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Research Article

COST-EFFECTIVE GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *AVICENNIA ALBA* BLUME LEAVES AND THEIR ANTIBACTERIAL ACTIVITY

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ASTRACT

Introduction: In recent years, green synthesis of silver nanoparticles (AgNPs) has gained much interest from chemists and researchers and also increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine.

Objective: In the present study, AgNPs were synthesized from 1 mM $AgNO_3$ solution through the extract of *Avicennia alba* leaves. It is a cost-effective and eco-friendly technique. The nature of AgNPs synthesized was analyzed by UV-visible spectroscopy, Fourier transform infrared spectroscopy, and scanning electron microscopy. The antibacterial potential of synthesized AgNPs was compared with that of standard antibiotic by agar well diffusion method.

Results: The antibacterial activity results revealed that *A. alba* leaf AgNPs showed a significant zone of inhibition against the majority of tested bacteria than the streptomycin. *Arthrobacter protophormiae* and *Proteus mirabilis* were found to be two-fold sensitive to AgNPs of *A. alba* leaf than to positive control, streptomycin. *Rhodococcus rhodochrous* was found sensitive only to AgNPs but not to streptomycin. Other remaining sensitive bacteria exhibited more or less same susceptibility to AgNPs and streptomycin.

Conclusion: AgNPs of *A. alba* leaf showed broad spectrum antibacterial activity and may be a good alternative therapeutic approach in future.

Keywords: Green synthesis, Silver nanoparticles, *Avicennia alba* leaves, Fourier transform infrared spectroscopy, Scanning electron microscopy, Antibacterial activity.

INTRODUCTION

Nanotechnology is deals with nanometer sized objects. Nanomaterials, due to their small size, show unique and considerably changed physical, chemical, and biological properties compared to their macro scale counterparts [1]. There are several methods for the preparation of silver nanoparticles (AgNPs) including electrochemical method, laser ablation, microwave irradiation, thermal decomposition, and sonochemical synthesis. The development of new chemical and physical methods for the preparation of nanoparticles may produce pure and well-defined nanoparticles. However, these methods are quite expensive and potentially hazardous to the environment. Due to this, recently the studies are focused toward eco-friendly methods such as green synthesis of AgNPs from natural sources such as plants and microorganisms have grabbed researchers' focus. Moreover, this method has some advantages such as cost-effectiveness and in the process of production, it does not require high temperature, pressure, energy, and toxic chemicals. In general, metallic nanoparticles are mostly prepared by noble metals such as silver, platinum, and gold [2,3]. Biologically synthesized gold and AgNPs could be of immense use in medical and biomedical textiles for their efficient antibacterial and antimicrobial properties and also in other applications such as spectrally-selective coatings for solar energy absorption and intercalation material for electrical batteries and also useful as optical receptors and as catalysts in chemical reactions [4]. *Avicennia alba* is a species of tropical mangrove belonging to the family Acanthaceae (Fig. 1).

It is used for the treatment of several types of diseases such as sexual disorders, scabies, rheumatism, paralysis, snake bites, asthma, and ulcers [5]. In the present investigation, *A. alba* leaf aqueous extract was used to synthesize a simple, low-cost and green method of AgNPs. Prepared nanoparticles were characterized by UV/vis, Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The *in vitro* antimicrobial activity of AgNPs was assessed by green synthesized nanoparticles.

Fig. 1: *Avicennia alba* plant

METHODS

Plant material and preparation of the extract

Fresh *Avicennia alba* leaves were collected from Coringa mangrove forest, near Kakinada (Andhra Pradesh), India. The collected leaves were washed and air dried in the shade and crushed by a mechanical grinder to obtain fine powder. 15 g of this powder was added to 100 ml of deionized water and boiled for 15 minutes at 60°C. After cooling to room temperature, the extract was filtered using Whatman No.1 filter paper and stored at 4°C for further analysis.

Green synthesis and characterization of *A. alba* leaf AgNPs

The silver nitrate of AR grade used in this study was obtained from Sigma-Aldrich. To 20 ml of plant extract, 80 ml of 1 mM $AgNO_3$ solution was added and further heated up to 60°C for 15 minutes. Then, observed the color change formation. For the preliminary confirmation of AgNPs formation. The bio-reduction of silver ions in solution was monitored by measuring the UV-Vis spectrum of the reaction medium. The UV-Vis spectral analysis of the sample was done by Thermo scientific evolution 201 spectrophotometer at room temperature. Furthermore, the solution was centrifuged at 20000 rpm for 30 minutes. After centrifugation, separated nanoparticles settled at the bottom were collected and washed thrice with double distilled water, then dried in an oven at 60°C for 2 hrs. The stabilized powder form of the nanoparticles was stored for further characterization. The pellet of AgNPs obtained after oven drying was mixed with KBr and KBr-AgNPs mixture was subjected to FT-IR (Model-Thermo Nicolet Nexus 670) to ensure the formation of AgNPs with encapsulation of biomolecules of *A. alba* plant leaves. Scanning electron microscope (Model-SEM Hitachi - S520, Japan) was used to study the morphology of AgNPs. These well-characterized AgNPs were further used for the antibacterial assay.

Antibacterial activity of *A. alba* leaf AgNPs

The AgNPs synthesized from *A. alba* leaves were tested for their antibacterial activity against pathogenic bacteria including both Gram positive and Gram negative bacteria *viz*., *Micrococcus luteus* MTCC 106, *Arthrobacter protophormiae* MTCC 2682, *Rhodococcus rhodochrous* MTCC 265, *Enterococcus faecalis* MTCC 439, *Streptococcus mutans* MTCC 497, *Bacillus subtilis* MTCC 441, *Enterobacter aerogenes* MTCC 10208, *Alcaligens faecalis* MTCC 126, *Proteus vulgaris* MTCC 426, *Proteus mirabilis* MTCC 425, *Pseudomonas aeruginosa* MTCC 1688, and Salmonella enteric MTCC 3858 by agar well diffusion method [6]. Into each agar well, 100 µl of sample prepared by dissolving 100 µg of nanoparticle material in 1 ml of dimethyl sulfoxide (DMSO) was placed. In a separate well, DMSO was also dispensed to maintain the control. The plates were incubated at 37°C for 24 hrs. After incubation, the diameter of the zone of inhibition was measured. For each sample and bacterial species, triplicates were maintained.

RESULTS

UV-vis analysis

As the leaf extract was added to aqueous silver nitrate solution, the color of the solution changed from faint light to yellowish-brown to reddish-brown and finally to colloidal brown indicating AgNPs formation (Fig. 2).

Fig. 3 shows the UV-vis absorption spectrum of the synthesized AgNPs. The peak observed at 455 nm was the characteristic band for silver, apart from that no other peak was observed in the spectrum, which confirms that the synthesized products are of silver only.

SEM

The SEM image of *A. alba* leaf AgNPs is shown in Fig. 4. The morphology and size details of the AgNPs were verified by SEM. Different shapes of AgNPs were observed. This may be due to the availability of different quantity and nature of capping agents present in the leaf extracts. This is also supported by the shifts and difference in areas of the peaks obtained in the FTIR analysis.

FTIR

Typical FTIR spectrum of *A. alba* leaf AgNPs revealed the strong band at 3415 which is assigned to OH stretching in alcohols and phenolic compounds (Fig. 5). The absorption peak at 2920 may be assigned to CH stretching, the absorption peak at 1660 assigned to a carbonyl stretch in the amide linkages of the proteins, 2920 peak assigned to CH stretching. The FTIR results thus indicate that the secondary structure of the proteins is not affected as a consequence of reaction with the Ag+ ions or binding with the AgNPs.

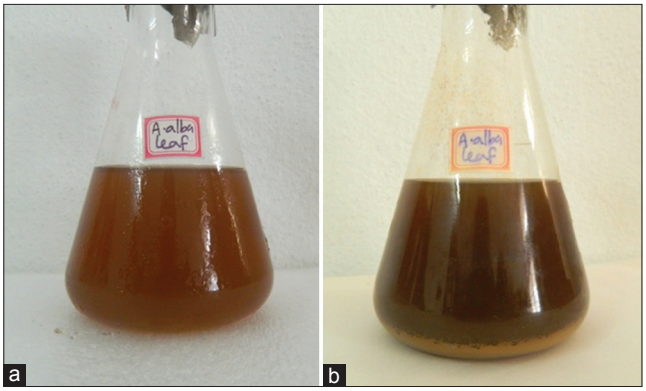

Fig. 2: (a) Leaf extract of *Avicennia alba*, (b) Brown solution of silver nanoparticles formed due to the reduction

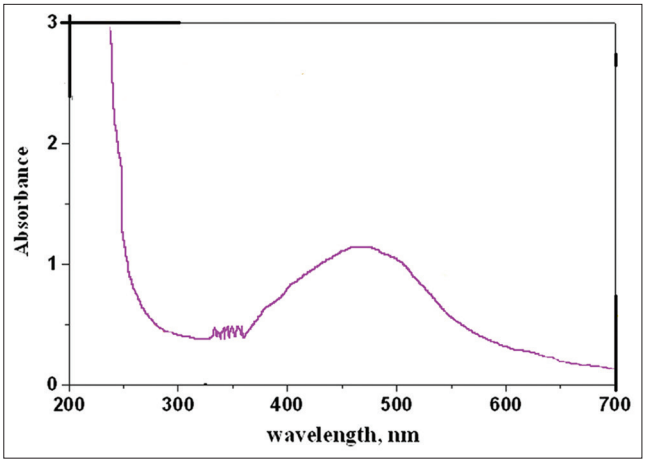

Fig. 3: UV-vis absorption spectrum obtained from silver nanoparticles of *Avicennia alba* leaves

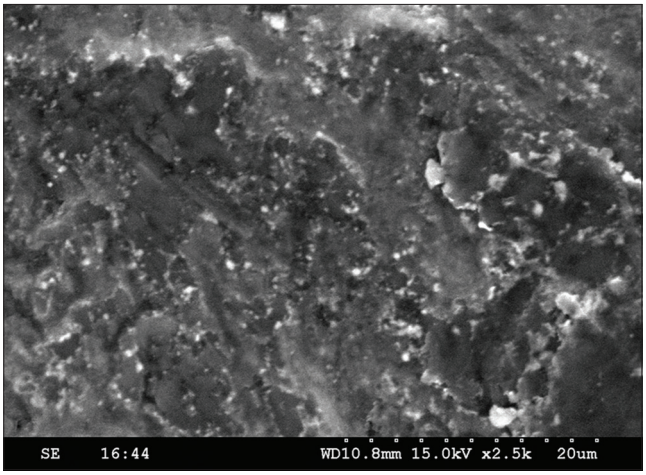

Fig. 4: Scanning electron microscopy image of silver nanoparticles synthesized from *Avicennia alba* leaves

Antibacterial activity

Silver is known as a very good antimicrobial agent against various pathogenic organisms, and it was used from ancient times. Further, the silver also used in water purification and air filtration system to eliminate the pathogenic microorganisms. The antibacterial activity of AgNPs of *A. alba* leaf demonstrated that both Gram-positive and Gram-negative bacteria were inhibited to different extents. Results (Fig. 6) revealed good antibacterial activity against the majority of

tested bacteria. Some photographs of zones of inhibition are given in Fig. 7. *A. protophormiae* and *P. mirabilis* exhibited a maximum zone of inhibition, i.e. two times larger than the positive control streptomycin. Streptomycin did not show any zone of inhibition against *R. rhodochrous,* and *E. aerogenes* but *A. alba* leaf AgNPs displayed good zone of inhibition than the positive control. *A. alba* leaf AgNPs showed a greater zone of inhibition than the positive control against *B. subtilis*, *A. faecalis*, *P. aeruginosa,* and *S. enterica*. However, *M. luteus*, *E. faecalis*, *S. mutans,* and *P. vulgaris* were also susceptible to *A. alba* leaf AgNPs but not as sensitive to the positive control.

DISCUSSION

The SEM analysis of synthesized AgNPs can provide morphological information on the submicron scale and elemental information at the micron scale [7]. The FTIR result suggests that the biological molecules could possibly perform a function for the formation and stabilization of Ag NP in an aqueous medium. It is well-known that proteins can bind to AgNP through free amine groups in the proteins [8]. Silver ions, as well as AgNps, were known to have strong antimicrobial activities [9,10]. The high bactericidal activity of AgNPs is due to their extremely large surface area, which provides better contact with microorganisms [11]. Similar type of results were observed with leaf AgNPs of *Citrus sinensis*, *Centella asiatica* against *Escherichia coli*, *Staphylococcus aureus,* and *Solanum tricobatum* leaf AgNPs against *K. pneumonia* [12]. The varying degrees of antibacterial activity exhibited by *A. alba* leaf AgNPs against tested bacteria is due to the variation in the cell wall structural composition. Several mechanisms were explained for the bactericidal activity of AgNPs against bacteria. One explanation is the disruption of cell wall permeability, and another one is causing damage to DNA and proteins by direct binding to phosphate and sulfur groups, respectively [13].

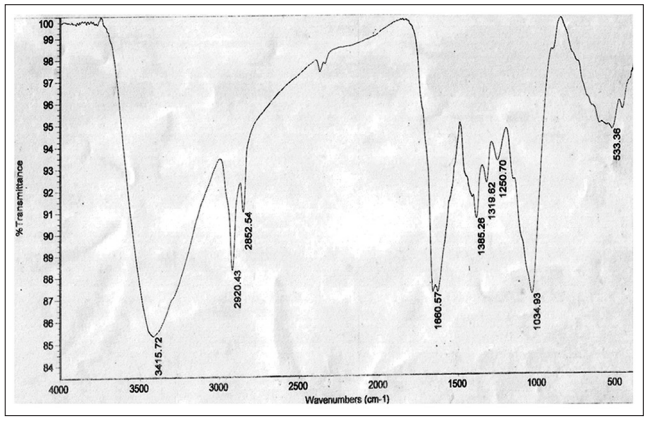

Fig. 5: Fourier transform infrared spectroscopy analysis of *Avicennia alba* silver nanoparticles

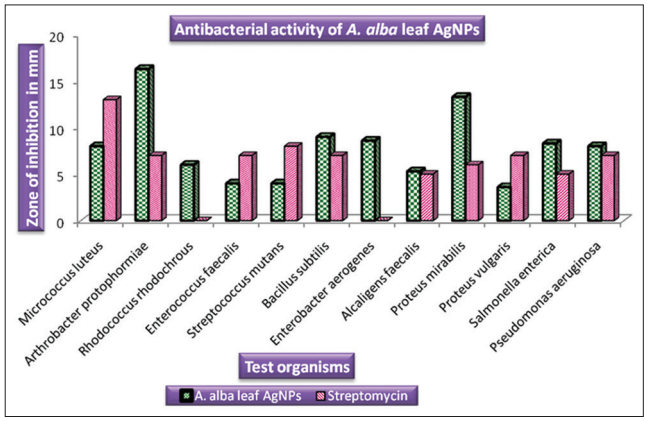

Fig. 6: Antibacterial activity of *Avicennia alba* silver nanoparticles against different bacteria

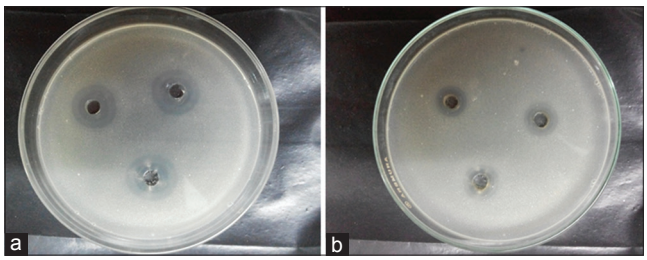

Fig. 7: (a) Leaf silver nanoparticles against *Arthrobacter protophormiae.* (b) Leaf silver nano particles against *Proteus mirabilis*

CONCLUSION

The Green synthesis method used in this study for the preparation of AgNPs using *A. alba* leaves is a quite fast and of low-cost technique. The results revealed that the biologically synthesized AgNPs could be of immense use in the medical field for their efficient antimicrobial function.

ACKNOWLEDGMENTS

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