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Potential antibacterial activity of crude extracts and silver nanoparticles synthesized from *Sargassum wightii*

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ABSTRACT
The present work investigates the antibacterial activity of silver nanoparticles (Ag-NPs) synthesized by biological method using *Sargassum wightii*. The fresh live seaweed was collected from the Mandapam coast of Tamilnadu, India. Solvent extract was prepared using acetone, petroleum ether and methanol. Aqueous extract of the seaweed was also used for the synthesis of silver Ag-NPs. Seaweed extract is used as a reducing agent of 2mM silver nitrate solution for the synthesis of Ag-NPs. Periodical monitoring of reaction mixture was done using UV-vis spectroscopy at 300-750 nm. The scanning electron microscopy (SEM) of the sample confirms the presence of Ag-NPs. The antibacterial activity of solvent extract was done by Minimal inhibitory concentration (MIC) assay. The methanol extract of the seaweed at a concentration of 250µg/ml exhibited potent antimicrobial activity against the test microorganism. The zone of inhibition ranging from 8-14 mm was observed with different extracts. The antibacterial activity of the synthesized Ag-NPs against the organism was also done by MIC test. The MIC of Ag-NPs was found to be 130µg/ml for all pathogenic microorganisms selected for the study. The zone of inhibition against *Bacillus cereus, Bacillus anhtracis, Staphylococcus aureus* and *Vibrio alginoyticus* were found to be 10, 8, 10 and 9 mm, respectively. The synthesized Ag-NPs exhibited significant antimicrobial activity against the selected microorganisms than the solvent extract of seaweed.

***Key Words*:** Seaweed, NPs, drug, SEM, pathogen, medicine.

INTRODUCTION

Seaweeds are the important marine resources and have been used as a source of food and medicine (Rajasekar *et al*., 2014). It produces variety of primary and secondary metabolites. Over 2,400 secondary metabolites have been isolated and many of which have been reported to have excellent biological activities (Faulkner *et al*., 2002) such as antibacterial, anti-cancer, anti-diabetic, anti-tumor, anti-coagulant and antioxidant (Lakshmanasenthil *et al*., 2014).

Seaweeds are the important sources of the primary producers and supply considerably to the carbon budget of the marine ecosystem. Over the past several decades seaweeds have generated an enormous amount of interest in the pharmacological industry with enormous medicinal properties. They are rich in antioxidants such as alkaloids, flavonoids, pigments, carotenoids, enzymes and polysaccharides (Heo *et al*., 2009).

The significant features of nanotechnology are development and synthesis of nanoparticles (NPs). Nanoparticles are fundamental building blocks of nanotechnology. The progress of biologically stimulated experimental process for synthesis of nanoparticles is evolving into an important aspect of nanotechnology. Nanoparticles have been extensively researched for anti-cancer, drug deliverv, antiviral, anti-cancer and antibacterial activity (Johnston *et al*., 2010).

In recent years, Ag-NPs have been widely used in various applications because of their well-known effectiveness in biomedical (Cao *et al*., 2010), electronic, catalysis and optical applications (Hayward *et al*., 2000). In particular, the outstanding antimicrobial properties of Ag-NPs have led to the development of variety of silver nanoparticle products including nanosilver-coated bandages, surgical instruments, contraceptive devices and dental implants (Lohse *et al*., 2012).

The present study was aimed to evaluate the bioactive compounds and synthesis of nanoparticles from brown seaweed *Sargassum wightii* against human bacterial pathogens.

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MATERIALS AND METHODS

Collection and identification of seaweed

The fresh live seaweed sample of *Sargassum wightii* was collected from Mandapam cost of Tamil Nadu, India. The collected seaweed was washed with fresh water to remove epiphytes. Then the sample was brought to laboratory in polythene bags and washed thoroughly with fresh water to remove salt and other extraneous material. The washed sample was shade dried and powdered using an electric mixer. Collected seaweed was identified on the basis of pigmentation, morphology and authenticated (*Voucher specimen No: S3/09/2012*) by Dr. P. Anantharaman, Associate Professor, CAS in Marine Biology, Chidambaram, Tamil Nadu.

Collection of bacterial culture

The pathogenic pure cultures of *Bacillus cereus, B. anthracis, Stapylococcus aureus* and *Vibrio alginolyticus* were obtained from Medical college, Annamalai University, Tamil Nadu. The pure cultures were then sub cultured on nutrient agar slants and preserved under refrigeration for further use.

Preparation of crude extract of the seaweed
From the powdered sample, 100 g was taken and extraction was done by maceration method using three different organic solvents - acetone, petroleum ether and methanol. Dried bio mass was taken in 500 ml conical flasks with 100 ml of solvents and the flasks were continuously stirred in Orbitek® shaker at 200 rpm for 48 hrs. The contents were subjected to centrifugation at 10000 rpm for 30 min. The supernatant was collected and evaporated at room temperature.

Synthesis of Ag-NPs
The synthesis of Ag-NPs from crude extract was done by taking 10 ml of the sample and mixing with 90 ml of 2 mM silver nitrate solution ($AgNO_3$). The mixture was then stirred using a magnetic stirrer in dark at room temperature for 2 hours. The synthesis of NPs was observed by a change in color of the silver nitrate solution. Periodical UV-visible spectroscopic monitoring of the sample mixture was done at 300-750 nm.

SEM analysis
The Ag-NPs solution obtained was purified by repeated centrifugation at 5000 rpm for 15 minutes. Ag-NPs so obtained were shade dried at room temperature and powdered. It was submitted for Scanning Electron Microscopic analysis (Hitachi S-4500 SEM machine) at South Indian Textiles Research Association, Coimbatore, Tamil Nadu. The size of the synthesized Ag-NPs from *S. wightii* was characterized using SEM.

Antibacterial activity of crude extract and Ag-NPs
The antibacterial activity of the seaweed was studied by well diffusion method. Acetone, petroleum ether and methanol extracted compounds of the seaweed was dissolved in DMSO (0.0075 g in 1 ml of DMSO). The log phase culture of the pathogenic microorganism namely B. *anthracis, B. cereus, S. aureus* and *V. alginolyticus* was prepared in nutrient broth and adjusted to 0.5 Mcfarlands opacity. Muller Hinton agar was prepared, sterilized and wells of 5mm diameter were cut on the agar. The extracts of *S. wightii* was then dispensed into the wells in varying quantity such as 4, 9, 18, and 35µl to give the final the concentration of 30, 61, 125, and 250µg respectively. The plates were then incubated at 37°C for 24 hrs in an incubator. The same method was also followed for studying the antibacterial activity of the synthesized Ag-NPs.

Statistical analysis
The experiments were conducted in triplicates and the results were expressed as means ± S.E.M. Statistical analysis were performed with Sigma plot® 12.5 software, Systat, USA.

RESULTS

Identification of seaweed
The collected seaweed sample was identified as *S. wightii* on the basis of pigmentation, morphology and authenticated by Dr. P. Anantharaman, Associate Professor, CAS in Marine Biology, Chidambaram.

Silver nanoparticle synthesis
Ag-NPs were synthesized by using the aqueous extract of *S. wightii.* The formation of nanoparticles was confirmed by visual assessment. The reaction mixture turning to dark brown color from brownish-yellow color indicated the synthesis of Ag-NPs and the excitation of surface plasmon resonance effect and reduction of silver nitrate. UV-visible spectrum of reaction mixture at different wave length ranging from 300-800 nm showed strong absorption peak at 450 nm indicate the formation of Ag-NPs (figure 1) and this wide peak is due to surface plasmon resonance property of Ag-NPs.

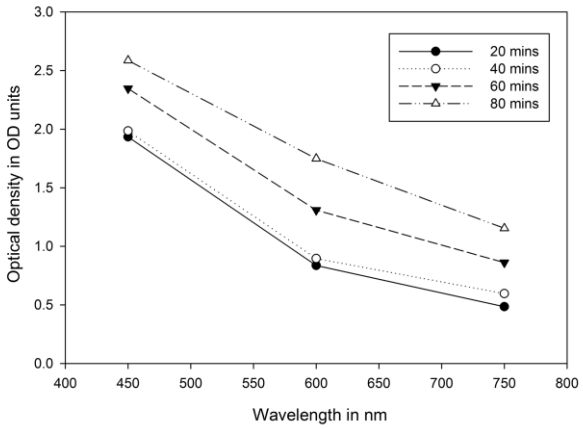

Figure 1: UV-Vis spectra of biologically synthesized silver nanoparticle at different time intervals.

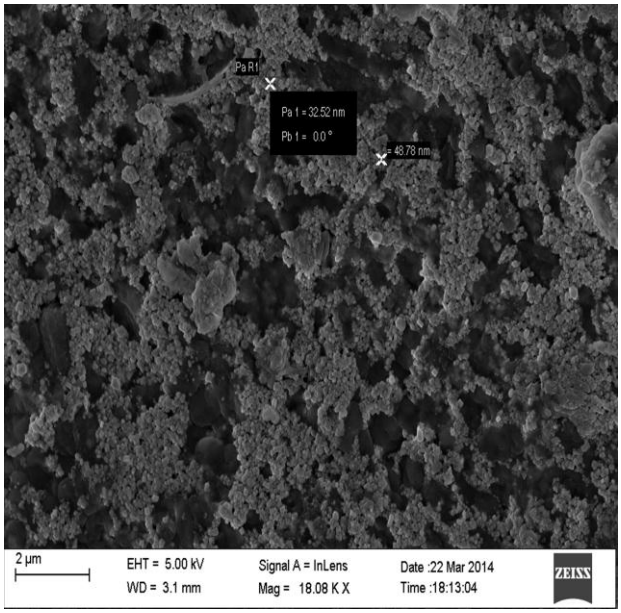

Figure 2: SEM micrograph of silver nanoparticles synthesized by reaction of 2mM silver nitrate with *S. wightii.*

SEM Analysis
The biosynthesized silver nanostructure from *S. wightii* extract was further confirmed by the SEM image. The SEM image shows the high density silver Ag-NPs synthesis. The SEM micrograph of nanoparticle showed that they are spherical shaped and well distributed without aggregation in solution (figure 2) and the average mean size of the Ag-NPs was found to be 48.78 nm.

Antibacterial activity of the solvent extract of *S. wightii*
The crude solvent extract (acetone, petroleum ether, and methanol) of seaweed sample were screened for anti-

Table 1: Antibacterial activity of various solvent extracts on pathogenic microorganisms.

Microorganisms	Zone of inhibition (mm)											
	Acetone Extract (µg)				Petroleum ether (µg)				Methanol (µg)			
	30	61	125	250	30	61	125	250	30	61	125	250
B. cereus	-	-	-	8±0.03	-	-	9±0.06	12±0.02	-	8±0.02	10±0.03	12±0.03
B. anthracis	-	-	-	8±0.02	-	8±0.07	10±0.04	13±0.06	-	9±0.08	11±0.05	12±0.08
S. aureus	-	-	9±0.05	11±0.07	-	-	8±0.03	10±0.03	-	9±0.07	12±0.04	14±0.04
V. alginolyticus	-	-	8±0.03	11±0.03	-	-	8±0.05	10±0.05	-	-	-	9±0.05

Table 2: Antibacterial activity of silver nanoparticles.

Microorganisams	Concentration of Ag-NPs (1µg/µl)	Diameter of zone of inhibition (in mm)
B. cereus	52	8±0.04
	78	11±0.08
	104	12±0.03
	130	15±0.09
B. anthracis	52	9±0.05
	78	10±0.06
	104	12±0.03
	130	13±0.09
S. aureus	52	8±0.06
	78	10±0.05
	104	13±0.05
	130	15±0.02
V. alginoyticus	52	8±0.09
	78	9±0.07
	104	13±0.09
	130	14±0.04

bacterial activity against pathogenic organisms namely *B. cereus, B. anthracis, S. aureus* and *V. alginolyticus.* Acetone extract exhibited the maximum activity against *S. aureus* (13mm at the concentration 250 µg) and moderate activity against *V. alginolyticus* (11mm at the concentration 250 µg) least activity observed against *B. cereus* and *B. anthracis.* The petroleum ether extract of the seaweed showed the highest antibacterial activity against *B. cereus* and *B. anthracis* (12 and 13 mm respectively) and moderate activity against *S. aureus* and *V. alginolyticus* (14 mm in diameter). The methanolic extract of seaweed showed highest zone against *S.aureus* (14 mm for 250 µg concentration). It gave moderate activity against *B. cereus* and *B. anthracis* (12 mm in diameter for 125 µg) and minimum zone against *V. alginolyticus* (table 1).

Antibacterial activity of Ag-NPs

The evaluation of antibiotic resistant pathogenic bacteria has stimulated the search for effective antibacterial agent from various sources. The nanoparticle synthesized from *S. wightii* was found highly active against the test bacteria. Potent antibacterial activity was observed against *B. cereus, B. anthracis, V. alginolyticus, S. aureus* at a concentration of 130 µg in 130 µl (table 2).

The Ag-NPs synthesized from the seaweed at a concentration of 130 µg showed very good antibacterial activity against all pathogenic microorganisms subjected for the study. The Diameter of zone of inhibition against *B. cereus, B. anthracis, S. aureus* and *V. alginoyticus* were found to be 15, 13, 15 and 14 mm respectively.

DISCUSSION

The silver ions reduction was observed when the silver nitrate was contacted with *S. wightii* extract and it becomes colorless to yellowish brown due to the formation of Ag-NPs. Literature confirms that Ag-NPs can exhibit a size-dependent characteristic surface plasmon resonance which can be deliberated using UV-Vis spectroscopy (Sukdeb *et al.*, 2007).

UV-visible spectrum of reaction mixture showed strong absorption peak at 450 nm with progressive increase in absorbance upon increasing time until 80 min as shown in figure 2. The observed band in this range has been associated with Ag-NPs confirming the synthesis of spherical Ag-NPs with narrow size distribution (Kumar *et al.*, 2012). The microstructure characterization of Ag-NPs was carried out using SEM showed that the Ag-NPs were in narrow size range, with most of the particles with size of 32 and 43nm.

In this present study, we found that *S. wightii* extract can be a good mediator for the synthesis of Ag-NPs. This green chemistry approach towards the synthesis of Ag-NPs has many advantages such as ease of scale up, economic viability and independent of use of energy, temperature and toxic chemicals. Applications of such pollution free nanoparticles in medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterials) (Dubay, 2009).

The results indicate that *S. wightii* can be beneficially used in the nanobiotechnology- based industries for bioinspired synthesis of Ag-NPs. Further studies are underway to characterize the synthesized Ag-NPs.

CONCLUSION

In this present investigation, environment friendly synthesis of Ag-NPs using macro algae was carried out. This method of Ag-NPs synthesis does not involve any toxic reagents and an alternative for Chemical synthesis methods that lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Macro algae mediated synthesis is an ecofriendly or nontoxic system and thus has the potential for use in biomedical applications.

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