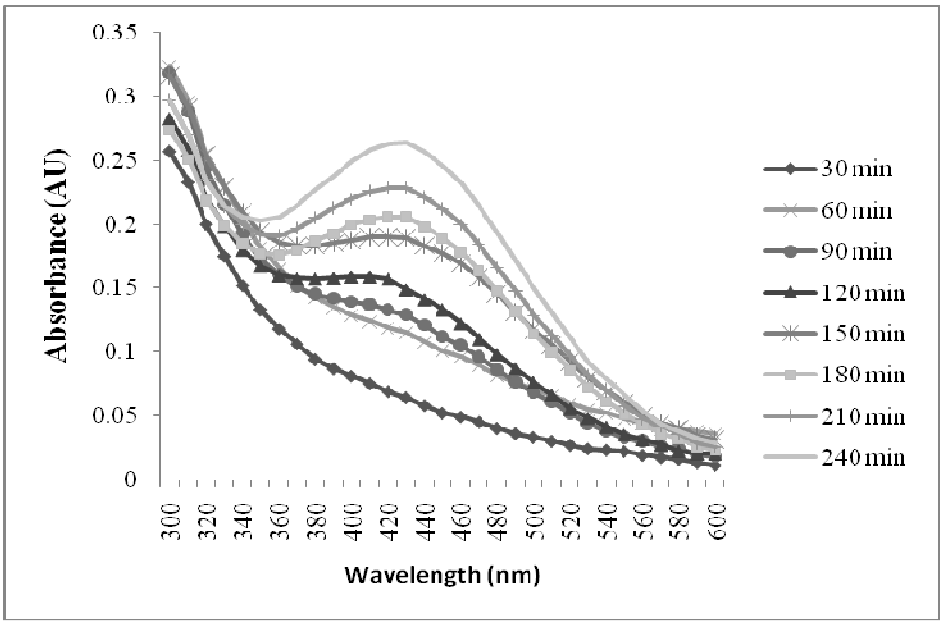

Fig. 1: UV-vis spectra of formation of AgNPs recorded as a function of time

Spherical nanoparticles ranging from 6 nm to 15 nm were observed in TEM and AFM micrographs (Fig. 2).

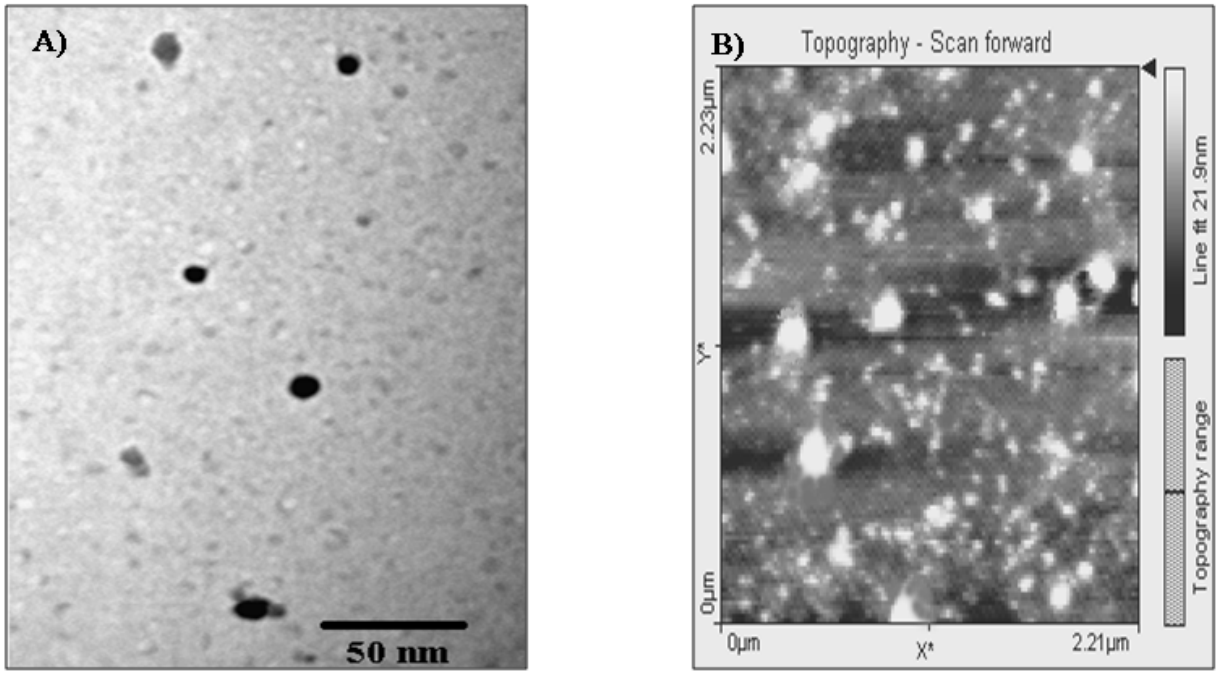

Fig. 2: Micrographs of (A) TEM and (B) AFM images

The ζ-potential value of the nanoparticles at the end of the reaction was determined to be −42.63 mV. The negative surface charge could be due to the adsorption of bioactive components present in the aqueous extract onto the nanoparticles surface. The particles in suspension with ζ-potentials more positive than +30 mV or more negative than -30 mV are considered stable [17]. Higher values of ζ-potential suggest a greater electrostatic repulsion between the nanoparticles and therefore a lower occurrence of agglomeration or clumping in the colloidal suspension. From Table 1 it is evident that the colloidal suspension was stable for one year.

Table 1: Zeta potential of silver nanocolloids at different time intervals

	Time period	Zeta potential (mV)
Silver nanocolloids	4 hrs	-42.63
	1 day	-42.42
	7 days	-41.83
	1 month	-41.28
	2 months	-41.42
	4 months	-41.19
	6 months	-41.12
	1 year	-41.10

The combination of silver nanocolloids along with gentamicin and chloramphenicol was investigated against the pathogenic bacteria using the disk diffusion method. The diameter of the inhibition zone (mm) around the different antibiotic disks with and without AgNPs against test strains is shown in Table 2. The antibacterial activity of gentamicin and chloramphenicol increased in the presence of AgNPs against test strains. The antibacterial activity of gentamicin against all the test strains was more in the presence of Ag-NPs as compared to chloramphenicol. The maximum antibacterial activity for gentamicin in combination with silver nanocolloids was observed against *S. aureus* followed by *B. cereus*, *P. mirabilis* and *E. coli* with a percentage fold increase of 44.44 %, 25 %, 14.28 % and 11.11 % respectively (Table 2). In case of, chloramphenicol in combination with silver nanocolloids the maximum activity was observed against *B. cereus* with 22.72 % fold increase in antibacterial activity. *B. cereus* and *E. coli* demonstrated 19.04 % and 11.11 % fold increase while a much smaller increase (7.7 %) was observed for *P. mirabilis*.

Table 2: Inhibition zones (mm) of antibiotics alone and in combination with AgNPs against bacterial strains

Microorganisms	Zone of inhibition (mm) Mean ± Standard error					
	Gentamycin (a)	Antibiotic + AgNPs (b)	Percentage (%)* increase [(b– a)/a]× 100	Chlorampinecol (a)	Antibiotic + AgNPs (b)	Percentage (%)* increase [(b– a)/a]× 100
Bacillus cereus	20 ± 0.23	25 ± 0.84	25	23 ± 0.45	27 ± 0.41	22.72
Staphylococcus aureus	18 ± 0.34	26 ± 0.45	44.44	21 ± 0.65	25 ± 0.18	19.04
Proteus mirabilis	27 ± 0.51	30 ± 0.32	11.11	26 ± 0.81	28 ± 0.92	7.7
Escherichia coli	28 ± 0.25	32 ± 0.28	14.28	27 ± 0.16	30 ± 0.47	11.11

* Percentage fold increases of individual antibiotics were calculated using the formula ((b − a)/a) × 100.

The antibacterial activity of gentamicin and chloramphenicol increased in the presence of AgNPs against the bacterial strains. The increase in synergistic effect may be due to the bonding reaction between antibiotics and silver nanoparticles. Further the nanoparticles have large surface area which allows them to closely interact with antibiotics. The antibiotic molecules contain active groups like hydroxyl and amido groups, which can easily react with AgNPs by chelation [18]. Recently, Fayaz et al. showed that the silver nanoparticles in combination with ampicillin exhibited higher antibacterial against Gram positive and Gram negative bacteria. They proposed a mode of action; the active groups of ampicillin molecule interact with the AgNPs. The ampicillin-AgNPs complex comes in contact with the cell wall and inhibits the formation of cross links in the peptidoglycan layer leading to cell wall lysis. Thus increases the penetration of the complex into the bacterium. The AgNPs-ampicillin complex reacts with DNA and prevents the unwinding of DNA, which results in serious damage to bacterial cells [19]. In another report by Dhar et al. the AgNPs along with streptomycin demonstrated enhanced antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans* [14]. The enhanced synergistic effect of biologically synthesized silver nanocolloids and antibiotics against pathogenic bacteria could be potentially applied in the development of new therapeutic agents.

CONCLUSION

We have demonstrated a facile and green process for the synthesis of silver nanoparticles from *Rhizophora apiculata* Blume at room temperature. Within four hours of interaction time, spherical shaped nanoparticles below 20 nm size were formed. The colloidal solution remained stable for over one year. Antibiotic resistance by pathogenic bacteria and fungi has been continuously increasing over the past decade; hence, there is a need. In the present scenario, AgNPs have appeared as a promising antibacterial 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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