*Research Article*

# Free radical scavenging activity of methanolic extract of marine red algae *Actinotrichia fragilis*

**Baskaran Krishnan*, Rathi M. A., Nirmaladevi N.**

*Department of Biochemistry, Sree Narayana Guru College, Coimbatore, Tamilnadu, India*

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**Abstract**

**Objective:** Antioxidants in seaweeds have attracted increasing interest for its role in protecting human health. The present study was designed to assess the antioxidant and reactive oxygen species (ROS) scavenging activities of 70% methanol extract of the red algae *Actinotrichia fragilis*. **Material and Methods:** The evaluation of antioxidant properties was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis 3ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay (NO), reducing power, hydroxyl, superoxide radical scavenging (SOD), hydrogen peroxide radical assay, the metal chelating activity as well as phosphomolypdenum assay. **Results:** Among the tested red algae, the maximum antioxidant activity was recorded in the methanol extract of *Actinotrichia fragilis*. Whereas methanol crude extract of red algae and diatom showed good antioxidant potential. **Conclusion:** This study suggests that methanol crude extracts contain different potential antioxidant compounds capable to scavenge different types of free radicals.

***Keywords:*** *Actinotrichia fragilis*, Free radical scavenging, Antioxidant activity

## Introduction

Antioxidants perform multiple functions which include defending against oxidative damage and cell signaling. One major function of antioxidants in biological system is to prevent the damage of cellular components by reactive oxygen species. Various free radicals with different biological, chemical and physical properties such as hydroxyl, alkoxyl, peroxyl, superoxide, nitric oxide, sulfur and nitrogen centered radical damages cells by chain reactions. As a result the oxidative stress generates and causes neural disorders, cardiovascular diseases, cancer, diabetes and arthritis (Devasagayam et al., 2004). Natural antioxidants with a multifunctional potential area of high interest as alternatives for synthetic antioxidants to prevent oxidation in complex food systems like muscle food. Aquatic plants are also gaining interest as a potential source of antioxidants. Algae are grouped into two main categories; the green algae, found in both benthic and littoral habitats and also throughout the ocean waters as phytoplankton, and the green algae or seaweeds, which occupy the littoral zone, and can be classified as red (Rhodophyta), brown (Phaeophyta) or green (Chlorophyta), depending on their nutrient and chemical composition (Dawczynski et al., 2007; Gupta and Abu, 2011).

Recently, the potential antioxidant compounds were identified as some pigments (fucoxanthin, astaxanthin, carotenoid e.g.) and polyphenols (phenolic acid, flavonoid, tannins e.g.). Those compounds are widely distributed in plants or seaweeds and are known to exhibit higher antioxidant activities. Seaweeds are noted to contain not only labile antioxidants (i.e. ascorbate, glutathione) when fresh (Kakinuma et al., 2001), but also, more stable molecules such as carotenoids (Yan et al., 1999), mycosporine-like amino acids (Nakayama et al., 1999) and a variety of polyphenols (e.g. catechins, phlorotannins) (Yoshie et al., 2000). More reports that are recent revealed seaweeds to be a rich source of antioxidant compounds (Lim etal., 2002; Park et al., 2004; Kuda et al., 2005; Duan et al., 2006).

Many synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroxyquinone (TBHQ), ascorbic acid (AA) have been widely used in different food products. However,

***Address for Corresponding Author:***
Dr. K. Baskaran, M.Sc., M.Phil., Ph.D.
Assistant Professor
Department of Biochemistry, Sree Narayana Guru College, Coimbatore-641105 Tamilnadu, India
Email: baskar.bio86@gmail.com

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because of the potential health hazards, their use as food additives is under strict regulation in many countries. Marine algal extracts have also been demonstrated to have strong antioxidant properties (Yuan and Walsh, 2006). The Phaeophyta (brown seaweeds) shows comparatively higher antioxidant activity than green and red algaes (Al-amoudi et al., 2009).

*Actinotrichia fragilis* is red or yellow with cylindrical branches about 1 mm in diameter that are covered with closely spaced rings of short, dark, stiff hairs, Figure.1. This lightly calcified seaweed is from 2 to 6 cm high and grows in tide pools, on reef flats, and in deeper subtidal habitats. *Actinotrichia fragilis* is a small (1±5–5 cm high) calcified, dichotomously divided multiaxial species, with an Indo-Pacific tropical distribution (Itono, 1979). Tetrasporophytes and dioecious gametophytes are isomorphic.

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities (Cox et al., 2010) with antiviral, antibacterial and antifungal activities (Delval et al., 2001) which acts as potential bioactive compounds of interest for pharmaceutical applications (Solomon et al., 2008). Most of these bioactive substances isolated from marine algae are chemically classified as brominated, aromatics, nitrogen-heterocyclic, nitrosulphuric-heterocyclic, sterols, dibutanoids, proteins, peptides and sulphated polysaccharides (Kolanjinathan et al., 2011). Therefore, the present investigation was attempted to study the antioxidant properties of methanolic crude extracts of marine diatom red algae *Actinotrichia fragilis.*

## Materials and methods

### Chemicals

DPPH, ABTs and FRAP were obtained from Sigma Aldrich (Steinheim, Germany). Methanol was of HPLC grade (Lab-Scan, Dublin, Ireland). All the other reagents were of analytical grade and obtained from Merck (Darmstadt, Germany).

### Algal materials

*Actinotrichia fragilis* red algae were collected from the Rameswaram area on January 5th, 2018. The freshly collected seaweeds were washed with clean seawater to remove salt, epiphytes and sand attached to the surfaces of the samples and transported to the laboratory. The samples were carefully rinsed with tap water, wiped with paper towel. For *Actinotrichia fragilis* the stipes and hapteres were removed and the new and old parts of the blades were separated. The samples were lyophilized for 72 h, pulverized into powder and stored at 80ºC prior to extraction.

### Preparation of sample extract

Take 5 g of *Actinotrichia fragilis* powdered seaweed was extracted overnight with 100 ml methanol at room temperature and centrifuged at 2800 rpm for 10 min. The supernatant was collected in a separate bottle after passing through a filter paper and the residue was re-extracted three times under the same conditions as mentioned above. The combined extracts were freeze dried. These extracts were kept at 80 ºC until analysis. The freeze dried extracts were redissolved in methanol and used for the analysis.

**Figure 1.** Marine red algae *Actinotrichia fragilis*

### DPPH radical scavenging activity

Various concentrations of *Actinotrichia fragilis* of the sample (4.0 mL) were mixed with 1.0 mL of methanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM (Subhashini et al., 2011). The mixture was shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517nm. AA was used as a control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\% \text{ decolorization} = [1-(ABS_{sample}/ABS_{control})] \times 100$$

$IC_{50}$ values (mg extract/mL) where the inhibitory concentration at which DPPH radicals were scavenged by 50%. AA was used for comparison.

### $ABTS^+$ scavenging activity

Samples were diluted to produce 200-1000 µg/mL. The reaction was initiated by the addition of 1.0 mL of diluted $ABTS^+$ to 10 mL of different concentrations of *Actinotrichia fragilis* of the sample or 10 mL methanol as control (Huang et al., 2011). The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation

$$I = A1/A0 \times 100$$

Where $A_0$ is the absorbance of control reaction and $A_1$ was the absorbance of test compound.

**Ferric-reducing antioxidant power assay (FRAP)**

A stock solution of 10mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40mM HCL, 20Mm $FeCl_3.6H_2O$ and 0.3M acetate buffer (pH 3.6) was prepared (Xu et al., 2009). The FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution, and 25 mL acetate buffer. It was freshly prepared and warmed to 37ºC. FRAP reagent (900 mL) was mixed with 90 mL water and 30 mL *Actinotrichia fragilis* of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37ºC for 30 minutes and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyltriazine ($Fe^{3+}$ -TPTZ) complex was reduced to ferrous ($Fe^{2+}$) form. The absorption at 540 nm was recorded.

**Nitric oxide radical activity**

Nitric oxide radical generated from sodium nitroprusside was measured (Saket et al., 2010). Briefly, the reaction mixture (5.0 mL) containing sodium nitroprusside (5mM) in phosphate-buffered saline (pH 7.3), with *Actinotrichia fragilis* sample at different concentration was incubated at 25ºC for 3 hours. The nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion which was assayed at 30 minute intervals by mixing 1.0 mL of incubation mixture with an equal amount of Griess reagent. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm.

**Reducing Power Assay**

The reducing power was determined as described (Yen and Chen, 1995). Briefly, 0.13 mL of *Actinotrichia fragilis* different concentration (200-1000 µg/mL) in phosphate buffer (0.2 M, pH 6.6) were mixed with 0.125 mL of potassium ferricyanide (1%, w/v) and incubated at 50ºC for 20 min. Afterwards, 0.125 mL of TCA (10%, w/v)were added to the mixture to terminate the reaction. Then, the solution was mixed with 1.5 mL ferric chloride (0.1%, w/v) and the absorbance was measured at 700 nm.

**Hydroxy radical activity**

The reaction mixture 3.0 mL contained 1.0 mL of 1.5mM $FeSO_4$, 0.7 mL of 6mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate, and varying concentrations of *Actinotrichia fragilis* sample (Naskar et al., 2010). After incubation for 1 hour at 37ºC, the absence of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as:

$$\text{Scavenging activity} = [1-(A1 - A2)/A0] \times 100\%$$

Where A0 was the absorbance of the control (without extract), A1 was the absorbance in the presence of the extract, and A2 was the absorbance without sodium salicylate.

**Superoxide anion radical scavenging activity**

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition (Chou et al.,2009). The 3 mL reaction mixture contained 50 mL of 1M NBT, 150 mL of 1M NADH with or without sample, and Tris buffer (0.02M, pH 8.0). The reaction was started by adding 15 mL of 1M PMS to the mixture and the absorbance change was recorded at 560 nm after 2 minutes. Percent inhibition was calculated against a control without the extract.

**Hydrogen peroxide radical**

*Actinotrichia fragilis* against $H_2O_2$ was measured according to the method (Delpour et al., 2009). A solution of 40 Mm $H_2O_2$ was prepared in phosphate buffer (pH-7.4). Next, 1.4 mL of different concentrations (200-1000 µg/mL) of the *Actinotrichia fragilis* was added to 0.6 mL of the $H_2O_2$ solution. The assay mixture was allowed to stand for 10 minutes at 25°C and the absorbance measured against a blank solution at ƛ max 230 nm. The *Actinotrichia fragilis* on $H_2O_2$ scavenging capacity index was calculated as follows:

$$\text{Scavenging capacity index} = \frac{A_{Blank} - A_{Test}}{A_{Blank}} \times 100$$

*Actinotrichia fragilis* was expressed as $IC_{50}$, which is defined as the concentration (mg/mL) of the *Actinotrichia fragilis* required to scavenge 50 % of $H_2O_2$. A is the absorbance.

**Metal ion Chelating activity**

The reaction mixture contained 1.0 mL of various concentrations of the *Actinotrichia fragilis* sample, 0.1 mL of 2mM $FeCl_2$, and 3.7 mL methanol (Smirnoff and Cumbes, 1989). The control contained all the reaction reagents except the sample. The reaction was initiated by the addition of 2.0 mL of 5mM ferrozine. After 10 minutes at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance of the reaction mixture indicated a higher iron chelating ability. The capacity to chelate the ferrous ion was calculated by

$$\%\ \text{chelation} = [1 - (ABS_{sample}/ABS_{control})] \times 100.$$

**Phosphomolybdenum assay:**

The phosphomolybdenum assay used for determining the antioxidant capacity is based on the reduction of $M_0$ (VI) – $M_0$ (V) by the antioxidants and subsequent formation of a green phosphate/$M_0$ (V) complex at acid pH 0.3 mL of *Actinotrichia fragilis* sample is taken in a tube and mixed with 3 mL of reagent solution containing 0.6 M sulphuric

acid, 28 mM sodium phosphate and 4 mM ammonium molybdate and incubated at 95°C for 90 min. AA is utilized as a reference standard. The absorbance of the mixture is then measured at 695 nm with methanol blank. The antioxidant activity is expressed as the number of gram equivalents of AA (Sahaa et al., 2008).

**Statistical analysis**

All the assays were carried out in triplicate. Experimental results are expressed as mean ± standard deviation. The results were analyzed using one-way analysis of variance and the group means were compared using Duncan's multiple range tests using SPSS version 16.

**Results and discussion**

DPPH have been used extensively as a free radical to evaluate reducing substances (Cotelle et al., 1996; Revathi et al., 2015). This purple color generally fades/disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e., 2, 2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517nm. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract. The DPPH radical assay is a suitable model for estimating radical scavenging activities of antioxidants. *Actinotrichia fragilis* exhibited a significant dose dependent inhibition of DPPH activity. This had a lesser activity then the standard of Ascorbic acid (AA). The results are presented in figure.2 the $IC_{50}$ value of AA and *Actinotrichia fragilis* was 65.57 µg/mL, 71.26 µg/mL, respectively.

ABTS assay is a simple indirect method for determining the activity of natural antioxidants. In the absence of phenolics, ABTS radical is rather stable, but it reacts energetically with an H-atom donor such as phenolics, being converted into a non-colored form of ABTS (Roginsky and Lissi, 2005; Pandian et al., 2015). The ABTS radical cation-scavenging assay performed showed that the antioxidant activity increases with an increase in the concentration. The inhibition capacity of the radical $ABTS^{+}$ and the AA% values, expressed in *Actinotrichia fragilis,* for the different concentration were presented in figure.3 the IC50 value of AA and *Actinotrichia fragilis* was 65.57 µg/mL, 69.51 µg/mL, respectively.

Ferric-reducing power is an important indicator of the antioxidant potential of a compound or an extract (Vinayak et al., 2011).The ability to reduce ferric ions indicates that the antioxidant compounds are electron donors and could reduce the oxidized intermediate of lipid peroxidation processes, thus acting as primary and secondary antioxidants (Matanjum et al., 2008; Yen and Chen,1995).The antioxidant activity of the methanolic crude extract determined by FRAP assay varied as seen in

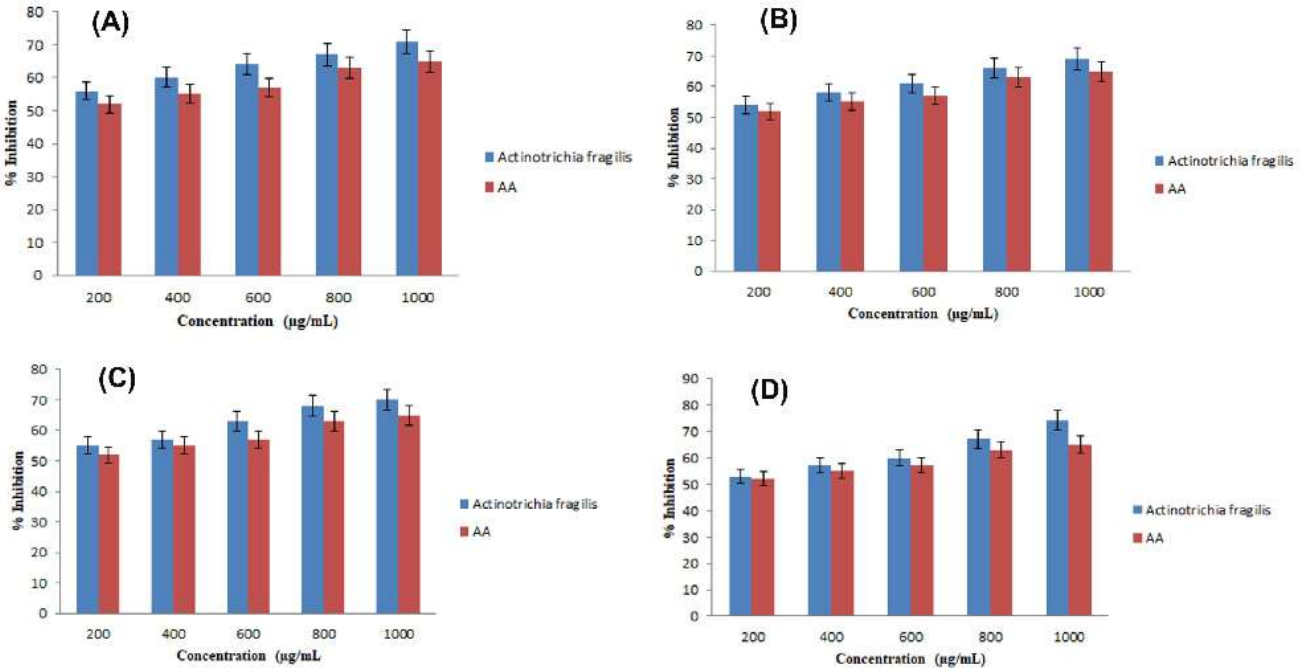


**Figure 2.** Figure shows radical scavenging activity of *A. fragilis* : (A) DPPH radical scavenging activity, (B) ABTS radical scavenging ability, (C) FRAP radical scavenging ability, (D) Nitric Oxide Radical scavenging. Each value is expressed as mean ± standard deviation (n=3)

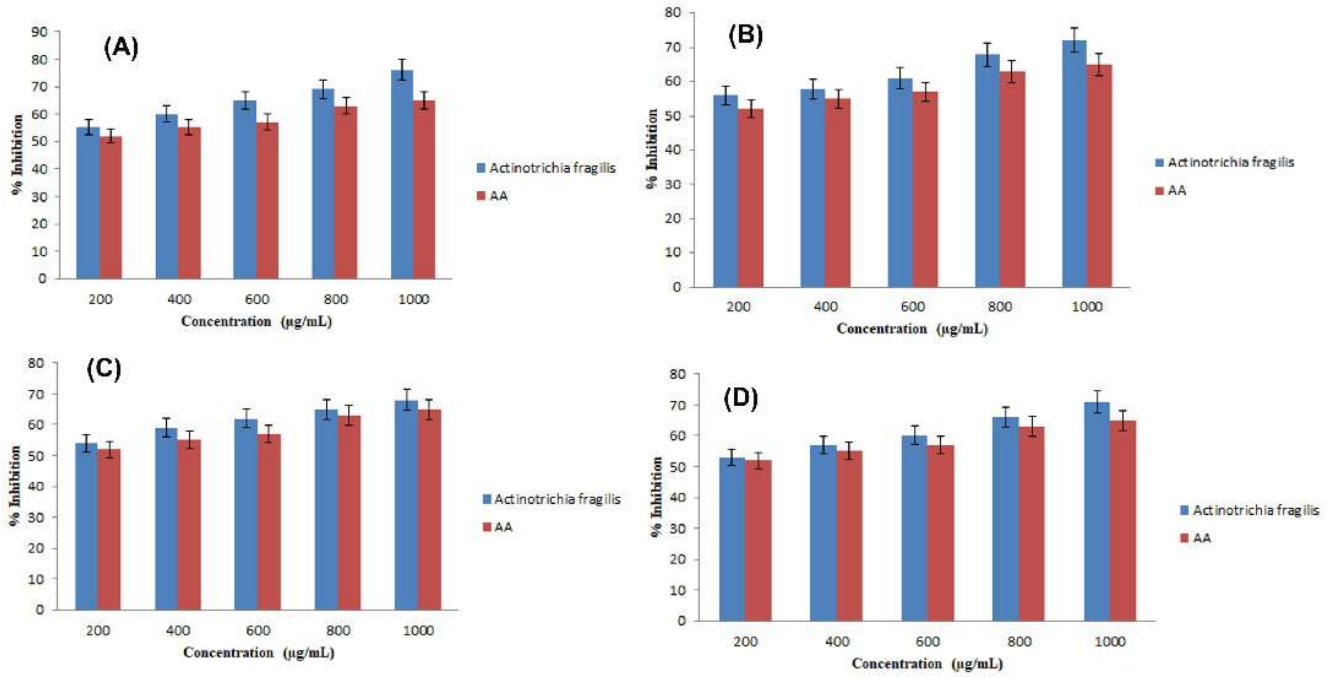


**Figure 3.** Figure shows radical scavenging activity of *A. fragilis* in different assays: (A) Reducing Power Radical scavenging ability, (B) Hydroxy Radical scavenging ability, (C) Superoxide Anion Radical scavenging ability, (D) Hydrogen Peroxide Radical scavenging ability Each value is expressed as mean ± standard deviation (n=3).

figure 4. The reducing power were found to be higher in methanolic extract. At concentration of 70.43 µg/mL of *Actinotrichia fragilis* 50% of FRAP generated by incubation was scavenged. The $IC_{50}$ value of AA was 65.57 µg/mL.

Active oxygen species and free radicals are involved in a variety of pathological events nitric oxide radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with $O_2$- radicals to form peroxynitrite, which damages biomolecules like proteins, lipids and nucleic acids (Moncada et al., 1991). Nitric oxide is generated when sodium nitroprusside reacts with oxygen to form nitrite. Seaweeds inhibit nitrite formation by competing with oxygen to react with nitric oxide directly. These compounds alter the structure and function of many cellular components. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage (Sanja et al ., 2009). The nitric oxide radical scavenging assay was also performed with methanolic crude extract of the seaweed samples. The scavenging of nitric oxide by Actinotrichia fragilis was increased in a dose-dependent manner as illustrated in Figure.5. At concentration of 74.65 µg/mL of Actinotrichia fragilis 50% of nitric oxide generated by incubation was scavenged. The IC50 value of AA was 65.57 µg/mL.

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound or sample (Nakayama et al., 1999). The presence of reductants (i.e. antioxidants) causes the reduction of the $Fe^{3+}$/ferricyanide complex to the ferrous form. Therefore, by measuring the formation of Perl's Prussian blue at 655 nm, the amount of $Fe^{2+}$ can be monitored. Higher absorbance indicated higher reducing power (Rashmi et al., 2010). Figure.6 elucidated the reductive capabilities of *Actinotrichia fragilis* compared to

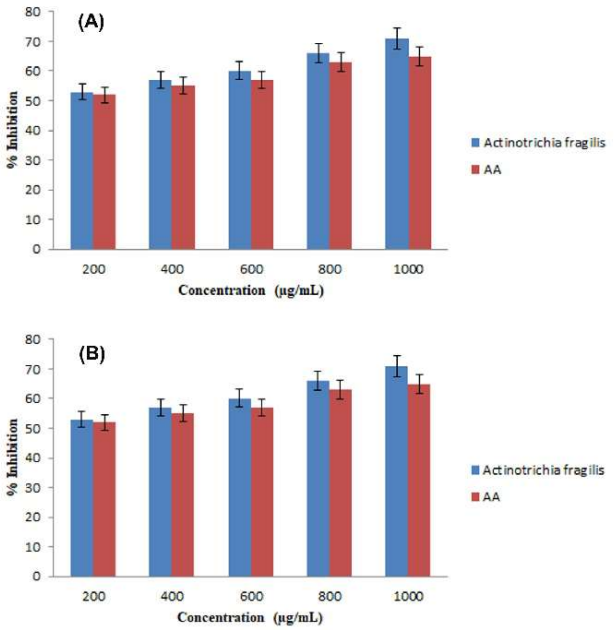


**Figure 4.** (A) Metal Chelating ability, (B) Phosphomolybdenum Radical scavenging ability of *A. fragilis*. Each value is expressed as mean ± standard deviation (n=3).

AA. The reducing power of *Actinotrichia fragilis* was increased with quantity of sample *Actinotrichia fragilis* could reduce the most $Fe^{3+}$ ions, which had a lesser reductive activity than the standard of AA. The $IC_{50}$ value of *Actinotrichia fragilis* and AA was 76.08 µg/mL and 65.57 µg/mL respectively.

Hydroxyl radical are the major active oxygen causing lipid peroxidation in enormous biological damage. The highly reactive hydroxyl radical can cause oxidative damage to DNA, lipid and protein (Baskaran et al., 2014; Subashini and Prasanth, 2014). In this study, *Actinotrichia fragilis* was found to scavenge $O_2$ significantly and in dose dependent manner and may protect the DNA, protein and lipid from damage. The results for hydroxyl scavenging assay are shown in figure 4. The concentrations for 50% inhibition was found to be 72.46 and 65.57 µg/mL for the *Actinotrichia fragilis* and AA respectively.

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated after oxygen is taken into living cells. Superoxide anion changes to other harmful ROS and free radicals such as hydrogen peroxide and hydroxyl radical, which induce oxidative damage (Srikanth et al., 2010). The decrease the absorbance at 560 nm with the *Actinotrichia fragilis* thus indicates the consumption of superoxide anion in the reaction mixture. The antioxidant activity of the methanol crude extract determined by superoxide anion radical assay varied. The reducing power were found to be higher in methanol extract. At concentration of 68.65 µg/mL of *Actinotrichia fragilis* 50% of Superoxide anion radical generated by incubation was scavenged. The $IC_{50}$ value of AA was 65.57µg/mL, respectively.

Many species of seaweed possess scavenging ability of hydrogen peroxide (Siriwardhana et al., 2010). It can cross membranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells. The $H_2O_2$ radical scavenging assay was also performed with the methanolic crude extract of the seaweed samples. From the results, *Actinotrichia fragilis* showed concentration dependent activity and the $H_2O_2$ scavenging effect at a concentration was 71.01µg/mL. This activity was comparable to the scavenging effect at the concentration of AA 65.57 µg/mL.

The metal iron chelating ability of seaweeds may be attributed to the presence of endogenous chelating agents, mainly phenolics because certain phenolic compounds have properly oriented functional groups, which can chelate metal ions (Wang et al., 1992). Ferrozine can quantitively form complexes with $Fe^{2+.}$ In the presence of chelating agents, the complex formation is disrupted with the result that red color of the complex is decreased. The metal chelating activity of *Actinotrichia fragilis* was 77.09µg/mL. This was comparable to the scavenging effect at the concentration of AA was 65.57 µg/mL.

Phosphomolybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the extract demonstrated high electron-donating capacity showing its ability to act as chain terminators, transforming relative free radical species into more stable non-reactive products (Zheng et al.,1992; Baskaran et al.,2015). In the ranking of the antioxidant capacity obtained by this method. This revealed that increased phosphomolybdenum reduction of *Actinotrichia fragilis* to the quantity of the sample. The $IC_{50}$ value of *Actinotrichia fragilis* was 75.82µg/mL and 65.57 µg/mL as a standard AA.

## Conclusions

In the present study the methanolic crude extracts of seaweeds at varying concentrations were shown as a potential DPPH, ABTS, FRAP, nitric oxide, reducing power, hydroxyl, superoxide radical scavenging, hydrogen peroxide radical assay, the metal chelating activity as well as phosphomolypdenum assay. Some extracts showed a higher antioxidant activity when compared to the commercial antioxidants. From the present study it can be concluded that the solvent extracts of marine macro algae exhibit good antioxidant activity. The methanolic extract showed a good result when compared to the AA. The results shown here indicate that the seaweed extracts can be a good source of natural antioxidant. Further investigation is needed to isolate and identify the specific class of compound that is responsible for the antioxidant activity.

## Conflict of interest

The authors declare that they don't have any conflict of interest.

## References

Al-Amoudi OA, Mutawie HH, Patel AV, Blunden G. 2009. Chemical composition and antioxidant activities of *Jeddah cornice* algae. Saudi Journal of Biological Sciences, 16:23–9.

Baskaran K, Kodukkur VP, Ramalingam S. 2014. Free radical scavenging activity of ethyl acetate extract of *Cardiospermum halicacabum* by In vitro assays. American Journal of Biological and Pharmaceutical Research, 2:300-8.

Baskaran K, Pugalendi KV, Saravanan R. 2015. Chrysoeriol a bioactive flavonoid, in *Cardiospermum halicacabum L.* leaves and their free radical scavenging activities. Journal of Pharmacy Research, 9(7):419-427.

Chou HG, Kuo JT, Lin ES. 2009. Comparative antioxidant properties of water extracts from different parts of

Beefsteak plant (*Perilla frutescens*). Journal of Food and Drug Analysis, 17:489-96.

Cotelle N, Bemier JL, Catteau JP, Pommery J, Wallet JC, Gaydou EM. 1996. Antioxidant properties of hydroxyl-flavones. Free Radical Biology & Medicine, 2:35–43.

Cox S, Abu-Ghannam N and Gupta S. 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. International Food Research Journal, 17:205-220.

Dawczynski C, Schubert R, Jahreis G. 2007. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chemistry, 103(3): 891–899.

Del Val AG, Platas G, Basilio A, Gorrochategui J, Suai I, Vicente F, Portillo E, del Rio MJ, Reina GG, Pelaez F. 2001. Screening of antimicrobial activities in red, green and brown macroalgae from *Gran Canaria* (Canary Islands, Spain). International Microbiology, 4:35-40.

Delpour AA, Ebrahimzadeh MA, Nabawi SF, Nabavi SM. 2009. Antioxidant activity of the Methanol extract of *Ferula asafetida* and its essential oil composition. Grassy Aceites, 60:405-12.

Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi S, Lele RD. 2004. Free radicals and antioxidants in human health: current status and future prospects. Journal of the Association of Physicians of India, 52:794–804.

Duan XJ, Zhang WW, Li XM, Wang BG. 2006. Evaluation of antioxidant property of extract and fractions obtained from red alga *Polysiphonia urceolata*. Food Chemistry, 95:37–43.

Gupta S, Abu-Ghannam N. 2011. Recent developments in the application of seaweeds or seaweed extracts as ameans for enhancing the safety and quality attributes of foods. Innovative Food Science and Emerging Technologies 12:600–609.

Huang MH, Huang SS, Wang BS. 2011. Antioxidant and antiinflammatory properties of *Cardiospermum halicacabum* and its reference compounds ex vivo and in vivo. Journal of Ethnopharmacology, 133:743-50.

Itono H. 1979. *Actinotrichia robusta*, a new species of the Chaetangiaceae (Nemaliales, Rhodophyta). Japanese Journal of Phycology, 27:137–141.

Kakinuma M, Park CS, Amano H. 2001. Distribution of free L-cysteine and glutathione in seaweeds. Fisheries Science, 67:194–196.

Kolanjinathan K, Ganesh P, Govindarajan M. 2009. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. European Review for Medical and Pharmacological Sciences, 13:173-177.

Kuda T, Tsunekawa M, Goto H, Araki Y. 2005. Antioxidant properties of four edible algae harvested in the *Noto peninsula* Japan. Journal of Food Composition and Analysis, 18:625–633.

Lim SN, Cheung P CK, Ooi VEC, Ang PO. 2002. Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. Journal of Agricultural and Food Chemistry, 50:3862– 3866.

Matanjun P, Mohamed S, Mustapha NM, Muhammad K, Ming CH. 2008. Antioxidant activities and phenolic content of eight species of seaweed from North Borneo. Journal of Applied Phycology 20:367-73.

Moncada S, Palmer RM, Higgs EA.1991. Nitric oxide: physiology, pathophysiology and pharmacology. Pharmacological Reviews 43:109-142.

Nakayama R, Tamura Y, Kikuzaki H, Nakatani N.1999. Antioxidant effect of the constituents of susabinori (*Porphyra yezoensis*). Journal of the American Oil Chemists' Society, 76: 649–653.

Naskar S, Islam A, Mazumder UK. 2010. In vitro and in vivo antioxidant potential of Hydromethanolic extract of *phoenix dactylifera* fruits. Journal of Scientific Research, 2:144-57.

Pandian A, Baskaran K, Suba Shini R. 2015. Antioxidant and free radical scavenging capacity of red seaweed *Hypnea Valentiae* from Rameshwaram Coast, Tamil Nadu, India. International Journal of Pharmacy and Pharmaceutical Sciences, 7(8): 232-237.

Park PJ, Shahidi F, Jeon YJ. 2004. Antioxidant activities of enzymatic extracts from and edible seaweed *Sargassum horneri* using ESR spectroscopy. Journal of Food Lipids 11: 15–27.

Rashmi C, Vinayak Sabu AS, Anil Chatterji. 2010. Bio prospecting of a little brown seaweeds for their cytotoxic and antioxidant activities. Evidence Based Complementary and Alternative Medicine 9: 23-32.

Revathi D, Baskaran K, Suba Shini R. 2015. Free radical scavenging and antioxidant properties of marine green algae *Hypnea Musciformis*. International Journal of Pharmacy and Pharmaceutical Sciences 7(8): 227-231.

Roginsky V, Lissi EA. 2005. Review of methods to determine the chain breaking antioxidant activity in food. Food Chemistry, 92:235–54.

Sahaa MR, Hasana SMR, Aktera R, Hossaina MM, Alamb MS, Alam MA, Mazumder MEH. 2008. In vitro free radical scavenging activity of methanol extract of the leaves of *Mimusopselengi Linn*. Bangladesh Journal of Veterinary Medicine 6(2): 197–202.

Sakat SS, Juvekar AR, Gambhire MN. 2010. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. International Journal

of Pharmacy and Pharmaceutical Sciences, 2:146-55.

Sanja SD, Sheth NR, Patel NK, Patel D, Patel B. 2009.Characterization and evaluation of antioxidant activity of Portulaca oleracea International Journal of Pharmacy and Pharmaceutical Sciences, 1(1): 74-84.

Siriwardhana N, Lee KW, Kim SH, Ha JW, Jeon YJ. 2003. Antioxidant activity of *Hizikia fusiformis* on reactive oxygen species scavenging and lipid peroxidation inhibition. Food Science and Technology International, 9(5):339–46.

Smirnoff N, Cumbes QJ. 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry, 28:1057-60.

Solomon RDJ, Santhi VS. 2008. Purification of bioactive natural product against human microbial pathogens from marine seaweed *Dictyota acutiloba*, J Ag. World J Microbiol Biotechnology, 24:1747- 1752.

Srikanth G, Babu SM, Kavitha CHN, Roa MEB, Vijaykumar N, Pradeep CH. 2010. Studies on in-vitro antioxidant activities of *Carica papaya* aqueous leaf extract. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 1:59-65.

Subashini R, Prasanth R. 2014. Studies of free radicals scavenging potential of seaweed *Enteromorpha sp*. Chemical Science Review and Letters, 3(12):931-40.

Subhashini N, Nagarajan G, Kavimani S. 2011. In vitro antioxidant and anticholinesterase activities of *Garcinia combogia*. International Journal of Pharmacy and Pharmaceutical Sciences, 3:129-32.

Vinayak RC, Sudha SA, Chatterji A. 2011. Bio screening of a few green seaweeds from India for their cytotoxic and antioxidant potential. Journal of the Science of Food and Agriculture, 91:2471-6.

Wang T, Jónsdóttir R, Ólafsdóttir G. 2009. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. (116):240–8.

Xu W, Zhang F, Luo Y, Ma L, Kou X, Huang K. 2009. The antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. Carbohydrate Research, 344:217-22.

Yan XJ, Chuda Y Suzuki M, Nagata, T. 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. Bioscience, Biotechnology, and Biochemistry, 63:605–607.

Yen GC, Chen HY.1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry, 43:27–32.

Yen GC, Chen HY.1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. Journal of Agricultural and Food Chemistry, 43:27-32.

Yoshie Y, Wang W, Petilo D, Suzuki T.2000. Distribution of catechins in Japanese seaweeds. Fish Science, 66: 998–1000.

Yuan YV, Walsh NA. 2006. Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds. Food and Chemical Toxicology 44:1144-1150.

Zheng GO, Kenney PM, Lam LK.1992. Anethofuran, carvone and limonene: potential chemopreventive agents from dill weed oil and caraway oil. Plants Medica, 58:338-41.