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Biosynthesis and characterization of silver nanoparticles from *Sargassum wightii* and its antibacterial activity against multi-resistant human pathogens

S Suganya[a], B Dhanalakshmi[*a] & S Dinesh Kumar[b]

[a]PG and Research Department of Zoology, Nirmala College for women (Autonomous), Coimbatore, Tamil Nadu – 641 018, India

[b]Marine Planktonology & Aquaculture Lab., Dept. of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu – 620 024, India

*[E-mail: dr.dhanalakshmi02@gmail.com]

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In association with human health there is a growing need to develop nanomaterials using biological approach which must be ecofriendly, non-toxic, and cheaper. Therefore, the present research analyses were framed to synthesis and characterize silver nanoparticles from the extracts of seaweed *S. wightii*. The synthesized silver nanoparticles were subjected to UV-Visible Spectroscopy, Fourier Transform Infrared Spectroscopy, Scanning Electron Microscopy, High Pressure Liquid Chromatography, X-Ray Diffraction and antibacterial activity of agar well diffusion method. The formation of Ag-NPs was confirmed through the presence of an intense absorption peak at 420 nm using UV-Visible spectroscopy. The HPLC profile showed the presence of six secondary metabolites. The FT-IR spectra of biosynthesized AgNp of present biomolecules are amino acids, esters, polysaccharides, phenols, alkanes, chlorophyll, protein amide-I band, protein amide II band, carboxylic group, glycosidic linkage, carbohydrate, and proteins etc. The morphology of biosynthesized AgNps as reported to be spherical in shape was documented by SEM. The nanoparticles are crystalline in structure was confirmed by XRD. The results of antibacterial activity against the gram positive and gram-negative bacteria of human pathogens confirmed and elucidate that the seaweed synthesized nanoparticles could play a profound and reliable role in nano based medicine therapy.

[**Keywords**: Antibacterial activity, Biosynthesis, *Sargassum wightii*, Silver nanoparticles]

Introduction

Marine macro alga are considered as ecologically and biologically important component[1] in the modern ocean because they create essential habitat in near shore system, support native biodiversity, convert inorganic to organic carbon to fuel food webs and influence biogeochemistry for its breeding and feeding[2]. Some of the researchers already worked in *S. wightii* to synthesis silver nanoparticles using aqueous extract and investigated their antibacterial activity[3-6]. Nanotechnology is an intriguing form of applied science which has opened avenues for novel technological advances by the integration of Materials Science, Biological Science, Medical Science and Technology in the 21st century. Seaweeds, the large marine benthic, brackish, unicellular algae (macro algae)[7] or thalloid plants[8] can synthesize nanosized materials[9] called nanoparticles. These nanoparticles play a top role in nanomedicine fields such as health care, medicine, diagnostic, screening purposes, drug delivery systems, gene therapy applications, tissue engineering and nanorobots configuration[10]. Presently, biological nanoscience has drawn increasing attention due to its avant-garde nature and the efficiency of produced nanoparticles in industrial, biomedical and electronic applications as catalyst[11], cancer detection[12] and bioimaging[13]. The main objective of our study is to synthesis silver nanoparticles from aqueous extract of *Sargassum wightii* and to characterize the biosynthesized AgNPs investigate the in-vitro antibacterial activity of human multi-resistant pathogens.

Materials and Methods

Collection of macroalgae

Seaweed was collected during the lowest tide of chart datum from the seaweed infested locations along the South East coast of India, Mandapam (9.288º N and 79.313º E), Rameswaram, Tamil Nadu, India. The collected samples are transported to the laboratory in polythene bags under ice at 20 ºC to

avoid decomposition and loss of metabolites for identification and further work

Preparation of powder and aqueous seaweed extract

The collected seaweeds were rinsed with sterile distilled water thrice to remove the extra adhered sand and dust materials. Seaweeds were cut into small pieces, shade dried for two weeks and the samples were made into coarse powder by grinding them in a lab electric mixer grinder. The powdered seaweed material 2 g was mixed in 200 mL of distilled water in a conical flask and kept in mechanical shaker (Labotec Scientific Orbital Shaker, SA) for 48 h at room temperature. Then the extract was filtered using a Buchner funnel and Whatman No. 1 filter paper and sterile cotton wool. The supernatant was stored at 4 ^{o}C for further process.

Synthesis of AgNPs form aqueous seaweed extract

The resultant aqueous filtrate was treated individually with 90 mL aqueous solution of (1 mM) $AgNO_3$ for reduction in to Ag^+ ions. The mixed solution was subjected to vigorous stirring for 3 hrs using a magnetic stirrer. The reaction mixtures were continuously monitored for its color change from yellowish green to brown. The mixture was incubated at room temperature and the color change in the reaction solution was noted by visual observation.

Characterization of silver nanoparticles

Spectrophotometric analysis was done using Shimadzu UV-2450 spectrophotometer with a resolution of 1 nm between 200 and 600 nm possessing the scanning speed of 500 nm/min. The HPLC analysis of water extract of *Sargassim wightii* was performed on a Shimadzu LC-10AT VP HPLC system, equipped with a model LC-10AT pump, UV-visible detector SPD-10 AT. FTIR were analyzed in Shimadzu spectrophotometer set within the range of 600 and 4000 cm^{-1}. To identify the most probable biomolecules and the functional group of proteins present in sample which may be responsible for Ag+ reduction was done for the spectral analysis. The SEM has been taken for synthesized nanoparticles to know the morphology. XRD analysis was done to know the crystal structure as well as size of silver nanoparticle produced. The 2θ values ranged from 0-100.

Antibacterial assay

The antibacterial bioassay of the seaweed synthesized nanoparticle was carried out using the agar well diffusion method. For testing antibacterial activity, the following (Gram +ve: *Enterococcus*, *Staphylococcus aureus* and Gram –ve: *Pseudomonas aeruginosa*, *E. coli*) bacterial strains were selected, 6 mm holes wells were punched in nutrient agar medium (Hi Media Laboratories Pvt Ltd) using a cork borer in nutrient agar plates inoculated with the test microorganisms. The seaweed aqueous extracted silver nanoparticle of the collected test samples was tested in five dose levels of 20 µl, 40 µl, 60 µl, 80 µl, 100 µl respectively. To prevent drying all plates were covered with sterile plastic bags. The petri dishes were incubated under 37 ^{o}C for 24 hrs. Assays were run in triplicates. After incubation the inhibition zones around the wells were measured (mm) on the underside of petri dishes and expressed in nearest millimeter. Pure solvent was used as a negative control and Ciprofloxacin antibiotics disc as positive control was[14] tested against the silver nanoparticles to compare with.

Results and Discussion

Green synthesis of silver nanoparticles (AgNPs)

With 1 Mm of silver nitrate ($AgNO_3$) solution aqueous extract of *Sargassum wightii* (1 % w/w) was mixed at room temperature. Within few hours, development of AgNPs was confirmed visually by identifying color change from pale yellow to brown when adding the algal extract into the silver ion solution and incubating for 15 min. After 24 hrs incubation a deep brown color was formed which was similarly to the reported by early workers[15,16] which specifies the reduction of the Ag ions to nanoparticles.

UV- Vis spectroscopy classification

The biological i.e. structural and optical characterization of green synthesized AgNPs is directly determined by reduction of $AgNO_3$ to Ag particles using UV-Vis spectroscopy. It is observed that the absorption spectra scanned after a time interval of 24 hrs from the initiation of reaction. A strong characteristic surface Plasmon resonance peak of Ag nanoparticle at 440 nm was obviously observed upon 18 - 20 hrs of reaction, signifying the confirmation of AgNPs formation at different wavelength ranging from 200 nm to 600 nm are shown in Figure 1. The wavelength of electro-magnetic interaction with radiation it may be smaller than the Ag nanoparticle size. It is clearly indicating that the free conduction electrons for polarization in order to the string ionic Ag nanoparticles cores. Consequently, the surface plasmon absorption band

was resulted by strong electron dipolar orientation[18]. On adding seaweed extract with colorless solution of $AgNO_3$ a change into yellowish brown will be noted. Thus, seaweed mediated AgNPs of the present study was noted to be highly stable in the solution. The change of silver ions into AgNPs was found to be 90 - 95 % within 8 - 10 nm at 37 ^{o}C. A characteristic smooth and narrow absorption band of almost spherical nanoparticles at 440 nm is observed.

HPLC analysis of biosynthesized AgNPs

For the separation and identification of constituents present in the *Sargassum wightii* the aqueous extract prepared by hot extraction was subjected to HPLC. Following which six compounds were separated at different retention time of 1.108 min, 1.233 min, 1.475 min, 1.617 min, 1.875 min, and 2.910 min, respectively. The retention time of 2.910 min, 1.875 min, 1.617 min and 1.475 min shows bulging peak followed by reasonable peaks at the retention time of 1.23 min and 1.108 min. At a retention time of 1.617 min one prominent peak was observed which was represented in Figure 2. This present research results offers a platform of using methanol extracts of *Sargassum wightii* as an alternative for various diseases.

FT-IR analysis of biosynthesized AgNPs

The aqueous extract and dried AgNPs showed 400 - 4000 cm^{-1} range in FT-IR analyses and also huge number of peaks which explaineda multifarious nature of *S. wightii* (Fig. 3). The band at 3903.92 cm^{-1} was ascribed to the free O-H and N-H extending ambiances of the amino acids. The O-H functional group beaks at 3753.48 cm^{-1} and 3657.04 cm^{-1}

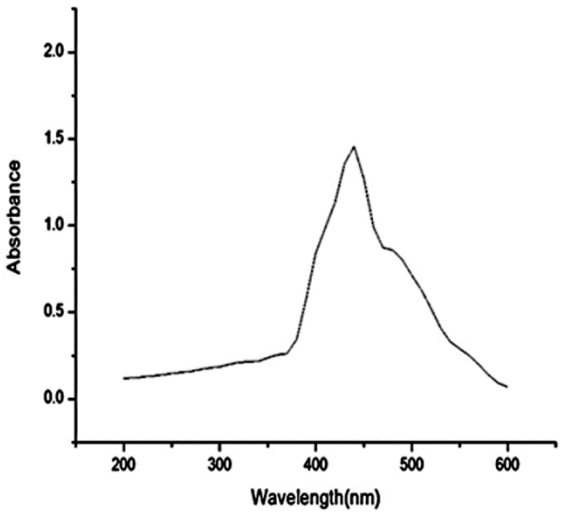

Fig. 1 — UV-Vis Spectroscopy of *Sargassum wightii* synthesized silver nanoparticles

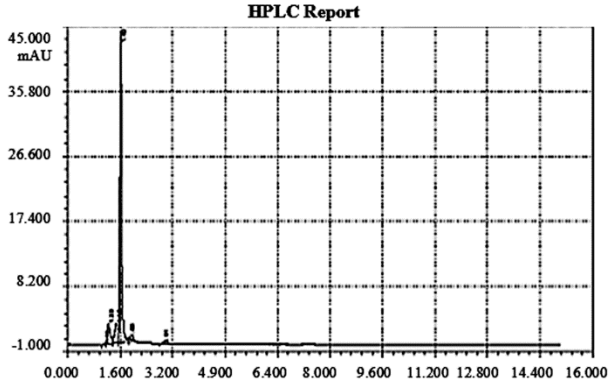

Fig. 2 — HPLC profile of methalonic extract of *Sargassum wightii*

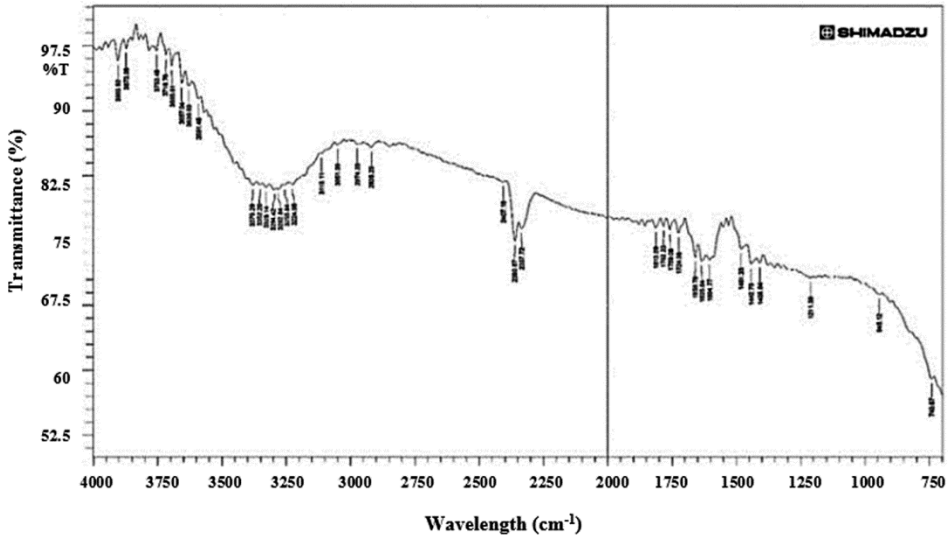

Fig. 3 — FT-IR spectroscopy of biosynthesized AgNPs of *Sargassum wightii*

describe the silica stretching of alcohol[17]. The peak at 1724.36 cm^{-1} and 1759.08 cm^{-1} explains occurrence of C=O and they authorizes esters vibrations. The reduction of O-H groups in polysaccharides may leads to silver reduction to NPs synthesis which confirmed by the band intensity decreasing from 3903.92 cm^{-1} to 3591.46 cm^{-1}. The band intensity was decreased while peak were shifting in large manner while wave numbers such as 3352.28 cm^{-1}, 3329.14 cm^{-1}, 3294.42 cm^{-1}, 3282.84 cm^{-1}, 3255.84 cm^{-1} and 3224.98 cm^{-1} observed and they represent O-H pf phenols and C-H of alkanes. A FT-IR report from Shanmugam *et al.*[18] showed some supplementary small peaks supporting our findings and their Ag-NPs were also quite related to the present study.

The presence of chlorophyll groups were confirmed with short peaks observed in 2974.23 cm^{-1} and 2920.23 cm^{-1} and it corresponds to C-H stretching vibrations[19]. The absorption peak in broad size at 1658.78 cm^{-1} was dispersed for C=O extending vibration of protein amide-I band[20]. The peak 1481.33 cm^{-1} from explains the N-H bending vibration of amines or amide groups and C-N stretching protein amide II band[21]. The presence (C-O) of COO groups, carboxylic group, protein and bending of methyl carboxylic acid were confirmed by peaks observed in 1408.04 cm^{-1}(ref. 22). The weak absorption band observed at 1211.30 cm^{-1} is due to C-O stretching vibration. The glycosidic linkages revealed peaks at 945.12 cm^{-1}. The weak absorption band centered at 740.67 cm^{-1} can be ascribed to the C-H bending vibration, which also confirmed the presence of carbohydrate.

Assessment of biosynthesized AgNPs in Scanning Electron Microscopy

The SEM image of *Sargassum wightii* synthesized AgNPs is shown in Figure 4. The morphology of the brown algae biosynthesized silver nanoparticles was described to be spherical in shape. The synthesized AgNPs size ranges differ from 80 to 100 nm. The predominance of spherical shape nanoparticles on the surface of the cell was observed at magnification of 1 µm x 37.08 KX. Under careful observation, it is noted that the silver nanoparticles are surrounded by a faint thin layer of other materials, which is the capping organic material from the algal extract.

X-Ray Powder Diffraction analysis of biosynthesized AgNPs

Figure 5 shows the X-Ray Powder Diffraction pattern using marine macroalgae *Sargassum wightii* after 120 hr of incubation of AgNPs reduced from silver nitrate. As a result of the present analysis four intense diffraction strong peaks due to AgNPs 30.15°, 50.02°, 60. 26° and 64.07° indexed at (110), (200), (220) and (311) lattice planes which confirmed the face centered cubic structure (FCC) were clearly observed of the formed AgNPs. All the above four peaks 110, 200, 220 and 311 of Bragg reflection formed in XRD (X- Ray Powder Diffraction pattern) pattern synthesized by AgNPs was observed and compared with standard powder diffraction card of JCPDS Card No 04-0783(refs. 26,27). In addition, few intense additional, unassigned peaks too were observed in the vicinity of the characteristic peaks which may be due to fewer biomolecules of stabilizing agents of enzymes or proteins in the seaweed extract. In general, the high intensity of face centered cubic material noted in the sample is (110) reflection, which confirmed the lattice structure. The crystalline size was calculated using Debye-Scherrer equation from full width half maximum of diffraction peaks. The size of the particle derived from X-Ray Powder Diffraction spectra was around 10 nm.

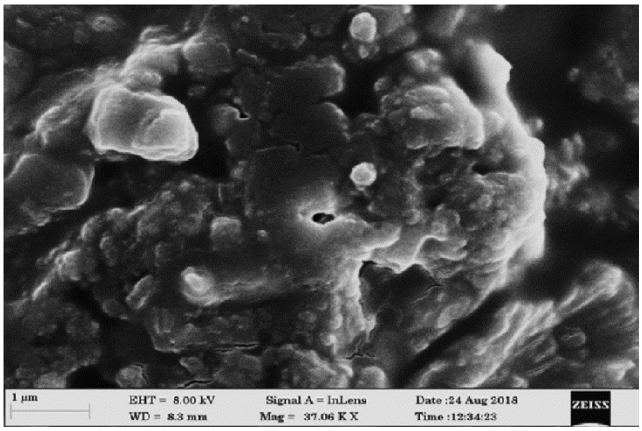

Fig. 4 — SEM microscopy of silver nanoparticles synthesized from the aqueous extract of *Sargassum wightii*

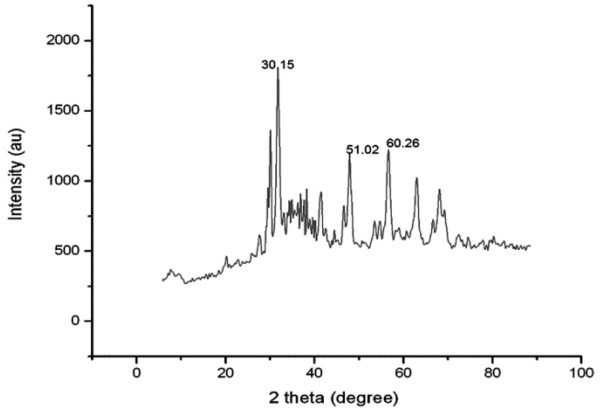

Fig. 5 — XRD pattern of crystalline structure of *Sargassum wightii* aqueous extract synthesized silver nanoparticles

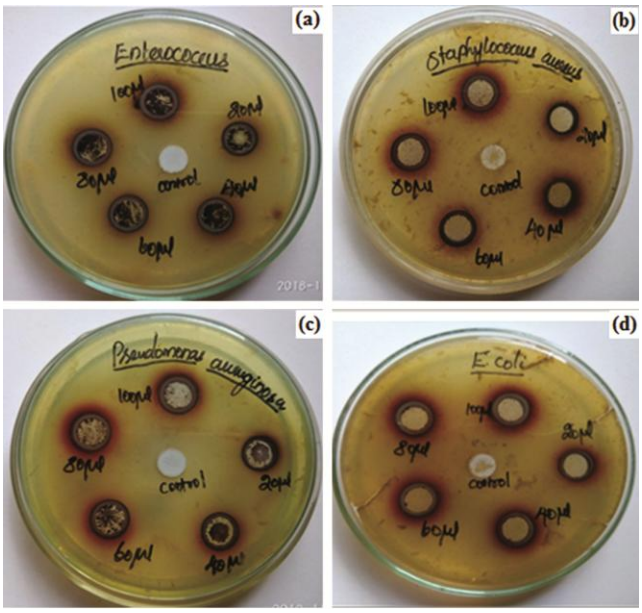

Fig. 6 — Antibacterial activity of *Sargassum wightii* synthesized silver nanoparticles against gram-positive

Antibacterial activity effect of AgNPs by well diffusion method

Gram positive bacteria

The output of this study proved that the aqueous extract of synthesized silver nanoparticles showed maximum zone of inhibition 5 mm at 100 µl concentration for *Enterococcus* sp. and *Staphylococcus* sp. and minimum 2 mm for *Enterococcus* and 1 mm for *Staphylococcus* at 20 and 40 µl concentration of *Sargassum wightii* aqueous extract synthesized silver nanoparticles in different selected concentrations (20 µl, 40 µl, 60 µl, 80 µl and 100 µl) (Fig. 6).

Gram negative bacteria

The results of the antibacterial activity against tested multi-resistant human pathogen was tabulated and presented in form of Figure 7. Of the various concentrations used, maximum zone of inhibition against *E.coli* at 100 µl (6 mm) was noticed and minimum zone of inhibition at 60 µl (3 mm) was noticed. The control antibiotic Ciprofloxacin inhibited the tested pathogen by showing clear zone of inhibition diameter of (3 mm). Padmakumar and Ayyakkannu[25] stated that organically extracted Indian seaweed exhibits antimicrobial activity against gram negative and gram-positive biomedical pathogens. Similarly maximum zone of inhibition against *Pseudomonas aeruginosa* (5 mm) was recorded at 80 and100 µl and minimum zone of inhibition (3 mm) was observed at 20, 40 and 60 µl.

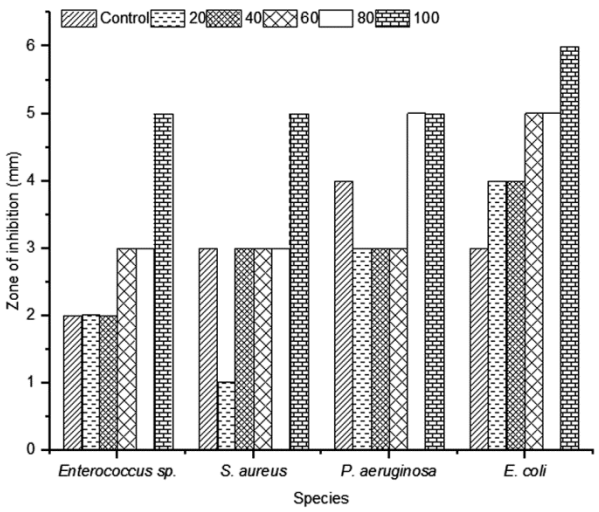

Fig. 7 — Showing the antibacterial activity of *Sargassum wightii* synthesized silver nanoparticles

Conclusion

Applying chemical methods to overcome many of the obstacles substituted with biological approach in synthesis of NPs have shown inadequate success in therapeutic field. Therefore, from this study we came to know synthesizing silver nanoparticles using brown macroalgae has paved way to environmentally approvable synthesis and safe process when compared to additional methods. Moreover, synthesis of AgNPs using marine green resources like macroalgae was considered a remedial substitute to chemical synthesis. In addition, from the results marine brown seaweeds *S. wightii* is efficient in producing silver nanoparticles extracellular and these nanoparticles are quite long term usable in solutions due to present of biomolecules and secondary metabolites. With these novel biogenic and ecofriendly method as the synthesized nanoparticles are quite stable for long duration with no lucid change it proves to play an ideal role in the field of medicine and pharmaceuticals. As the selected seaweed synthesized nanoparticles showed best antibacterial activity against multi-resistant human pathogens like gram positive bacteria *Enterococcus*, *Staphylococcus aureus* and gram-negative bacteria *E. coli*, *Pseudomonas aeruginosa* along with the standard commercially prescribed antibiotics it could be further analyzed against other contagious and most dreadful diseases causing pathogens in future as a possible alternative.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

SS: Conceptualization, methodology, sample analyses, and writing - original draft; BD: Conceptualization, methodology, resources, writing – original draft, review & editing; and SDK: Writing - review & editing the draft, statistical analyses, plotting graphs.

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