ALGAE MEDIATED GREEN FABRICATION OF SILVER NANOPARTICLES *Sargassum prismaticum* V.D. CHAUHAN AND ITS BIOLOGICAL APPLICATION POTENTIAL

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Abstract: The aim of the present study was to evaluate seed germination, embryonic axis length, toxicity, and antioxidant efficacy of silver nanoparticles synthesized (SNPs) from seaweed extract of *Sargassum prismaticum* V.D. Chauhan. The seaweed *S. prismaticum* extract was mixed with silver nitrate, change in colour from yellow to dark brown. The formation of SNPs is confirmed by the appearance signatory brown colour of the solution and a characteristic peak at 411 nm in the UV-Vis spectrum. FTIR spectrum shows 11 peaks. The *S. prismaticum* extract was subjected to XRD analysis for further confirmation of its crystalline nature. FESEM images were found that the SNPs were uniformly distributed and size ranging between 69.99 - 99.15 nm with spherical and cuboidal morphology. The obtained zeta potential value of SNPs through DLS method was had an average size of 94.73 nm. The seedling growth was positively affected by certain concentration of SNPs. For DWt (Dry Weight) of embryonic axis, All the SNPs concentration (25, 50 and 100%) significantly increases EAL (Embriyonic Axis Length) and DWt in all the three plant seeds. The highest percentage inhibition was 79.8% observed in algal extract at the concentration of 500 µg/mL. The IC_{50} value of algal SNP was 238.21 µg/mL and algal extract was 110.63 µg/mL. *S. prismaticum* extract SNPs react with earthworm *Lampitomauritii* in the mortality rate of 25% and 100% for 3 minutes. Overall, the biotransformation of silver nanoparticles utilizing *S. prismaticum* bioreagent is a low-cost, environmentally friendly approach with enormous biotechnological potential.

Key words: *Sargassum prismaticum*; algae; biosynthesis; aqueous; embryonic axis length; earthworm.

INTRODUCTION

Nanoparticles have taken up a lot of space in the field of marine bio-nanotechnology in recent years. Nanoparticles are thought to be clusters of atoms with sizes ranging from 1 to 100 nm [68]. Nanotechnology has progressively more expanded as a promising field of applied science due to its various applications, which involve many aspects of human life using nanoparticles [16, 22, 60].

Metal nanoparticles have magnetic, electronic, and optical properties, allowing them to be used in a variety of fields such as medicine,agriculture, and electronics [49]. Due to their physicochemical characteristics, silver nanoparticles have attracted the most attention among the metal nanoparticles [15]. Silver nanoparticles are used in a variety of applications, including water filters [20], biosensors [5], antibacterial activity [54], anti-HIV activity [14], and plant pathogen control [34]. Seed germination was positively affected by treatment with SNPs in *Boswellia ovalifoliolata* plant [13] and *Pennisetum glaucum* [14]. In contrast to conventional chemical and physical methods, the biosynthetic method using marine seaweed extract has drawn more attention because it is straightforward, environmentally friendly, and time-efficient.

Algae are also used in the synthesis of metallic nanoparticles as "bio-factories." Seaweeds have distinct advantages over other bioreductant genera due to their high metal uptake capacity and low cost [7]. It is possible to produce silver nanoparticles quickly using extracellular biosynthesis from seaweeds [22, 29, 47, 64, 67]. Seaweeds, also known as marine algae, are a commercially important renewable marine living resource. The last three decades have been spent primarily on screening biologically active compounds in various seaweeds against various human pathogenic viruses, bacteria, and fungi [8, 20, 27, 46, 48, 49, 54, 58, 65].

Seaweeds have been studied all over the world and found to have enormous therapeutic potential due to their anti-oxidant, neuroprotective, antifungal, antiviral, anti-inflammatory, anticancer, anti-HIV, antimutagenic, scavenging free radicals, and antimicrobial properties. Seaweeds also contain vitamins A, Bl, B12, C, D, E, riboflavin, niacin, pantothenic acid, and folic acid, as well as minerals Ca, P, Na, and K [13]. The nutrient content and bioactive compounds of seaweeds vary with seasonal cycles and environmental factors such as ecological distribution, salinity, temperature, light, and so on [17, 19, 25,33]. *S. prismaticum* have antimicrobial [73], anti-inflammatory [26], antiviral [3], antioxidant [10], anticancer activities [34]. Hence, the results prove that this algal species is an excellent source of bioactive compounds with a wide variety of application.

Sargassum prismaticum is brown seaweed in the order Fucales of the family Sargassaceae. This species is found throughout the world's tropical and subtropical regions. Most species produce metabolites with therapeutic properties such as terpenoids, polysaccharides, polyphenols, steroids, glycerides, and so on [72]. The results showed that *S. prismaticum* showed higher phytochemicals than the others. Therefore, the present study elucidates silver nanoparticles can play a vital role in nano-based therapy in future. Here, we have focused on effect of SNPs on seed germination that exposed to different concentrations of SNPs. Moreover, using optimum dose of nanoparticles might minimize the risk of nanoparticles in plant environment.

MATERIALS AND METHODS

Algae collection and identification

The fresh seaweed *Sargassum prismaticum* V.D. Chauhan (Fig 1) was collected from Kanyakumari coast, Tamil Nadu, South India. The latitude of Puthalam, Kanyakumari, Tamil Nadu, and India is 8.106106N, and the longitude is 77.466888E. The samples were brought to the laboratory in polythene bag and cleaned thoroughly with fresh water to remove adhering debris and associated biota. The algae was identified and authenticated by Botanical Survey of India, Southern Region, Coimbatore 641013 with the letter number S1/SRC/5/23/2014-15/Tech/851 Dated 22nd August 2014 and was dried for further use in the shade at room temperature.

Figure 1. *Sargassum prismaticum*

Preparation of seaweed extract

The seaweed*Sargassum prismaticum* V.D. Chauhan were washed thoroughly with Milli Q water to remove extraneous materials and log of the washed seaweed was finely cut into small pieces and mixed with 100 mL. The sterile Milli Q water for 1min and kept in a water bath at 60ºc for 20 minutes [49]. Finally, Whatmann no. 1 filter paper was used to filter the extract. The filtrate serves as a stabilizer and reducing agent.

Biosynthesis of Silver nanoparticles (SNPs)

For the biosynthesis of SNPs, 50 mL of aqueous *Sargassum prismaticum* seaweed extract was combined with 50 mL of a 1 Mm $AgNO_3$ solution, which was then incubated at room temperature and in the dark under static conditions for 1 minute. A control setup without seaweed extract was also kept. The bioreduction of $AgNO_3$ into SNPs can be confirmed visually by the change in colour of thesolution after 24 hrs of incubation [49].

Characterization techniques

UV-Visible spectroscopy analysis

The reduction process for the formation of SNPs in solution was monitored on a Perkin-Elmer UV-VIS Spectrometer Lambda – 35 to know the kinetic behavior of the SNPs. With a scan speed of 480 nm/min, the reaction of the solution was examined at various reaction times in the wavelength ranges between 200 and 800 nm. The Spectrophotometer was equipped with “UV Winlab” software to record and analyze data. Baseline correction of the Spectrometer was carried out by using a blank reference. The UV-Vis absorption spectra of the extract were recorded along with the resulting data recorded in graphical format headings were taken for all the concentrations mentioned. To study the effect of time duration on SNP formation the reaction solution was incubated at 24 hrs for each of theconcentrations.

X-ray diffraction measurement(XRD)

The phase evolution of cleaned powder as well as that of sintered sample was studied by X-ray Diffraction Techniques (Philips PAN Analytical, The Netherland) using Cu kα radiation. The voltage and current of the generator were set to 35 KV and 25 Ma, respectively. The Ag sample was scanned in the 2θ ranges 15 to 70ºC range in continuous scan mode. The scan rate was 0.04/sec.

Microscopy field emission scanning electron microscopy (FESEM)

The morphology and mean particle size of the SNPs were characterised using FESEM, which generates clear images with spatial resolution down to 11/2 nm and is electrostatically less distorted, or between 3 and 6 times better than conventional SEM. At electron accelerating voltages compatible with energy dispersive X-ray spectroscopy, smaller contamination spots can be examined. Low kinetic energy electrons with reduced penetration probe closer to the immediate material surface. Images of high quality and low voltage are captured with barely any electrical charging of the sample (accelerating voltages range from 0.5 to 30 kV). SNPs solution samples in the form of powder and freeze-dried samples were sonicated with distilled water before a small drop of the sample was applied to a glass slide and left to dry. Athin layer of platinum was coated to make the sample conductive Jeol. JSM-6480 L V FESEM machine was operated at a vacuum for the order of 10-5 torr. The microscope's accelerating voltage was maintained between 10 and 20 keV.

Fourier Transform Infraredanalysis (FTIR)

In order to identify the chemical responsible for the synthesis of SNPs, FTIR measurements were conducted on the dried biomass of the extract treated with $AgNO_3$ to look into and predict any physicochemical interactions between various components in a formulation. FTIR measurements were taken for the SNPs synthesized after 0hr, 6 hrs, 12 hrs and 24 hrs of reaction. These measurements were carried using a FTIR Perkin Elmer Spectrum 100 instrument with a wavelength range of 4000 to 400 nm where the sample were incorporated with KBr pellets to acquire the spectra. In order to compare the results, the functional peaks were shifted.

Energy Dispersive X-ray (EDX) analysis

The JEOL -2100 High Transmission Electron Microscope was used to conduct EDX in order to both confirm the presence of Ag in the particles and to identify their other primary chemical compositions. The composition of the synthesised nanoparticles was determined by drop coating a very small portion of the sample onto film.

Particle size (DLS- Diffuse Light Scattering Method) with Zeta potential analysis

To ascertain the powder's particle size distribution, a laser diffraction method with a multiple scattering technique was employed. It was based on Mie-Scattering Theory [63]. This theory provides rigorous solutions for light scattering by an isotropic sphere embedded in a homogeneous medium. In order to find out the particles size distribution the Ag powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLPI). The data on particle size distribution was extracted in Zeta sizer version 6.20 Mall052893, Malven Instrument.

Applications

Seed germination assay

Experiment was made to evaluate the effect of seaweed extract *Sargassum prismaticum* SNPs on the germinability of the candidate seeds *Vigna unguiculata* (L.) Walp, *Vigna radiata* (L.) Wilczek and *Cicer arietinum* L. in a fully randomized plan with duplicates. The treatments in the experiment were taken in 25%, 50%, 75% and 100% concentration of SNPs. This setup was kept in room temperature of 28 ºC for 24 hrs. To ensure the sterility of the seed surface, the seeds were dipped in a 5% sodium hypochlorite solution for 15 minutes. They were then soaked in a solution containing silver nanoparticles overnight. As a control, the seeds were also soaked in regular tap water overnight. Then, each piece of filter paper was wetted with 5 mL Silver nanoparticles solution and placed in the Petriplates. On filter paper inside Petri plates, the treated seeds were kept. Petri plates were then covered and kept at room temperature for incubation. The percentage of germination, mean germination time, germination index, relative root elongation, relative seed germination, and germination rate were calculated after germination had stopped after 24 hrs. The following equations [60-62] were used to calculate the germination parameters. (Gf/n) 100; Germination Percentage (GP%) (1) Where Ni is the total number of seeds that have germinated up to the I^{th} day, Di is the number of days since the experiment began, and n is the total number of seeds that have germinated [24].

Germination Rate (GR) = (Σ Ni) / (Σ Ti Ni) (3)

where Ni is the number of newly germinated seeds at time Ti.

GR = (a/1) + (b-a/2) +(c-b/3)+ (n-n-1/N) (4)

Relative root elongation (E) = [(Mean root length with NPs) / (Mean root length with control)] × 100

Germination index(GI) = [(Relative seed germination) × (Relative root elongation)] / 100

where, Relative seed germination = [(Seeds germinated with NPs) / (Seeds germinated with control)] ×100

Embryonic axis length (EAL) study

The embryonic axis length, fresh and dry weight of the embryonic axis and percentage increase/decrease over the control germinated seeds of *Vigna unguiculata* (L.) Walp, *Vigna radiata* (L.) Wilczek and *Cicer arietinum* L. were calculated in 25%, 50%, 75% and 100% concentrated SNPs solution of seaweed extract *Sargassum prismaticum*.

Toxicity dtudy of earthworm

The seaweed extract *Sargassum prismaticum* silver nanoparticles (SNPs) was allowed to interact with earthworm *Lampito mauritii* at 25%, 50%, 75% and 100% concentrations of SNPs solution and incubated at a room temperature of 28ºC. The amount of time needed for the earthworm to die was noted and tallied. The controls included silver nitrate solution, methanol, ethanol, rectified spirit, and these substances.

Antioxidant activity

1,1 Diphenyl 1-2-picrylhydrazyl free radical dcavenging sctivity

The DPPH radical scavenging activity of different concentration (100, 200, 300, 400 and 500 µg/mL) of algal extract and green synthesized SNPs of the seaweed extract *Sargassum prismaticum* sample was measured according to the method [37]. The test sample (100-500 mL) was mixed with 0.8 mL of Tris-HCl buffer (pH 7.4) to which 1 mL DPPH (500mM in ethanol) was added. After giving the mixture a vigorous shake, it was set aside for 30 minutes. The resulting solution's absorbance was assessed using a UV-Visible Spectrophotometer at 517 nm (UV-160A; Shimadzu C.o.). The concentration at which 50% of the DPPH radicals are scavenged is known as the IC_{50} value, which is how the potential for radical scavenging was expressed. Using the following equation, the DPPH radical's scavenging activity was determined.

Inhibition percentage equals $(A_{control}\text{-}A_{sample}) \times 100$. A sample is the DPPH solution absorbance with sample, and a control is the blank absorbance. Ascorbic acid, a synthetic antioxidant, served as a positive control.

RESULTS

UV-Vis spectroscopy analysis of *Sargassum prismaticum* SNPs

S. prismaticum is a medicinal macro alga. Biosynthesis of SNPs using aqueous extract of *S. prismaticum* with $AgNO_3$ was proved through this study. The fresh extract of *S. prismaticum* was yellow in colour. Nevertheless, after addition of silver nitrate and allowed it for 24 hrs at room temperature, the emulsion turned to dark brown. The colour change confirmed the SNP synthesis in the mixture solution.The absorption spectrum of synthesized SNPs in the range of 242-411 nm for the reaction of silver was represented in the figure 2.

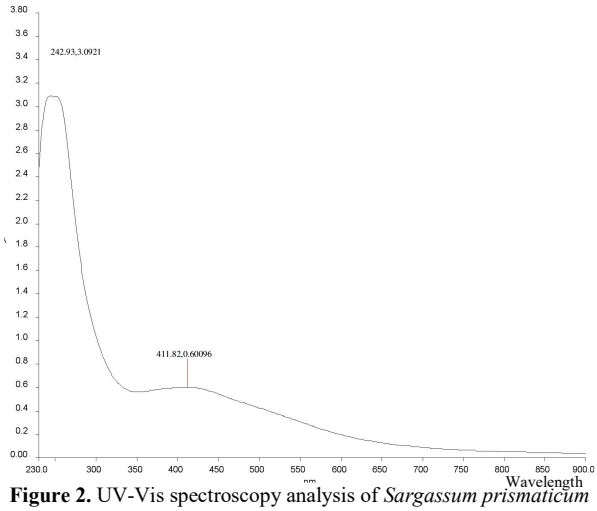

Figure 2. UV-Vis spectroscopy analysis of *Sargassum prismaticum* SNPs

FTIR spectroscopy analysis of *S. prismaticum* SNPs

The FTIR study of *S. prismaticum* was carried out to know the biomolecules for capping and stabilization of the metal nanoparticles synthesized. The spectrum of SNPs was displayed in the figure 3 and the peak values, type of vibrations and corresponding functional groups were presented in the table 1. Absorbance bands in the region of 400-4000 cm^{-1} are 3913, 3873, 3469,3433, 2767, 2671, 2362, 2037, 1640, 1442, 1302 and 676 cm^{-1}. The absorbance peaks are known to be associated with stretching vibrations for O=H stretch (alcohol), N-H stretch (secondary amine), C-H stretch (alkane and aldehyde), O=C=O stretch (carbondioxide), C-C stretch (cyclic alkene), S-O stretch (sulfonyl chloride, O-H stretch (phenol) and C-Br stretching (halo compounds) respectively (Table 1).

XRD analysis of *S. prismaticum* SNPs

The XRD analysis was performed in the range 20-70º at 2θ angles to know the crystalline nature and morphology of the SNPs synthesized from the sea weed *S. prismaticum.* The diffractogram and data obtained from the analysis was displayed in the figure 4 and table 2. Only one high intensity peak of SNPs was observed at around 38.13° (11) with FWHM value 0.2. Apart from this some unassigned peaks were also observed which may due to presence of some impurities in the sample. Through the Debye-Scherer equation the average size of particles was noticed as 39.7 nm.

The FESEM micrograph of *S. prismaticum* provided information about the morphological characters of the synthesized SNPs. The photograph confirmed that the particles were spherical, cuboidal in shape (Fig 5). The size of the particles range between 69.99 to 99.15 nm. Source of the SNPs clusters were also seen apart from uniform distribution. The size is suitable for the drug delivery.

Table 1. FTIR analysis of *S. prismaticum* SNPs

S.No.	Peak values	Appearance	Functional group	Compound Class
1	676	strong	C-Br stretching	halo compound
2	1302	medium	O-H bending	phenol
3	1442	strong	S=O stretching	sulfonyl chloride
4	1640	medium	C=C stretching	cyclic alkene
5	2037	strong	O=C=O stretching	carbon dioxide
6	2362	medium	C-H stretching	aldehyde
7	2671	medium	C-H stretching	alkane
8	2767	medium	N-H stretching	secondary amine
9	3433	strong, broad	O-H stretching	alcohol
10	3469	strong, broad	O-H stretching	alcohol
11	3873	strong, broad	O-H stretching	alcohol
12	3913	strong,broad	O-H stretching	alcohol

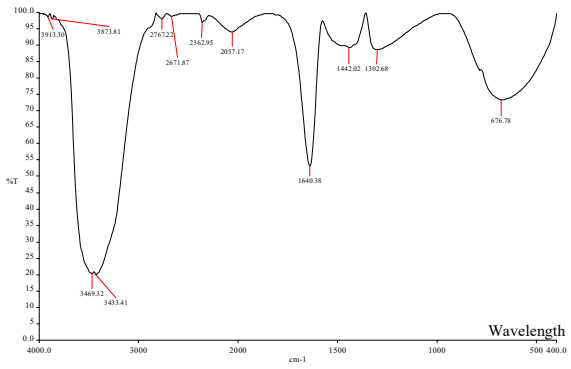

Figure 3. FTIR spectroscopy analysis of *S. prismaticum* SNPs

Table 2. XRD analysis of *S. prismaticum* SNPs

Nos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
38.13(5)	11(12)	0.2(2)	2.35836	100.00

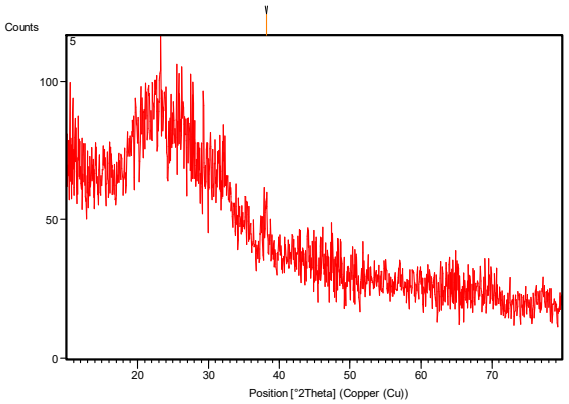

Figure 4. XRD analysis of *S. prismaticum* SNPs

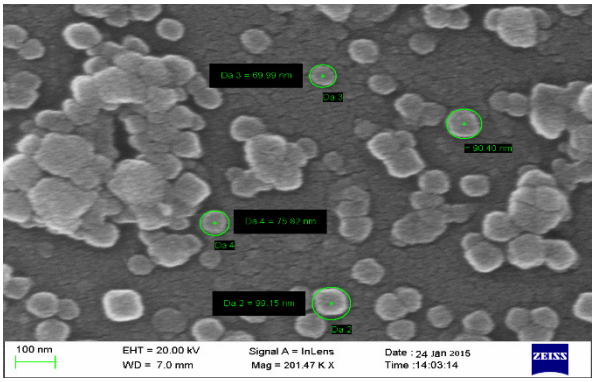

Figure 5. FESEM analysis of *S. prismaticum* SNPs

EDX analysis of *S. prismaticum* SNPs

The EDX spectra analysis results of *S. prismaticum* SNPs was displayed in the figure 6. EDX analysis of SNPs confirmed the presence of elemental silver signal of the SNPs. The X-ray counts displayed with vertical axis and the horizontal axis showed the energy in kev. In *S. prismaticum* extract silver nanoparticles strong optical absorption peaks were observed approximately at 3kev. Some of other peaks are C, Na, Al, Cl, O, Mg, Si and Ca. The weak signals were observed for oxygen, sodium, magnesium, calcium, silicon, carbon, and aluminum are due to the presence of various biochemical molecules in the sample responsible for the SNPs synthesis.

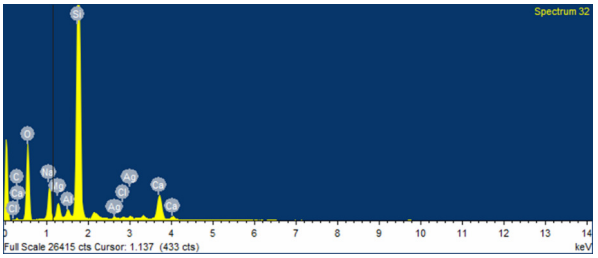

Figure 6. EDX analysis of *S. prismaticum* of SNPs

Particle size analysis of *S. prismaticum* SNPs

The average size of bioreduced SNPs obtained from *S. prismaticum* extract was calculated by using dynamic light scattering analysis in triplicates. The results of DLS analysis was displayed in the table 3. As per the table values the size of the particle was 93.93,

Table 3. Summary of particle size analysis – *S. prismaticum* SNPs

Record No.	Count rate(kcps)	Z-Average(d.nm)	Size (d.nm)	Intensity (%)	St.Dev. (d.nm)	PdI/Intercept
1	344.7	93.93	95.06 5410	98.2 1.8	33.86 301.8	0.302/0.912
2	338.6	93.37	100.4 5162	97.6 2.4	42.07 494.2	0.275/0.910
3	345.7	96.91	104.6 5101	96.8 3.2	38.16 546.12	0.279/0.914

Table 3a. Summary of Zeta potential analysis–*S. prismaticum*SNPs

Record No.	Count rate(kcps)	Z-Potential(mV)	Mean (mV)	Area (%)	St.Dev (mV)	Z-Deviation(mV)	Conductivity (mS/cm)
1	322.2	-27.9	-27.9	100.0	9.93	9,93	0.302

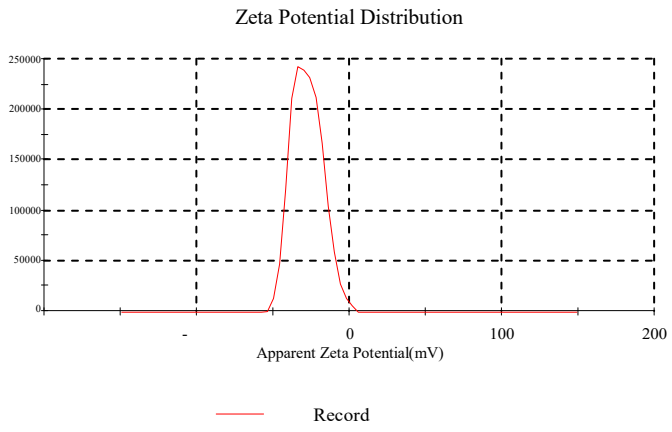

Figure 7. Zeta potential distribution of *S. prismaticum* SNPs

93.37 and 96.91 nm. The average particle size was 94.73 nm. On the basis of the DLS study with triplicate values, the zeta potential was calculated as -27.9. The curve of zeta potential and values were presented in the figure 7 and table 3a.

Effect of *S. prismaticum* SNPs on seed germination potential

The seed germination assay of *S. prismaticum* SNPs, all the concentration shows increased in germination percentage of *Vigna unguiculata* seeds over the control. The data are +33.33, +50.0, +33.33 and 66.66% against 25, 50, 75, and 100% of SNPs concentrations respectively. The higher seed germination percentage (100%) was obtained in 100% SNPs concentration treatment (Table 4; Fig 8). Where as in *V. radiata* seed germination, all the concentrations induced 100% seed germination except at 75%. The increase in percentage germination over the control was +35, +25, +12.5 and 25% against the concentration 25, 50, 75, and 100%. (Table 4; Fig 8). In *Cicer arietinum*, lower concentrations (25 and 50%) of SNPs did not show any increase in seed germination percentage when compared to control. But the higher concentrations (75 and 100%) of SNPs showed good increase in percentage of germination (+40 and 50%) over the control. Among the four concentrations the highest seed germination percentage was achieved (80%) in 100% SNPs treatment (Table 4; Fig 8).

Effect of *S. prismaticum* SNPs on embryonic axisstudy

The effect of *S. prismaticum* SNPs on EAL, FWt, DWt and percentage increase or decrease over control of *Vigna unguiculata, V. radiata and Cicer arietinum* L. seeds were tested and results depicted in table 5. All the SNPs concentration (25, 50 and 100%) significantly increases EAL and DWt in all the three plant seeds. The percentage of increase in *V. unguiculata* was +69.71, +25.71, +46.85, +29.29 and for *V.radiata*+34.46,+12.84, +0.68, +20.27 and for *C. arietinum* +108.42, +61.05, +68.42, +20.0 over the control. The DWt increase was for *V. unguiculata* was +12.90, +27.41, +35.48 and for *V. radiata* +40.0, +31.42, +35.48 and for *C. arietinum* +34.12, +28.51, +38.88, +10.71 when compared to the control (Table 5).

Table 4. Seed germination assay of*S. prismaticum* SNPs

Algae name	Plant name	Concentration	Germination (%)	Increase / Decrease over Control
***S. prismaticum* SNPs**	*Vigna unguiculata*	Control	60 ± 2	
		25%	80 ± 3	+33.33
		50%	90 ± 2	+50
		75%	80± 5	+33.33
		100%	100 ± 4	+66.66
	Vigna radiata	Control	80 ± 5	
		25%	100 ± 1	+25
		50%	100 ± 2	+25
		75%	90 ± 2	+12.5
		100%	100 ± 5	+25
	Cicer arietinum	Control	50 ± 3	
		25%	50 ± 5	0
		50%	50 ± 5	0
		75%	70 ± 3	+40
		100%	80 ± 1	+50
Cd SEd(P<0.05)			2.73252 5.46587	

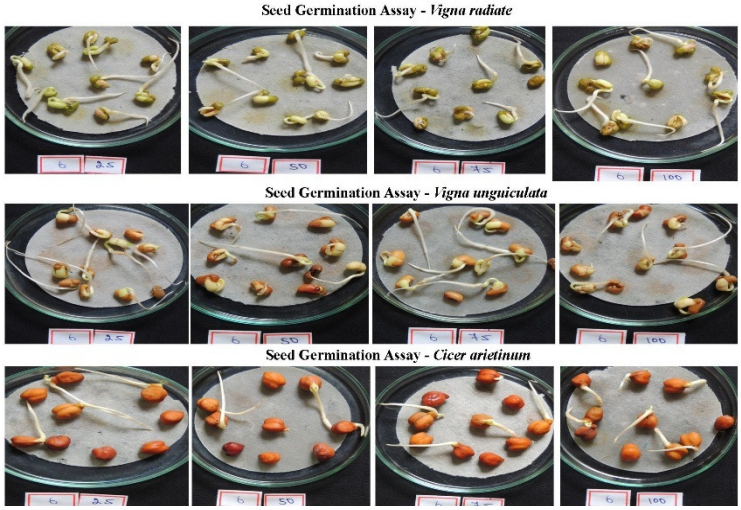

Figure 8. Seed germination assay of *S. prismaticum* SNPs

Antioxidant activity

The green synthesized *S. prismaticum* SNPs and algal extract sample were subjected to DPPH antioxidant assay. Among the five concentrations of SNPs and algal extract examined (100 to 500 µg/mL), the highest inhibition percentage 58.7 and 79.8% was observed at 500 µg/mL concentration against SNPs and algal extract respectively. The obtained IC_{50}value was 238.21 µg/mL for algal SNPs and 110.63 µg/mL for algal extract. The lowest IC_{50} value was observed in algal extract alone (Table 6).

Toxicologystudyof *S. prismaticum* SNPs on *Lampito mauritii* (Earthworm)

The earthworm death time was noticed in the 25, 50, 75, and 100% SNPs concentration. Among these concentrations, earthworm death occurred within 3 min of exposure at 25% concentration, whereas in control, no death has occurred up to 120 min. Furthermore, the dose-dependent death time was noticed, i.e., an increase in the concentration decrease in the death time of earthworms. In this study, the death of earthworm occurred within 3 min of incubation at a lower concentration. This might be due to the higher SNP concentration (25, 50, 75, and 100%) (Table 7; Fig 9). At the same time, the solvent control death time was 1.39, 2.05, 2.25, and 3 min against methanol, sprit, ethanol, and $AgNO_3$, respectively. So, the present result is equal to the $AgNO_3$ death time of earthworms (Table 8) [35].

Table 5. Embryonic axis study of *S. prismaticum* SNPs

Algal sample	Plant name	Conc. of SNPs/ mean	EAL cm mean	% Increase/ decrease of EAL overC	FWt g mean	DWt g mean	Water g mean	% Increase / decrease of DWt over C
S. prismaticum	*Vigna unguiculata*	Control	3.5 ± 0.1		0.150	0.062	0.088	
		25%	5.94 ± 0.031	+69,71	0.181	0.062	0.119	0
		50%	4.4 ± 0.2	+25.71	0.195	0.070	0.125	+12.90
		75%	5.14 ± 0.01	+46.85	0.209	0.079	0.13	+27.41
		100%	4.42 ± 0.1	+26.29	0.197	0.084	0.113	+35.48
	Vigna radiate	Control	2.96 ± 0.02		0.175	0.035	0.14	
		25%	3.98 ± 0.01	+34.46	0.144	0.035	0.109	0
		50%	3.34 ± 0.1	+12.84	0.141	0.049	0.092	+40
		75%	2.98 ± 0.01	+0.68	0.151	0.046	0.105	+31.42
		100%	3.56 ± 0.1	+20.27	0.134	0.040	0.094	+14.28
	Cicer arietinum	Control	1.9 ± 0.44		0.585	0.252	0.333	
		25%	3.96 ± 0.02	+108.42	0.678	0.338	0.34	+34.12
		50%	3.06 ± 0.02	+61.05	0.639	0.324	0.315	+28.57
		75%	3.2 ± 0.1	+68.42	0.540	0.350	0.19	+38.88
		100%	2.28 ± 0.01	+20.0	0.586	0.279	0.307	+10.71
Cd SEd(P<0.05)				0.27673 0.55355				

Values are mean ÷ SD of three triplicates

Table 6. DPPH radicalscavenging activity of *S. prismaticum* SNPs

Algal sample	Components	Concentration (µg/mL) / OD value					IC_{50}
		100	200	300	400	500	
S. prismaticum	SNPs	1.152 ± 0.15	1.086 ± 0.01	1.027 ±0.03	0.998 ±0.18	0.886 ± 0.23	238.21
	%	46.3	49.4	52.1	53.5	58.7	
	Extract	1.007 ± 0.00	0.947 ± 0.21	0.899 ± 0.17	0.761 ±0.11	0.703 ±0.19	110.63
	%	53.1	54.6	58.1	64.5	79.8	
Control		2.148	0.15	0.15	0.15	0.15	
Cd SEd(P<0.05)				142.76718 282.67624			

Values are mean ÷ SD of three triplicates

Table 7. Earthworm toxicology study of *S. prismaticum* SNPs

Algal sample	Death time (min)				
	25%	50%	75%	100%	C
S. prismaticum	1 ± 1	1.55 ± 0.28	2 ± 1.53	2 ± 1	110 ± 40.71
Cd SEd(P<0.05)			4.65296 9.23137		

Values are mean ÷ SD of three triplicates

Table 8. Solvent death rate

S.No	Name	Death time (min)
1	Methanol	1.39 ± 0.26
2	Sprit	2.05 ± 0.67
3	Ethanol	2.25 ± 0.61
4	$AgNO_3$	3 ± 0.45

Values are mean ÷ SD of three triplicates

Figure 9. Earthworm toxicology study of *S. prismaticum* SNPs

DISCUSSION

The present study *S. prismaticum* is a medicinal macro alga. The fresh extract of *S. prismaticum* was yellow in colour. Nevertheless, after addition of silver nitrate and allowed it for 24 hrs at room temperature, the emulsion turned to dark brown. Due to the release of excess intracellular SNP or reduction of Ag ions by polysaccharides or enzymes secreted by algal thallus [57]. After 48 hours, the reaction mixture's colour had changed to a brownish-yellow, which was visually indicative of $AgNO_3$ reduction. The length of the incubation period directly correlated with an increase in the brown color's intensity. The reduction of $AgNO_3$ and the activation of the Surface Plasmon Resonance (SPR) effect could be the cause of this [42].

In the present investigation, UV- Vis spectra of SNPs synthesized using algal extract evidenced the blue shift of the observation band around 411nm. This information confirmed that these SNPs haveformed in the extracts, where the Ag^+ has been reduced to Ag^0. *Sargassum longifolium* [10], *S. muticum* [2] exhibited intense peaks as 420 nm.

The peak values around 11 were observed in the FITR analysis of *Sargassum prismaticum* SNPs. Among the eleven peaks three strong and broad bands, (3923, 3783, and 3540 cm-^{1}) five medium bands (3316, 2803, 2627, 1649 and 1330 cm^{-1}) and three strong peaks (2350, 1402,and669 cm^{-1}) were observed. The strong observation bands at 3923, 3783, 3540 with O-H stretching, 3316 with N-H stretching, 2803 C-H stretching vibration respectively having the characteristic of the presence of amino acids [51]. The medium band around 1649cm^{-1} represents C=C stretching vibration indicates the lignin presence [37]. The absorption peak at 1402 cm^{-1} is due to S=O sulphate esters [59]. In the present study *S. prismaticum* contain a strong absorption bound at 1402 due to S=O stretching vibration indicates the presence of starch and polysaccharides. This is in agreement with the study of crude extract of *S.wightii* and *Gracilaria corticata* at the absorption band at 1034 cm^{-1} [30].

The XRD analysis of *Sargassum prismaticum*, the pattern shows that the particles are crystalline with small size. The formation of spherical green synthesized SNPs were noted from the X-ray diffraction spectrum. The average size of the particles was 39.7 nm. Almost similar results were obtained in *S. muticum* [2]. Apart from this some unidentified peaks were also observed. This type of unidentified crystalline peaks is also noticeable in many previous reports [1, 38, 46]. This predicts that the synthesized SNPs by using the *S. prismaticum* extract are pure and crystalline in nature. The observed peak broadening and noise were may be related to the effect of nanoparticles and the presence of different crystalline biological macromolecules [16].

In *S. prismaticum* SNPs FESEM micrographs exhibited the spherical and cubical morphology of the silver with the size less than 99 nm. Whereas in *Turbinaria conoides* the shape is spherical and triangles with the size less than 60 nm [66]. Apart from uniform distribution of the nanomaterials some SNP clusters were also seen in the micrograph. This is in agreement with the shape and size of the earlier report on *Tephrosia villosa* extract SNPs [50]. However in *Sargassum tenerrimum* the transmission electron microscope (TEM) studies, the morphology of SNPs observed to be spherical with an average size of 20 nm [37]. The spherical shaped SNP synthesized by *Sargassum muticum* [2], *S. longifolium* [10], *Sargassum plagiophyllum* [16] was reported.

Dispersive energy X-ray spectroscopy is an analytical technique used to analyse a sample's elemental composition or chemical characterization. It is determined by the interaction of the X-ray excitation source and the sample. The fundamental principle is that each element has a unique atomic structure, which results in a unique set of peaks on its emission spectrum [21]. EDX analysis of SNPs obtained from macro alga *Sargassum prismaticum*, extract was performed for the confirmation of silver nanoparticles. The EDX analysis confirmed the presence of silver in SNPs. Strong signal at 3 kev confirmed the presence of SNPs formation in the solution. EDX study spectrum of *Sargassum wightii* showed the presence of sodium, magnesium, silicon, phosphorus, sulphur, chloride, potassium, calcium, manganese, and zinc [9]. Whereas in the present study, EDX analysis of SNPs obtained from *S. prismaticum* spectrum exhibited the presence of carbon, sodium, aluminum, chloride, oxygen, magnesium, silicon, and calcium. Devi (2013) also confirmed the presence of elemental silver in biogenic SNP in *Sargassum longifolium* through EDX analysis [13].

The particle size of SNP data obtained from the

DLS analysis of *S. prismaticum* are 93.93, 93.37 and 96.91 nm with average size of 94.73 nm with polydispersity index of 0.302, 0.275 and 0.279. On the basis of triplicate DLS values, the zeta potential was calculated as -27.9 mv. Kumar (2012) reported that the *S. tenerrium*SNPs DLS intensity was 45 nm and zeta potential value of -27 mV [39]. Whereas the same values were obtained in *S. plagiophyllum* SNP also [27]. The DLS study also decided the polydispersity index (PDI) or heterogencity of biosynthesized SNPs (PDI < 0.5). Indeed, it is an indicator of aggregation and particle stability. The PDI value more than 0.5 denotes less stability and precipitation, whereas it is closer to zero it indicates the high stability of particles. In the present study, the obtained PDI values of SNPs were less than 0.5, showing the stability of the synthesized nanoparticles. This is in agreeing and comparable result of previous studies using *L. majuscula, L. valderiana, L. tenis, R. risparium* and *S. submaxima.* SNP and GNP study PDI was less than 0.5 [57].

The biosynthesis of SNPs from sea weeds viz. *Sargassum prismaticum*, were subjected to phytotoxicty studies by testing its effect on *Vigna unguiculata*, *Vigna radiata* and *Cicer arietinum* L. seed germination. After the synthesis of SNPs, the SNPs solution at the concentration of 25, 50, 75 and 100% was used for the seed germination test. Before the SNPs treatment the seeds were presoaked in SNP solution for 12 hrs. The seed germination percentage was measured after 24 hrs of incubation. The alga SNPs increased the germination percentage of *Vigna unguiculata* seeds almost in all the concentrations over the control. Almost the alga SNPs increased the percentage of germination upto +66.66% when compared to the control. Minimum increase percentage over control was +16.66. Decrease in the percentage germination was not observed in the *S. prismaticum* SNPs. In previous studies, it had been reported that *Sargassum cinctum* sea weed mediated SNPs had positive and friendly effect and enhanced the growth of seedlings as well as seed germination percentage of *Abelmoschus esculantus* [54].

Result suggested that the *Vigna radiata* seeds treated with different concentrations of SNPs of the algal extract enhanced the seed germination upto +25 percent over the control. In this treatment the percentage germination was lower -87.5 over the control at 25% concentration, whereas all other concentration there was no increase in the germination percentage. *Sargassum ilicifolium* SNPs exhibited good promoting effect on germination of *Abelmoschus esculentus* and *Raphanus*seeds [56].

Among the sea weed, the lower IC_{50} value of DPPH antioxidant activity was observed in *Sargassum prismaticum* extract was 110.63 µg/mL, where as SNPs obtained from the crude extract IC_{50}value was 238.21 µg/mL. One of the most effective antioxidant in brown algae is phenolic compounds; its content is 20-30% algal dry weight [4,6].

Farideh (2013) observed the total phenolic contents of *S. muticum* methanol extract were 78.95 mg per 100 g dried plant [18]. Phlorotannins are the main phenolic compound present in brown algae has 10-100 times higher more potent and stable antioxidants than other polyphenols [45]. Higher DPPH radical scavenging activity was reported in *S. wightii* having more phenolic content [15, 44]. Ye (2009) reported that the higher DPPH free radical scavenging activities of ethyl acetate and n. Butonol fraction of *S.pallidum* [71]. The higher antioxidant activities of crude extract and SNPs of the present investigation might be the presence of polyphenols in *Sargassum* species.

In the present study, eco-friendly synthesis of SNPs was successfully demonstrated through the use of seaweed *Sargassum prismaticum* extract as natural reducing and stabilizing agents. Various characterization techniques (UV-vis, FTIR, XRD, SEM, EDX, and DLS zeta potential) confirmed the formation of phytochemicals-capped SNPs. The SNPs can be applied as nanopriming agent for enhancing seed germination and embryonic axis length of *S. prismaticum* SNPs. It was evident that SNPs can internalize seed coat and support water uptake inside seeds, leading to promote seed germination and embryonic axis length. In this context, the mortality of earthworms does not appear to be greatly affected by NPs dispersed in the soil. In the future, our research could lead to the development of nanopriming applications for sustainable agricultural practises and the agri-seed industry. In prospect, it is suggested that the bioactive compounds responsible for the activities be studied and that applied research on this extract of the studied species be expanded for additional therapeutic and medicinal uses.

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