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Research Article

ANTIMICROBIAL POTENTIAL OF SILVER NANOPARTICLES SYNTHESIZED USING *ULVA RETICULATA*

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ABSTRACT

Production and application of silver nanoparticles (AgNPs) is an emerging field of research. The present study was demonstrated with rapid synthesis of AgNPs using marine macroalgae-*Ulva reticulata*. The observation of prominent colour change at 121˚C within 10 mins indicates the formation of AgNPs. The synthesized AgNPs were characterized by UV-Vis spectrum, Energy Dispersive Spetrum (EDS), Fourrier Transform Infra Red spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). The aim of the study is to evaluate the antibacterial and antifungal activity of synthesized AgNPs. Antibacterial activity was tested against Gram positive *Staphylococcus aureus*, and Gram negative *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus sp.*, *Klebsiella pneumoniae*. Antifungal activity was tested against *Candida albicans*, *Candida parapsilosis* and *Aspergillus niger*. AgNPs were fairly toxic to *Bacillus sp.*and *Staphylococcus aureus* with the inhibition zone of 26 and 25mm. AgNPs revealed higher antifungal activity against *Candida albicans*, *Candida parapsilosis* and *Aspergillus niger* with inhibition zone of 36, 30 and 30mm.

Keywords: Silver nanoparticles, Marine maro algae-*Ulva reticulata*, antibacterial, antifungal activity.

INTRODUCTION

Metal nanoparticles, which have a high specific surface area and a high fraction of surface atoms have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antimicrobial activity, and magnetic properties [1].

Silver or silver ions have long been known to have strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities [2-5]. Several proposals have been developed to explain the inhibitory effects of silver ion/silver metal on bacteria. It is generally believed that heavy metals react with proteins by combining the thiol (SH) groups, which leads to the inactivation of the proteins [6]. Recent, microbiological and chemical experiments implied that interaction of silver ion with thiol groups played an essential role in bacterial inactivation.

For several years, silver has been known for its antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, protozoa and certain viruses [7]. Silver, as antimicrobial agent, is used in wound dressings, burn treatments, creams and as coatings on different medical devices [8, 9]. The antimicrobial activities of AgNPs are related to their size and shape. Baker *et al*. observed that smaller particles with a larger surface area available for interaction have a high bactericidal effect [10].

The ability of pathogenic bacteria to resist antimicrobial agents has emerged in recent years and is a major health problem [11, 12].

Candida sp. is ubiquitous fungi and an important opportunistic pathogen of immunocompromised hosts because of cancer chemotherapy, or organ or human immunodeficiency virus infections [13]. They produce a wide spectrum of diseases [14]. Treating infection caused by *Candida sp*. becomes a hectic problem due to serious side effects like renal and liver dysfunction associated with amphotericin B and nystatin [15] and increased reisistance to fluconoazole [16].

Several kinds of the biological organisms like microbes, plants and seaweeds have been employed and well studied for the synthesis of Ag nanoparticles [17]. Of them, utilization of plants for the synthesis of Ag nanoparticle is advantageous over other biological methods.

Today, seaweed research has been increased considerably for the search of new and effective medicines of natural origin. Several compounds include primary and secondary metabolites synthesized by seaweeds are promising source for medical, industrial and biotechnological applications [18].

MATERIALS AND METHODS

Synthesis of AgNPs

The macroalgae-*Ulva reticulata* was collected from Mandapam coastal regions, Tamil Nadu, Southeast coast of India. Sample was washed thoroughly with sterile water to remove debris and salt on the surface and ground to fine powder. Collected samples were transferred to the lab in a cool bag, lyophilized, powdered and stored in -4˚C. About 5gm of the powdered material were extracted with 1000ml of sterilized double distilled water and the filtrate was used for the synthesis of AgNPs at 121˚C within 10 min.

Characterization of AgNPs

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium. Aliquots of the reaction solution were measured using a UV-1601 Schimadzu spectrophotometer operated at a resolution of 1nm. The interaction between protein-silver nanoparticles was analyzed by Fourrier Transform Infrared spectroscopy (FT-IR). Energy Dispersive Spectrum (EDS) was performed to confirm the presence of elemental silver. The EDX observations were carried out in STIC, CUSAT, Kerala (JOEL Model JED-2300). Scanning Electron Microscopy (SEM, JOEL Model JSM-63 90 LV) and Transmission Electron Microscopy (TEM, TENCAI 10 Philips instrument) analysis was employed to visualize the size and shape of silver nanoparticles.

Antibacterial activity of AgNPs

The antibacterial activity of AgNPs was tested against the following microorganism by disc diffusion method: *Escherichia coli, Bacillus sp., Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The samples were procured from MTCC and were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of each isolate (1×10^5cfu/ml) was used to prepare bacterial lawns. Antibacterial activity of the synthesized AgNPs was determined using the agar well diffusion assay method [19]. Approximately 20

ml of molten and cooled Mueller Hinton agar media was poured in sterilized petri dishes. The plates were left overnight at room temperature to check for sterility. The samples were swabbed onto the plates. Agar wells of 5 mm diameter were prepared with the help of a sterilized cork borer. As a preliminary qualitative assay, two wells were bored, one well containing the extract alone and the other well loaded with the synthesized AgNPs. The plates were examined for evidence of Zone of inhibition (ZOI) which appear as a clear area around the wells.

As a quantitative method, four wells were prepared in Mueller Hinton agar plate. Different concentrations (20µg/ml, 40µg/ml, 60µg/ml and 80µg/ml) of the AgNPs were prepared in DMSO and added to the respective wells and a control was maintained with the fourth well. Then the plates were incubated at 37˚C for 24 hrs, where upon inhibitory activity was observed as a zone of clearing around the wells. The diameter of the clearing zones was measured in mm using the ruler scale. The experiment was done in triplicate for each pathogenic bacterium and compared with the standard antibiotic sensitivity chart.

Antifungal activity of AgNPs

Antifungal activity of the synthesized AgNPs was determined using the agar well diffusion assay method [20]. Stock cultures of *Candida albicans, Candida parapsilosis* and *Aspergilus niger* were prepared and maintained in Sabouraud Dextrose Agar (SDA) slants at 4˚C. A 100 ml nutrient broth culture of each isolate (1×10^5cfu/ml) was used to prepare bacterial lawns. Prior to experiment, SDA broth was prepared, inoculated and incubated with pure culture at 25 ˚C for 3 days. 20ml of molten and cooled SDA media was poured in sterilized petridishes. The plates were overnight at room temperature to check for its sterility. The samples were swabbed onto the plates. Agar wells of 5 mm diameter were prepared with the help of a sterilized cork borer. Three wells were prepared in the agar plates. The wells were labeled as A, B, C. 'A' well was loaded with 50µl of AgNPs, 'B' well was loaded with 50µl of distilled water and 'C' well was loaded with 50µl of positive control drug (Amphotericin B).The plates containing the fungal and AgNPs were incubated at 25ºC for 3 days to measure the zone of inhibition. The plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells [21]. The diameter of such zones of inhibition was measured using a meter ruler. Mean value was calculated by performing the experiments in triplicates.

Results and Discussions

The present study was conducted to screen the marine macro-algae, *Ulva reticulata* for the synthesis of AgNPs and its antibacterial and antifungal activity against pathogenic organisms. Reduction of silver ions into AgNPs during exposure of the seaweed extracts could be followed by color change when kept at 121°C for 10min [22]. The reaction mixture turning brown after the addition of silver nitrate solution is a clear indication of the formation of AgNPs [23]. The colour change is due to the presence of the surface Plasmon vibrations of the AgNPs well dispersed in the solution [24]. Simultaneously, control without silver ions was also run along with the experimental flask. A single broad peak was observed at 430 nm (Figure 1), which corresponds to plasmon excitation of the AgNPs. A control without the addition of the silver nitrate solution was also recorded. Several investigators have observed absorption maxima of colloidal silver solution between 410 to 440 nm, which is assigned to surface plasmon of various metal nanoparticles [25]. The observed band in this range has been associated with Ag nanoparticles confirming the synthesis of Ag nanoparticles with narrow size distribution [20]. Similarly, biological synthesis of shape-controlled Ag nanoparticles can be achieved by varying temperature [26]. It is known that proteins and amino acids have a tendency to reduce Ag^+ ions to Ag^0. However, it is not yet clear which protein or compound is responsible for bioreduction of silver.

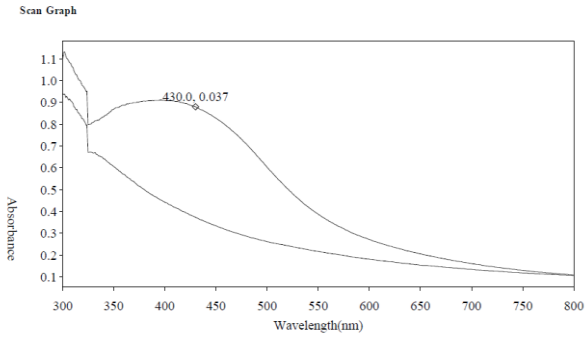

Fig. 1. UV-Vis spectrum of the aqueous extract of *Ulva reticulata* Vs. AgNPs.

Synthesized AgNPs were assayed for antibacterial activity against Escherichia *coli, Bacillus sp., Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa* by using standard ZOI assay, with a well size of 5mm diameter and 50µl of samples. Ampicillin of 50µl concentration was used as a control antimicrobial agent. AgNPs were found to be fairly toxic to *Bacillus sp.* and *Staphylococcus aureus* with the inhibition zone of 26 and 25mm. However AgNPs exhibited low toxicity against *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas aeruginosa* with the zone of 18, 20 and 16mm (Table 1). Diluted solution of AgNPs have been used to treat various infections and burns [27]. In this present study, different concentrations such as 20, 40, 60 and 80 µl from 10mg/ml were checked for antibacterial activity and zone of inhibition were tabulated in Table 2. The AgNPs synthesized showed inhibition zone against all the studied bacteria. A number of theories for antimicrobial actions of colloidal silver solution have been proposed, viz. the generation of free radicals responsible for the damage of membrane, dissipation of the proton motive force resulting in the collapse of the membrane potential; however exact mechanism has not been fully deciphered [28]. Tripathi et al studied effect of silver nano balls on *E. coli, Salmonella typhimurium, Bacillus subtilis* and *P. aeruginosa* by colony forming units (CFU) and growth curve at 40 µg/ml concentrations. The study showed significant reduction of bacterial population and their growth pattern at different concentrations. AgNPs attach to the sulphur containing proteins of the cell membrane, thereby causing membrane damage and depleting the levels of intracellular ATP of the microorganism [29]. Silver can also interact with the DNA of microorganisms, preventing cell reproduction [30].

Table 1: Screening of antibacterial activity of AgNPs with standard-Ampicillin

Organisms	Concentration (µl)	Zone of inhibition (mm)	
		Sample	Standard
Staphylococcus aureus	50	25	23
Bacillus sp.	50	26	21
Klebsiella pneumonia	50	18	19
Pseudomonas aeruginosa	50	16	Nil
Escherichia coli	50	20	21

Table 2: Screening of antibacterial activity with different concentrations of AgNPs

Concentrations (µl)	Zone of inhibition (mm)				
	E. coli	*K. Pneumoniae*	*Ps. aeruginosa*	*Staph. aureus*	*Bacillus sp.*
20	20	Nil	Nil	Nil	17
40	24	16	Nil	16	20
60	24	17	15	17	22
80	26	18	18	18	28

Table 3: Screening of antifungal activity of AgNPs with standard-Amphotericin B.

Organisms	Concentration (µl)	Zone of inhibition (mm)	
		Sample	Standard
Candida albicans	50	36	42
Candida parapsilosis	50	30	39
Aspergillus niger	50	30	28

Table 4: Screening of antifungal activity with different concentrations of AgNPs

Concentrations (µl)	Zone of inhibition (mm)		
	Candida albicans	*Candida parapsilosis*	*Aspergillus niger*
10	19	19	18
20	18	20	19
30	18	22	21
40	17	23	22

The antifungal activity of AgNPs was tested against *Candida albicans, Candida parapsilosis* and *Aspergillus niger* as shown in Fig. The AgNPs was found to be highly toxic against three fungal pathogens at a concentration of 0.5mg. Different concentrations such as 10, 20, 30 and 40 µl were checked for antifungal activity. AgNPs revealed higher antifungal activity with inhibition zone of 36, 30and 30mm (Table 4). We observed a good antifungal activity as compared with standard reference antifungal drug (Amphotericin B) (Table 3). Fungal cells when exposed to AgNP solution results in formation of pits on the surface of membrane resulting in destruction of its cell integrity [31].

It is a well known fact that antimicrobial activity of Ag nanoparticles is likely to be well correlated with its decreased size and shape owing to increased surface area with enhanced antimicrobial effect [32]. Antifungal activity of AgNPs has been reported by some authors [33].

CONCLUSION

In this study, we have discussed about the synthesis of AgNPs using a marine macro algae-*Ulva reticulata* its characterization and its antimicrobial application. The AgNPs were characterized by UV-Vis spectrum, Energy Dispersive Spectrum (EDS), Fourrier Transform Infra Red spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). The synthesized AgNPs were tested for antibacterial activity against *Escherichia coli, Bacillus sp., Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa* by using standard ZOI assay and against *Candida albicans, Candida parapsilosis* and *Aspergillus niger* for its antifungal activity. The AgNPs showed a good antibacterial and antifungal activity, therefore they could be recommended for production of a new range of antimicrobial agents.

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