

Antioxidant Properties of Green Synthesized Silver Nanoparticles from *Sargassum wightii*

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Abstract

Natural antioxidants and their association with health benefits have gained unprecedented attention in recent years. They have multiple functions in biological systems and mainly defense against oxidation that produce free radicals in food, chemicals and in living systems. During the past decades, synthesized nanoparticles from marine organisms and many of these substances have been demonstrated to possess exciting biological activities. With this background, *S. wightii* and its AgNPs were analyzed for their antioxidant properties. Green synthesis of silver nanoparticles was carried out using the aqueous extract of *S. wightii*. The aqueous extract of *S. wightii* showed significant potential for the quick reduction of silver ions. The antioxidant properties of green synthesized AgNPs was carried out and it showed significant activity when compared with standard. The present work of green synthesis of silver nanoparticles using *S. wightii* appears to be cost effective, eco-friendly, and an alternative to conventional method of synthesis. In the present study, the aqueous extract and its synthesized AgNPs showed high antioxidant activity to the best of our knowledge. Hence, green synthesized AgNPs of *S. wightii* can be used as a potential therapeutic drug for various diseases due to its bioactive properties.

Keywords: DPPH, Super Oxide Anion, Metal Chelating, Nitric Oxide, *S. wightii*, AgNPs.

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INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanotechnology is emerging as a cutting edge technology interdisciplinary with biology, chemistry and material science [1]. Nano-medicine is an emerging field expanding rapidly because of the development and incorporation of new nano composites into a range of products and technologies [2]. In recent years, the application of nanoparticles (NPs) in medicine has increased and expanded to the fields of molecular imaging [3], drug delivery [4], diagnosis and treatment of cardiovascular diseases [5], wound healing [6], anticancer [7, 8] and development of materials and medical devices with antimicrobial properties [9]. New applications of nanoparticles and nanomaterials are emerging rapidly in biomedical sciences [2, 10].

Seaweeds contain some valuable nutrients as well as pharmacologically active substances, and now-a-days there is a lot of interest in seaweed meals,

functional foods and nutraceuticals for human consumption [11]. *Sargassum*, one of the marine macro algae belonging to the class *Phaeophyceae*, is widely distributed in tropical and temperate oceans; it is large, cost-effective, important and ecologically dominant brown algae present in much of the tropics. It is found to be the most diverse genus among *Phaeophyta* in India and is represented by 38 species [12].

Sargassum wightii is one of the important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species [13]. Since *Sargassum wightii* is available in large quantities, it appears to be the most suitable raw material for commercial exploitation. Based on the literature, in the present study, the antioxidant activity was assessed by DPPH, super oxide anion radical scavenging activity, metal chelating activity and nitric oxide radical scavenging activity.

MATERIALS AND METHODS

Green Synthesis of Silver Nanoparticles (AgNPs) from *Sargassum wightii*

Green synthesis of AgNPs was carried out by the method of Song and Kim [14]. Best AgNPs synthesis was observed best in the aqueous extract of *S. wightii*, while other solvents extracts showed poor synthesis of AgNPs, and this may due poor solubility of solvent extracts and silver nitrate dissolved in water as reported by Jayaprakash *et al.*, [15]. Based on the results, antioxidant potential of aqueous extract and its green synthesized AgNPs from *S. wightii* was evaluated.

Evaluation of Antioxidant Potential

DPPH Assay

The antioxidant potential was determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity by the modified method of McCune and Johns [16]. The reaction mixture (3.0 ml) consisting of 1.0 ml DPPH in methanol, 1.0 ml methanol and 1.0 ml different concentrations of aqueous extract, synthesized AgNPs and standard BHT (20, 40, 60, 80 and 100 µg/ml) was incubated in dark for 10 min., after which the absorbance was measured at 517 nm against blank. For control, 1.0 ml of methanol was used in place of aqueous and synthesized AgNPs. Percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ is the absorbance of control and A₁ is the absorbance of sample.

Super Oxide Anion Radical Scavenging Assay

Super oxide anion radical scavenging activity was estimated according to the method of Jing and Zhao [17]. To 1.0 ml of NBT solution, 1.0 ml of NADH solution, 0.1 ml of PMS solution and 20, 40, 60, 80 and 100 µg/ml of aqueous extract, synthesized AgNPs and standard rutin were added together in their respective tubes and incubated at 25°C for 5 min. After 5 min. the absorbance was read at 560 nm. The percentage of scavenging activity was calculated by the following formula:

$$\text{Scavenging activity (\%)} = \frac{AC - AS}{AC} \times 100$$

Where, AC was the absorbance of the control (deionized water, instead of sample), and AS was the absorbance of the test sample mixed with reaction solution.

Metal Chelating Assay

Metal chelating activity was done according to the method of Soler-Rivas *et al.*, [18]. Different concentrations (20, 40, 60, 80 and 100 µg/ml) of

aqueous extract, synthesized AgNPs and standard EDTA were dissolved in DMSO. To this, 50 µl of ferrous chloride solution and 200 µl of ferrozine solution were added. The solutions were mixed thoroughly and incubated in dark at room temperature for 10 min. The absorbance was read at 562 nm. The percentage of metal chelating activity was calculated by the following formula:

$$\text{Chelating rate (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A₀ was the absorbance of the control (blank without extract) and A₁ was the absorbance in the present of the extract. Then IC₅₀ value was calculated from the formula on the graph.

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical inhibition was estimated by Griess Illosvoy reaction method of Green *et al.* (1982) [19]. A volume of 0.5 ml of sodium nitroprusside in phosphate buffered saline was mixed with 1.0 ml of aqueous extract, AgNPs and standard BHT (20, 40, 60, 80 and 100 µg/ml) in their respective tubes and incubated at 25°C for 180 min. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the samples but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. A volume of 150 µl of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 546 nm using a SpectraMaxPlus UV-Vis microplate reader (Molecular Devices, GA, USA). BHT was used as the positive control. The percentage nitrite radical scavenging activity of the sample and BHT were calculated using the following formula:

$$\text{Nitric oxide radical scavenging effect (\%)} = \frac{AC - AS}{AC} \times 100$$

Where, AC is the absorbance of control sample and AS is the absorbance of samples.

Statistical Analysis

The data with five replicates for antioxidant potential assays were subjected to statistical analysis and the mean value along with its respective standard error was calculated. The per cent change between control and experimental data were calculated. The data were analyzed statistically using Two Way Analysis of Variance (ANOVA). The data along with tables and graphs/bar diagrams are presented in appropriate places in the results section [20].

RESULTS

Evaluation of Antioxidant Potential

DPPH Activity

DPPH radical scavenging activity of aqueous extract and AgNPs of *S.wightii* and standard BHT are presented in Table-1. The per cent inhibition of DPPH

activity in aqueous and AgNPs was directly proportional to the concentration of the samples. When compared, the inhibition was high in AgNPs than that of the aqueous extract; the values being from -10.66 to -49.38 in aqueous extract and from -20.65 to -64.83 in AgNPs. When compared with that of standard BHT, the per cent inhibition of DPPH in AgNPs was more or less similar to that of the standard. Statistical treatment of

the data by two-way ANOVA revealed that all the values were significant at 5% level. The mean inhibitory concentration (IC_{50}) of aqueous extract, AgNPs and BHT was found to be 102.59 $\mu\text{g/ml}$, 59.67 $\mu\text{g/ml}$ and 53.94 $\mu\text{g/ml}$, respectively. The results clearly indicate that AgNPs have profound reducing activity against stable free radicals (Fig-1).

Table-1: DPPH radical scavenging activity of aqueous extract and AgNPs of *S. wightii*

Concentration	Aqueous Extract	AgNPs	BHT
Control	100 \pm 0	100 \pm 0	100 \pm 0
20 $\mu\text{g/ml}$	0.876 \pm 0.004* (-10.66)	0.965 \pm 0.003* (-20.65)	0.984 \pm 0.002* (-18.92)
40 $\mu\text{g/ml}$	0.712 \pm 0.001* (-27.42)	0.866 \pm 0.002* (-29.69)	0.758 \pm 0.002* (-37.56)
60 $\mu\text{g/ml}$	0.617 \pm 0.002* (-36.93)	0.641 \pm 0.001* (-50.35)	0.541 \pm 0.004* (-55.41)
80 $\mu\text{g/ml}$	0.548 \pm 0.003* (-44.17)	0.492 \pm 0.001* (-54.93)	0.358 \pm 0.003* (-70.54)
100 $\mu\text{g/ml}$	0.497 \pm 0.002* (-49.38)	0.384 \pm 0.004* (-64.83)	0.195 \pm 0.002* (-83.96)
IC_{50} ($\mu\text{g/ml}$)	102.59	59.67	53.94

Values are mean \pm S.E. of five individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control.

* Denotes that values are significant at $p < 0.05$.

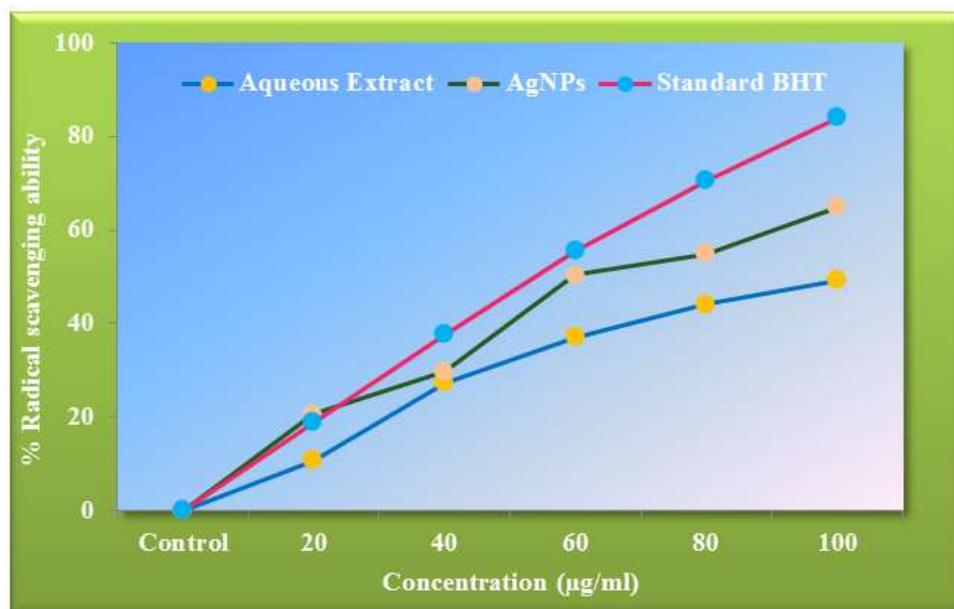


Fig-1: DPPH radical scavenging activity of aqueous extract and AgNPs of *S. wightii*

Super Oxide Anion Radical Scavenging Activity

Super oxide radical scavenging activity of aqueous extract and AgNPs of *S. wightii* depicted a per cent inhibition by SOD that was directly proportional to the concentration of the samples; the values being from -7.30 to -59.27 in aqueous extract and from -7.48 to -64.31 in AgNPs (Table-2). The per cent inhibition of SOD in AgNPs was more or less similar to that of the

standard and the values were significantly different from each other at 5% level when analyzed by two-way ANOVA. The median inhibitory concentration (IC_{50}) of aqueous extract, AgNPs and rutin was found to be 82.59 $\mu\text{g/ml}$, 59.08 $\mu\text{g/ml}$ and 55.72 $\mu\text{g/ml}$, respectively. The result pragmatically shows that AgNPs have profound super oxide activity against stable free radicals (Fig-2).

Table 2: Super oxide radical scavenging activity of aqueous extract and AgNPs of *S. wightii*

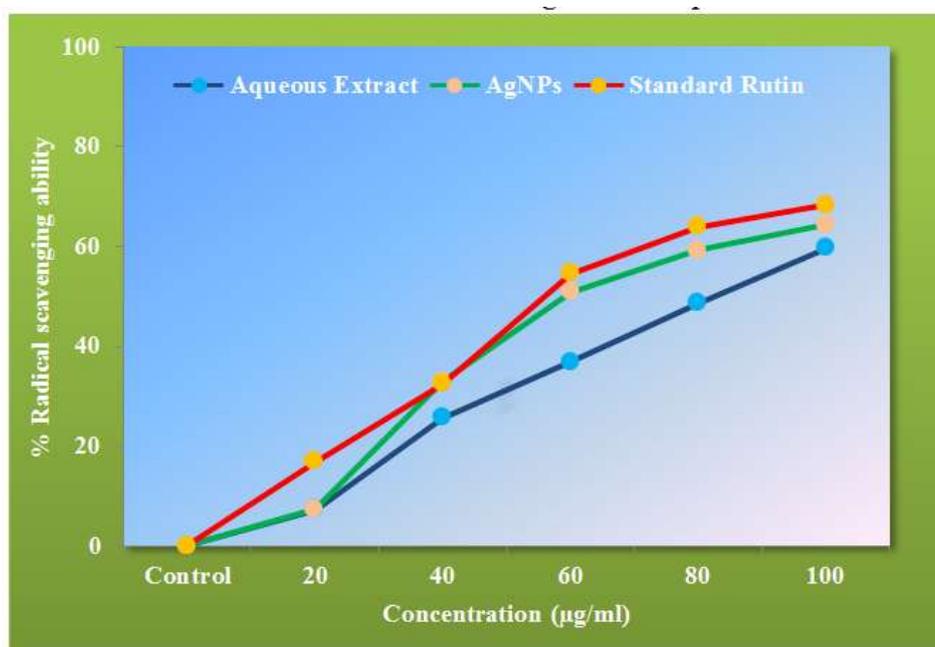
Concentration	Aqueous Extract	AgNPs	Rutin
Control	100 ± 0	100 ± 0	100 ± 0
20 µg/ml	0.681 ± 0.001* (-7.30)	0.73 ± 0.001* (-7.48)	0.741 ± 0.001* (-16.99)
40 µg/ml	0.547 ± 0.003* (-25.58)	0.529 ± 0.003* (-32.91)	0.601 ± 0.005* (-32.58)
60 µg/ml	0.464 ± 0.002* (-36.87)	0.388 ± 0.003* (-50.82)	0.405 ± 0.002* (-54.59)
80 µg/ml	0.378 ± 0.001* (-48.62)	0.322 ± 0.004* (-59.19)	0.322 ± 0.002* (-63.94)
100 µg/ml	0.299 ± 0.002* (-59.27)	0.282 ± 0.003* (-64.31)	0.282 ± 0.002* (-68.42)
IC ₅₀ (µg/ml)	82.59	59.08	55.72

Values are mean ± S.E. of five individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control.

* Denotes that values are significant at p<0.05.

**Fig-2: Super oxide radical scavenging activity of aqueous extract and AgNPs of *S. wightii***

Metal Chelating Activity

Metal chelating activity of aqueous extract and AgNPs of *S. wightii* and EDTA showed a per cent inhibition of -12.61, -14.99 and -20.37, respectively at 200 µg/ml concentrations. As the concentration increased, the per cent inhibition also increased giving a value of -61.11, -75.92 and -79.19 in all the three samples at 1000 µg/ml. All the values were found to be

significant at 5% level when analyzed by two-way ANOVA. The median inhibitory concentration (IC₅₀) of aqueous extract, AgNPs and standard EDTA was found to be 757.5 µg/ml, 634.22 µg/ml and 542.99 µg/ml respectively. The results altogether depicts that AgNPs have profound metal chelating activity against stable free radicals (Fig-3).

Table-3: Metal chelating activity of aqueous extract and AgNPs of *S. wightii*

Concentration	Aqueous Extract	AgNPs	Standard EDTA
Control	100 ± 0	100 ± 0	100 ± 0
200 µg/ml	0.330 ± 0.006* (-12.61)	0.321 ± 0.001* (-14.99)	0.301 ± 0.001* (-20.37)
400 µg/ml	0.278 ± 0.002* (-26.45)	0.252 ± 0.002* (-33.24)	0.238 ± 0.004* (-36.95)
600 µg/ml	0.005 ± 0.002* (-41.18)	0.202 ± 0.001* (-46.56)	0.169 ± 0.004* (-55.21)
800 µg/ml	0.18 ± 0.002* (-52.38)	0.126 ± 0.004* (-66.67)	0.099 ± 0.002* (-73.72)
1000 µg/ml	0.147 ± 0.002* (-61.11)	0.091 ± 0.002* (-75.92)	0.0787 ± 0.002* (-79.19)
IC ₅₀ (µg/ml)	757.5	634.22	542.99

Values are mean ± S.E. of five individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control.

* Denotes that values are significant at p<0.05.

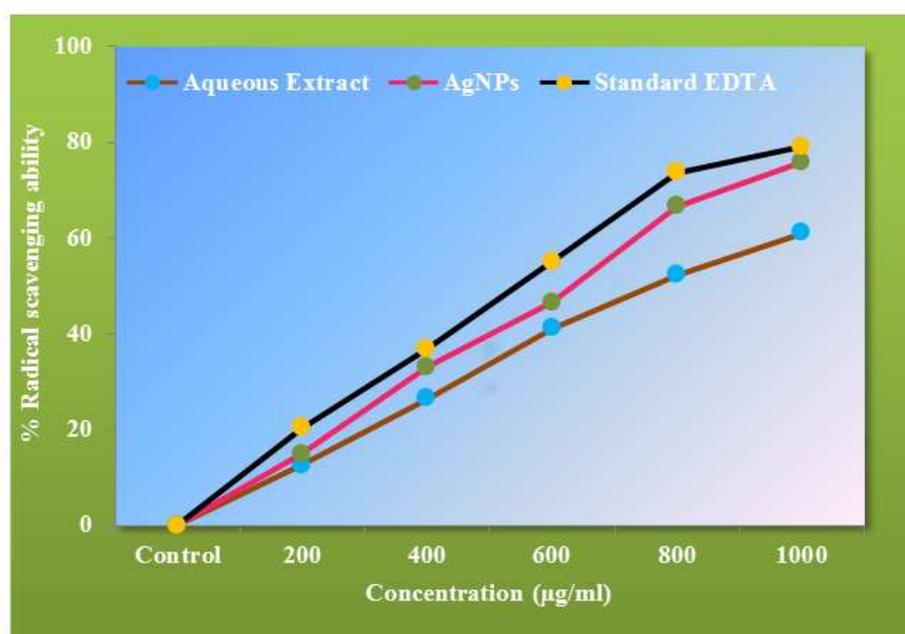


Fig-3: Metal chelating activity of aqueous extract and AgNPs of *S. wightii*

Nitric Oxide Radical Scavenging Activity

Nitric oxide radical scavenging activity of aqueous extract and AgNPs of *S. wightii* and BHT are presented in Table 4. The per cent inhibition in nitric oxide radical scavenging increased as the concentration of the samples increased; the values being -7.61 to -52.78 in aqueous extract, -14.39 to -67.78 in AgNPs and -17.85 to -81.88 in standard BHT. The per cent inhibition was higher in AgNPs than that of the aqueous

extract, thus revealing that AgNPs have profound antioxidant potential. Statistical analysis of the data by two-way ANOVA showed that all the values were significant at 5% level. The half maximal inhibitory concentration (IC₅₀) of aqueous extract, AgNPs and BHT was found to be 94.29 µg/ml, 69.05 µg/ml and 63.37 µg/ml, respectively. This proves that AgNPs have profound nitric oxide radical scavenging activity against stable free radicals (Fig-4).

Table-4: Nitric oxide scavenging activity of aqueous extract and AgNPs of *S. wightii*

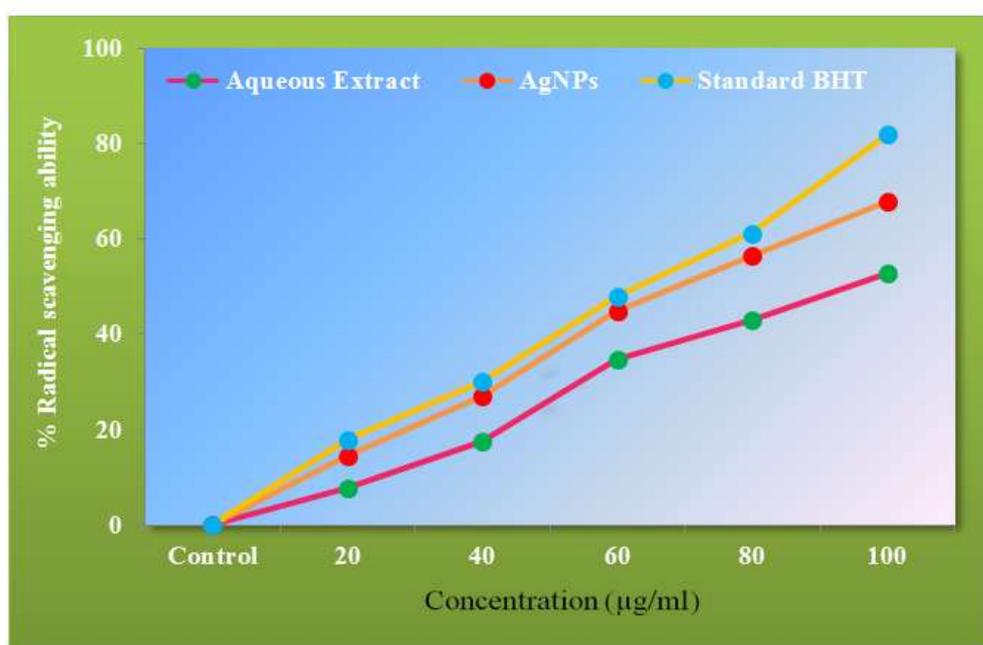
Concentration	Aqueous Extract	AgNPs	Standard BHT
Control	100 ± 0	100 ± 0	100 ± 0
20 µg/ml	0.648 ± 0.002* (-7.61)	0.591 ± 0.681* (-14.39)	0.707 ± 0.002* (-17.85)
40 µg/ml	0.579 ± 0.001* (-17.36)	0.504 ± 0.586* (-26.91)	0.604 ± 0.006* (-29.89)
60 µg/ml	0.459 ± 0.004* (-34.57)	0.382 ± 0.443* (-44.68)	0.45 ± 0.003* (-47.73)
80 µg/ml	0.399 ± 0.001* (-43.03)	0.301 ± 0.346* (-56.42)	0.334 ± 0.002* (-61.21)
100 µg/ml	0.331 ± 0.003* (-52.78)	0.222 ± 0.259* (-67.78)	0.156 ± 0.003* (-81.88)
IC ₅₀ (µg/ml)	94.29	69.05	63.37

Values are mean ± S.E. of five individual observations.

Values in parentheses are per cent change over control.

- Denotes per cent decrease over control.

* Denotes that values are significant at p<0.05.

**Fig-4: Nitric oxide scavenging activity of aqueous extract and AgNPs of *S. wightii***

DISCUSSION

According to Gupta and Abu-Ghannam [21], antioxidants are secondary metabolites that inhibit oxidation by transforming radicals into non-radicals by donating electrons and hydrogen, chelating transition metals and dissolving generated peroxidation compounds. The antioxidant compounds produced by plants include phenolic compounds such as flavonoids, cinnamic acid, benzoic acid, gallic acid, phlorotannins and quercetin. Among marine organisms, seaweeds represent one of the richest sources of antioxidants. *S. wightii* also shows a good amount of flavonoids in support and its antioxidant activity [22] indicating that this species is an ideal target for investigating the activity of the biomolecules present in *S. wightii* for various medical and industrial applications as opined by

Iswarya Devi *et al.*, [23]. Radical scavenging effect of natural antioxidants could be evaluated using DPPH, a free radical donor under *in vitro* conditions as pointed out by Matsukawa *et al.*, [24] and Jao and Ko [25].

Several authors have worked on antioxidant potential of seaweeds. Badrinathan *et al.*, [26] evaluated the radical quenching activity of *Sargassum myriocystum* by DPPH assay, deoxy ribose radical scavenging activity, super oxide anion radical scavenging, reducing power and total antioxidant activity. It was presumed by the authors that from the phenolic contents, algal extracts have some other novel bio-molecules, which are responsible for OH- radical quenching activity. Similarly, Siti *et al.*, [27] determined the antioxidant activity of brown algae *Sargassum* species like *Sargassum polyceratum*,

Sargassum angustifolium, *Sargassum filipendula*, *Sargassum hystrix* and *Sargassum cinereum*. The antioxidant properties were evaluated by using 2,2-Diphenyl-1-Picrylhydrazyl Radical Scavenging Activity (DPPH-RSA) and Ferrous Ion-Chelating (FIC) ability. The authors are of the opinion that the antioxidant property of *Sargassum* sp., was because of presence of high levels of phenolic and flavonoid content. In the present study also, the antioxidant property of *S. wightii* and its AgNPs might be due to the presence of phenolic and flavonoid content in *S. wightii*, thus finding support from the above authors.

Super oxide dismutase (SOD) is a metallo-enzyme that catalyzes the dismutation of super oxide radical into hydrogen peroxide (H₂O₂) and molecular oxygen (O₂) and consequently provides an important defense mechanism against super oxide radical toxicity as pointed out by Nishikimi *et al.*, [28]. Liochev and Fridovich [29] have stated that super oxide anion radicals are formed in cellular oxidation reactions, and these radicals can result in the production of hydrogen peroxide and hydroxyl radicals through dismutation and other chemical reactions. Super oxide anions have a longer life time and can move over greater distances than other oxygen radicals; hence, they are more damaging. Therefore, the ability to scavenge super oxide anions is an important biological property for a therapeutic compound.

Singlet oxygen (O₂-) is formed in almost all the viable and living cells through several metabolic reactions [30] and its effect can be magnified because it produces other types of free radicals and oxidizing agent that can induce cell damage, which leads to apoptosis or necrosis [31]. Antioxidants in algal extracts may inhibit both oxygen consumption and hydrogen peroxide formation. It has been shown that hydrogen peroxide together with reactive oxygen species (ROS) can damage several cellular components [32]. Hydroxyl radical has the highest 1-electron reduction potential (2310 mV) and can react with anything in living organisms at the second order rate constants of 109 to 1010 mol /L /s [33], among various free radical compounds. It can react with lipids, polypeptides, saccharides, nucleotides and organic acids, especially thiamine and guanosine and thereby cause cell damage [34]. The results obtained by Badrinathan *et al.*, [26] concluded that *Sargassum myriocystum* showed highest inhibition against OH-radicals than other reactive oxygen species, that apart from the phenolic contents, algal extracts have some other novel bio-molecules, which are responsible for OH- radical quenching activity.

Zhang *et al.*, [35] in *Sargassum graminifolium* assessed antioxidant activities of SGP by determining its reducing power, its ability to scavenge superoxide radicals, and its activity in the 1,1-diphenyl-2-

picrylhydrazyl (DPPH) assay. The scavenging of the DPPH free radical by antioxidants is due to their hydrogen-donating ability as opined by Bortolomeazzi *et al.*, [36] and Fagali and Catal [37]. Moreover, Wang *et al.*, [38] and Hassas-Roudsari *et al.*, [39] have stated that reducing power assays are used to evaluate the capacity of natural antioxidants to donate an electron. Natural antioxidants are believed to break free-radical chain reactions by donating an electron or hydrogen atom to free radicals. Therefore, the reducing power of a compound is a significant indicator of its potential antioxidant activity. In the present case also, the DPPH radical scavenging activity might be due to hydrogen-donating ability of the *S. wightii* and its ability to break free-radical chain reactions by donating an electron or hydrogen atom to free radicals.

Govindaraju *et al.*, [40] determined antioxidant activity of the biosynthesized AgNPs and analyzed using ultrasonic-induced lipid peroxidation in the liposomal membrane of *Sargassum vulgare*. The study revealed that the biosynthesized AgNPs exhibited the antioxidant activity in a dose-dependent manner. This is in agreement with the previous reports on the antioxidant property of the biosynthesized AgNPs by Veerapandian *et al.*, [41]. It is well known that the surface chemistry of the NP plays a vital role in their cellular responses as stated by Krishnamoorthy *et al.*, [42]. Previous study by Jain *et al.*, also demonstrated that AgNPs treatment reduce the lipid peroxidation and increase the glutathione and superoxide dismutase levels in primary fibroblast cells. However, the generation of free radicals by chemically synthesized AgNPs has also been reported by Vankayala *et al.*, [43]. These divisive results arise from the synthesis methods and the nature of reducing agent used for the preparation of AgNPs.

Antioxidant property of biosynthesized AgNPs was due to the following reasons: alginate, being a natural antioxidant material, acted as a reducing and stabilizing agent in the biosynthesis, resulting in the surface modification of AgNPs and the fact was supported by Zubia *et al.*, [44], Kanagalakshmi *et al.*, [45], Premanathan *et al.*, [46], Veerapandian *et al.*, [41] and Govindaraju *et al.*, [40]. The similar type of observations were seen in the biosynthesized AgNPs using *Trichoderma viride* by Fayaz *et al.*, [47] and *Brevibacterium casei* by Kalishwaralal *et al.*, [48]. Hence, the surface modification of AgNPs during biosynthesis plays a vital role in their intracellular response. In conclusion, the biosynthesized AgNPs inhibited the lipid peroxidation-mediated reactive oxygen species generation thus preventing the irradiation-related carcinogenesis.

In the present study, the antioxidant activity was assessed by DPPH, super oxide anion radical scavenging activity, metal chelating activity and nitric

oxide radical scavenging activity. The reports of the above authors support the observations of the present study. In our study also, a similar mechanism of surface modification of AgNPs leading to inhibition of lipid peroxidation-mediated reactive oxygen species generation, thus preventing the irradiation-related carcinogenesis might have operated leading to the inhibition of DPPH, super oxide radical scavenging, metal chelating and nitric oxide radical scavenging activity in aqueous and AgNPs of *S. wightii*. Moreover, the AgNPs might have endocytosed into the cells and damaged the DNA leading to apoptosis.

CONCLUSION

In the present study, the aqueous extract and its synthesized AgNPs showed high antioxidant activity to the best of our knowledge. Hence, green synthesized AgNPs of *S. wightii* can be used as a potential therapeutic drug for various diseases due to its bioactive properties.

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