INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 16, No. 3, p. 468-481, 2020

RESEARCH PAPER OPEN ACCESS

Antioxidant and antiproliferative activity of phytoconstituents identified from *Sargassum binderi* seaweed extracts cultivated in Bangladesh

A.K.M. Asaduzzaman[1*], Imtiaj Hasan[1], M. Habibur Rahman[1], Abu Rayhan Mohammad Tareq[2]

[1]*Department of Biochemistry & Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh*

[2]*Chemistry Division, Atomic Energy Centre, 4 Kazi Nazrul Islam Avenue, P.O Box-164, Shahbag, Dhaka-1000, Bangladesh*

Key words: Antioxidant, Anticancer, Cytotoxicity, Ehrlich ascites carcinoma cells, *Sargassum binderi.*

http://dx.doi.org/10.12692/ijb/16.3.468-481 Article published on March 28, 2020

Abstract

In the present study, seaweed *Sargassum binderi* was extracted by the solvent ethanol and methanol. As phytoconstituents of both extracts were analyzed by gas chromatography-mass spectrometry (GC-MS), eighteen and nineteen compounds have been found from the ethanolic extract and methanolic extract, respectively. 1,2-Benzenedicarboxylic acid, diisooctyl esters was found in higher amounts and Pentadecanoic acid, 14-methyl-, methyl ester was the other major compounds present in both extracts. Total phenolic and flavonoid compounds were found to be as 11.890 mg gallic acid equivalent (GAE)/g and 269.542 mg catechin (CE)/g from ethanolic extract and 11.495 mg GAE/g and 266.936 mg CE/g from the methanolic extract. Good percentages of scavenging activity of DPPH, ABTS, total antioxidant and ferric reducing power have been observed in both extracts. 88.33% and 87.27% cell growth inhibition was observed *in vitro* at 176 µg/ml, respectively. Both *S. binderi* extracts showed mild toxicity against brine shrimp nauplii with LC_{50} values of 106.66 µg/ml and 125.82 µg/ml, respectively. Therefore we can say the ethanolic and methanolic extracts of *S. binderi* are a promising source of new molecules with possible applications as antioxidants and in cancer therapeutics.

* **Corresponding Author:** A.K.M. Asaduzzaman ✉ jonyasad2005@ru.ac.bd

Introduction

Seaweeds have become a very versatile product widely used as food for direct human consumption. Almost everywhere in the world, from ancient times, people have been consuming seaweeds. In Japan, China, Korea and Philippine, people consider seaweeds as a food of great delicacy and included it in their diets centuries ago. It is often eaten as fresh salads or vegetables along with rice. It is also used for making fish curries and meat dishes as well as soups. Seaweeds have plenty of essential nutrients, especially trace elements and several other bioactive substances and are considered as delicious upper class dishes in many eastern and western countries, but its medicinal values to cure a few diseases is on the rise now in many countries (Ahmed and Taparhudee, 2005). Besides a delicious food item, seaweeds have long been applied and recognized widely as alternate medicines in Japan, China, Thailand and Korea. It is also an ingredient for cosmetics industries and is used as an animal feed additive, and for freshwater treatment (Wang and Chiang, 1994). Seaweeds are also commonly used as decorative plants in salt water aquaria (Williams, 1990) and are valuable sources of bio-chemicals and pharmaceuticals. Presence of light and high concentration of oxygen is favorable for the growth of seaweeds but in this condition, photo damaging and free radical production may also result. Since seaweeds possess anti-oxidative compounds, they can protect themselves from stress resulting from free radicals and serious photodynamic damages. Generally, seaweeds can be divided into three groups based on their pigmentation: brown seaweed (Phaeophyceae), red seaweed (Rhodophyceae) and green seaweed (Chlorophyceae). Phaeophyta have been summarized to contain more active and comparatively higher contents of antioxidants than green and red seaweed (Al-Amoudi *et al.,* 2009; Kang *et al.,* 2014).

Recently, studies of seaweed compounds are getting much attention since they are rich in natural bioactive compounds required by the human body in order to stay healthy (Fung *et al.,* 2013). Antioxidant activities are attributed to various reactions and mechanisms: prevention of chain initiation, binding of transition metal ion catalysts, reductive capacity and radical scavenging (Huang and Wang, 2004; Tierney *et al.,* 2010). Several free radicals like superoxide anion, hydrogen peroxide, nitric oxide, singlet oxygen, lipid peroxide and hydroxyl radical, collectively called as reactive oxygen species (ROS), are produced from the metabolism of oxygen in aerobic conditions (Wang *et al.,* 2006). ROS can easily react with most biological molecules including protein, lipid, lipoprotein, and DNA to generate oxidative stress and play a role in the onset of human disorders such as rheumatoid arthritis, diabetes mellitus, inflammatory conditions, heart, genotoxic diseases, cancer as well as ageing (Valko *et al.*, 2004). The antioxidant containing drug is being used for the prevention and treatment of some oxidative stress related diseases during the last three decades. Cancer is a serious disease for people worldwide and it is characterized by uncontrolled cellular growth, local tissue invasion and distant metastases (Ford, 1991) and it has already been reported that free radicals are associated in carcinogenesis (Dreher and Junod, 1996). Along with this supportive result, many researchers have been reported plant extracts containing antioxidant principles possess antiproliferative activity (Ruby *et al.,* 1995; Kim, 2011). So, algal species have attracted much attention as alternative materials to extract natural antioxidative and antiproliferative compounds.

Sargassum binderi or brown algae, belongs to the family Phaeophyta, and contains an abundance of bioactive compounds. They are known to show various biological activities, such as anticancer, antitumor, antioxidant, anti-allergic and anti-neurodegenerative disease activities (Kang *et al.,* 2011; Shao *et al.,* 2014; Yoshioka *et al.,* 2014). Furthermore, they have numerous unexplored bioactive compounds with a high value from an economical aspect and provide positive effects on human life. *S. binderi* is being cultivated in the Cox`s bazar region of Bangladesh at very limited areas and so far, it could not become very popular to be used as

foods. Not many people in this part of the world know about its medicinal values and the medicinal impact of *S. binderi* cultivated in Bangladesh is not reported yet. Therefore, the aim of this study was to explore the medicinal impact, especially the antioxidative and antiproliferative effect of *S. binderi* extracts cultivated in Bangladesh.

Materials and methods

Materials

Seaweed (*Sargassum binderi*) was collected from the Cox's bazar region of Bangladesh. Gallic acid, 2,2-Diphenyl-1-pircryhydrazyl (DPPH), 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and catechin were purchased from Sigma-Aldrich Corp. (USA). Ethanol and Methanol were of HPLC grade (Labscan, Thailand). All other chemicals and reagents used of this study were of the highest analytical grade.

Sample preparation

Sargassum binderi was washed with deionized water and air dried. Then it was powdered by mechanical blender and sieved through a 710 μm mesh. After that, it was stored at -20 ^{o}C until further use.

Solvent extraction

The powdered sample was mixed with ethanol and methanol at a ratio of 1:40 (w/v) in a 500 ml beaker and stirred for 12 h by a magnetic stirrer at 40 °C and 250 rpm. After extraction, the ethanol solution was filtered by a filter paper and then it was evaporated in a rotary vacuum evaporator (RE 200, Bibby Sterilin Ltd., UK) at 40 °C. The extracted sample was collected in a vial and stored at -20 °C until further analysis.

GC – MS analysis

GC-MS analysis of both extracts were performed using a Saturn 2200 mass spectrometer coupled with a Varian CP-3800 gas chromatograph fitted with a split-splitless injector 1177 and a VF-5 ms capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 300 ^{o}C, and the split ratio was 20. Oven temperature programming was done from 50 ^{o}C (holding at 1 min) and then 50 ^{o}C to 280 ^{o}C at 10 ^{o}C/min and it was kept at 280 ^{o}C for 20 min. Ionization mode was selected as electron impact ionization and the scanning range was from 40 amu to 500 amu.

Identification of phytoconstituents

Interpretation on Mass-Spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having huge data with their fragmentation pattern. The spectrum and fragmentation pattern of the unknown compound was compared with the spectrum of known components stored in the NIST library. The name, retention time and area percentage of the components of test materials were determined.

Total phenolic content (TPC) assay

Total phenolic contents of the ethanolic and methanolic extracts were determined by the modified Folin-Ciocalteu method described by Singleton and Rossi (Singleton and Rossi, 1995). Exactly, 0.5 mL of each sample was mixed with 2.5 mL of 0.2 M Folin-Ciocalteu reagent into the test tubes. This mixture was kept at room temperature for 5 min and then 2.0 mL of 7.5% sodium carbonate solution was added to it. The tubes were mixed well and allowed to stand for 30 min. After that the absorbance was measured at 760 nm against 70% methanol as blank. A calibration curve was plotted using a standard gallic acid. Total phenolic contents were expressed in terms of gallic acid equivalent, GAE (standard curve equation: $y = 0.117x+0.051$, $R^2 = 0.998$), mg of GAE/g of dry extract.

Total flavonoid content (TFC) assay

Total flavonoids content were estimated according to the method of Dewanto *et al* (Dewanto *et al.,* 2002). Exact, 1 ml solutions from both extracts were mixed in 5 ml of distilled water into the test tubes, followed by the addition of 0.3 ml of 5% $NaNO_2$. The mixture was allowed to stand for 5 min and 0.6 ml of 10% aluminum chloride was then added. The mixture was allowed to stand for another 5 min and 2 ml of 1M

NaOH solution and 1.10 ml of distilled water were then added, accordingly. The absorbance was measured at 510 nm against 70% methanol as blank. Catechin was used for calibration of the standard curve. Total flavonoid contents were expressed in terms of catechin equivalent, CAE (standard curve equation: $y = 0.005x+0.047$, $R^2 = 0.998$), mg of CAE/g of dry extract.

DPPH free radical scavenging assay

The determination of DPPH free radical scavenging was carried out according to the method described by Choi *et al* (Choi *et al.,* 2000) with slight modification. Briefly, 3.0 ml of 0.1 mM DPPH solution in methanol was added to the test tube containing 1.0 mL of each sample at various concentrations. The mixture was rotated using a vortex mixer for 10 s and kept at room temperature for 30 min in the dark. Absorbance values of all the sample solutions were measured at 517 nm against 70% methanol as a blank. Tests were carried out in triplicates. Catechin was used as the reference standard. The scavenging activity (%) on DPPH radicals was calculated using the formula:

SA% = [(Absorbance of control– Absorbance of sample) / Absorbance of control] ×100

ABTS free radical scavenging assay

$ABTS^+$ radical scavenging was carried out according to the method of Cai *et al* (Cai *et al.,* 2004) with a little modification. ABTS was dissolved in water to make a concentration of 7 mM/l. $ABTS^+$ was produced by reacting the equal volume of ABTS stock solution with 2.45 mM/l of potassium persulfate and allowing the mixture to stand in the dark at room temperature for 16 h before use. For the test of samples, the $ABTS^+$ stock solution was diluted with 80% methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Exactly, 3.0 ml of $ABTS^+$ solution was added to test tubes containing 1 ml of the test sample with various concentrations and mixed vigorously. The mixtures were kept in the dark at room temperature for 6 min and the absorbance was measured at 734 nm against 80% methanol as blank. Tests were carried out in triplicates. Catechin was used as the reference standard. The percentage of inhibition was measured by the same calculation used in the DPPH assay.

Total antioxidant capacity assay

The antioxidant potential of the samples was determined according to the method of Prieto *et al* (Prieto *et al.,* 1999) with little modifications. 0.5 ml of samples at different concentrations was mixed with 3.0 ml of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate into the test tubes. The test tubes were incubated at 95 °C for 10 min to complete the reaction. After cooling by cold distilled water, the absorbance was measured at 695 nm against 70% methanol as a blank. Tests were carried out in triplicates.

Ferric reducing antioxidant capacity assay

The reductive potential of the samples was determined according to the method of Narasimhan *et al* (Narasimahan *et al.,* 2013). 0.25 ml sample solution was taken in different concentrations. Then, 0.625 ml of 0.2 M phosphate buffer and 0.625 ml of 1% potassium ferricyanide $[K_3Fe(CN)_6]$ solution were added into the test tubes. The mixture was incubated for 20 min at 50 °C and after incubation, 0.625 ml of 10% TCA solution was added immediately. The total mixture was centrifuged at 4000 rpm for 10 min. An aliquot of 1.8 ml supernatant was withdrawn and followed by 1.8 ml of distilled water and 0.36 ml of 0.1% $FeCl_3$ solution was mixed to the solution. The absorbance was recorded at 700 nm against 70% methanol as a blank. Tests were carried out in triplicates.

MTT colorimetric assay

According to the method of Kabir *et al* (Kabir *et al.,* 2013) with slight modifications, MTT colorimetric assay was used to detect the proliferation of Ehrlich's ascites carcinoma (EAC) cells. 5×10^5 EAC cells in 200 µl RPMI-1640 medium were plated in the 96-well flat-bottom culture plate in the presence and absence of different concentrations of seaweed extracts (11–176 µg/ml) and incubated at 37 °C in a CO_2 incubator for 24 h. After removal of the aliquot from each well,

180 µL of PBS and 20 µL of MTT (5 mg/ml) was added and incubated at 37 °C for 4 h. Then the aliquot was removed again and 200 µL of acidic isopropanol was added into each well. The plate was agitated for 5 min and then the absorbance was taken at 570 nm using a titer plate reader.

Brine shrimp nauplii lethality assay

Brine shrimp nauplii (*Artemia salina*) were used to examine the lethality of the ethanolic and methanolic extract of *S. binderi* according to Kabir *et al* (Kabir *et al.,* 2011). In brief, 10 brine shrimp nauplii were placed in each vial containing 30.0, 60.0, 120.0 and 240.0 µg/ml of seaweed extract in 3.0 ml of artificial sea water and kept at 30 °C for 24 h under a continuous light regime. Three replicates were used for each experiment and the percentage of mortality of the nauplii was calculated by Probit analysis as described by Finney (Finney, 1971).

Statistical analysis

The experimental results are expressed as the mean ± SD (standard deviation). (n = 3, $P<0.05$). Data have been calculated by one-way ANOVA followed by Duncan test using SPSS software, version 16.

Results

Bioactive components

GC-MS chromatogram of ethanolic and methanolic extracts of *S. binderi* is shown in Fig. 1 (A) and 1 (B). Varieties of bioactive compounds have been identified in both *S. binderi* extracts. Eighteen (18) compounds were identified from the ethanolic extract of *S. binderi* shown in Table 1. Among the identified compounds, the higher area percentage of compounds were 1, 2-Benzenedicarboxylic acid, diisooctyl ester (69.23%); Pentadecanoic acid, 14-methyl-, methyl ester (8.60%); 17-(1, 5-Dimethylhexyl)-10, 13-dimethyl-hexahydro-1H-cyclopenta [a]-phenanthrene (2.89%); and 5-Cholesten-3B-ol, acetate (2.84%). On the other hand, nineteen (19) compounds were identified from the methanolic extract of *S. binderi* shown in Table 2. The most higher area percentage of identified compounds were 1,2-Benzenedicarboxylic acid, diisooctyl ester (31.98%); Pentadecanoic acid, 14-methyl-, methyl ester (21.46%); 17-(1,5-Dimethylhexyl)-10,13-dimethyl-hexahydro-1H-cyclopenta[a]-phenanthrene (12.00%); 5-Cholesten-3B-ol, acetate (6.85%); Tridecanoic acid, 12-methyl-, methyl ester (4.96%); and 9-Octadecenoic acid, methyl ester, (E) (4.78%).

Table 1. GC-MS analysis of phytoconstituents identified from ethanolic extract of *S. binderi.*

Serial No.	Compound name	Retention time (RT)	Area (%)
1	Cyclohexasiloxane, dodecamethyl-	10.420	0.44
2	No match	12.598	0.46
3	Phenol, 2,4-bis- (1,1-dimethylethyl), TMS	13.298	0.41
4	Oxirane, tetradecyl-	14.132	0.71
5	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-ol	15.299	0.89
6	Tridecanoic acid, 12-methyl-, methyl ester	15.790	1.99
7	3,7,11,15-tetramethyl-2-hexadecen-1-ol	16.979	0.60
8	2-Pentadecanone, 6,10,14-trimethyl-	17.046	1.71
9	7,9-Di-tert-butyl-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione	17.786	0.58
10	Pentadecanoic acid, 14-methyl-, methyl ester	17.893	8.60
11	Dibutyl phthalate	18.261	1.59
12	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	18.554	1.64
13	Benzene, 1-acetyl-3-ethyl-2-(2-ethenyl-6-ethylphenylazo)-	18.613	1.13
14	9-Octadecanoic acid (Z)-, 2,3-dihydroxypropyl ester	19.580	1.77
15	(1 Ar-(1aalpha, 5abeta, 9ar(*)))-5a,9,9-tri	20.678	2.52
16	1,2-Benzenedicarboxylic acid, diisooctyl ester	23.276	69.23
17	17-(1,5-Dimethylhexyl)-10,13-dimethyl-hexahydro-1H-cyclopenta[a]-phenanthrene	29.347	2.89
18	5-Cholesten-3B-ol, acetate	30.038	2.84

TPC

The total phenolic content (TPC) of the seaweed extract was calculated by using a modified Folin-Ciocalteu method. As shown in Table 3, the TPC content of *S. binderi* ethanolic and methanolic extract was 11.890 mg GAE/g and 11.495 mg GAE/g, respectively.

TFC

Total flavonoid content of ethanolic and methanolic *S. binderi* extracts is shown in Table 3. Flavonoids are the most important natural phenolics due to their chemical and biological activities, including antioxidant and free radical scavenging properties. Comparing to the total flavonoid compound found in the methanolic extract of *S. binderi* (266.936 mg CE/g), the TFC for the ethanolic extract of *S. binderi* was 269.542 mg CE/g, a little bit higher.

Antioxidant activities

DPPH free radical scavenging activity

DPPH has been used extensively as a stable free radical to evaluate reducing substances and is a useful reagent for investigating free radical scavenging activity of the components. DPPH free radical scavenging activity of ethanolic and methanolic *S. binderi* extracts at different concentrations is shown in Fig. 2A.

The highest DPPH free radical scavenging activity of ethanolic and methanolic extract of *S. binderi* was 50.91% and 50.81%, respectively, at 350 µg/ml concentration. DPPH free radical scavenging activity of both extract became amplified with the increase of sample concentration. The IC_{50} values of both extracts were 308.51 µg/ml and 302.07 µg/ml, respectively.

Table 2. GC-MS analysis of phytoconstituents identified from methanolic extract of *S. binderi*

Serial No.	Compound name	Retention time (RT)	Area (%)
1	No match	12.599	0.46
2	No match	14.619	1.18
3	(R)-(-)(Z)-14-Methyl-8-hexadecen-1-ol	15.300	1.60
4	Tridecanoic acid, 12-methyl-, methyl ester	15.788	4.96
5	3.7,11,15-Tetramethyl-2-hexadecen-1-ol	16.980	0.83
6	2-Pentadecanone, 6,10,14-trimethyl-	17.047	1.76
7	17-Pentatriacontene	17.474	1.42
8	Pentadecanoic acid, 14-methyl-, methyl ester	17.901	21.46
9	Phthalic acid, butyl hexyl ester	18.264	0.65
10	9-Octadecenoic acid, methyl ester, (E)-	19.585	4.78
11	2-Methyl-Z,Z-3,13-octadecadienol	19.684	1.83
12	Octadecanoic acid, methyl ester	19.810	1.66
13	(1 Ar-(1aalpha, 5abeta, 9ar(*)))-5a,9,9-tri	20.679	3.63
14	Hexadecanoic acid, 1-(hydroxymethyl)-1	21.329	0.82
15	3,3a-Epoxydicyclopenta [a,d]cyclooctan-4	21.700	1.26
16	No match	22.135	0.86
17	1,2-Benzenedicarboxylic acid, diisooctyl ester	23.262	31.98
18	17-(1,5-Dimethylhexyl)-10,13-dimethyl-hexahydro-1H-cyclopenta[a] phenanthrene	29.347	12.00
19	5-Cholesten-3B-ol, acetate	30.043	6.85

ABTS free radical scavenging activity

The ABTS scavenging activity was determined from both ethanolic and methanolic *S. binderi* extract. This has chain-breaking antioxidant property. As shown in Fig. 2B, our results revealed that ABTS scavenging activity was found to be slightly higher in the ethanolic extract of *S. binderi* (84.11%) comparing to the activity found in case of the methanolic extract of *S. binderi* (81.31%).

The percentage efficiency of ABTS scavenged by both *S. binderi* extracts was found to concentration-dependent. The IC_{50} values of both extracts were 7.66 µg/ml and 10.40 µg/ml, respectively.

Table 3. TPC and TFC content of *S. binderi* extract

Algae	Extract	TPC (mg GAE/g)	FC (mg CE/g)
S. binderi	Ethanol	11.890 ± 0.704	269.542 ± 5.143
	Methanol	11.495 ± 1.049	266.936 ± 3.518

Total antioxidant and ferric reducing antioxidant activity

Total antioxidant and ferric reducing antioxidant activity of both ethanolic and methanolic *S. binderi* extracts is shown in Fig. 3A and Fig. 3B. In case of total antioxidant activity, the absorbance became increased with increasing concentrations of *S. binderi* extracts. Among the extracts, absorbance values for the ethanolic extract were higher compared to those for the methanoilic extract. Similar observation was found in case of ferric reducing antioxidant activity.

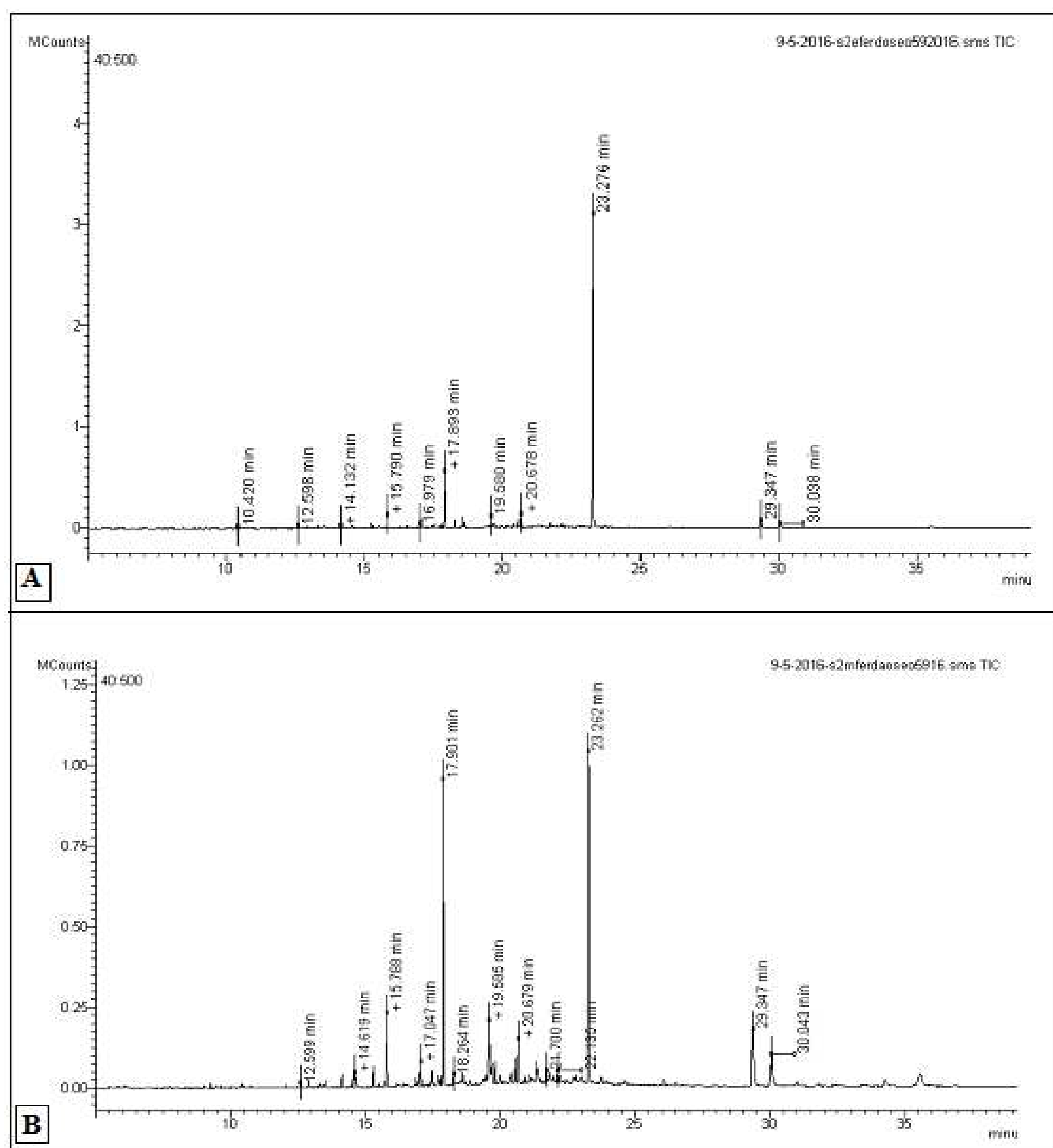

Fig. 1. GC-MS chromatogram of the ethanolic (A) and methanolic (B) extract of *S. binderi*.

Anticancer activity

MTT assay was used to investigate the effect of *S. binderi* extract *in vitro* on EAC cells. The *S. binderi*-induced EAC cell death was found to occur in a dose-dependent manner as shown in Fig. 4. At 11 µg/ml of *S. binderi* extract concentration, the inhibitory effect of the ethanolic extract was 6.67% whereas for the methanolic extract, it was 5.30%, respectively. When the concentration gradually increased, the inhibitory effect also increased and finally reached to 88.33% and 87.27% at 176 µg/ml of ethanolic and methanolic extract of *S. binderi*, respectively.

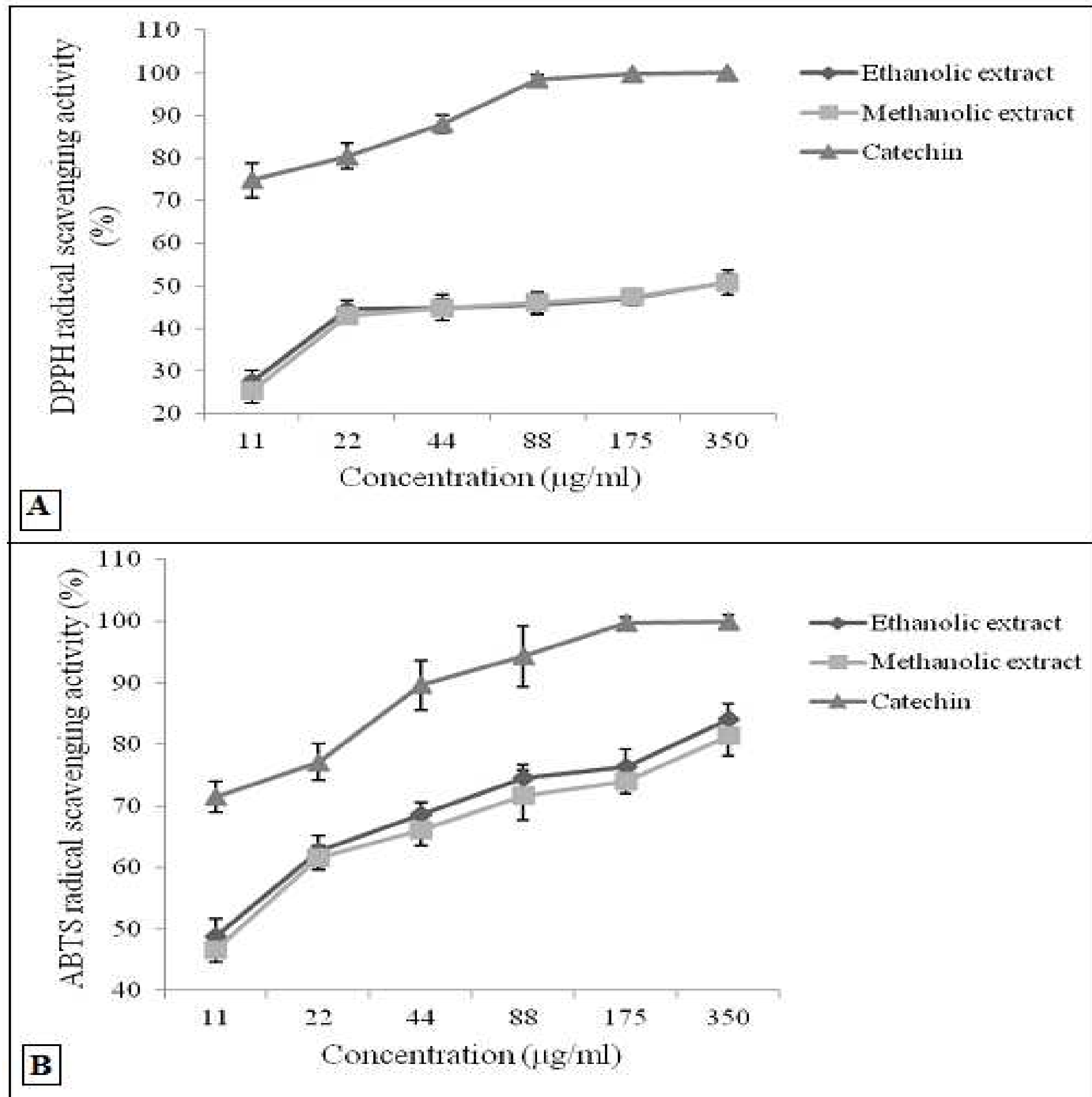

Fig. 2. Scavenging activity of ethanolic and methanolic extract of *S. binderi.* (A) DPPH and (B) ABTS. n = 3, mean ± S.D.

Cytotoxicity

In the cytotototoxicity assay, at the concentration range of 30-120 µg/ml, mortality rate of ethanolic *S. binderi* extract was found to be 3.33% to 70% and in case of the methanolic extract, the values were 6.67% to 46.67%. But the mortality rate became increased to 100% when the concentrations for both extracts rose to 240 µg/ml and the LC_{50} values were determined to be 106.66 µg/ml and 125.82 µg/ml for ethanolic and methanolic *S. binderi* extracts, respectively (shown in Fig. 5).

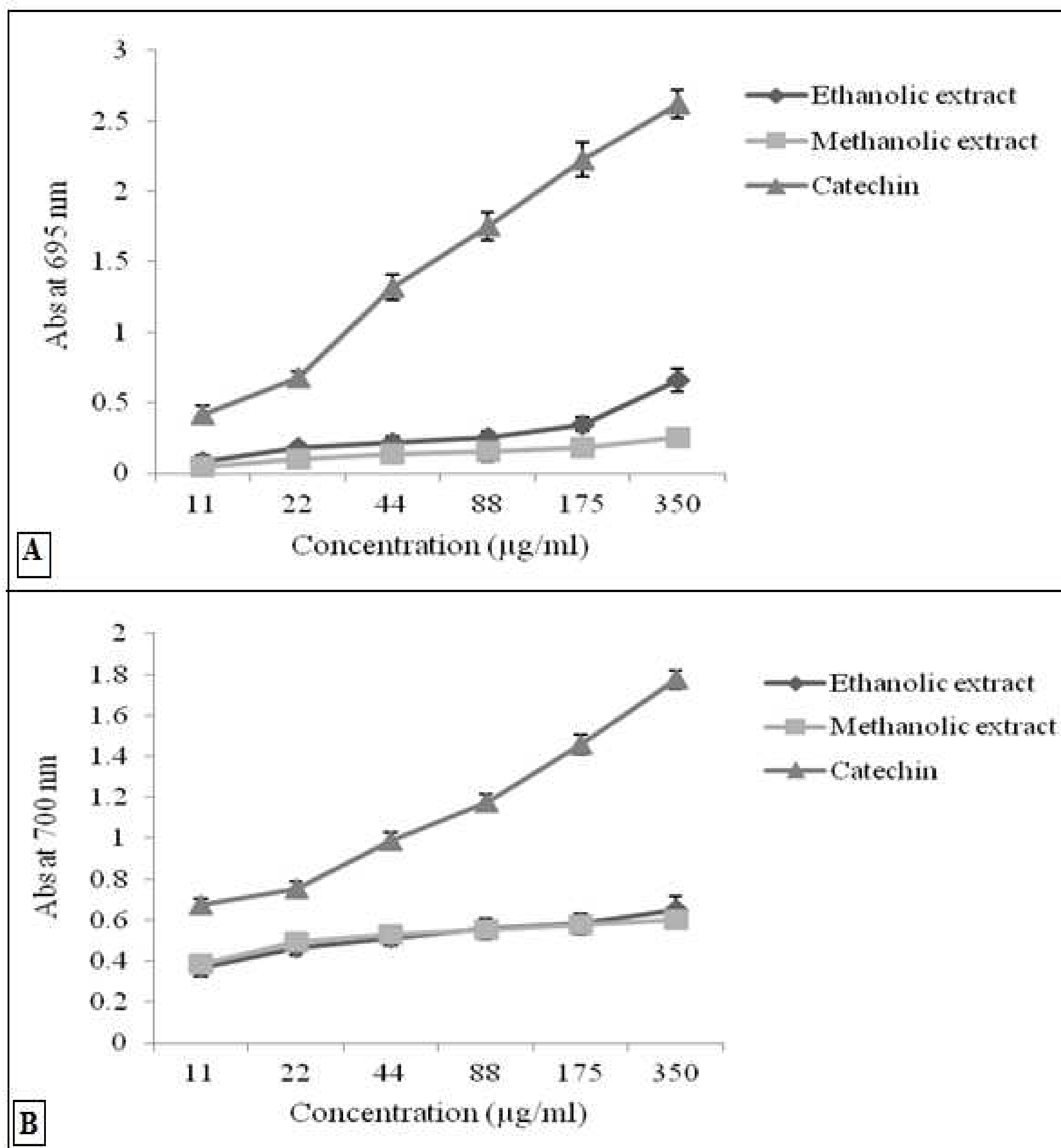

Fig. 3. Antioxidant capacity of ethanolic and methanolic extract of *S. binderi*. (A) total antioxidant and (B) ferric reducing. n = 3, mean ± S.D.

Discussion

Due to their biodiversity, seaweeds are well thought-out as attractive samples for the identification of biologically active compounds (Fung *et al.,* 2013; Lim *et al.,* 2016). In the present study we found many bioactive compounds from ethanolic and methanolic extracts. Among those, as reported by several studies, pentadecanoic acid, 14-methyl-, methyl ester had antioxidant activity, 9-Octadecenoic acid, methyl ester, (E) and 3,7,11,15-tetramethyl-2-hexadecen-1-ol compounds had anticancer activity and 1,2-Benzenedicarboxylic acid, diisooctyl ester had both antioxidant and anticancer activity (Rajeswari *et al.,* 2012; Khalil *et al.,* 2014; Elezabeth and Arumugam, 2014; Singaravadivel, 2014). Phenolic and flavonoid compounds have a major effect on antioxidative activities (Ganesan *et al.,* 2011) which was focused in many studies. The amount of phenolic and flavonoid compounds from the ethanolic and methanolic *S. binderi* extracts was found to be higher comparing to the amount obtained from *Ulva clathrata*, *Ulva flexuosa* and *Sargassum horneri* (Farasat *et al.,* 2014;

Shipeng *et al.,* 2015). This variation might be linked with some species and environmental factors like location, seasonal periods, temperature, salinity, and storage conditions. Several researchers reported increased antioxidant and anticarcinogenic activities resulting due to the presence of phenolic and flavonoid compounds (Ganesan *et al.,* 2011; Farasat *et al.,* 2014; Moussavou *et al.,* 2014; Shipeng *et al.,* 2015). At physiological concentrations, cellular components such as lipids, protein, and DNA might become damaged due to the excessive amount of free radicals (Melov *et al.,* 2000). Thus, free-radical scavenging activities of antioxidants can protect the human body from serious cellular or molecular damages by free radicals and retard the progress of many chronic diseases. The assay of DPPH, a stable free-radical, has been widely accepted assay to determine the free radical scavenging activity of antioxidants (Fenglin *et al.,* 2004).

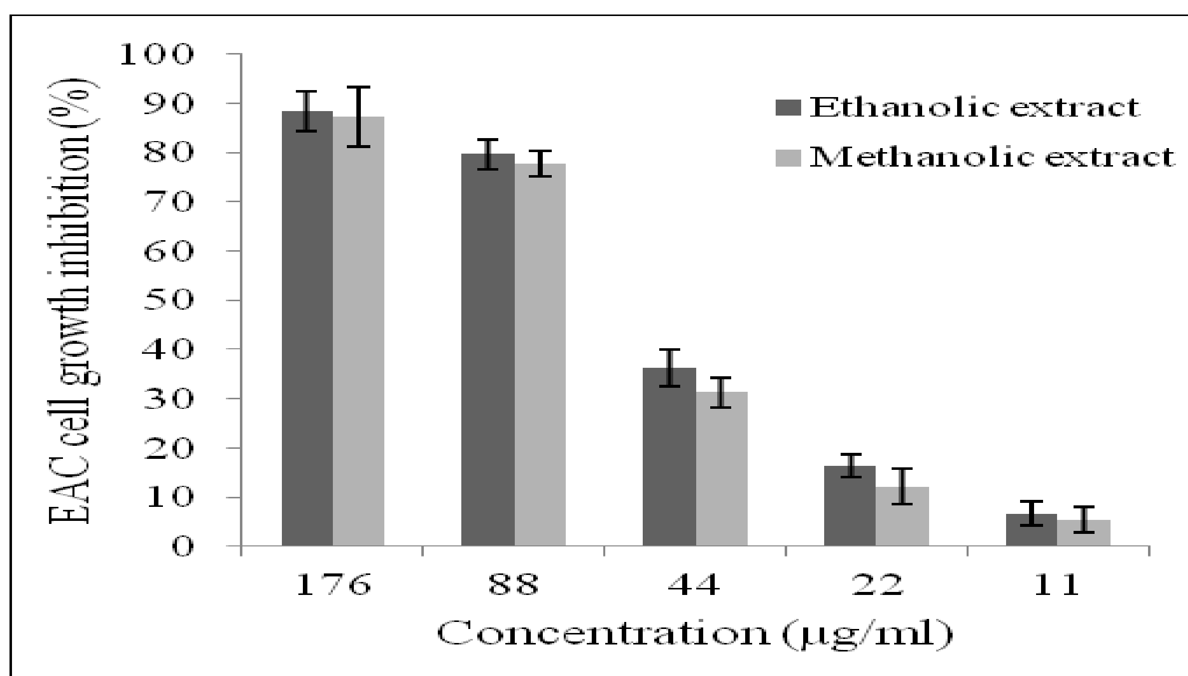

Fig. 4. Ethanolic and methanolic extract of *S. binderi* inhibited EAC cells growth. EAC cells were treated with various doses of *S. binderi* extract for 24 h in RPMI-1640 medium. The growth inhibition was measured by MTT assay (n = 3, mean ± S.D.).

In the present study, we found that the DPPH free-radical scavenging activities of ethanolic and methanolic extract of *S. binderi* (50.91% and 50.81% at 0.35 mg/ml) were much higher than EtOAc and n-BuOH fraction of *S. pallidum* (30.50% and 29.36% at 2 mg/ml) (Ye *et al.,* 2009). ABTS assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxyl radical) (Leong and Shui, 2002).

We found excellent ABTS scavenging activity of both ethanolic and methanolic *S. binderi* extracts with similar values whereas different ABTS scavenging activity values for various brown seaweeds had already been reported (Rattaya *et al.,* 2015). Total antioxidant and ferric reducing power indicated that all extracts were capable of donating electrons to the radicals, in which propagation could be terminated or retarded. Total antioxidant and ferric reducing effect of ethanolic and methanolic *S. binderi* extracts have been found to be lower when compared to any natural antioxidant like catechin. Results of these experiments showed that *S. binderi* ethanolic and methanolic extracts exhibited strong antioxidant properties, indicating their great potential to be used as a natural antioxidant.

Cancers are one of the major causes of death in humans and have high impact in industrialized countries (Smyrniotopoulos *et al.,* 2010). The search and development of new drugs became mandatory in last few decades, and nature became a relevant resource for the discovery of anticancer compounds. Till now, more than 60% of the commercially available anticancer drugs are evolved from naturally origin. Now a days, naturally derived anti-cancer drugs such as doxorubicin, bleomycin, daunomicin and vinblastine are playing important roles in curative cancer chemotherapy (Sithranga *et al.,* 2010). Several experiments have been carried out to study the anticancer effect of seaweed extract on different cancer cells *in vitro*. In this study, ethanolic and methanolic extracts of *S. binderi* inhibited 6.67% to 88.33% and 5.30% to 87.27% of EAC cells growth *in vitro* at concentrations ranging from 11 to 76 µg/ml, respectively. Similar results were reported for Egyptian marine algae extracts when applied on EAC cells *in vitro* (Ahmed *et al.,* 2011). Next, Alves *et al.* (2016) also reported that methanolic and dichlromethane extracts of *Asparagopsis armata* and *Sphaerococcus coronopifolius* (1000 µg/ml; 24 h) presented high cytotoxicity, 11.22 & 1.51% and 14.04 & 12.84%, against HepG-2 live cells, respectively.

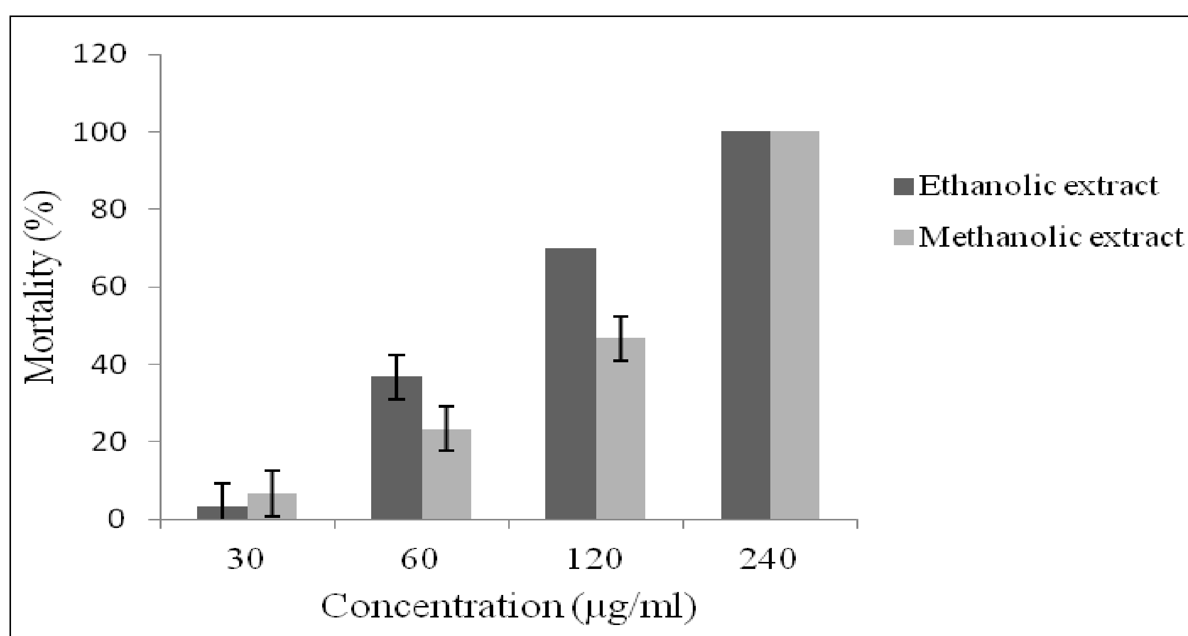

Fig. 5. Percentage of mortality of brine shrimp nauplii treated with *S. binderi* extracts at different concentrations with exposure for 24 h. n = 3, mean ± S.D.

As one of the standard widespread saltwater organism, *artemia*, a genus of aquatic crustaceans known as brine shrimp is quite commonly used for checking the toxicity of extract (Sreejamole and Greeshma, 2013). In the present study, the mortality rate of brine shrimp nauplii rose with the increase of ethanolic and methanolic *S. binderi* extract concentrations and 50% mortality (LC_{50}) of the nauplii occurred at 106.66 µg/ml and 125.82 µg/ml concentration, respectively. Several studies confirmed that LC_{50} values of different seaweed extracts (collected using different solvents) were also different. In accordance with the present study, Ayesha *et al.* (2010) reported that the ethanol extract of seaweed *Dictyota indica*, *Iyengaria stellata* and *Melanothamnus afaqhusainii* showed the LC_{50} value of 141 µg/ml, 186 µg/ml & 190 µg/ml, respectively. However, LC_{50} value of ethanol extracts were observed in *S. asperum* as 443 µg/ml, followed by *S. indica* (507 µg/ml), *S. marginatum* (612 µg/ml), *C. racemosa* (929 µg/ml), *S. swartzii* (928 µg/ml) and *S. binderi* (735 µg/ml) (Ara *et al.,* 1999). The LC_{50} value of hexane soluble fractions of *S. marginatum* and *S. swartzii* were 349 and 61 µg/ml, respectively, whereas the methanol soluble fraction of *S. binderi* and *S. asperum* were 121 and 415 µg/ml and water

extracts of *S. indica* and *C. racemosa* were 64 and 67 µg/ml respectively (Ara *et al.,* 1999). As a summary, we can say that the cytotoxic activity of our experimental seaweed extract was mild compared to above reported seaweed extract.

Conclusions

Bioactive phytoconstitutes in seaweed *S. binderi* extract showed the presence of higher phenolic and flavonoid compounds, good percentage scavenging activity of DPPH, ABTS, total antioxidant and ferric reducing power and excellent anticancer activity with mild cytotoxicity. Therefore we can say that *S. binderi* might be used as an antioxidant and anticancer drug in food and pharmaceutical industry though more research is considered necessary to establish that.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This research project was funded by the Faculty of Science, University of Rajshahi, Bangladesh, No. A-314/5/52/RU/Science-4/2015.

References

Ahmed RA, Ibrahim AM, Salem TA, Hamza HA. 2011. Anticancer activity of some Egyptian marine algae extracts. Egyptian Journal of Experimental Biology (Botany) **7,** 9–13.

Al-Amoudi Omar A, Mutawie HH, Patel AV, Blunden G. 2009. Chemical composition and antioxidant activities of jeddah corniche algae, Saudi Arabia. Saudi Journal of Biological Sciences **16,** 23–29.

Alves C, Pinteus S, Horta A, Pedrosa R. 2016. High cytotoxicity and anti-proliferative activity of algae extracts on an in vitro model of human hepatocellular carcinoma. Springer Plus **5,** 1339. http://dx.doi.org/10.1186/s40064-016-2938-2

Ara J, Viqar Sultana V, Ehteshamul-Haque S, Qasim R, Ahmad VU. 1999. Cytotoxic activity of marine macro-algae on artemia salina (Brine Shrimp). Phytotherapy Research **13,** 304–307.

Ayesha Hira Sultana V, Ara J, Ehteshamul-Haque S. 2010. *In vitro* cytotoxicity of seaweeds from karachi coast on brine shrimp. Pakistan Journal of Botany **42,** 3555–3560.

Cai Y, Luo Q, Sun M, Corke H. 2004. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences **74,** 2157–2184.

Chabner BA, Collins JM. 1990. Cancer chemotherapy: Principles an practice. Philadelphia, Lippincott JB.

Choi HY, Jhun EJ, Lim BO. 2000. Application of flow injection-chemilumineacence to the study of radical scavenging activity in plants. Phytotherapy Research **14,** 250–253.

Dewanto V, Wu X, Adom KK, Liu RH. 2002. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry **50,** 3010–3014.

Farasat M, Khavari-Nejad RA, Nabavi SMB, Namjooyan F. 2014. Antioxidant activity, total phenolics and flavonoid contents of some edible green seaweeds from northern coasts of the Persian Gulf. Iranian Journal of Pharmaceutical Research **13,** 163–170.

Finney DJ. 1971. Probit Analysis. London, Cambridge University press 333.

Fung, Adah, Nazimah H, Jun L. 2013. Fucoxanthin content and antioxidant properties of *Undaria Pinnatifida*. Food Chemistry **136,** 1055–1062.

Ganesan K, Kumar SK, Subba Rao PV. 2011. Comparative assessment of antioxidant activity in

three edible species of green seaweed, Enteromorpha from Okha, Northwest coast of India. Innovative Food Science and Emerging Technologies **12,** 73–78.

Hong Y, Chunhong Z, Yi S, Xin Z, Jun L, Qiuhui H, Xiaoxiong Z. 2009. Antioxidant activities *in vitro* of ethanol extract from brown seaweed *Sargassum pallidum*. European Food Research and Technology **230,** 101–109.

Hu FL, Lu RL, Huang B, Ming L. 2004. Free radical scavenging activity of extracts prepared from fresh leaves of selected Chinese medicinal plants. Fitoterapia **75,** 14–23.

Huang Lan H, Wang BG. 2004. Antioxidant capacity and lipophilic content of seaweeds collected from the qingdao coastline. Journal of Agricultural and Food Chemistry **52,** 4993–4997.

Jumeri Kim SM. 2011. Antioxidant and anticancer activities of enzymatic hydrolysates of solitary tunicate (Styela clava). Food Science and Biotechnology **20,** 1075–1085.

Kabir SR, Hossen A, Zubair A, Alom J, Islam F, Hossain A, Kimura Y. 2011. A new lectin from the tuberous rhizome of *Kaempferia rotunda*: isolation, characterization, antibacterial and antiproliferative activities. Protein Peptide Letter **18,** 1140–1149.

Kabir SR, Nabi MM, Haque A, Zaman RU, Mahmud ZH, Reza MA. 2013. Pea lectin inhibits growth of Ehrlich ascites carcinoma cells by inducing apoptosis and G_2/M cell cycle arrest *in vivo* in mice. Phytomedicine **20,** 1288–1296.

Kang Cheol M, Kim KN, Wijesinghe WAJP, Yang X, Ahn G, Jeon YJ. 2014. Protective effect of polyphenol extracted from ecklonia cava against ethanol induced oxidative damage in vitro and in zebrafish model. Journal of Functional Foods **6,** 339–347.

Kang Myung S, Kim KN, Lee SH, Ahn G, Cha SH, Kim AD, Yang XD, Kang MC, Jeon YJ. 2011. Anti-inflammatory activity of polysaccharide purified from amg-assistant extract of ecklonia cava in lps-stimulated RAW 264.7 macrophages. Carbohydrate Polymers **85,** 80–85.

Khalil NM, Shalaby EA, Ali DMIA, Ali EM, Aboul-Enein AM. 2014. Biological activities of secondary metabolites from Emericella nidulans EGCU 312. African Journal of Microbiology Research **8,** 2011–2021.

Knanzawa A. 1992. New directions in microdiet research in Japan. Isr J Aquacult **44,** 123–133.

Leong LP, Shui G. 2002. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry **76,** 69–75.

Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ. 2000. Extension of life-span with superoxide dismutase/catalase mimetics. Science **289,** 1567–1569.

Moussavou G, Kwak DH, Obiang-Obonou BW, Maranguy CAO, Boutamba SDD, Lee DH, Pissibanganga OGM, Ko K, Seo JI, Choo YK. 2014. Anticancer effects of different seaweeds on human colon and breast cancers. Marine Drugs **12,** 4898–4911.

Oyaizu M. 1986. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucose amine. The Japanese Journal of Nutrition and Dietetics **44,** 307–315.

Player T. 1982. Free radicals and cancer. London, Academic Press.

Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to

the determination of vitamin E. Analytical Biochemistry **269,** 337–341.

Rajeswari G, Murugan M, Mohan VR. 2012. GC-MS analysis of bioactive components of *Hugonia mystax L.* (Linaceae). Research Journal of Pharmaceutical, Biological and Chemical Sciences (RJPBCS) **3,** 301–308.

Rajeswari J, Rani S. 2015. GC-MS Analysis of phytochemical compounds in the ethanolic extract of root of Lawsonia inermis Linn. International Journal of ChemTech Research **7,** 389–399.

Rattaya S, Benjakul S, Prodpran T. 2015. Extraction, antioxidative, and antimicrobial activities of brown seaweed extracts, Turbinaria ornate and Sargassum polycystum, grown in Thailand. International Aquatic Research **7,** 1–16.

Ruby AJ, Kuttan G, Babu KD, Rajasekaran KN, Kuttan R. 1995. Antitumor and antioxidant activity of natural curcuminoids. Cancer Letter **94,** 783–789.

Shao, Ping, Chen X, Sun P. 2014. Chemical characterization, antioxidant and antitumor activity of sulfated polysaccharide from *Sargassum horneri*. Carbohydrate Polymers **105,** 260–269.

Shipeng Y, Woo HC, Choi JH, Park YB, Chun BS. 2015. Measurement of antioxidant activities and phenolic and flavonoid contents of the brown seaweed *Sargassum horneri*: Comparison of supercritical co_2 and various solvent extractions. Fish and Aquatic Sciences **18,** 123–130.

Singleton VL, Rossi JA. 1996. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. American Journal of Enology and Viticulture **16,** 144–153.

Sithranga BN, Kathiresan K. 2010. Anticancer drugs from marine flora: an overview. Journal of Oncology **2010,** 1–18.

Smyrniotopoulos V, Vagias C, Bruyère C, Lamoral-Theys D, Kiss R, Roussis V. 2010. Structure and in vitro antitumor activity evaluation of brominated diterpenes from the red alga *Sphaerococcus coronopifolius*. Bioorganic and Medicinal Chemistry **18,** 1321–1330.

Sreejamole KL, Greeshma PM. 2013. Antioxidant and brine shrimp cytotoxic activities of ethanolic extract of red alga Gracilaria corticata (J. Agardh). Indian Journal of Natural Products and Resources **4,** 233–237.

Tierney, Michelle S, Croft AK, Hayes M. 2010. A review of antihypertensive and antioxidant activities in macroalgae. Botanica Marina **53,** 387–408.

Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. 2004. Role of oxygen radicals in DNA damage and cancer incidence. Molecular and Cellular Biochemistry **266,** 37–56.

Vijisaral ED, Arumugam S. 2014. GC-MS analysis of bioactive constituents of *Indigofera suffruticosa* leaves. Journal of Chemical and Pharmaceutical Research **6,** 294–300.

Wang L, Tu YC, Lian TW, Hung JT, Yen JH, Wu MJ. 2006. Distinctive antioxidant and antiinflammatory effects of flavonols. Journal of Agricultural and Food Chemistry **54,** 9798–9804.

Wang WL, Chiang YM. 1994. Potential economic seaweeds of *Hengchum Peninsula*, Taiwan. Economic Botany **84,** 182–189.

Williams SL. 1990. Experimental studies of Caribbean seagrass bed development. Ecological Monographs **60,** 449–469.

Yoshioka, Hisashi, Ishida M, Nishi K, Oda H, Toyohara H, Sugahara T. 2014. Studies on anti-allergic activity of sargassum horneri extract. Journal of Functional Foods **10,** 154–160.