Antibacterial and Antifungal Activity of Silver Nanoparticles Synthesized using *Hypnea muciformis*

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In this study silver nanoparticles (AgNPs) were synthesized using a marine macro red algae-Hypnea muciformis. To the algal extract 1mM aqueous silver nitrate was added and exposed to 121ºC for 10 mins. The synthesized AgNPs were characterized by observing a peak at 429nm using UV-visible spectroscopy. Antibacterial activity was investigated against *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The synthesized AgNPs were found to possess discrete antibacterial activity at a concentration of 5µl/ml. AgNPs exhibited more activity than silver nitrate (control) and standard antibiotic ampicillin. Antifungal activity against *Candida albicans*, *Candida parapsilosis* and *Aspergillus niger* was investigated along with standard control, Nystatin. The synthesized AgNPs exhibited a potent antifungal activity against tested fungal strains. Results of this study indicated that AgNPs have remarkable potential as an antimicrobial agent in treating infectious diseases.

Key words: Marine macro red algae-Hypnea muciformis, AgNPs, UV Vis, Antibacterial, Antifungal, Agar well diffusion.

In India seaweeds are mainly exploited by industries for phycocolloids but very poorly explored for their beneficial application in pharmacology. Numerous reports show macro algae to present a broad range of biological activities such as antibacterial[1] , antifungal[2] and antiviral activities. Seaweed mediated synthesis of AgNPs is preferred as it is cost effective, eco-safe and suitable for human therapeutic use. In the recent years, resistance of fungal infections has emerged as major health problem[3]. *Candida* spp. represents one of the most common pathogens which are responsible for causing hospital acquired sepsis with an associated mortality rate upto 40%[4]. During the past three decades, seaweed research has been increased considerably for the search of new and effective medicines of natural origin. Hence, in the present study, we report the synthesis of AgNPs, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *Hypnea muciformis*. Furthermore, these biologically synthesized nanoparticles were found to produce a high bactericidal and fungicidal activity.

MATERIALS AND METHODS

1gm of seaweed powder was extracted with water and filtered. 1mM silver nitrate solution was added to the filterate and agitated in the dark conditions under normal p^H. The extract is used as reducing and stabilizing agent for 1mM of Silver nitrate. Simultaneously control without silver ions was also run along with the experimental flask. The synthesized nanoparticles were characterized by UV-Vis spectroscopy.

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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 diameter of the clearing zones was measured in mm using the ruler scale and compared with standard Ampicillin disc (positive control) and silver nitrate solution (negative control). 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. Stock cultures of *Candida albicans, Candida parapsilosis* and *Aspergilus niger* were prepared and maintained in Sabouraud Dextrose Agar (SDA) slants at 4ºC. A positive control drug (Nystatin) was also done parallel. The plates were examined for evidence of zone of inhibition, which appear as a clear area around the wells[5]. 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

It is well known that AgNPs exhibit brown colour in water which arises due to excitation of surface plasmon vibrations in the metal nanoparticles[6]. The synthesized AgNPs were characterized using UV-Vis spectroscopy and subjected to antimicrobial activity.

Antibacterial activity was carried out using five different strains, viz. *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The results of the investigation showed that AgNPs synthesized from *Hypnea musciformis* possess discrete antibacterial activity against pathogenic bacteria at a concentration of 5µg/ml. The AgNPs were compared favourably with silver nitrate solution and standard antibiotic ampicillin at a concentration of 5µg/ml (Table 1&2). The AgNPs exhibited more activity than silver nitrate and standard antibiotic. AgNPs were fairly toxic to *Bacillus sp.*, *E. coli* and *Staphylococcus aureus* with the inhibition zone of 26, 22 and 24mm. SN attach to the sulphur containing proteins of the cell membrane, thereby causing membrane damage and depleting the levels of intracellular ATP of the microorganism. Silver can also interact with the DNA of microorganisms,

Table 1. Antibacterial activity of AgNPs determined by agar well diffussion

Organism	Concentration (mg)	Zone of inhibition (mm)		
		Control	Standard	Sample
Klebsiella pneumoniae	50	Nil	17	17
Bacillus subtilis	50	Nil	29	30
Staphylococcus aureus	50	Nil	27	25
Pseudomonas aeruginosa	50	Nil	15	00
Escherichia coli	50	Nil	20	22

Table 2. Antibacterial activity of AgNPs at different concentrations

Organism	Zone of inhibition (mm) at different Concentrations (µl)			
	20	40	60	80
Klebsiella pneumonia	13	14	14	15
Bacillus subtilis	18	18	20	22
Staphylococcus aureus	14	18	20	24
Pseudomonas aeruginosa	Nil	Nil	Nil	10
Escherichia coli	18	20	24	26

preventing cell reproduction[7]. The red alga *Hypnea musciformis* showed narrow spectrum of activity as it successfully prevented the growth of gram positive bacteria more significantly than the gram negative strains. *H. musciformis* extract inhibited the growth of gram positive strains to the extent of 66.0% at 30ºC, whereas all the gram positive bacteria were susceptible at 20ºC[8]. It is generally believed that heavy metals react with proteins by combining the thiol (SH) groups, which leads to the inactivation of the proteins. Recent, microbiological and chemical experiments implied that interaction of silver ion with thiol groups played an essential role in bacterial inactivation[9].

The antifungal activity of AgNPs against *Candida albicans*, *Candida parapsilosis* and *Aspergillus niger* was investigated using antifungal drug-Nystatin as a comparable control. AgNPs exhibited a potent antifungal activity against fungal strains. Different concentrations such as 10, 20, 30 and 40 µl were checked for antifungal activity. AgNPs revealed higher antifungal activity with inhibition zone of 24, 26 and 30mm (Table 4). Kim *et al.*[10]reported that spherical AgNPs showed potent activity against *Candida albicans* compared with that of commercially available antifungal agents. Treating infection caused by fungi becomes a hectic problem due to serious side effects like renal and liver dysfunction associated with amphotericin B and nystatin[11]. Ag+ also forms complexes with bases contained in DNA and is a potent inhibitor of fungal DNAases[12]. The rate of biosynthesis of Ag nanoparticles from seaweeds is cost effective and does not use toxic chemicals. 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.

Table 3. Antifungal activity of *H. muciformis* with control and standard

Organism	Concentration (mg)	Zone of inhibition (mm)		
		Control	Standard	Sample
Aspergillus niger	50	Nil	22	20
Candida albicans	50	Nil	42	32
Candida parasilopsis	50	Nil	37	26

Table 4. Antifungal activity of *H. muciformis* at different concentrations

Organism	Zone of inhibition (mm) at different Concentrations (µl)			
	20	40	60	80
Klebsiella pneumonia	23	25	25	27
Bacillus subtilis	22	23	26	26
Staphylococcus aureus	17	18	20	21

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