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ORIGINAL ARTICLE

Synthesis, characterization and biological potential of silver nanoparticles using *Enteromorpha prolifera* algal extract

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Abstract
Aqueous extract of seaweed *Enteromorpha prolifera* was used to synthesize Silver Nanoparticles (AgNPs). The reaction mixture turns brown when AgNPs are formed in it. UV–VIS, FTIR, XRD, EDAX and DLS with Zeta potential techniques were used to characterize the green synthesized AgNPs. AgNPs exhibited an absorbance band at 448 nm in the UV–Vis spectrum. FTIR spectrum registered 11 intense peaks with distinct functional groups. AgNPs display an XRD pattern with 8 peaks, and the average grain size was 17.8 nm. FESEM analysis shows that AgNPs are polydispersed and spherical. EDX results revealed a strong signal peak at 3 keV due to silver presence. The Zeta potential of AgNPs was 30.88 mV, which indicates their stability. The growth of the seedling was positively affected by certain concentrations of AgNPs. In DPPH, the lowest "IC50" value against algae extract was 133.33 g/mL, compared to 142.0 g/mL for algal AgNPs. Increased AgNPs concentrations reduce the earthworms' death time in toxicology studies.

Keywords Biosynthesis · AgNP · *Enteromorpha prolifera* · Toxicology · Earthworms · Algal extract

Introduction

Biomedical applications of nanotechnology have leapfrogged the current state of the art. It has excellent electrical, magnetic and optical properties. Its innovative size-dependent properties are the main reason for its success in the medical field. As the particle is at the Nanoscale, it can interact with the matter at a sub-molecular and atomic level. So, the fsensitivity increases enormously. The nanoparticles synthesized biologically are upgraded techniques compared to the conventional methods. These methods could be made more eco-friendly by replacing these relatively powerful reductants (Muthukumar et al. 2021). A variety of biological methods are commonly used, including extracts from plants and fruits, fungi and even insects (Jakinala et al. 2021; Yassin et al. 2021; Le et al. 2021). As a result of the harsh nature of chemical methods, biological organisms are now used to reduce silver ions due to the cost and short lifespan of silver nanoparticles made by chemical methods (Song and Kim 2009). Silver nanoparticles, due to their unique properties, have proven to be of interest for applications including antimicrobial materials, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, pharmaceutics, medicine and dentistry. In comparison to chemical preparation, biological AgNPs are more stable, solubilized and yield-enhancing (Gurunathan et al. 2015; Arivalagan et al. 2018). They are lucid, hasty, harmless, reliable and environmentally friendly. AgNP bioactivity is determined by several factors including particle size, surface chemistry, architecture, formulation, topping,

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aggregation, termination rate, particle reactivity in solution, ability to release ions cell composition. It is crucial to determine whether or not AgNPs will be cytotoxic by considering the reducing agents used during the synthesis (Carlson et al. 2008). The physical and chemical properties of nanoparticles can enhance the uptake, distribution and penetration of therapeutic agents through inhibitory factors through enhanced assimilability of curative agents both systemically and locally as well as enhance their effects in treating diseases (Jo et al. 2015; Duan and Li 2013).

The macroalgae *Enteromorpha prolifera* belongs to the order Ulvales, family Ulvaceae. Several types of seaweeds have been cultivated by countries in Asia, including *E. prolifera*. The nutrient-rich polysaccharides and nutritional compounds in *E. prolifera* make it a good source of nutrition that has multiple biological activities. These compounds have applications in the food, cosmetic and pharmaceutical industries as well as in microbiology and biotechnology. This species also have very good antioxidant and antimicrobial properties (Zhao et al. 2016). An important marine alga, it contains a wide range of bioactive agents, of which polysaccharides are the most important constituents. It contains natural compounds such as carotenoids, fucoidans, and cosmeceutical, functional and pharmaceutical foods. The polysaccharides present in this species have high hypolipidemic activity, antioxidant activity, immunomodulatory properties, antibacterial activities and also enhances the activities of some enzymes (Tang et al. 2013; Wei et al. 2014; Lu et al. 2014). To our knowledge, and according to our literature search, there are no reports on silver nanoparticles from *E.prolifera.* Accordingly, this study aims to synthesize silver nanoparticles by a green biological route using an extract obtained from green algae *Enteromorpha prolifera*, and characterization of the synthesized nanoparticles utilizing UV–visible spectroscopy (UV–vis), scanning electron microscope (SEM), energy-dispersive X-ray spectroscopy (EDX), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR) analysis. Furthermore, these silver nanoparticles (AgNPs) are tested for their seed germination characteristics, their effects on earthworms, as well as their antioxidant properties.

Materials and methods

Collection and documentation

The sea weeds *E. prolifera* (O.F. Mull.) J. Agardh, (Fig. 1) were collected from sea shore of Kanyakumari District, Tamil Nadu, India. Seaweeds were carried to the laboratory and thoroughly cleaned with water in order to remove debris and unwanted organisms. The algal specimen was identified and validated by Botanical Survey of India, Southern Region, Coimbatore (S1/SRC/5/23/2014-15/Tech/851) and shade dried for subsequent experiment (Fig. 1).

Fig. 1 *Enteromorpha prolifera*—Habit

Preparation of seaweed extracts

The *E. prolifera* sea weeds were washed thoroughly with Milli Q water. Seaweed was washed and chopped into small pieces before being mixed with 100 mL sterile distilled water for 1 min and kept at 40 °C for 20 min. Filtration was accomplished using Whatman no. 1 filter paper.

Biosynthesis of silver nanoparticles (AgNPs)

Aqueous seaweed extract of *E. prolifera* was mixed well with 50 mL of 1 Mm $AgNO_3$ solution and then incubated in a water bath at 60 ºC for 1 h. After that, the mixture was incubated at room temperature for another hour. The change in colour of the solution confirmed bio-reduction of $AgNO_3$ into AgNPs.

UV- Visible spectroscopy investigation

Perkin-Elmer UV–VIS Spectrometer Lambda—35 was used to monitor the reduction process for the formation of AgNPs in solution. With 480 nm/min scan speed, different reaction times of the solution were studied at different wavelengths between 200 and 800 nm. Data collection and analysis was carried out using the software "UV Winlab" on the Spectrophotometer. The absorption spectra for the extracts were documented as well as the graphically displayed results for all the concentrations listed. AgNP formation was studied by incubating each concentration of reaction solution for different periods of time. The intervals of time were 0, 1, 6, 12 and 24 h.

X-ray diffraction measurement (XRD)

By using Cu kα radiation, X-ray diffraction methodology were used to study phase transitions in cleansed powder and sintered samples. 35 kV and 25MA were specified as generator voltage and current, independently. Detections were conducted in the ranges from 15 to 700 °C using continuous scanning on Ag samples.

Microscopy field emission scanning electron microscopy

The size and shape of AgNPs were determined using FESEM. By comparison, FESEM produces three to six times less distortion from electrostatic stress than conventional SEM, producing images with a spatial resolution of 11/2 nm. The spectroscopic examination of smaller contamination spots may be possible when electron-accelerated voltages are agreeable with energy-dispersive X-ray spectroscopy. By reducing penetration, probes can probe within a smaller range of materials. With a small voltage charge applied to the samples (corresponding to an accelerating voltage between 0.5 and 30 kV), the resulting images are of high quality and low voltage. A droplet of the AgNPs powder and freeze-dried powder from the AgNPs solution was placed on a glass slide to dry after being sonicated with distilled water. To make the samples conductive, thin layers of platinum were applied. We maintained an accelerating voltage between 10 and 20 kV on our microscopes.

Fourier transform infrared (FTIR) measurement

For the purpose of determining the compound responsible for the synthesis of AgNPs, FTIR measurements were performed on the dried biomass of the extract prepared with $AgNO_3$. FTIR measurements were taken for the AgNPs synthesized after 0 h, 6 h, 12 h and 24 h of reaction. These measurements were carried using a FTIR PERKIN ELMER instrument with a wavelength range of 4000–400 nm where the samples were incorporated with KBr pellets to acquire the spectra. Based on the shift in functional peaks, the results were compared.

Energy dispersive x-ray (EDX) analysis observation of AgNPs

In order to confirm the presence of Ag in the particles, EDX was carried out using the JEOL -2100 High Transmission Electron Microscope. Very small amount of the sample was drop coated film and analysed for the composition of the synthesized nanoparticles.

Particle size (diffuse light scattering method- DLS) with zeta potential analysis

Laser diffraction was used to determine the particle size distribution of powder based on Mie-Scattering Theory. In this theory, isotropic spheres embedded in homogeneous media provide rigorous solutions for light scattering. Particle size distribution data were extracted via Zeta sizer version 6.20 Mall052893, Malven Instruments.

Applications

Seed germination analysis

To analyse the effect of seaweed extract of *E. prolifera* and synthesized AgNPs on the sprouting of *Vigna unguiculata* (L.) Walp, *Vigna radiata* (L.) Wilczek and *Cicer arietinum* L. seeds experiment was conducted in a randomized design. Different concentrations 25%, 50%, 75% and 100% of AgNPs were used in this treatment. The seeds were immersed in sodium chloride solution for 15 min to fortify the surfaces were sterile. The seeds were then immersed in AgNP solution overnight. The seeds were also immersed in water as a control overnight. Then the soaked seeds were kept on the AgNP solution wetted filter paper present in the petriplates and incubated for 12 h at normal temperature. After this, various parameters were evaluated and presented. Calculation of germination parameters was done with the equations given by Thakkar et al. (2010).

Embryonic axis length (EAL) study

EAL, fresh and dry weight of the embryonic axis, and the percentage difference and 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% AgNPs concentration of seaweed extract of *Enteromorpha prolifera*.

Toxicity study of earthworm

The seaweed extract *E. prolifera* AgNPs was permitted to associate with earthworm *Lampito mauritii* at 25%, 50%, 75% and 100% concentrations of AgNPs solution and incubated at a room temperature. An assessment was made of the duration until the earthworms perished and a table was made. Methanol, Ethanol, Rectified spirit and AgNP solution served as the controls.

Antioxidant activity

1,1 Diphenyl 1–2-picrylhydrazyl free radical scavenging activity

At various concentrations (100, 200, 300, 400 and 500 g/mL), algae extracts and green synthesized AgNPs in *E. prolifera* extract samples were tested for DPPH activity (Kulkarni et al. 2004).

For the determination of DPPH percentages, assay samples (200–500 mL) were diluted with buffer (pH 7.4) and DPPH solution and thoroughly mixed for 30 min. Scavenging potential of radicals was expressed as IC_{50}, the concentration at which 50% of the DPPH radicals were scavenged. Using the following equation, you can calculate the activity. Percentage inhibition = (A control-A sample) × 100 A control where a sample is the Absorbance of DPPH solution with sample, Positive control testing was performed using synthetic antioxidant Ascorbic acid.

Results and discussion

Nanotechnology holds tremendous future in diverse fields of life sciences. A material with components that have smaller dimensions than 100 nm is used. Due to the drawbacks of chemical synthesis, the demand of green synthesis of nanoparticles is increasing day by day (Roy 2017). This technique is an important tool for development of ecofriendly, reliable methodology for synthesis of nanoscale material using biological source. Nano silver is an additive in Indian Siddha, Ayurveda medicines due to its ability to counter the disease spreading organism (Shankar et al. 2004). Plant-based nanoparticles (green synthesis) under biological synthesis are environment friendly, cost effective, devoid of toxic chemicals, demonstrate zero energy consumption, less time consumption and non- requirement of stabilizers. These AgNPs are greatly suitable for pharmaceutical and other biological applications (Tagad et al. 2013).

The marine organism diversity has become a motivation for researches to identity new novel marine products that could ultimately be developed into pharmaceutical and therapeutic products. Indeed many diverse natural products isolated from marine organisms are reported to have an array of bioactivities mainly anticancer, antidiabetic, antioxidant and anti-inflammatory activities (Schwartsmann 2000; Schwartsmann et al. 2001). More than 3000 new marine natural substances have been isolated from seaweeds of subtropical and tropical population, exhibiting the potential of the ocean as a source of novel chemical compounds (Schweitzer et al. 1991).

UV–Vis spectroscopy analysis of *Enteromorpha prolifera* AgNPs

The formation of AgNPs was apparent by the colour change in the reaction mixture. The AgNPs synthesized by using *E. prolifera* were analy***zed through UV–Vis spectroscopy. This was done to determine the characteristic of the peak spectrum of the AgNP wavelength prepared for various AgNO3 concentrations. The spectrum of AgNPs was depicted in Fig. 2. The reduction of the silver ion with silver particles occurred after mixing with plant extract and is followed by the change in colour from yellow to brown colour. Silver nanoparticles undergo this colour change through surface plasma vibrations. The UV visible spectroscopy of the synthesized AgNPs was in the range of 233–449 nm. The formation of absorption spectra of AgNPs in the reaction has an absorption peak at 206–420 nm, and widening of peak evidenced that the AgNPs are polydispersed. AgNP production was nearly spherical shaped as indicated by peak absorption at 410–430 nm, directly correlated with surface plasma resonance (SPR) (Lü et al. 2014). *Caulerpa racemosa* and the *Ulva flexousa* AgNPs displayed absorbance peaks at 430–440 nm, 440 nm and 430 nm, respectively (Rahimi et al. 2014; Kathiraven et al. 2015). Silver nanoparticles have two bands that are close to each other where electrons can move easily. Surface plasmon reverberation band (SPR) assimilation is caused by the aggregate oscillation of electrons within AgNPs in reaction with the light wave (Patri et al. 2006). For various metal nanoparticles of sizes from 2 to 100 nm, the peak observation to a surface plasmon is recorded (Sadeghi and Gholamhoseinpoor 2015). AgNPs have a wavelength range of 400–600 nm, depending on their size and shape (Vasireddy et al. 2012). An observation band around 449 nm was detected in the UV–VIS spectrum of AgNPs synthesized using an extract of seaweed *E. prolifera* in the present study. This information confirmed that these AgNPs have formed in the extracts, where the Ag + has been reduced to Ag0. Biologically active secondary metabolites and proteins found in the extract emerged as key determinants for both reducing and capping mechanisms of AgNP formation (Marslin et al. 2018). The synthesized AgNPs were highly stable which was also confirmed by the zeta potential study. The spherical shaped AgNPs synthesized from *Ulva reticulata* and *Enteromorpha compressa* exhibited intense peaks as 420 nm (Dhanalakshmi et al. 2012).

FTIR spectroscopy analysis of *Enteromorpha prolifera* AgNPs

FTIR analysis of *E. prolifera* confirmed the presence of alcohol, thiol, carbon dioxide, and ketanine, alkene, carboxylic acid and amine and alkene compound class. In the

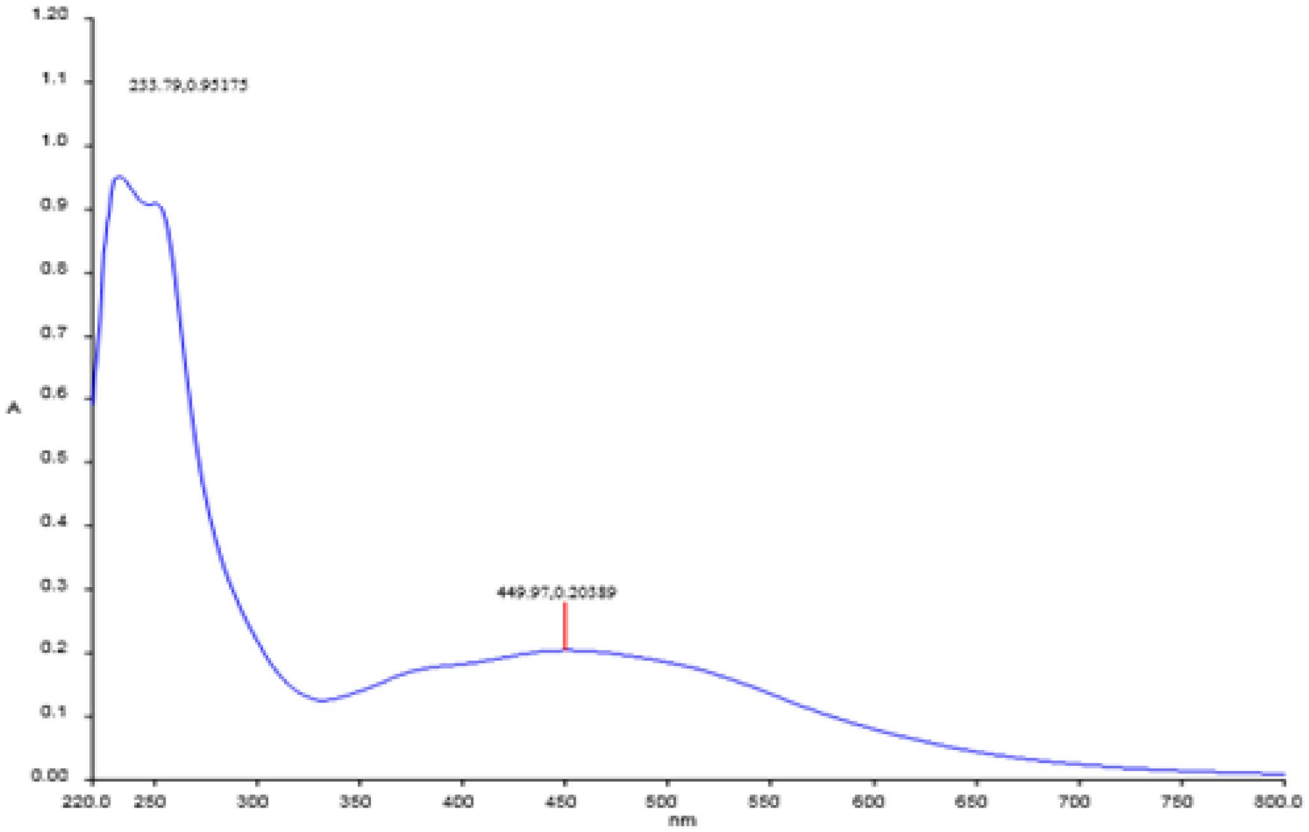

Fig. 2 UVspectram of *Enteromorpha prolifera* AgNPs

Table 1 FTIR analysis of *Enteromorpha prolifera* AgNPs

S. No	Peak values (cm^{-1})	Appearance	Functional group	Compound Class
1	675	Strong	C=C bending	Alkene
2	1234	Medium	C-N stretching	Amine
3	1437	Medium	C-O bending	Carboxylic acid
4	1642	Strong	C=C stretching	Alkene
5	2083	Strong	N=C=S stretching	Ketemine
6	2347	Strong	C=O=C stretching	Carbon dioxide
7	2661	Weak	O=H stretching	Thiol
8	3470	Strong, Broad	O=H stretching	Alcohol
9	3918	Strong, Broad	O=H stretching	Alcohol

spectrum, nine peaks were observed. The peak values, type of vibrations and functional groups obtained through FTIR analysis of *E. prolifera* AgNPs were represented in Table 1 and the corresponding FTIR spectrum profile was illustrated in Fig. 3. In these, three peaks 3918, 3479 and 2661 cm^{-1} showed O–H functional groups, while 2347 cm^{-1} with C=O=C group, 2085 cm^{-1} with N=C=S group, 1642 with C=C stretching, 1437 with C-O bending, 1234 with C-N group and 675 cm^{-1} with C=C bending. Infantrisa and Radhika (2018) also mentioned the occurrence of the strong peak at 3854 and 3955 cm^{-1} in FTIR spectroscopy which confirmed the presence of alcoholic groups on the surface of the AgNPs synthesized by *Padina antillarum*. The presence of protein and peptides in the seaweeds *Scenedesmus* is mainly responsible for the formation of AgNPs synthesized was confirmed by Jena et al. (2014).

XRD analysis of *Enteromorpha prolifera* AgNPs

The XRD analysis of synthesized AgNPs from *E. prolifera* algal extract has eight peaks at 27.93, 32.4, 38.7, 44.4, 46.33, 50.24, 64.6 and 780 at 2θ angles, whereas the curve has the highest peak at 38.20 (129), which was determined according to the standard JCPDS # 001Ago card. The diffractogram and data obtained through XRD analysis are depicted in Fig. 4 and Table 2. The obtained grain size (D) was 17.8 nm. This proves the formation of crystal AgNPs (Kumar et al. 2012). The peaks obtained in X-ray

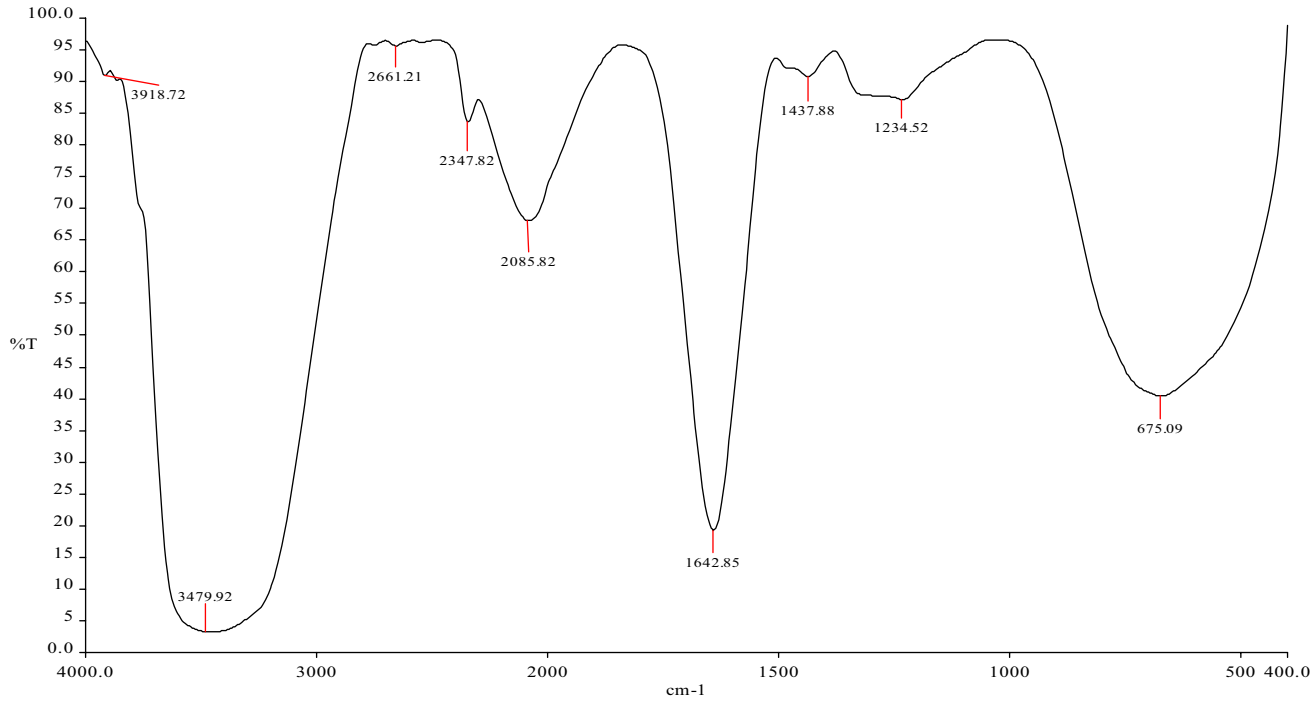

Fig. 3 FTIR analysis of *Enteromorpha prolifera* AgNPs

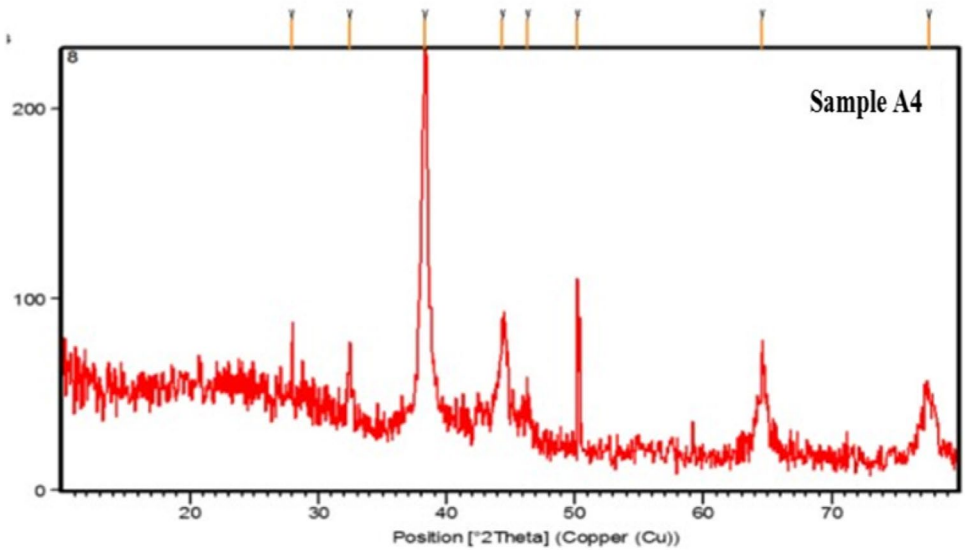

Fig. 4 XRD analysis of *Enteromorpha prolifera* AgNPs

diffractrometer spectrum of AgNPs clearly explained that the formation of spherical biosynthesized SNPs as the peaks have broader base and the narrower apex. This is the indication of the presence of reduced crystal size SNPs (Roy and Anantharaman 2018),whereas in *E. linza* only six diffraction peaks were reported at 27.83, 32.25, 46.19, 54.84, 57.55 and 76.74. The obtained AgNPs had a high purity due to the non-appearance of peaks of the XRD pattern of Ag_2O and other substances. The obtained peak broadening and noise were possibly related to the nanoparticles and the presence of biological macromolecules in the algal extracts (Dhanalakshmi et al. 2012).

FESEM study of *Enteromorpha prolifera* AgNPs

FESEM provided further insight into the morphology and size details of AgNPs obtained through *E. prolifera* extract. The FESEM study of *E. prolifera* was displayed in Fig. 5.

Table 2 XRD data of *Enteromorpha prolifera* AgNPs

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
27.93(3)	14(16)	0.2(1)	3.19220	10.80
32.4(2)	37(67)	1(1)	2.76257	28.87
38.2(3)	129(290)	1(1)	2.35192	100.00
44.4(2)	35(74)	0.5(4)	2.03794	27.54
46.33(5)	16(37)	0.3(1)	1.95803	12.49
50.247(3)	124(10)	0.081(9)	1.81430	96.13
64.6(2)	30(155)	0(2)	1.44123	23.31
78(16)	19(781)	1(28)	1.23047	14.83

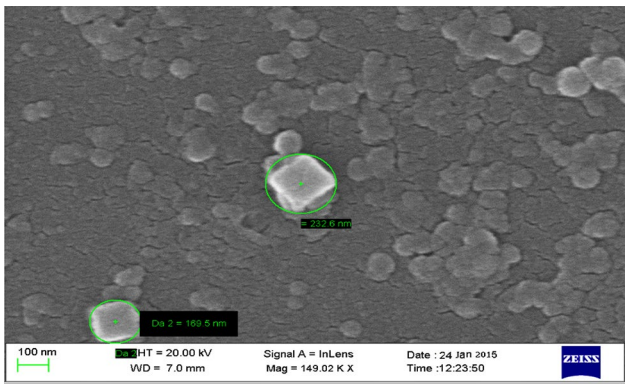

Fig. 5 FESEM image of *Enteromorpha prolifera* AgNPs

The AgNPs were spherical and well separated without aggregation. The size of the AgNP was ranged between 169.5 and 232.6 nm. Previous reports also indicate the size and shape of AgNPs obtained in *Enteromorpha compressa* (Dhanalakshmi et al. 2012; Ramkumar et al. 2017). The size of the AgNPs of the present study was almost similar to that of *Chaetomorpha antennina* (Roy and Anantharaman 2017) *Turbinaria conoides* (Sri Ramkumar Vijayan et al. 2014) and *Padina* sp AgNPs (Bhuyar et al. 2020).

EDX analysis of *Enteromorpha prolifera* AgNPs

EDX spectra recorded from the silver nanoparticles obtained from *E. prolifera* extract were shown in Fig. 6. The EDX analysis confirmed the presence of silver in *E. prolifera* AgNPs. A strong signal at 3 keV confirmed the presence of AgNPs' formation in the solution. The presence of a strong signal for silver atoms in the sample specified the purity of particles. The weak signals were observed for oxygen, sodium, magnesium, calcium, potassium, silicon, carbon and aluminum are due to the presence of various biochemical molecules in the sample responsible for the AgNPs synthesis. The EDX analysis of *Padina tetrastromatica* (Rajeshkumar 2017), *Chaetomorpha* sp. (Haq et al. 2019) confirmed the presence of silver. Ramkumar et al. (2017) also confirmed the presence of a strong signal in AgNP biosynthesized by *Enteromorpha compressa*.

Particle size analysis of *Enteromorpha prolifera* AgNPs

DLS and Zeta potential analysis of *E. prolifera* AgNPs revealed the average diameter and surface charge and also predicting the stability of the nanoparticles. The average

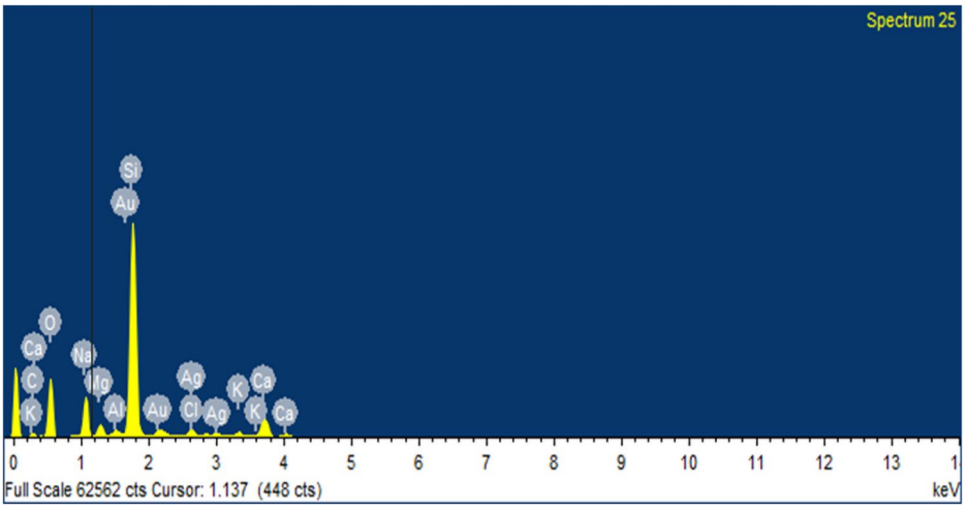

Fig. 6 EDAX report of *Enteromorpha prolifera* AgNPs

diameter of 103.2 nm was observed in the present study. The triplicate values of DLS analysis were 102.4, 101.8 and 105.6 nm (Table 3). The obtained graph and values of zeta potential are presented in Table 4 and Fig. 7. As per the table, the zeta potential value of synthesized AgNPs was − 30.88. Kingslin and Ravikumar (2016) reported that the average diameter of AgNPs obtained from *Padina tetrastromatica* was 88.39, whereas in *Valenopsis pachynema* was 103.3 nm (Kingslin and Ravikumar 2018). Zeta potential was associated with the mobility and stability of the colloidal suspension of AgNPs. A negative zeta potential of about -30.88 mV confirmed the ideal surface charge, repulsion amongst particles and high Energy barriers, which institute particle stability (Gnanakani et al. 2019).

Effect of *Enteromorpha prolifera* AgNPs on seed germination potential

The biosynthesis of AgNPs from seaweed viz. *E. prolifera* was subjected to phytotoxicity studies by testing its effect on *Vigna unguiculata*, *Vigna radiata* and *Cicer arietinum* L. seed germination. Figure 8 and Table 5 represent the data obtained through the seed germination assay of green synthesized AgNPs of *E. prolifera*. All the AgNPs' concentrations increased the germination percentage of *Vigna unguiculata* seeds over the control. The algal AgNPs increased the percentage of germination up to + 66.66% when compared to the control. The minimum increase percentage over control was + 33.33. It shows that all the concentrations of the algal AgNPs induced the germination rate in *V. unguiculata* seeds. In previous studies, it had been reported that *Sargassum cinctum* seaweed-mediated AgNPs had a positive and friendly effect and enhanced the growth of seedlings as well as seed germination percentage of *Abelmoschus esculantus* (Roy and Anantharaman 2017). In the present study also *E. prolifera* seaweed extract AgNPs exhibited a positive effect on *V. unguiculata* seed germination. In comparison to water, biosynthesized AgNPs showed a better effect on seed germination which may be due to the availability of biochemical components, minerals and antioxidants in

Table 3 Summary of particle size analysis of *Enteromorpha prolifera* AgNPs

Record No	Count rate (kcps)	Z-Average (d.nm)	Size (d.nm)	Intensity (%)	St. Dev. (d.nm)	PdI/Intercept
1	246.2	102.4	122.7	100.0	46.86	0.277/0.917
2	247.2	101.8	128.8	98.0	67.22	0.287/0.913
			5088	2.0	556.6	
3	248.3	105.6	139.9	93.9	59.38	0.273/0.914
			23.79	4.9	5.282	

Table 4 Summary of Zeta potential analysis of *Enteromorpha prolifera* AgNPs

Record No	Count rate (kcps)	Z-Potential (mV)	Mean (mV)	Area (%)	St.Dev (mV)	Z-Deviation (mV)	Conductivity (mS/cm)
1	185.1	− 30.8	− 16.2	51.8	9.78	25.4	0.298

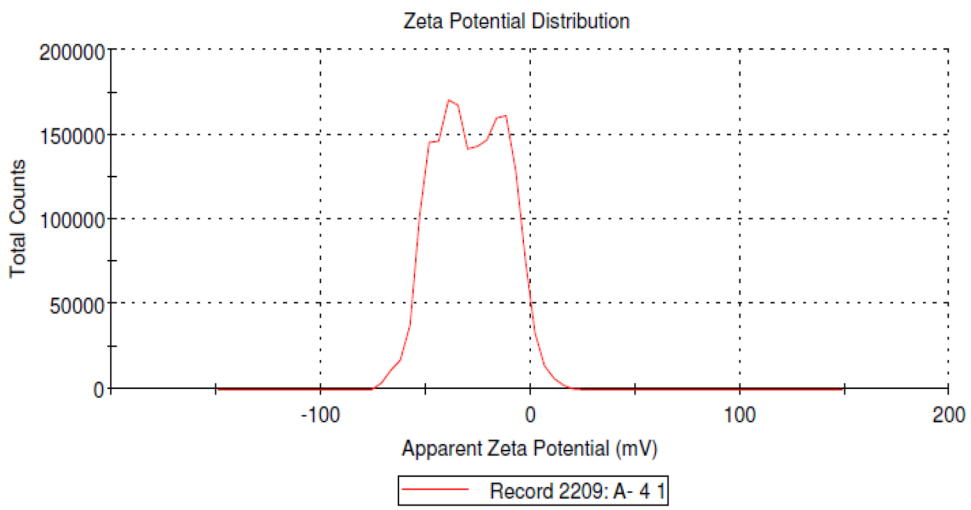

Fig. 7 Zeta potential of *Enteromorpha prolifera* AgNPs

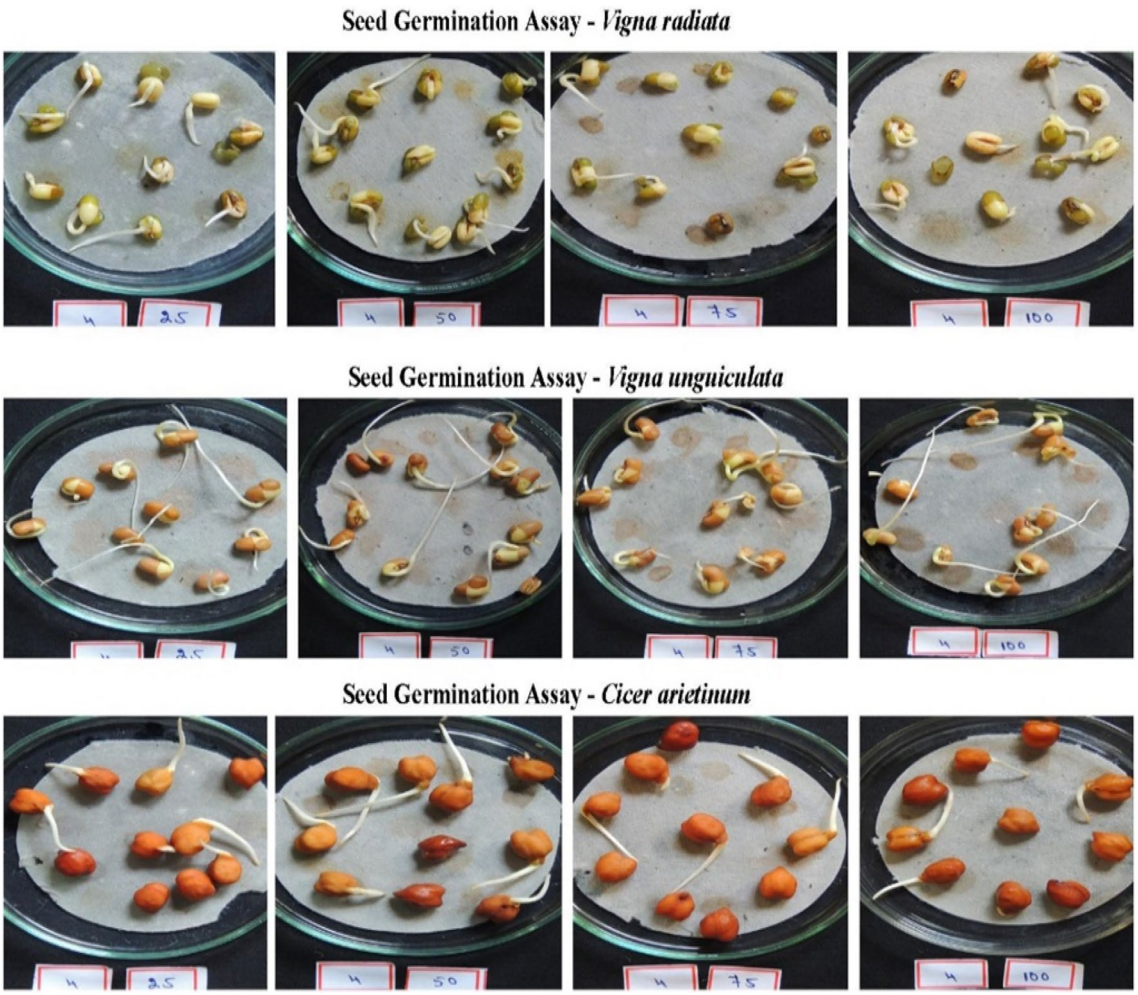

Fig. 8 Seed Germination assayof *Enteromorpha prolifera* AgNPs

the algal extract which is used for the synthesis of AgNPs (Roy and Anantharaman 2017), whereas the AgNPs synthesized from *Amphiroa anceps* also increased the seed germination percentage 75% and 80% in *Abelmoschus esculentus* and *Raphanus sativus* seeds respectively (Roy and Anantharaman 2018). The enhanced seed germination might be due to the high efficiency with which AgNPs absorbed moisture and nutrients, as the AgNPs penetrated the seed coat and triggered the embryo. By penetrating AgNPs, many new pores are created that contribute to effective nutrient absorption and faster germination (Srinivasan and Saraswathi 2010).

The results suggested that the *Vigna radiata* seeds treated with different concentrations of AgNPs extract enhanced the seed germination up to + 25% over the control. But in 75% AgNP concentration the seed germination was affected up to − 25%. The enhanced seed germination was mainly due to the seeds' ability to absorb water and nutrients. The AgNPs obtained from the various algal extracts induced water and mineral uptake leads to a higher germination rate. An almost similar observation was obtained from AgNPs effect on *Solanum lycopersicum* (Noshad et al. 2019) and *Abelmoschus esculentus* seeds (Roy and Anantharaman 2017).

Some of the previous reports explained that the AgNPs had a dose-dependent inhibiting effect on seed germination. The inhibitory effect of AgNPs might be influenced by the factors like size and concentrations of nanoparticles, temperature, duration, etc. (Amooaghaie et al. 2015). Some of the other studies showed that the AgNPs had little effect on *Cucumis sativus* or *Lactuca sativa* seeds (Barrena et al. 2009). From this study, macroalgae-mediated AgNPs had a positive effect on *Vigna radiata* seeds.

The AgNP-treated seed germination of *Cicer arietinum* has a significant increase in the germination percentage as well as in the percentage over control (Table 5 and Fig. 8). Seaweed extract AgNPs positively increases the seed germination percentage up to + 60% over the control. This is in agreement with the study of Kingslin and Ravikumar

Table 5 Seed germination assay of *Enteromorpha prolifera* AgNPs

Algae name	Plant name	Concentration	Germination (%)	Increase/ Decrease over Control
A4	*Vigna unguiculata*	Control	60 ± 5	
		25%	100 ± 3	+ 66.66
		50%	80 ± 2	+ 33.33
		75%	100 ± 8.50	+ 66.66
		100%	90 ± 5	+ 50
	Vigna radiata	Control	80 ± 2	
		25%	100 ± 3	+ 25
		50%	100 ± 4	+ 25
		75%	60 ± 4	− 25
		100%	80 ± 2	0
	Cicer arietinum	Control	50 ± 4	
		25%	80 ± 2	+ 60
		50%	80 ± 5	+ 60
		75%	60 ± 2	+ 20
		100%	70 ± 4	+ 40
Cd SEd($P < 0.05$)	3.12931 6.25958			

(2016) in *Padina tetrastromatica* AgNPs' role on *Cicer arietinum* seed germination. The increase in the percentage in AgNP-treated seeds of *C. arietinum* may be due to the role of AgNPs dosage in increasing seed water absorption capabilities (Zheng et al. 2005). *Sargassum ilicifolium* AgNPs exhibited good promoting efficacy on germination of *Abelmoschus esculentus* and *Raphanus* seeds (Roy and Anantharaman 2018). Yin et al. (2012) also recorded that the seed germination and plant growth were not adversely affected by silver-soluble in $AgNO_3$.

Effect of *Enteromorpha prolifera* AgNPs on embryonic axis study

Embryonic Axis Length (EAL), fresh weight (FWt) and dry weight (DWt) of the embryonic axis, and percentage increase/ decrease over the control of germinated seeds of *Vigna unguiculata*, *V. radiata* and *Cicer arietinum* against *E. prolifera* seaweed extracts synthesized AgNPs at different concentrations (25, 50, 75 and 100%) were studied. The effect of *E. prolifera* AgNPs on *Vigna unguiculata* seed tested for its EAL, FWt and DWt. The mean EAL was 3.34, 4.42, 4.96 and 4.82 cm and increase or decrease percentage of EAL over control was − 4.57, + 26.29, + 41.71 and + 37.71 across the AgNPs concentration 25, 50, 75 and 100% respectively. An increase or decrease in the percentage of DWt was observed as + 16.12, − 6.45, + 4.84 and + 9.67 over the control for the concentrations 25, 50, 75 and 100% respectively (Table 6). EAL study of *Vigna unguiculata* exhibited an increase in the EAL percentage over the control. The increase in the EAL percentage is mainly due to the increased water uptake. This is in agreement with the earlier study of AgNPs on maize plants (Mahakham et al. 2016). Thus, the higher water uptake may make seeds germinate faster and be able to develop EALs (Mahakham et al. 2017). Regarding DWt and FWt of the embryonic axis, there was a significant increase in the DWt over the control was noted in algal extract AgNPs' concentrations, whereas algal extract AgNPs concentration exhibited a decrease in the DWt over the control. This may be due to the toxic effect of AgNPs on embryonic axis length. Previous studies on the biological effects of AgNPs revealed higher toxicity (Yin et al. 2011). In plants, toxicity and uptake of AgNPs are influenced primarily by particle physicochemical properties, including the shape, size, surface coating and conditions of exposure, such as treatment length, time and plant area (Amooaghaie et al. 2015).

The EAL of Vigna radiata did not enhance by the *E. prolifera* AgNPs concentration when compared to control; it was decreased to -31.08, -11.49, -35.81 and -40.54 against four concentrations, whereas DWt of EAL was increased to + 25.21, + 14.28, + 400 and + 8.57% based on AgNPs concentrations. In *Cicer arietinum* seeds EAL, all the four concentrations of AgNPs exhibited a positive result with an increase percentage of + 84.21, + 72.63, + 44.73 and + 33.68, respectively.

But the DWt was increased to + 39.90 and + 23.41 for 25 and 50% concentration and decreased to − 14.28 and − 15.07% for 75 and 100% concentration over the control (Table 6). In *V. radiata* the various concentrations of AgNPs decreased the EAL percentage over control. Furthermore, AgNPs increased the DWt over control, whereas in *Cicer*

Table 6 Embryonic axis study of *Enteromorpha prolifera* AgNPs

Algal Sample	Plant Name	Conc. of AgNPs/ Mean	EAL cm Mean	% Increase / Decrease of EAL over C	FWt g Mean	DWt g Mean	Water g Mean	% Increase / Decrease of DWt over C
	Vigna unguiculata	Control	3.5 ± 0.26		0.150	0.062	0.088	
		25%	3.34 ± 0.06	− 4.57	0.194	0.063	0.131	+ 1.612
		50%	4.42 ± 0.03	+ 26.29	0.163	0.058	0.105	− 6.45
		75%	4.96 ± 0.02	+ 41.71	0.181	0.065	0.116	+ 4.84
		100%	4.82 ± 0.48	+ 37.71	0.182	0.068	0.114	+ 9.67
	Vigna radiate	Control	2.96 ± 0.02		0.175	0.035	0.14	
		25%	2.04 ± 0.02	− 31.08	0.141	0.044	0.097	+ 25.71
		50%	2.62 ± 0.02	− 11.49	0.151	0.040	0.111	+ 14.28
		75%	1.9 ± 0.44	− 35.81	0.154	0.049	0.105	+ 40
		100%	1.76 ± 0.1	-40.54	0.150	0.038	0.112	+ 8.57
	Cicer arietinum	Control	1.9 ± 0.44		0.585	0.252	0.333	
		25%	3.5 ± 0.25	+ 84.21	0.605	0.345	0.26	+ 39.90
		50%	3.28 ± 0.1	+ 72.63	0.665	0.311	0.354	+ 23.41
		75%	2.75 ± 0.1	+ 44.73	0.661	0.288	0.373	− 14.28
		100%	2.54 ± 0.1	+ 33.68	0.617	0.290	0.327	− 15.07
Cd	0.15440							
SEd($P < 0.05$)	0.30886							

Values are mean ÷ SD of three triplicates

arietinum embryonic axis length increased against all the concentrations of algal AgNPs. Additionally, the DWt of the algal AgNPs decreased over the control. In *E. prolifera* the lower concentration 25 and 50% increased the DWt whereas the higher concentration (75 and 100%) decreases the DWt over the control. The effect of AgNPs on embryonic axis growth for the seaweed appeared to be not related to the seed germination effect. Generally, the increase in seed germination percentage EAL also increased. But in some cases, the seed germination percentage is not related to EAL and DWt. Most of the AgNPs induced seed germination and also increase the length of the embryonic axis. But increase in EAL is not related to DWt increase or decrease. Similar results were obtained from watermelon and Zucchini plants by AgNPs (Almutairi and Alharbi 2015). Increase EAL with increased DWt over control was in agreement with a study in *Vicia faba* (Abdel-Azeem and Elsayed 2013) and *Eruca sativa* (Vannini et al. 2013) root length. The increase in dry weight and fresh weight of EAL in the present study was similar to the study of corn, watermelon and Zucchini plants seedling fresh weight and dry weight (Almutairi and Alharbi 2015).

Antioxidant activity

The antioxidant activity of aqueous extracts of *E. prolifera* AgNPs and algal solutions was analysed by using DPPH scavenging activity. The result of the DPPH assay and graphical representation of AgNPs of *E. prolifera* and algal extract samples were presented (Table 7). In both algal extract and AgNP samples, the DPPH values were a result of increasing doses. The higher inhibition percentage of DPPH for *E. prolifera* algal AgNPs was 60.2%, whereas the algal extract alone has 67.9% at the concentration/mL. The lowest IC50 value was 133.33 µg/mL against algal extract when compared to algal AgNPs 142.0 µg/mL (Table 7). Generally, the green algae possess polydisperse hetero-polysaccharides. *E. prolifera* polysaccharides had high lipid-lowering property and this might be the reason for antioxidants (Tang et al. 2013), whereas Cho et al. (2011) recorded that the strong antioxidant activity of *E. prolifera* extract is mainly from the chlorophyll compound. *E. prolifera* contains many secondary metabolites among which the compounds with polysaccharides being the most abundant (Zhao et al. 2016). In this observation, there was a steady increase in inhibition of radicals with the consequent increase in the concentration of the extract and AgNPs showed the dose dependence in scavenging DPPH radicals. Similar results were observed in *E. prolifera* methanolic extract (Sivaramakrishnan et al. 2017). The previous report revealed that the extracts obtained from *Enteromorpha intestinalis* (Mole and Sabale 2013), *Gracilaria corticata* (Ismail and Hong 2002) are having high scavenging activity to control against free radicals. The present data are in agreement with the report for brown macroalgae *Padina tetrastromatica* and green macroalgae *Valoniopsis pachynema* silver nanoparticles' antioxidant activities (Kingslin and Ravikumar 2016, 2018).

Table 7 DPPH radical scavenging activity of *Enteromorpha prolifera* AgNPs

Algae No	Components	Concentration (µg/mL) / OD value					
		100	200	300	400	500	IC50
A4	AgNPs	1.102 ± 0.10	1.008 ± 0.11	0.995 ± 0.06	0.950 ± 0.11	0.854 ± 0.26	142.0
	%	48.6	53.0	53.6	55.7	60.2	
	Extract	1.037 ± 0.02	1.022 ± 0.02	1.016 ± 0.01	1.000 ± 0.44	0.925 ± 0.03	133.33
	%	51.7	52.4	52.7	53.4	67.9	
Control		2.148	0.15	0.15	0.15	0.15	
Cd SEd($P < 0.05$)	0.14205 0.28126						

Values are mean ÷ SD of three triplicates

Toxicology study of *Lampito mauritii*

Toxicology study of *Enteromorpha prolifera* AgNPs on *Lampito mauritii* (earthworm)

Earthworms provided a suitable model for analyzing the metal hazards present in soils as well as studying the process of regeneration in soil. The present study has examined the effect of in vitro exposure of earthworms to *E. prolifera* algal extract AgNPs through the petridish filter paper method. The earthworm death time was noticed in the AgNPs' concentration 25, 50, 75 and 100%. Among these concentrations, the death of the earthworm occurred within 3 min of exposure at 25% concentration of the algal extract AgNPs, whereas in the control no death was occurred up to 120 min (Fig. 8). Furthermore, almost in all the AgNP concentrations, the dose-dependent death time was noticed, i.e. increase in the concentration decrease in the death time of earthworms. Coleman et al. (2010) performed a toxicity test by using Al_2O_3 nanoparticles and found that up to 10,000 mg/kg of Al_2O_3 nanoparticle, there was no death of earthworm. Further, they also stated that the earthworms avoid the NP enriched soil more than 5000 mg/kg of Al_2O_3 nanoparticles. In the present study, the death of earthworm occurred within 3 min of incubation at a lower concentration. But in the control, the death occurred after 120 min. This is might be due to the higher concentration of AgNPs (25, 50, 75 and 100%), whereas 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 the earthworm (Fig. 9).

Conclusion

Our experimental study revealed the noteworthy amalgamation of AgNPs utilizing macroalgae *E. prolifera* aqueous extract. The bioactive compounds of the algae are played a major role in this AgNP production. Silver nanoparticles were confirmed to form from crystals by XRD. Also, there is remarkable antioxidant activity was observed from these AgNPs. Silver nanoparticles created using this method do not contain any toxic reagents, making them potentially useful to biomedical applications.

Fig. 9 Toxicology study of *Enteromorpha prolifera* AgNPs

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Declarations

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.

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