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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles from *Chaetomorpha antennina* (Bory) Kützing and its Antibacterial Activity

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ABSTRACT

A simple method for the green synthesis of silver nanoparticles (AgNPs) from Green algae *Chaetomorpha antennina* present in the coastal region of Kerela. The formation of silver nanoparticles was characterized by UV–vis and FTIR. The UV absorption spectra at 430 nm revealed the characteristic spectra of the silver nanoparticles. The Fourier Transform Infrared (FTIR) spectra indicated the presence of polyphenols or protein, alkenes, amide II and amide III of aromatic rings. Synthesised silvernano particles were tested for antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae, Streptococcus aureus* and *Pseudomonas aeruginosa*. At 100µl concentration, the largest zone of inhibition (18mm) was found in *E. coli* and *P. aeruginosa* while the least zone of inhibition (16mm) was observed in *K. pneumoniae* and *S. aureus.* This type of research could also serve as a model for the future development of nanomedicines or focused algal drug delivery.

Keywords: *Chaetomorpha antennina*, Green algae, Ultraviolet visible spectroscopy, FTIR, Antibactrial activity

INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nano scale level [1]. Silver nanoparticles (AgNPs) are non-toxic to humans and are most effective at low concentrations against bacteria, viruses, and other eukaryotic microorganisms. AgNPs have potential applications in the biomedical field and has several advantages over physical and chemical methods due to its cost effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production [2]. Bio-extracts from a varied set of microorganisms, ranging from bacteria to fungi and algae, are used in the green production of nanoparticles as a reducing and sometimes capping agent [3]. AgNPs have been successfully synthesized using several plant extracts [4]. India is blessed with a longest coastline. So it gives an opportunity to investigate the vast bio resources especially seaweeds. In India 1,553 species of seaweeds belonging to 271 genera are recorded [5]. Seaweeds are natural and renewable living resources found in the marine ecosystem, and they are used for food, feed, and medicine. More than 60 elements, macro and micronutrients, proteins, carbohydrates, vitamins, and aminoacids can be found in seaweeds [6]. Some of the seaweeds are used for nanoparticles synthesis and their various medical applications especially antibacterial activity [7,8] and water filters, bio sensors, in controlling plant pathogens and antifungal activity [9].With current antibiotic therapy in treating infectious diseases, antimicrobial drug resistance is the most serious problem all over the world [10]. Recently, a lot of attention has been paid to seaweeds in terms of isolating and creating novel antimicrobial compounds. Many unique bioactive chemicals have been identified from marine organisms over the past four decades [11]. *Chaetomorpha antennina* is a marine green alga, which belongs to the family Cladophoraceae. Literature survey showed that there is no data available regarding silver nanoparticles synthesis in *Chaetomorpha antennina* in kerela costal area. The current study aimed to evaluate the antibacterial activity of green synthesised Silver nano particles synthesized from the green seed weed.

MATERIALS AND METHODS

Collection of plant material

The green alga *C. antennina* was collected from Chavakkad coast, Thrissur, kerala and the algae was identified by PG and Research Department of Botany, NGM College.

Preparation of Seaweed extracts

C. antennina fresh seaweed was shade dried and powdered. 25gm of powder were mixed with 500 ml of distilled water and boiled for 30-40 minutes. Filter the content with Whatman no.1 filter paper and stored it on room temperature for synthesis of SNPs.

Silver Nanoparticle synthesis

Ammonium solution (2.5ml) was added to 5ml of 1mM $AgNO_3$ solution, followed by addition of algal extract 1-10 ml and the final volume was adjusted to 50 ml by adding the appropriate amount of de-ionized water in Erlenmeyer flask. The Erlenmeyer flask was incubated at 37^0C under agitation (200rpm) for 24-72 hrs [12].

Characterization of silver nanoparticles

UV-VIS Spectra analysis

The silver nanoparticles were confirmed by measuring the wave length of reaction mixture in the UV-vis spectrum of the Labman spectrophotometer at a resolution from 300-700 nm.

FTIR analysis of nanoparticles

Perkin-Elmer spectrometer FTIR spectrum in range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} is used for detection of various functional bonds in nanoparticles being synthesized. The samples were mixed with KBr and thin sample disc

was prepared by pressing with the disc preparing machine and placed in the Fourier transform infrared spectroscopy [FTIR] for the analysis of the nanoparticles.

Antibacterial activity of synthesized silver nanoparticles

The AgNPs synthesized from *C. antennina* was tested for their antibacterial activity by well diffusion method [13] against human pathogenic organisms such as *E. coli*, *Klebsiella pneumonia, Streptococcus aureus* and *Pseudomonas aeruginosa*. Clinically isolated these microorganisms were obtained from Department of Microbiology, PSG institute of Medical Sciences & Research, Peelamedu, Coimbatore.The pure culture of all these stains was subcultured on nutrient broth at 35ºC on rotary shaker at 200 rpm. The petriplates were sterilized using autoclave at 121^{0}C for 15-20 minutes. Freshly prepared nutrient agar medium was poured into sterilized petriplates and allowed to cool. Each strain was spread uniformly on the individual plate using sterile cotton swabs. Wells of size 6mm was made on nutrient agar plates using gel puncture. Using micropipette 50µl and 100µl of the sample of nanoparticles solution was poured into wells on all plates and negative control ampicillin 25µl (1mg/L) and Positive control distilled water was added to all plates. After incubation at 35ºC for 18 hours, the different levels of zone of inhibition were measured.

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The aqueous extract of *Chaetomorpha antennina* (10 ml) was added to the 90 ml aqueous solution of Silver Nitrate in 250 ml conical flask and kept in room temperature for 72 hours. The colour of solution turned from yellow to brown indicates the formation of silver Nanoparticles (Figure-1). The solution colour changed from pale yellow to brown colour due to the excitation of surface plasmon vibrations. Similar results were reported in *Sargassum muticuml*[14]; *Caulerpa racemosa* [15]; *P. boergesenii*[16]. Due to the excitation of surface plasmon vibration, the brown seaweed *P. boergesenii* showed colour change from brownish to pale yellow colour [16]. In the present study, biosynthesis of silver nanoparticles was evidenced by the colour change of the reaction mixture (algae extract and silver nitrate). It confirmed the presence of AgNPs in the *Chaetomorpha antennina* with colour change.

UV-VIS spectra analysis

The formation of metal nanoparticles was confirmed by one of the important technique UV-Vis spectrum. In the present study synthesized silver nanoparticles of *Chaetomorpha antennina* was treated with UV-Visible spectrophotometer for confirming the presence of silver nanoparticles. An absorbance peak at 440 nm was recorded and it indicated the synthesis AgNPs (Figure-2). The formation of AgNPs in the present study was confirmed through comparison with Rajeshkumar *et al*. [17]. They reported that the brown algae *Padina tetrastromatica* silver nanoparticles exhibited a single absorbance band at 440 nm at 15 minutes and steadily increased in intensity at 24 hrs without any shift in the peak. The formation of AgNPs in the present study was also confirmed through comparison with Roy and Suparna report [18]. They also reported the surface plasmon resonance band corresponding to formation of AgNPs was occurred at 435.5 nm for *Chaetomorpha antennina.* There is some inappropriateness when compared with the observations of Vishnu kiran and Murugesan [15] in which the bands corresponding to the surface plasmon resonance of AgNPs arised at 420 nm for red algae *Halymenia porphyroides*.

FTIR analysis of the leaf extract and nanoparticles

An important biomolecule responsible for the reduction of silver ions to silver nanoparticles was identified using FT—IR analysis. Figure-3 shows the FT—IR spectrum of *Chaetomorpha antennina* assisted silver nanoparticles. The band at 3423.65 cm^{-1} represents O—H stretching groups in polyphenols (or) protein enzymes (or) polysaccharides. Our result was agreed with Rajeshkumar *et al*. [17]. The band at 1633.71 cm^{-1} corresponds to C=C stretching groups of conjugated alkenes. The peak at 1384.89 cm^{-1} corresponding to amide II and amide III of aromatic rings either may by poly phenols. The band at 2061.90 - 2081.19cm-1. Corresponds to N=C=S stretching groups of isothiocyanate. The band at 501.49 - 545.85 cm^{-1} corresponds to C- Cl / C- Br stretching groups of halogens. The similar result was

observed in *Padina boergesenii*, the band at 1383.88 cm^{-1} and 1627.92 cm^{-1} assigned to the C-H and C=C stretch alkanes group respectively. The band at 2207.58 cm^{-1} corresponding to the S-H bend Mercaptans group. The band seen at 3448.72 cm^{-1} corresponding to the O-H stretching carboxylic acids group [16]. FTIR spectrum of the SNPS of *Sargassum wightii* showed peaks at 3411, 2925, 1584, 1425 and 1033 cm−1. The peaks at 3411 cm−1 (H–bonded hydroxyl groups), 2925 cm−1 (–OH stretching), 1584 and 1425 cm−1 (asymmetrical and symmetrical vibration of carboxylate ions) and 1033 cm−1 (C–O stretching of alcoholic groups) [19]. In present study showed some appropriateness and inappropriateness groups of molecules were observed.

Antibacterial activity of plant extracts *Chaetomorpha antennina*

The silver nanoparticles were synthesized from *C. antennina* and tested for their antibacterial activity by well diffusion method against pathogenic organism like *E. coli*, *Klebsiella pneumoniae*, *Streptococcus aureus* and *Pseudomonas aeruginosa*. The result was compared with the standard broad spectrum antibiotic Ampicillin (10 mg/ml), which was used as positive control and the distilled water served as negative control. The zone of inhibition was measured all the tested organisms and tabulated. (Table-1) The maximum zone of inhibition was observed in *E. coli* (18 mm) and *P. aeroginosa* (18mm) and minimum zone of inhibition was observed *K. pneumonia* (16) and *S. aureus* (16) at 100µl concentration. The maximum zone of inhibition was observed in *E. coli* (17mm) and minimum zone of inhibition was observed in *Streptococcus aureus* (14 mm) at 50µl concentrations. Figure 4 showed the antibacterial activities of different concentration of synthesized silver nanoparticles were lower than of positive control ampicillin except *Pseudomonas aeruginosa*. No zone of inhibition was observed in negative control (distilled water). Roy and Anantharaman [18] reported that the biosynthesized SNPs from *Chaetomorpha antennina* was used for antibacterial study against six pathogenic bacteria. The zone of inhibition showed in *E. coli*, *P. aeroginosa, K. pneumonia and P. mirabilis* were 11.6, 8.1, 4.4 and 2 mm respectively. In this study zone of inhibition showed more significant in *E. coli* (17mm), *P. aeroginosa* (15), *K. pneumonia* (15) at low concentration. Shanmugam *et al*. [20] reported that synthesized SNPS of *Sargassum whitii* showed the zone of inhibition in *E. coli* (2mm), *P. aeroginosa* (5mm) and *S. aureus* (12mm). In our present study antibacterial activity at 50 µl concentration showed more remarkable activity than SNPS of *Sargasssum whitii.* Synthesized SNPS of fresh water green alga *Pithophora oedogonia* showed the maximum zone of inhibition against *P. aeroginosa* (17.2mm) and followed by *E. coli* (16.8mm) [21]. Similar result was obtained in SNPS of *C. antennina*. The above result concluded that the algal mediated AgNPs shows a wide range of biological activity against microorganisms which can be applied in the medical field in future.

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Table 1: Antibacterial activity of silver nanoparticles from *Chaetomorpha antennina*

S.No	ORGANISM	ZONE OF INHIBITION(mm)			
		Synthesized silver nanoparticles		Ampicillin 25 µl (10 mg/ml)	Distilled water (50µl)
		50 µl	100µl		
1	*Escherichia coli*,	17	18	20	0
2	*Klebsiella pneumoniae*	15	16	20	0
3	*Streptococcus aureus*	14	16	19	0
4	*Pseudomonas aeruginosa*	15	18	18	0

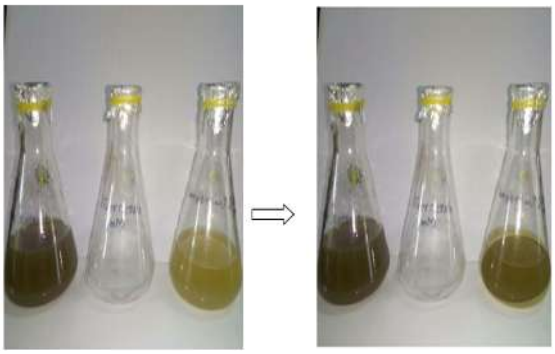

Fig. 1: 1 mM $AgNo_3$ with seaweed extract solution turned from yellow to bright yellow and turned to brown

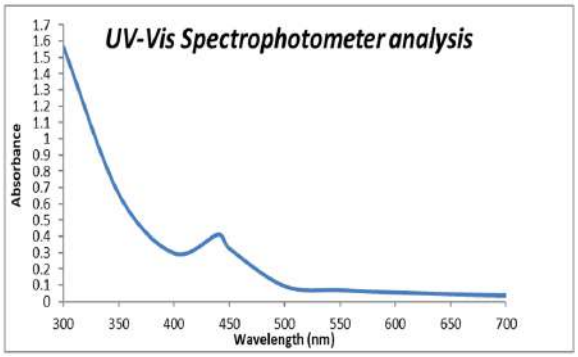

Fig. 2: UV-Vis spectra of SNPs synthesized from the extract of *Chaetomorpha antennina*

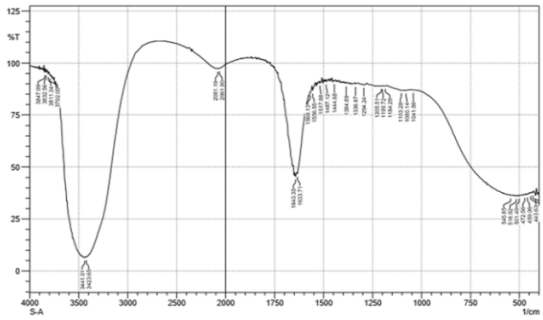

Fig. 3: FT-IR spectra of SNPs synthesized from the seaweed extract of *Chaetomorpha antennina*

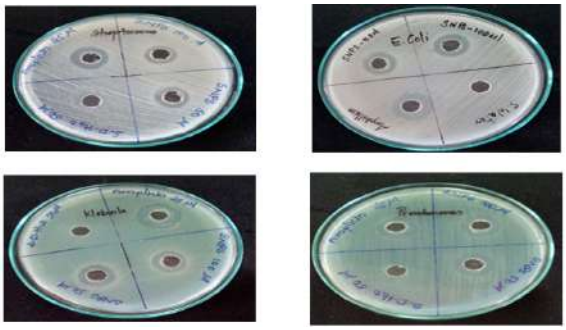

Fig. 4: Antibacterial activity of synthesized silver nanoparticles from *Chaetomorpha antennina* seaweed extract