Biosynthesis of Silver Nanoparticle From Seaweed Extract And Its Antibacterial Activity Against Human Pathogens

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Abstract- *In the present study, we report the four different seaweed (Spatoglossum asperum, Enteromorpha compressa, Caulerpa scalpelliformis and Padina gymnospora) extract to produce silver nanoparticles (AgNPs) by reduction of silver nitrate. It was noted that synthesis process was considerably rapid and silver nanoparticles were generated within few minutes of silver ions coming in contact with the seaweed extract. A peak at 419, 418, 390 and 392 nm corresponding to the plasmon absorbance of AgNPs which was noted in the UV–vis spectrum of the seaweed extract that contained silver ions. Fourier transform infrared spectroscopic analysis of the nanoparticles indicated the presence of protein which was regarding a capping agent surrounding the AgNPs. Among the four seaweed the antibacterial activity of synthesized nanoparticles from Caulerpa scalpelliformis shows maximum inhibitory activity against four tested pathogenic bacteria.*

Keywords- Silver nanoparticle, Antimicrobial activity, Seaweed, Padina gymnospora

I. INTRODUCTION

Nanoparticles are bunch of atoms in the size range of 1–100 nm (Williams, 2008).a Number of methods like physical, chemical and biological have been established and accepted to synthesis nanoparticles of different size, stability and functions. The earliermethods have augmented the concern of environmental conditions by generating hazardous by-products and economically expensive (Ramkumar et al., 2016). Biological methods towardsmetallic nanoparticle synthesis using microorganisms, enzymes, plants or plant extracts have been recommended as possible eco-friendly alternate to chemical and physical methods (Armendariz et al., 2004). In particular, plants were found to be faster than microorganisms in the rate of metal ions reduction and formation of stable NPs (Iravani, 2011). Only limited reports are available for synthesis of nanoparticles from marine plants (Govindaraju*et al.,* 2009; Nabikhan*et al.,* 2010; Venkatpurwar and Pokharkar, 2011).

The metal nanoparticles are most capable due to their extraordinary electromagnetic, optical, catalytic, magnetic and antibacterial properties (Jeong et al., 2005). Among the metalssilver has been respected as the effective antimicrobial agent, non-toxic to humans and other organisms(Ramkumar et al., 2016).Silver nanoparticles are recognised for their antimicrobial properties, being active against pathogens,which elucidate their potential for several biotechnological applications, in addition to their electrical, thermal, magnetic, and catalytic characteristics (Chen et al., 2008; Konwarh et al., 2011; Mohantyet al., 2012; MubarakAli et al., 2011; Thakkar et al., 2010). Silver nanoparticles have been successfully synthesized using several plant extracts (Shankar *et al.,* 2003; Amkamwar*et al.,* 2005; Chandran*et al.,* 2006; Venkatpurwar and Pokharkar 2011). Algae are used as a “bio-factories” for synthesis of metal nanoparticle (Kannan et al., 2013).

Among the genera seaweed has individual advantage due to their high metal uptake and low cost (Davis et al., 2003). Seaweeds constitute commercially important marine renewable resources which provide valuable idea for the development of new drugs against cancer, microbial infections and inflammations (Rebecca et al., 2012). Silver nanoparticles attracted great attention because of their wide range of applications includingantibacterials (Venkatpurwar and Pokharkar 2011; Wang et al., 2008) and therapeutics (Elechiguerra et al., 2005), catalysis (Crooks et al., 2001), biosensors (Chen et al., 2007), and in plant growth metabolism (Krishnaraj et al., 2012). Based on the antibacterial properties of SNPs, various nano silver products and surgical instruments have been developed (Lohse and Murphy 2012; You et al., 2012).This study envisioned to put on a biological technique for the synthesis of Silver nanoparticles and observe its antibacterial properties against various pathogenic bacteria. In respect to that the seaweeds was used for bioconversion of silver ions to nanoparticles.

II. MATERIALS AND METHODS

Collection of seaweed sample

Four seaweeds were collected from area of Tuticorin and Mandapam using sterile plastic bags and were immediately transfer to laboratory.

Identification of species

The collected four samples were identified as *Spatoglossum asperum, Enteromorpha compressa, Caulerpa scalpelliformis* and *Padina gymnospora* and were shown in (Fig1).

Preparation of seaweed extract

The seaweed samples were washed thoroughly without any attached debris and sand. The seaweeds were shade dried completely and grind fine using mortar and pestle. 5 gram of seaweed powder was mixed with 100 ml of distilled water and was boiled for 30 minutes at 60 ^{o}C. Then the boiled sample was filtered through Millipore filter unit and the extract was stored in the sterile container for further process.

Preparation of silver nitrate

1mM silver nitrate solution was prepared using distilled water and was stored in dark container.

Synthesis of silver nanoparticle

Ag+ ions were reducedby adding10 ml of seaweed extract to 90 ml of distilled water and was kept in shade light for 30 to 45 minutes with continuous shaking after 45 minutes the mixture was incubated for 24 hours in dark condition. The change in colour which indicates the formation of silver nanoparticles by bioreduction of ions was monitored using UV visible spectrophotometer.

Characterization of synthesized silver nanoparticle
UV-Visible spectrophotometer

The formation of nanoparticle was detected Using shimadzhu 1800 at the resolution of 1nm from 300 to 700 nm and monitoring the resultant peak between 380 to 420 nm.

FT-IR analysis

The characterization of functional groups in the AgNPs by seaweed extracts were investigated by FTIR analysis (Shimadzu) and the spectra was scanned in the range of 4000– 400 cm−1 range at a resolution of 4 cm−1. The samples were prepared by dispersing the AgNPs uniformly in a matrix of dry KBr compressed to form an almost transparent disc. KBr was used as a standard analyse the samples**.**

Antibacterial activity

The clinical pathogenic strains such as *Bacillus flexius, Flarobacterium*sp.,*Enterobacteraerogens and Streptococcus* sp. were obtained from Raja Muthaiya Medical Collage and Hospital, Annamalai University, Tamilnadu. Well diffusion method was performed to evaluate the antibacterial activity of synthesized silver nanoparticle against pathogens. Pathogens were inoculated in nutrient broth and incubated for 24 hours and was spread onto Muller Hinton agar plates. Four wells were made on the agar plates using gel puncture. 100µl of Gentamycin (positive control), distilled water (negative control), Silver nitrate (positive control) and synthesized nanoparticles were added in the four wells respectively. The plates were incubated at 37 ^{o}C for 24 hours. The activity were detected by the formation of zone of inhibition around each well and was measured in mm.

III. RESULTS

Synthesis of silver nanoparticles

The reduction of silver ion was visually identified by changes in colourless solution to reddish brown in colour.

Characterization of synthesized nanoparticle
UV-Vis spectrophotometer

The reddish-brown colour formed due to excitation of surface plasmon vibrations with an absorbance maxima at 419 nm was observed in *Spatoglossum asperum* (Fig 2), 418 nm was observed for the extract of *Enteromorpha compressa* (Fig 2), *Caulerpa scalpelliformis* extract shown 390 nm (Fig 3) and 392 nm was observed in *Padina gymnospora* (Fig 3) extract respectively when reacted with silver nitrate.

FT-IR spectrophotometer

In FTIR *Spatoglossum asperum* shows peak at 1035 cm^{-1} and 1089cm^{-1}of alcohol group C=C C-Ostretch.1382cm^{-1}C-H bend which respond to alkanes and alkyls group. The peak shows at Amides group 1517cm^{-1} which is N-H bend. 2260 cm^{-1}R-C≡CR' and C≡C stretch alkynes (Fig 4). *Enteromorpha compressa* shows peak at 1093 cm^{-1}and 1128 cm^{-1}responsible C-O stretch in alcohol group. 1382 cm^{-1}–CH (CH3)2 (two bands)-(CH3)3 bend of alkanes and alkyls group. 474 cm^{-1}peak respond to alkyl halides of C-I stretch 530 cm^{-1}C-Br stretch of alkyl halides and 2106 cm^{-1} peak C≡C stretch of alkynes group (Fig 4). *Caulerpa scalpelliformis* shows peak at 468 cm^{-1} made strong bond of C-I stretch in Alkyl halides, C-Br stretch at the peak 532 cm^{-1}, 607 cm^{-1}, 665 cm^{-1} which is responsible to Alkyl halides, the peak at 831 cm^{-1} made strong

bondbetween C-Cl stretch of Alkyl halides, the peak at 1631 cm^{-1} responsible to C=O stretch of Amides, 2854 cm^{-1} peak is C-H stretch of alkanes and alkyls and 2767 cm^{-1} peak belong to the group of Carboxylic Acids with broad O-H stretch (Fig 5). *Padina gymnospora* shows peak at 2922 cm^{-1} and 2854 cm^{-1} which is responsible to C-H stretch of alkanes and alkyls. Peak at 3404 cm^{-1} responsible to Amines of weak N-H symmetric & asymmetric Stretch, the peak at 1797 cm^{-1} of Esters group responsible to C=O stretch, alkanes and alkyls group at the peak of 1382 cm^{-1} which is C-H bend (Fig 5).

Antibacterial activity

An activity of synthesized silver nanoparticles using extract of seaweed against human pathogen. Nanoparticle synthesized from *Caulerpa scalpelliformis* showed more actiity in bacillus flexus when compard to other seaweed extract for flarobacterium and *Streptococcus* sp also *caulerpa scalpelliformis* showed more activity. *Padina gymnospora* shows more activity in *Enterobacter aerogens*. On the Whole *caulerpa scalpelliformis* shows more activity against all pathogenic bacteria when compared to other seaweed extracts which was shown in (Fig 6)

IV. DISCUSSION

The formation of AgNPs by reduction of aqueous silver nitrate during exposure to the aqueous extract of seaweeds showed change in colour from pale yellow to reddish-brown, suggested the formation of AgNPs in solution which agreement with the statement reporting that AgNPs display reddish-brown in water [Boulch et al., 1998]. Formation of peak at 419 for *Spatoglossumasperum*, 418 for *Enteromorpha compressa*, 390 for *Caulerpa scalpelliformis* and 392 nm for *Padina gymnospora* extract when reacted with silver nitrate which was suppoted by Senthil Kumar and Sudha 2013 in which the extracr of *Dictyota Bartayresiana* Surface plasmon band in the AgNPs solution remains close to 419 nm throughout the reaction period, suggesting that the AgNPs were dispersed in the aqueous solution with no evidence for aggregation of them in UV-Vis absorption spectrum. In our study 392 nm for *Padina gymnospora* extract when reacted with silver nitrate which was supported by Harekrishna et al., 2009 in which *Padina tetrastromatica* surface plasmon absorption bands are noticed at 425 nm and rising of nanoparticles size can also affect the SPR band broadening.

In FTIR *Spatoglossum asperum* shows peak at 1035 cm^{-1} and 1089$cm^{-1}$of alcohol group C=C C-Ostretch.1382cm^{-1}C-H bend which respond to alkanes and alkyls group. The peak shows at Amides group 1517cm^{-1} which is N-H bend. 2260 cm^{-1}R-C≡CR' and C≡C stretch alkynes.Which was supported by Mukherjee et al. 2008 in which the peak located at around 2359 cm−1 was accredited to the N–H stretching vibrations or the C═O stretching vibrations like that the peak at 3280 cm−1 was possibly related with stretching in alcohol and phenolic constituents (Sharbidre and Kasote 2013). *Enteromorpha compressa* shows peak at 1093 $cm^{-1}$and 1128 cm^{-1}responsible C-O stretch in alcohol group. 1382 cm^{-1}–CH (CH3)2 (two bands)-(CH3)3 bend of alkanes and alkyls group. 474 cm^{-1}peak respond to alkyl halides of C-I stretch 530 cm^{-1}C-Br stretch of alkyl halides and 2106 cm^{-1} peak C≡C stretch of alkynes group.Peaks at 1027–1092 cm−1 link to the C–N stretching vibration of aliphatic amines or to alcohols or phenols, representing the presence of polyphenols (Song et al. 2009).

Caulerpas calpelliformis shows peak at 468 cm^{-1} made strong bond of C-I stretch in Alkyl halides, C-Br stretch at the peak 532 cm^{-1}, 607 cm^{-1}, 665 cm^{-1} which is responsible to Alkyl halides, the peak at 831 cm^{-1} made strong bond between C-Cl stretch of Alkyl halides, the peak at 1631 cm^{-1} responsible to C=O stretch of Amides, 2854 cm^{-1} peak is C-H stretch of alkanes and alkyls and 2767 cm^{-1} peak belong to the group of Carboxylic Acids with broad O-H stretch. Salari et al., 2016 noted a Strong broad O–H stretch carboxylic bands in the region 3423 cm^{-1} and carboxylic/phenolic stretching bands in the region 2927 cm^{-1}. The peaks locating in the region 1645 cm^{-1} are attributed to the stretching vibration of the [NH] C,O group that is characteristic of proteins shifted from 1645 cm^{-1} after the synthesis of Ag-NPs. *Padina gymnospora* shows peak at 2922 cm^{-1} and 2854 cm^{-1} which is responsible to C-H stretch of alkanes and alkyls. Peak at 3404 cm^{-1} responsible to Amines of weak N-H symmetric & asymmetric Stretch, the peak at 1797 cm^{-1} of Esters group responsible to C=O stretch, alkanes and alkyls group at the peak of 1382 cm^{-1} which is C-H bend.

The distinct band at 1654 cm−1 represents the involvement of C=N in plane vibrations of amino acids and 1023– 1227 cm−1 represents the involvement of C-N in plane vibrations of aliphatic amines. The band at 1023 cm−1 can be assigned to absorption peaks of \C\O\C. (Huang et al., 2007; Philip, 2009a, b).A strong IR band at 1509 cm−1 in the spectrumof silver nanoparticle is due to the stretching vibrations of the C-C chain (Schulz and Baranska, 2007).

Caulerpa scalpelliformis shows maximum activity in all human pathogens and the zone of inhibition was around 20 mm which was supported by (Mohandass et al., 2013) in which nanoparticle synthesized from *S. cinereum* showed good activity against the pathogenic bacteria *Enterobactor aerogens* and in general *S. cinereum* extract exhibited the zone of inhibitions from 10 to 29 mm. In Valentin bhimba and Raja

kumara, 2014 report AgNP's of *U. lactuca* were highly toxic to *Escherichia coli*, *Bacillus sp*. and *Staphylococcus aureus* with the inhibition zone of 22, 25 and 25 mm; low toxic against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* with the inhibition zone of 17 mm.

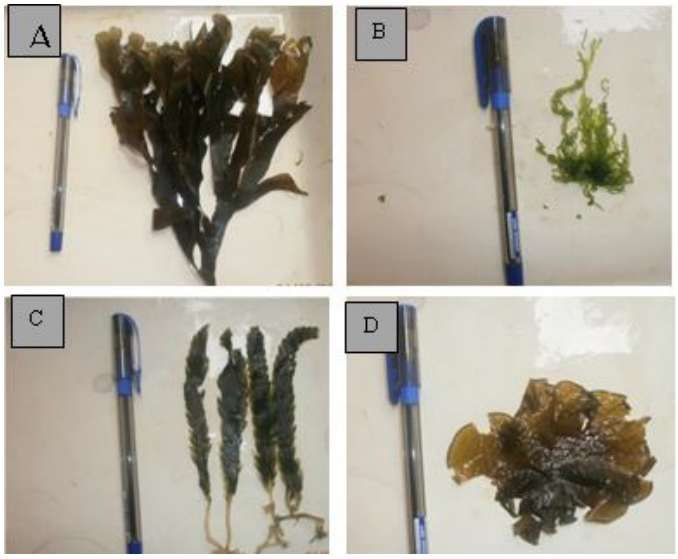

Figure 1: Study material seaweeds (A) *Spatoglossum asperum*(B) *Enteromorpha compressa*,(C) *Caulerpa scalpelliformis* and (D) *Padina gymnospora.*

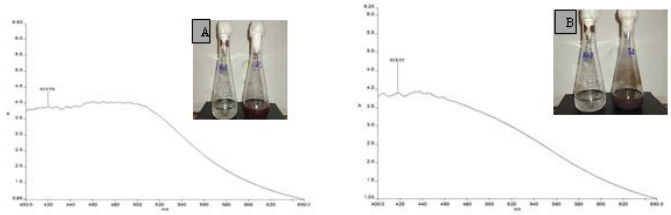

Figure 2: UV-Vis spectrophotometer of *Spatoglossum asperum* and *Enteromorpha compressa* extract reacted with silver nitrate

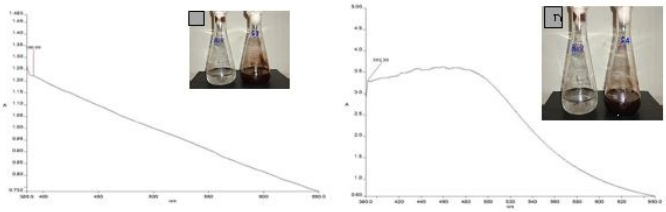

Figure 3: UV-Vis spectrophotometer of *Caulerpa scalpelliformis* and *Padina gymnospora* extract reacted with silver nitrate

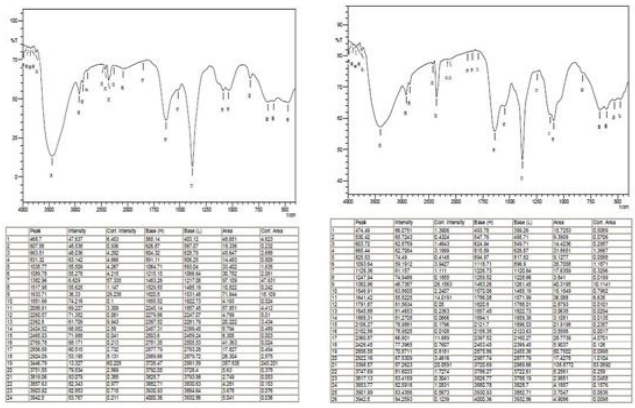

Figure 4: FTIR analysis of synthesized silver nanoparticle from seaweed extract of *Spatoglossum asperum* and *Enteromorpha compressa*

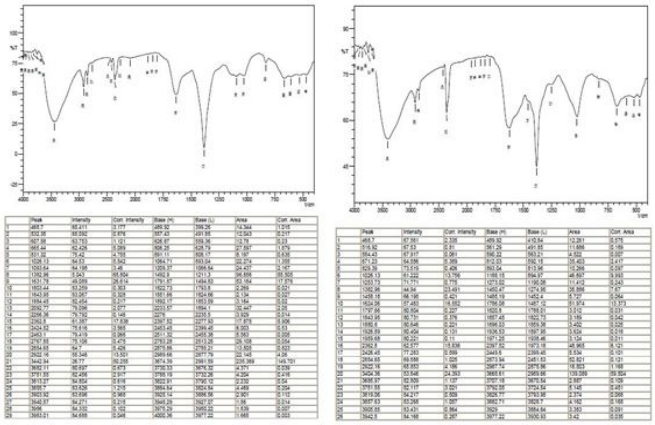

Figure 5: FTIR analysis of synthesized nanoparticle from seaweed extract of *Caulerpa scalpelliformis* and *Padina gymnospora*

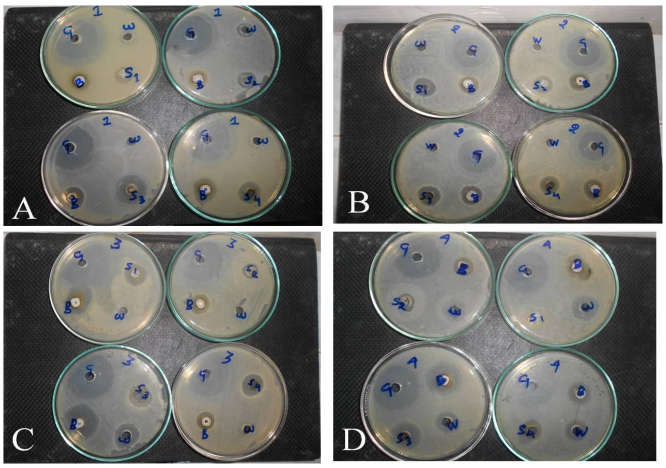

Figure 6: Antimicrobial activity of silver Nanoparticle against human pathogen (A) *Bacillus flexus*, (B) *Flarobacterium* sp, (C) *Enterobacter aerogens* and (D) *Streptococcus* sp. G-positive control, W-negative control, B- Silver nitrate and S1-*Spatoglossum asperum*, S2- *Enteromorpha compressa*, S3-*Caulerpa scalpelliformis* and S4-*Padina gymnospora* nanoparticle synthesized from the extract.

V. CONCLUSION

Due to their wealth and easy accessibility, seaweeds are good and gainful sources for the synthesis of metallic nanoparticles. When compared to terrestrial plants and microbes it has been less studied in seaweeds. In this study four seaweed samples were collected and its extracts were used for synthesis of silver nanoparticle.Among the four seaweed samples *Caulerpa scalpelliformis* shows maximum activity for all four pathogens and the zone of inhibition was maximum in all studied pathogens. Further in vivo studies are essential to be carried out to authorise the benefits of this AgNPs.

VI. ACKNOWLEDGEMENT

We are grateful thank to our Research Guide and also thanks to dean and director centre of advance study in Marine Biology. Annamalai University, Parangipettai, Tamilnadu, India.

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