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SYNTHESIS AND CHARACTERIZATION OF CYTOTOXIC SILVER NANOPARTICLES USING MARINE BROWN SEAWEED *SARGASSUM JOHNSTONII* SETCHELL & N.L.GARDNER

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ABSTRACTS

The present study was aimed to optimize the protocol for the synthesis of silver nanoparticles using aqueous extracts of *Sargassum johnstonii* Setchell & N.L.Gardner and evaluate cytotoxic potentials using brine shrimp bio-assay activity and Trypan blue dye exclusion method. The reduction of pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the solution at 200-900 nm using Shimadzu spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups of *S. johnstonii* SNPs. To know the cytotoxic potentials, the brine shrimp bio-assay was performed. To reveal the anticancer potentials of *Sargassum johnstonii* SNPs, Trypan blue dye exclusion method was employed against DLA cell lines. On mixing the *S. johnstonii* aqueous extract with 1 mM $AgNO_3$ solution, the colour of the solution changes from pale yellowish brown to dark brown colour indicates the presence of silver nanoparticles. The AgNP's synthesised from aqueous extracts of *S. johnstonii* showed the optical peak at 430 nm with the absorption of 0.771. The results of FTIR analysis of *S. johnstonii* confirmed the presence of alkyl halides, aliphatic amines, alkynes, alcohols, amines and phenols with the peak values from 559.25 to 3401.82. The cytotoxic potential of *S. johnstonii* silver nanoparticles showed LC_{50} value at 656.89 µg/ml followed by aqueous extracts of *S. johnstonii* showed LC_{50} value at 805.45 µg/ml. The AgNPs of *S. johnstonii* showed their best growth inhibition activity with CTC_{50} value 293.66 µl/ml.

KEYWORDS: Silver nanoparticles; Seaweeds; *Sargassum.*

INTRODUCTION

Nanoparticles are essential to construct the nanomaterials and play a vital role in the nanotechnology. Among the various nanoparticles the green nanoparticles are occupied an important place in the nanotechnology due to their potential application as anti-bacterial, anti-fungal, anticancer agents.[1] Cao et al.[2] and Hayward et al.[3] pointed out the application of nanoparticles in biomedical, electronic, catalysis and optical applications. Among the various biomedical applications, antimicrobial properties of silver nanoparticles showed the way for development of variety of silver nanoparticle products viz., nanosilver-coated bandages, surgical instruments, contraceptive devices and dental implants.[4] The seaweed *Sargassum* is one of the economically important and ecologically dominant brown algae and widely distributed in the tropics. *Sargassum* is one of the most diverse genus among Phaeophyta in India and harbours nearly 38 species.[5] At the global level, a number of reports are availble for the synthesis of AgNP's from *Sargassum* viz., *S. plagiophyllum,* [6] *Sargassum ilicifolium*,[7] *S. cinerecum*,[8] S. *muticum*,[9] *S. longifolium*,[10,11] *S. wightii*,[12-14] *S. plagiophyllum*[12] and *Sargassum duplicatum*.[15] But there is no report on the silver nanoparticle synthesis using aqueous extracts of *Sargassum johnstonii*. Nanoparticles encapuslated with other agents may act as cell growth inhibitor and have potential to control the cancer cell growth.[16-18] *Sargassum* possess cytotoxic compound such as fucoxanthin, fucoidans, laminarians and terpeniods, which have anticancer, antitumour and antiproliferative properties.[19] More over the extracts of *Sargassum* species showed cytotoxic activity against various cancer cell line.[20-21] Still huge opportunities are available to explore new anticancer agents from *Sargassum* species. With this knowledge the present study was aimed to optimize the protocol for the synthesis of silver nanoparticles using aqueous extracts of *Sargassum johnstonii* Setchell & N.L.Gardner and evaluate cytotoxic potetnials using brine shrimp bio-assay activity and Trypan blue dye exclusion method.

MATERIAL AND METHODS

Collection of Plant Materials

The mature and healthy thallus of *Sargassum johnstonii* Setchell & N.L.Gardner were collected from Manapad, Tirunelveli district, Tamil Nadu. The collected seaweed materials were washed with tap water followed by distilled water to remove the unwanted debris.

Synthesis and characterization of silver nano particles

The aqueous extracts were prepared directly by boiling the 10 g of *Sargassum johnstonii* thallus with distilled water for 3 h and filtered using Whatman No.1 filter paper. The aqueous extract of *S. johnstonii* was used for the synthesis of silver nanoparticles. $AgNO_3$ was dissolved in 100 ml distilled water (10^{-3} M). The extracts were added to $AgNO_3$ solution in 1: 10 ratio for reduction of Ag^+ ions. After reduction incubated solution was centrifuged at 10,000 rpm for 15 min.

UV-Vis analysis

The aqueous extracts of *S. johnstonii* was centrifuged at 3000 rpm for 10 min and then filtered through Whatman No. 1 filter paper using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The crude extract containing the bioactive compound was analyzed spectroscopically for further confirmation. The extracts were scanned in the wavelength ranged from 200-1100 nm using Shimadzu Spectrophotometer and the characteristic peaks were detected. Each and every analysis was repeated twice and confirmed the spectrum. The supernatant containing silver nanoparticles of *S. johnstonii* was analyzed spectroscopically for further confirmation. The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the solution at 200-900 nm using Shimadzu spectrophotometer and the characteristic peaks were detected.

FTIR analysis

FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The AgNP's of *S. johnstonii* was passed into the FTIR and the peak values were recorded. Each and every analysis was repeated twice and confirmed the spectrum.

Cytotoxic activity

25 mg of dried aqueous extract was taken in 10 ml beaker and 500 µl DMSO was added it. Finally the volume (5 ml) was adjusted by distilled water. The concentration of this solution was 5 µg / µl. Artificial sea water (38 g NaCl / 1000 ml tap water) was taken in small tank and shrimp eggs were added to one side of the divided tank and the side was covered. The shrimps were allowed for 48 hrs to hatch and mature as nauplii. During this period constant oxygen supply, temperature (around 37˚C) and light supply was maintained. The hatched shrimps were taken for bioassay.

30 clean test tubes were taken and separated by 10 ml in each test tube. 25 were used for the samples in five different concentrations (five test tubes for each concentration) and 5 tubes for control. With the help of a Pasteur pipette, 10 living shrimps were dropped into each test tube.[22] The aqueous and silver nanoparticles of *S. johnstonii* was taken in different concentrations for SNP (50, 100, 150, 200, 250 µl / 50 ml and for aqueous 50, 100, 150, 200, 250 µg / 50 ml to the sample tubes.

Control group was added in cytotoxic activity to validate the test method and result obtained due to the cytotoxic activity of the test agents. 50 µl of DMSO was added to the control tubes containing 5 ml of mother solution and 10 shrimp nauplii to use as control groups. No extract were added to prepare control solution. After 24 hours, the tubes were inspected using a magnifying glass and the number of survived nauplii in each tube was counted and the LC_{50}, 95% confidence limit, LCL and UCL were calculated.

Anticancer activity of aqueous and SNPs of *S. johnstonii* to against Dalton's lymphoma ascites cells

Dalton's lymphoma ascites (DLA) cells were used for short term *in vitro* cytotoxicity experiments. For the cytotoxicity analysis, aqueous and SNPs of *Sargassum johnstonii* were taken in different concentrations viz., 10, 20, 50, 100, 200 µl / ml to the sample tubes. Cells were aspirated from the peritoneal cavity of tumor bearing mice and it was washed three times using PBS. The viability of cells were checked using trypan blue (cell viability should be above 98%). The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures was incubated for 3 h at 37°C and then 1 ml of trypan blue was added after incubation and the number of dead cell was counted using a haemocytometer. [23] The percentage cytotoxicity was calculated using the formula as follows.

% of cytotoxicity = Number of dead cells / Total number of cells (Dead and live cells) * 100

RESULTS

Synthesis of silver nanoparticles

On mixing the *S. johnstonii* aqueous extract with 1 mM $AgNO_3$ solution, the colour of the solution changes from pale yellowish brown to dark brown colour indicates the presence of silver nanoparticles (Fig. 1 A and B).

UV-Vis analysis

The reduction of silver nanoparticles in aqueous extracts of *S. johnstonii* was confirmed by measuring the UV-Vis spectrum of the reaction media (Fig. 1 C and D). The AgNP's synthesised from aqueous extracts of *S. johnstonii* showed the optical peak at 430 nm with the absorption of 0.771. The broadening of peaks indicated that the particles are polydispersed. The frequency and width of the surface plasmon absorption depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium.

FTIR analysis

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The aqueous and $AgNO_3$ extracts of *S. johnstonii* were passed into the FTIR and the functional groups of the components were separated based on its peak ratio. The results of FTIR analysis of *S. johnstonii* confirmed the presence of alkyl halides, aliphatic amines, alkynes, alcohols, 1°amines and phenols with the peak values from 559.255to 3401.82 (Table 1; Fig. 2-3). The occurrence of phenol and alcohols was confimred by the existence of band at 3370.96 and 3401.82 cm^{-1} corresponds to O–H stretching. The existence of primary amines was confimred by the occurrence of band at 1638.23 and 1643.05 cm^{-1} corresponds to N–H band. The peak at 1042.34 cm^{-1} corresponds to C–N stretching of aliphatic amine group. Therefore, the synthesized silver nanoparticles were capped by proteins and metabolites such as phenolic acid, carboxylic acid and flavonoids. The capping was confirmed by the existence of band at 1643.05 and 3401.82 cm^{-1} (Table 1).

Brine shrimp Lethality bioassay

The *in vivo* lethality test on a simple zoological organism, such as brine shrimp nauplii, has been used as a convenient tool for screening of bioactive natural products. The aqueous and silver nanoparticles of *S. johnstonii* showed different mortality rate of brine shrimp which increased proportionally with the increasing concentration of the extract. The inhibitory effect of the extract might be due to the toxic compounds present in the aqueous extracts and silver nanoparticles of *S. johnstonii*. The cytotoxic potential of *S. johnstonii* silver nanoparticles showed LC_{50} value at 656.89 µg/ml followed by aqueous extracts of *S. johnstonii* showed LC_{50} value at 805.45 µg/ml.

Anticancer activity

Cytotoxic activity of *S. johnstonii* SNP's against DLA cells was evaluated by Trypan blue dye exclusion method. In the present study DLA cells treated with silver nano particles of *S. johnstonii* showed growth inhibition in a dose dependent manner. As the concentration increases there is an increase in the cell growth inhibition. The AgNPs of *S. johnstonii* showed their best growth inhibition activity with CTC_{50} value 293.66 µl/ml.

Table 1: FT-IR Peak values of *S. johnstonii* – Aqueous and AgNP's extract

Peak	Bond	Functional Groups	*S. johnstonii*	
			Aqueous	AgNP's
559.255	C–Br stretch	alkyl halides	-	+
566.959	C–Br stretch	alkyl halides	+	-
597.825	C–Br stretch	alkyl halides	-	+
647.001	C–Cl stretch	alkyl halides	+	-
675.928	C–Br stretch	alkyl halides	-	+
1042.34	C–N stretch	aliphatic amines	+	-
1638.23	N–H bend	1° amines	+	-
1643.05	N–H bend	1° amines	-	+
2129.03	–C≡C– stretch	Alkynes	+	-
3370.96	O–H stretch, H–bonded	alcohols, phenols	+	-
3401.82	O–H stretch, H–bonded	alcohols, phenols	-	+

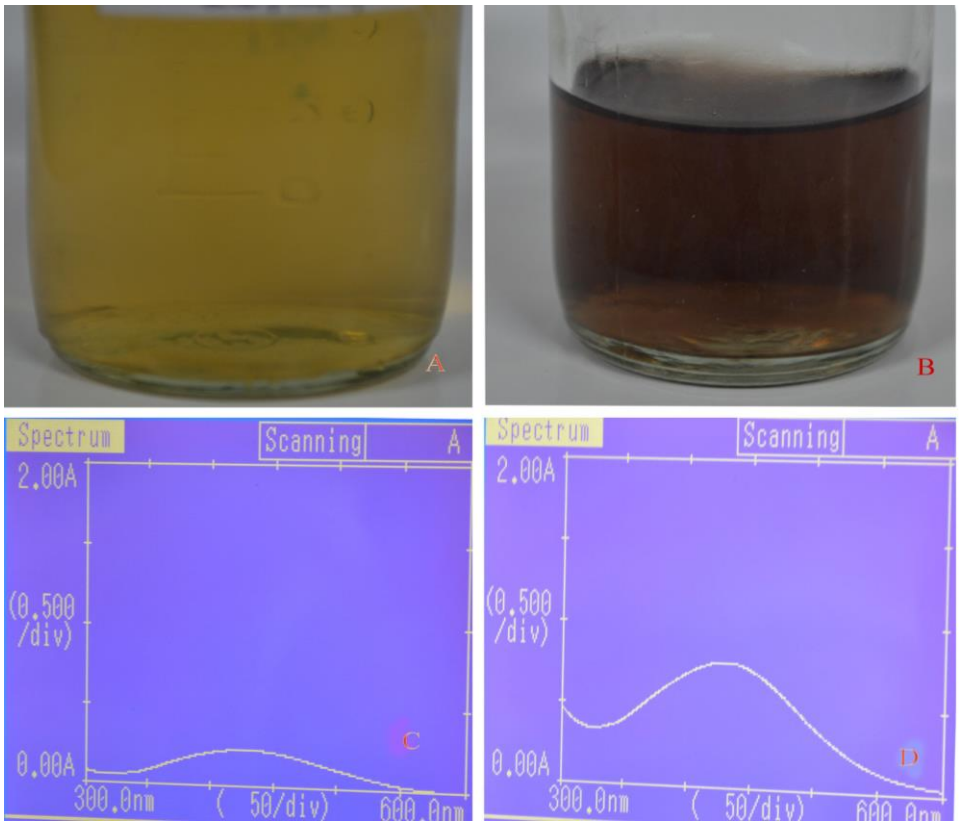

Fig. 1: Synthesis and characterization of Silver nanoparticles- *S. johnstonii*

A- aqueous extract; B- AgNP's of *S. johnstonii*; C- UV-Vis spectrum of *S. johnstonii* aqueous extract; D- UV-Vis spectrum of AgNP's *S. johnstonii*

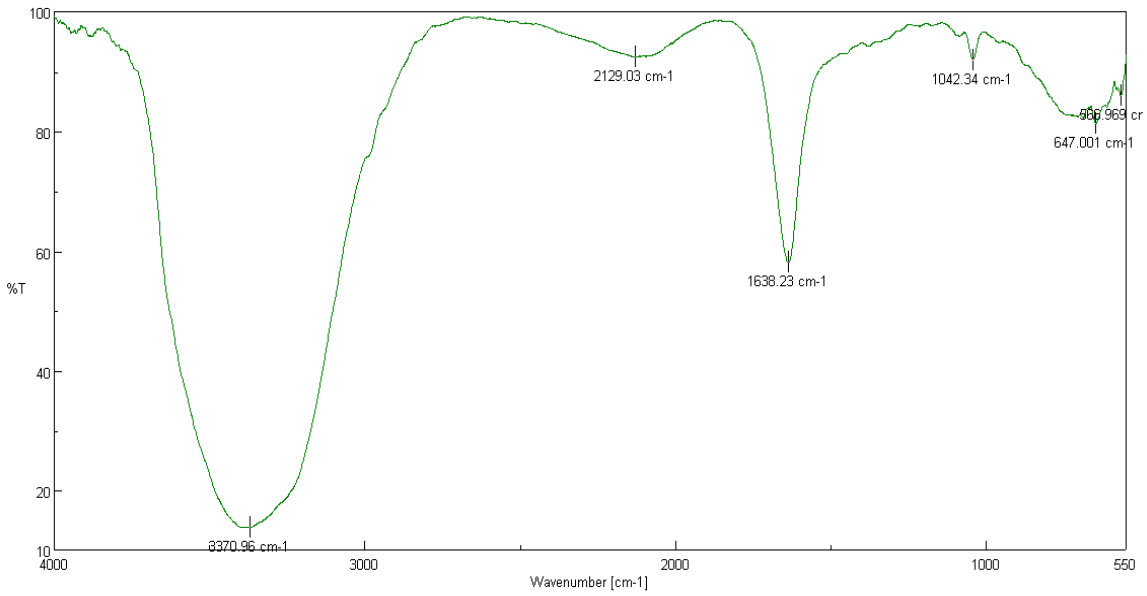

Fig. 2: FTIR spectrum of aqeuous extract of *S. johnstonii*

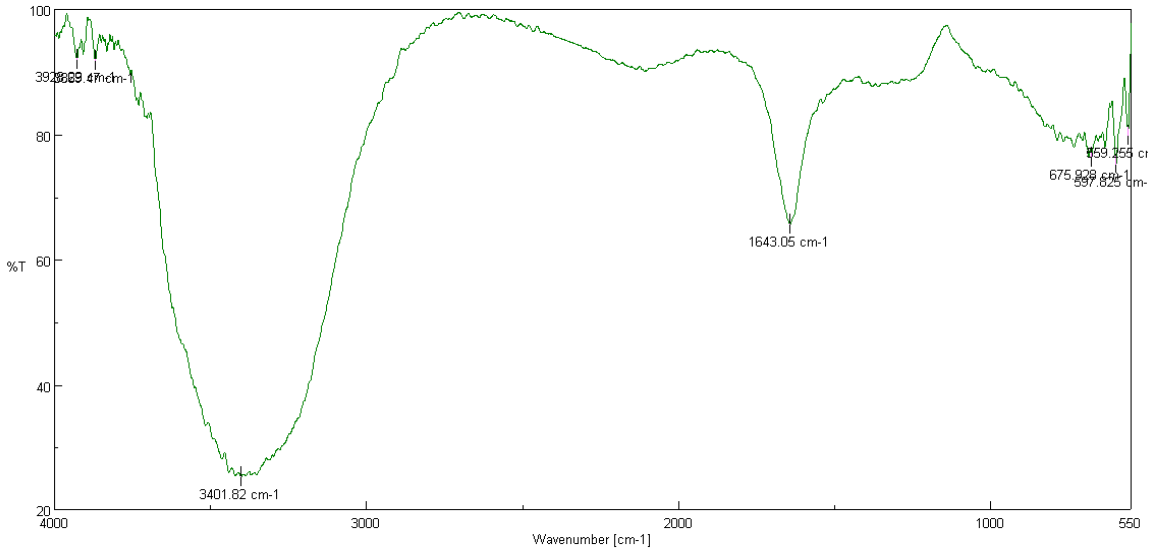

Fig. 3: FTIR spectrum of AgNP's of *S. johnstonii*

DISCUSSION

Recent times, the researchers are focused their attention on biogenic silver nanoparticles using seaweeds because seaweeds are called factories of nanoparticles. Few reports are available indicating the production of biogenic silver nanoparticles using seaweeds such as *Sargassum longifolium*,[10] *Sargassum cinereum*,[8] *Padina tetrastromatica*,[24] *Kappaphycus*

alvarezii,[25] *Sargassum tenerrimum*,[7] *Gracilaria edulis*,[26] *Laurencia pedicularioides*,[27] *Lobophora variegata*,[28] *Sargassum duplicatum*,[15] *Laurencia papillosa*,[29] *Spatoglossum* asperum[30] and *Stoechospermum marginatum*.[31] In the present study also the silver nano particles of *S. johnstonii* were synthesized from the aqueous extracts. The nanomaterials synthesised from chemical and physical agents caused the damage or injury to human cells. This leads to search for alternatives which may be ecofriendly and does not cause any damage or injury to human cells. Researcher's came out with an alternative method for the synthesis of nanoparticles using biological sources as reducing agents.[32-34] Researchers confirmed the nanoparticles synthesis by the colour change, UV-Vis, FT-IR, XRD analysis and size and shape of the materials are characterized by SEM, TEM, AFM etc.[35] In the present study also the aqueous extracts of *S. johnstonii* exhibit yellowish - brown colour due to excitation of surface plasmon vibrations in silver nanoparticles.

Linga Rao and Savithramma[36] confirmed the SNP's synthesis from *Boswellia ovaliofoliolata*, *Shorea tumbuggaia* and *Svensonia hyderobadensis* with absorbance peaks at 350 nm, 430 and 400 nm respectively. In the present study also the synthesis of *S. johnstonii* SNP's was confirmed by the optical peak at 430 nm with the absorption of 0.771. Trypan blue method provide the exact number of living and dead cells because trypan blue has the ability to penetrate in to the dead cells and give it blue colour.[37] In the present study also the dead cells are identified by penetration of trypan blue. The results of cytotoxicity studies using *Artemia salina* and DLA cells indicated that silver nanoparticles have cytotoxic potenitals with varied LC_{50} and CTC_{50} values. Cytotoxic studies against *Artemia salina* confirmed that silver nanoparticles are capable of rendering high cytotoxic activity and hence has a great potential in the preparation of anti-cancer drugs. The synthesized SNP's improve the therapeutic and medicinal values of *S. johnstonii*.

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