Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 2, 2013

Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF SELECTED SEAWEEDS FROM SOUTHEAST COAST OF INDIA

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Received: 18 Mar 2013, Revised and Accepted: 30 Apr 2013

ABSTRACT

Objective: To investigate the i*n vitro* antioxidant activities of three selected green, red and brown seaweeds – viz., *Chaetomorpha linum* (Muller) Kützing, *Grateloupia lithophila* Boergesen, *Sargassum wightii* Greville from Mandapam coast in Rameshwaram (Southeast Coast of India).

Methods: The acetone and ethanol extracts of the seaweeds were investigated for their Total antioxidant activity, Reducing power, DPPH radical scavenging activity, ABTS radical scavenging activity, Deoxyribose scavenging activity, H_2O_2 radical scavenging assay, Lipoxygenase activity and Nitric oxide radical inhibition assay. The antioxidant assay was performed at the concentration ranging from 200-1000µg/ml.

Results: Brown seaweed exhibited good antioxidant activity when compared to red and green seaweeds. The maximum antioxidant activity was shown by the ethanol extract of *S.wightii* by DPPH radical scavenging assay (79.1 ± 1.21%). Ethanol extract showed a good antioxidant activity when compared to the acetone extract.

Conclusion: Seaweeds have received special attention as a source of natural antioxidants and were found to be a good source of antioxidant. Further, there is a need for isolation and characterization of natural antioxidants having less or no side effects for use in foods or medicines to replace synthetic antioxidants.

Keywords: Antioxidant activity, Radical scavenging activity, Ethanol extract, Acetone extract, Seaweeds, *Grateloupia lithophila*, *Sargassum wightii*, *Chaetomorpha linum*

INTRODUCTION

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. It also represents a great challenge that requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. Seaweeds are considered to be a rich source of antioxidants [1]. 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 [2] , but also, more stable molecules such as carotenoids [3], mycosporine-like a mino acids [4] and a variety of polyphenols (e.g. catechins, phlorotannins) [5]. More reports that are recent revealed seaweeds to be a rich source of antioxidant compounds [6] [7] [8] [9].

Further, the evidence available in the literature suggests the potential protective effects of seaweeds against oxidative stress in target tissues and lipid oxidation in foods [10]. Some active antioxidant compounds from marine algae were identified as phylopheophylin in *Eisenia bicyclis* [1], phlorotannins in *Sargassum kjellamanianum* [11] and fucoxanthin in *Hijikia fusiformis* [12]. The sulphated polysaccharides of *Sargassum* act as a potent free radical scavenger and anticancer agent [13].

Studies have been carried out on the antioxidative potential of different seaweeds mainly from the waters of China, Korea and Japan [8] [14] [15]. The presence of antioxidant substances in seaweeds is found to be an endogenous defense mechanism as a protection against oxidative stress due to extreme environmental conditions [16]. The antioxidant activity of red seaweed extracts correlated with their polyphenol content [9]. Fucoxanthin, isolated from the brown seaweed *Wakame* exhibited various radical scavenging activities [17]. Water and ethanol extracts from the dried sample of brown alga (*Sargassum boveanum*) were prepared and exa mined for its phenolic compounds and antioxidant activity [18]. *In vitro,* antioxidant effects of the hexane and methanol extracts of *Sargassum baccularia* and *Cladophora patentiramea* were tested [19]. Antioxidant activity (AA), total phenolic content, and reducing power of the crude extract, fractions, and sub fractions derived from a red alga, *Polysiphonia urceolata*, were evaluated and determined [9].

Potential antioxidant activities of enzymatic extracts from seven species of brown seaweeds were evaluated using four different reactive oxygen species (ROS) scavenging assays containing DPPH free radical, superoxide anion, hydroxyl radical and hydrogen peroxide scavenging assay [15]. The antioxidant activity, by free radical scavenging (DPPH-decolorization method) and inhibition of lipid peroxidation (Fe^{2+}/Ascorbate), in three species of seaweeds *Sargassum dentifolium, Laurencia papillosa & Jania corniculata* (Egyptian isolates) were analyzed [20]. Supercritical CO_2 extraction, ultrasonic-aid extraction and membrane separation technology were applied to prepare *Sargassum pallidum* polysaccharides (SP) and to study its antioxidant and anti-proliferative activity [21]. *In vitro* antioxidant activities of three selected Indian red seaweeds – viz., *Euchema kappaphycus, Gracilaria edulis* and *Acanthophora Spicifera* and three selected Indian brown seaweeds *Sargassum marginatum*, *Padina tetrastomatica* and *Turbinaria conoides* were evaluated by [22] [23]. Pharmacological properties of several seaweed species are still unexplored and unidentified. The present study is focused to analyze the *in vitro* antioxidant activities of three selected seaweeds from Mandapam coast.

MATERIALS AND METHODS

Seaweed collection and processing

Three seaweed samples (i) *Chaetomorpha linum* (ii) *Grateloupia lithophila* (iii) *Sargassum wightii* were collected from Mandapam coast in Rameshwaram. The algal sample was handpicked and washed thoroughly with seawater to remove all the impurities, sand particles and epiphytes. It was kept in icebox containing slush ice, transported to the laboratory and washed thoroughly using tap water to remove the salt on the surface of the sample. The water was drained off and the algal material was spread on blotting paper to remove excess water. They were shade dried. The dried seaweeds were finally pulverized in the commercial grinder and the powdered seaweed samples were stored at 4°C and used for further analysis.

Solvent extraction

Soxhlet extraction of the seaweeds was carried out with acetone and ethanol organic solvents. 200 ml of the organic solvent was used for extraction and it was carried out in Soxhlet apparatus for a period of 12-24 hours until the solvent becomes colorless at 65±2°C. The solvent was evaporated using rotary vacuum evaporator to make the final volume one-fourth of the original volume [24]. The final concentrate after extraction was then dissolved in methanol and stored at 4°C. Stock Solutions of the crude extract was prepared by dissolving the extract using methanol as a solvent.

Antioxidant Assays

The three seaweed species were tested for their *in vitro* antioxidant activity by using eight different assays. Different concentration of the solvent extracted test samples were used *viz*, 0.2, 0.4 0.6, 0.8 and 1.0 mg/ml were used for the assay. Standards were also taken in their respective concentrations.

Total Antioxidant Activity

Total antioxidant activities of crude ethanol and acetone extract obtained from seaweeds were determined [25]. Briefly, 0.3 ml of sample was mixed with 63.0ml reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695nm. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

Reducing Power

Reducing power of crude ethanolic and acetone extract obtained from seaweeds was estimated [26]. Briefly, 1.0 ml of methanol containing different concentration of sample was mixed with 2.5 ml of phosphate buffer (0.2M, pH-6.6) and 2.5 ml potassium ferricyanide (1%). Reaction mixture was incubated at 50°C for 20 min. After incubation 2.5 ml of trichloro acetic acid (10%) was added and centrifuged (650 g) for 10 min. From the upper layer 2.5 ml solution was mixed with 2.5 ml distilled water and 0.5 ml $FeCl_3$ (0.1%). Absorbances of all the sample solutions were measured at 700nm. Increased absorbance indicates increased reducing power.

DPPH (2, 2- diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The scavenging effects of ethanol and acetone extract obtained from seaweeds were determined [27]. Briefly, 2.0 ml of 0.16mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 minutes and kept at room temperature for 30 minutes in the dark. The absorbance of all the sample solutions was measured at 517nm.The scavenging effect (%) was calculated by using the formulae [9]. Sample blank and control samples were performed according to the method. Scavenging effect of DPPH radical was calculated using the following equation

$$\text{DPPH radical scavenging activity [\%]} = [1- (A_{sample} - A_{sample\ blank} / A_{control}) \times 100]$$

Where A_{sample} is the Absorbance of DPPH solution & test sample, $A_{sampleblank}$ is the absorbance of the sample only without DPPH solution).Synthetic antioxidant Ascorbic acid was used as positive controls.

Deoxyribose Radical Scavenging Activity

Deoxyribose non-site specific hydroxyl radical scavenging activity of the crude ethanol and acetone extract obtained from seaweeds was estimated [28]. Briefly, 2.0 ml aliquots of sample were added to the test tube containing reaction mixture of 2.0 ml $FeSO_4.7H_2O$ (10mM), 0.2 ml EDTA (10mM) and 0.2 ml deoxyribose (10mM). The volume was made up to 1.8 ml with phosphate buffer (0.1M, pH-7.4) and to that 0.2 ml H_2O_2 (10mM) was added. The mixture was incubated at 37°C under dark for 4 hours. After incubation, 1 ml of TCA (2.8%) and TBA (1%) were added to the mixture, and then left to stand under boiling water bath for 10 min. After treatment absorbance was measured at 532nm. If the mixture was turbid, the absorbance was measured after filtration. Scavenging activity (%) was calculated using the equation [15].

$$\text{Deoxyribose radical scavenging activity [\%]} = [1- (A_{sample} / A_{control}) \times 100]$$

Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity

The ability of the seaweeds to scavenge H_2O_2 was determined [29] with the slight modification [30]. Briefly, 40mM H_2O_2 was prepared in phosphate buffer (pH-7.4) and the H_2O_2 concentration was determined spectrophotometrically by measuring the absorption with the extinction coefficient for H2O2 of $81M^{-1}cm^{-1}$. Extracts (100 µg/ml) in distilled water and ascorbic acid (20 – 100 µg/ml, positive control) were added to 0.6 ml of 40mM H_2O_2 solution and the absorbance of H_2O_2 was determined at 230 nm after 10 minutes incubation against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 was calculated as the percentage of scavenging of hydrogen peroxide was calculated using the following formula:

$$H_2O_2 \text{ radical scavenging activity [\%]} = [(A_0 - A_1) / A_0] \times 100$$

(Where A_0 – Absorbance of control; A_1 – Absorbance of sample)

Nitric Oxide (NO) Radical Scavenging Activity

Nitric Oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which was measured by the Griess reaction. Briefly, 3 ml of the reaction mixture containing 10mM sodium nitroprusside and the seaweed extract (100 µg/ml) in phosphate buffer were incubated at 25°C for 150 min. After incubation 0.5 ml of the reaction mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 minutes for complete diazotization. Then 1 ml of naphthyl ethylene dia mine dihydrochloride (0.1%) was added and the solution mixed and allowed to stand for 30 minutes at 25°C. A pink colored chromophore is formed in diffused light. The absorbance of these solutions was measured at 540nm against the corresponding blank solutions. Ascorbic acid (200-1000 µg/ml) was used as positive control. The Nitric oxide scavenging activity of the seaweeds extract is reported as % inhibition and calculated as below:

$$\text{Nitric Oxide radical scavenging activity [\%]} = [(A_0 - A_1) / A_0] \times 100$$

(Where A_0 – Absorbance of control; A_1 – Absorbance of sample)

Deter mination of Lipoxygenase Activity

The assay for lipoxygenase activity was carried out [31]. The reaction mixture contained 0.2M citrate-phosphate buffer pH-9.0, 0.25% Tween 20, 0.125mM linoleic acid, an enzyme solution (57µg protein) and 10 µL algal extract to a final volume of 1ml. 10 µL of aqueous or ethanol was used instead of the extract as a control. The enzyme reaction was carried out in the cuvette and monitored at 234nm until the reaction rate reached a steady state. This wavelength corresponds to the absorption of the hydroperoxides generated by the action of the lipoxygenase on linoleic acid. The percentage inhibition defined by the rate of increase in OD in the absence of algal extract to that measured with the extract.

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Assay

Free radical scavenging activity of the extract was also determined by ABTS (Sigma-Aldrich) radical cation decolorization assay [32]. ABTS radical cation was generated by mixing 20mM ABTS solution with 70mM potassium peroxodisulphate and allowing it to stand in dark at room temperature for 24 hours before use. 0.6 ml of extract (0.25 mg) was mixed with 0.45 ml of ABTS reagent and absorbance of these solutions was measured at 734 nm after 10 min.

$$\text{ABTS radical cation scavenging assay [\%]} = [(A_B - A_A) / A_A] \times 100$$

Where A_B = Absorbance of Sample blank, A_A = Absorbance of Sample blank

Analysis

All the data were expressed as means ± standard deviation (SD) Statistical analysis was calculated by one way ANOVA followed by Student's Newman Keul's test. Number of replicate experiments carried out were, n=3.

RESULTS

Total Antioxidant Activity

The total antioxidant activity of the seaweeds was determined. The maximum antioxidant activity was exhibited by the ethanol extract of *S.wightii*- 12.8±0.11 (12.81 mg of Ascorbic acid/g of seaweed extract) and the lowest activity was recorded in the green algae *C.linum.* Similarly findings also showed that the maximum total antioxidant activity was shown by the *Sargassum sp*. (brown algae) compared to the green algae [33] and brown algae *C. sinuosa* had a high total antioxidant activity of 60.3 and 57% [34] and maximum total antioxidant activity was seen in the brown algae *Sargassum marginatum* compared to the other brown algae [23]. The Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in milligram per gram of extract (Table-1). Ascorbic acid was taken in different concentration viz; 200-1000µg/ml. Graph was plotted using the O.D value of the extract against the standard Ascorbic acid (n=3).

Table 1: Total Antioxidant activity of Seaweeds

Seaweeds	Ethanol extract[#]	Acetone extract[#]
C. linum	3.7±0.20*	2.5±0.28*
G. lithophila	10.1±0.40*	9.0±0.13*
S. wightii	12.8±0.11*	11.1±0.85*

(#- mg of Ascorbic acid/g of Seaweed extract, *-Mean ± S.D, P>0.05)

Reducing Power

Reducing capacity is considered as a significant indicator of potential antioxidant activity of a compound or sample [4]. 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 [35]. The reducing power of the acetone and extract of the seaweeds were analyzed. Increase in the O.D deter mines the increase in the reducing power. In the present study the ethanol extracts of the brown algae *S.wightii* possessed a good reducing power where there was a steady increase in reductive potential of the brown seaweed with increase in the O.D in a dose dependent manner (increase in concentration) followed by that *G.lithophila* also showed a good reducing power. (Figure 1 A & B). Ascorbic was used as standard (n=3).

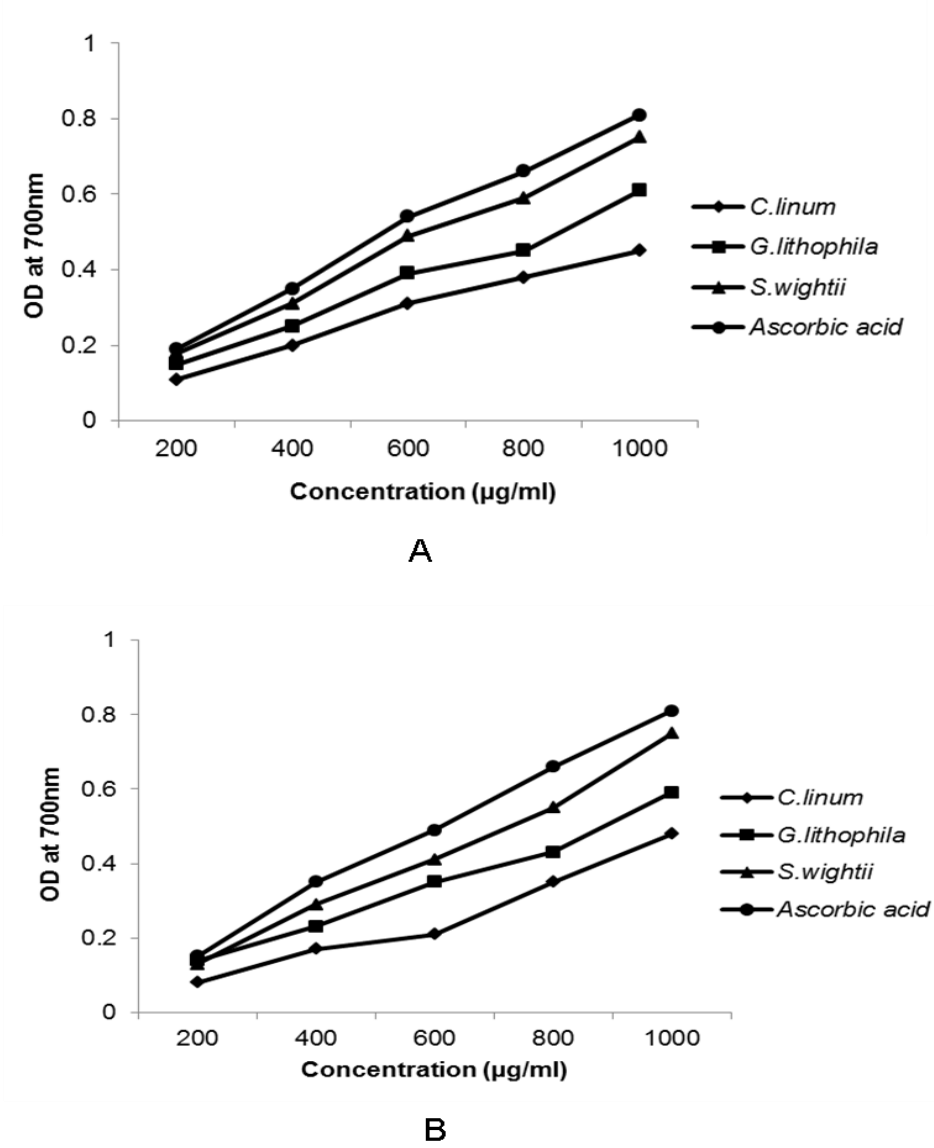


Fig. 1: Reducing power of (A) Ethanol extract and (B) Acetone extract of seaweed

DPPH Radical Scavenging Assay

DPPH have been used extensively as a free radical to evaluate reducing substances [36]. A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. 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 scavenging assay was performed with the acetone and ethanol extract of the Seaweed samples. The antioxidant activity increases with increase in the concentration. The maximum scavenging effect was shown by the ethanol extract of *S.wightii* (79.1±0.23 % inhibition) (Figure -2A) and acetone extract showed 78.8±0.18 % inhibition (Figure-2B). The lowest inhibition was shown by *C.linum* (16.2±0.31%).

Deoxyribose Radical Scavenging Assay

In this assay, the antioxidant activity was determined based on the ability of the antioxidant components in the samples to inhibit deoxyribose oxidation by reactive OH- generated from Fenton's type reaction. The Deoxyribose radical scavenging assay was performed showed that the maximum scavenging activity was exhibited by the ethanol extract of *S.wightii* (72.9±0.43 % inhibition) (Figure-3A) and acetone extract of *G.lithophila* showed 72.4±0.10 % inhibition. (Figure-3B). The lowest inhibition was shown by *C.linum* (14.4 ± 0.22%).

3.5 H_2O_2 Radical Scavenging Assay

Many species of seaweed possess scavenging ability of hydrogen peroxide [37]. 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 acetone and ethanol extract of the seaweed samples. The maximum scavenging activity was shown by the ethanol extract of *G.lithophila* (71.4±0.31 % inhibition) (Figure-4A) and acetone extract of *S.wightii* showed 70.8±0.19 % inhibition. (Figure-4B).The lowest inhibition was shown by *C.linum* (11.9±0.44%). The antioxidant activities of commercial enzyme extracts from *Sargassum sp*. exhibited more pro minent effects in hydrogen peroxide scavenging, which was approximately 90% at 2 mg/ml [15]. Similarly brown algae *C. sinuosa* showed a good inhibition of 70.7% and 56.6% with the DMSO and methanol extracts [34].

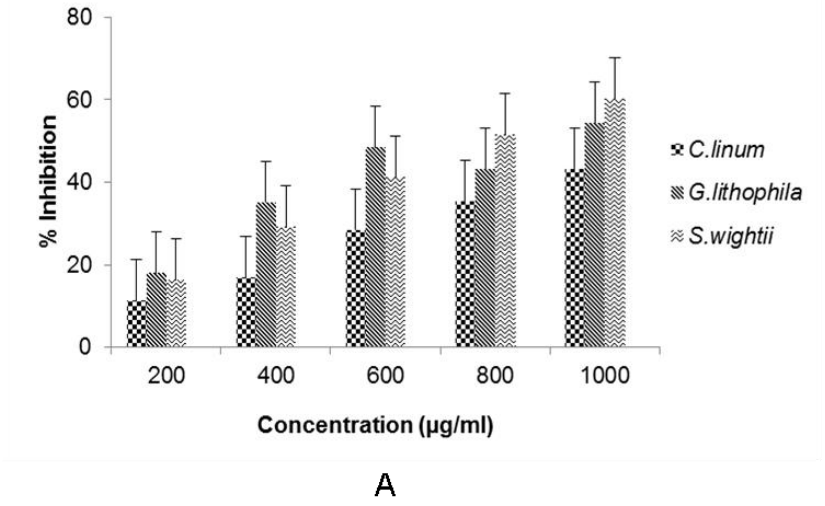
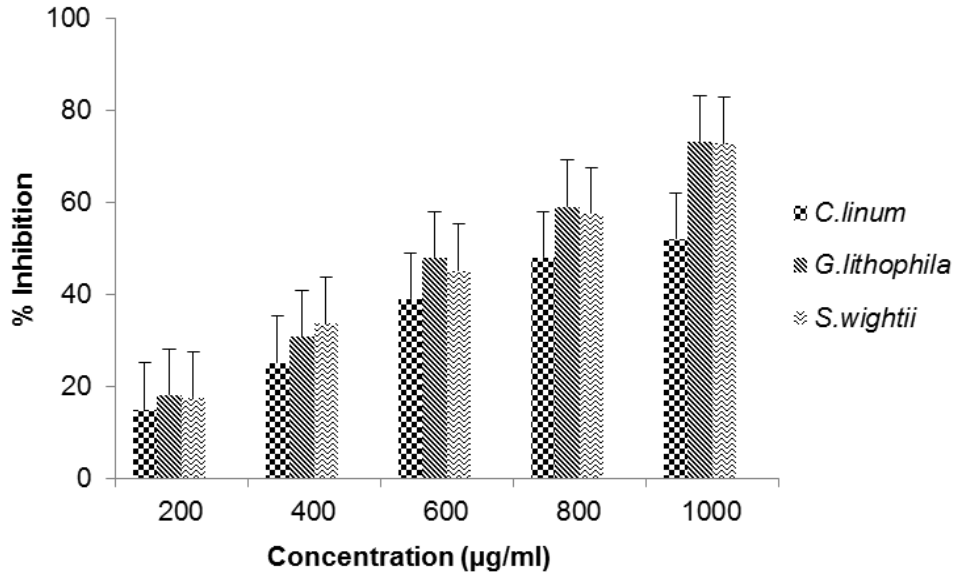
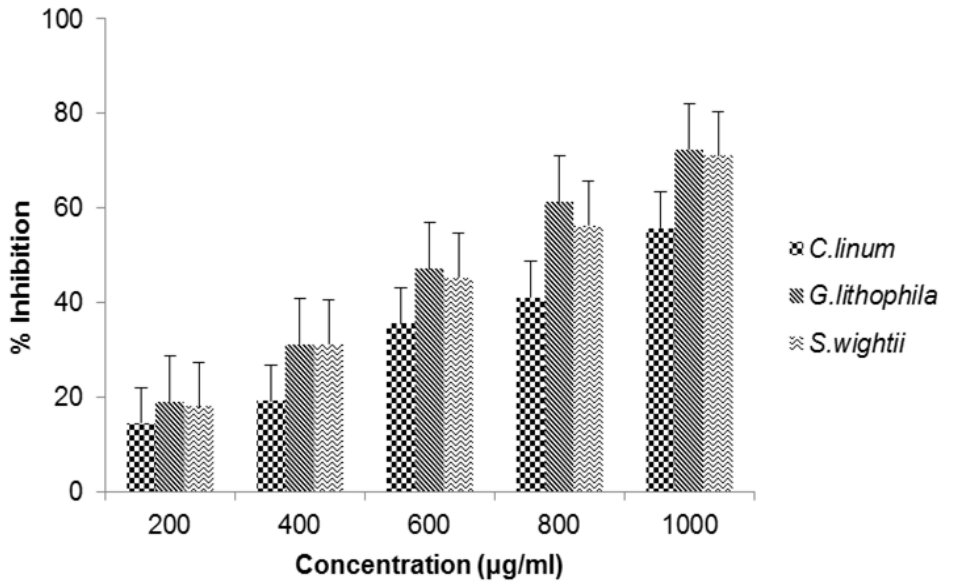


Fig. 2: DPPH radical scavenging activity of (A) Ethanol extract and (B) Acetone extract of seaweeds



A



B

Fig. 3: Deoxyribose radical scavenging activity of (A) Ethanol extract and (B) Acetone extract of seaweeds

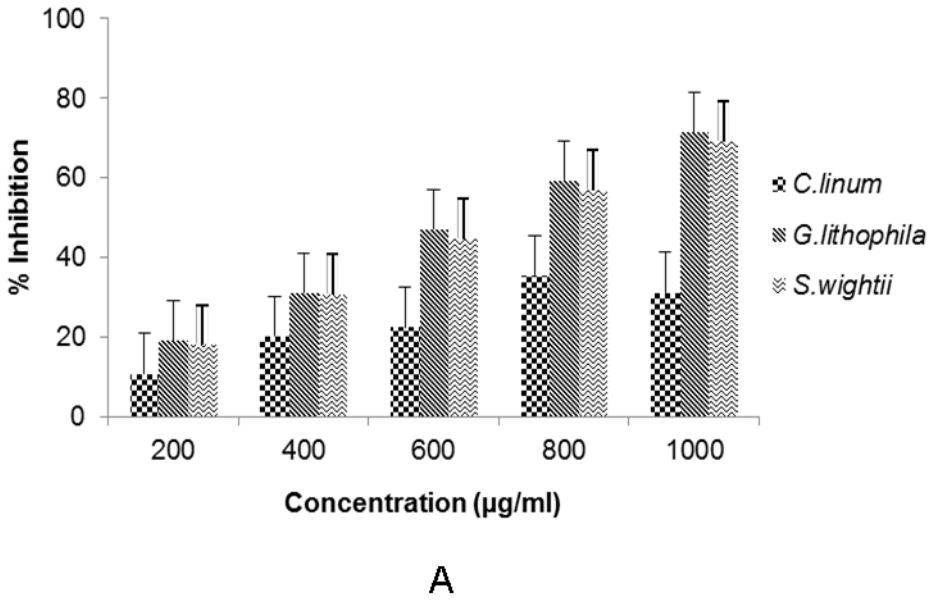
Nitric Oxide Radical Inhibition Assay

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 [38]. 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 [39]. The nitric oxide radical scavenging assay was also performed with solvent extract of the seaweed samples. The maximum scavenging activity was shown by the ethanol extract of *S.wightii* (60.22± 0.52% inhibition) (Figure 5A) and acetone extract showed 59.39±0.61 % inhibition (Figure 5B). The lowest inhibition was shown by *C.linum* (9.9±0.51%).

Lipoxygenase Activity

Increase in O.D denotes the good lipoxygenase activity. The lipoxygenase activities of the seaweed extract were also determined. Increase in O.D denotes the good lipoxygenase activity. The maximum scavenging activity was shown by the ethanol extract of *S.wightii* (Figure 6A) but there was not much difference in the acetone extract also. (Figure-6B). The lowest inhibition was shown by *C.linum*. The antioxidant activity (AOA) of water extract of *Sargassum boveanum* was high at about 90% inhibition of peroxidation of linoleic acid with 7 mg dry sample/ml solvent [18]. Red brown and green seaweeds were screened for lipoxygenase

activity and was found that the antioxidant activity in methanol extract of three Sargassum species (*S. horneri, S. macrocarpum & S. siliquastrum*), which exhibited the greatest lipoxygenase activity with ethanol extract [40].



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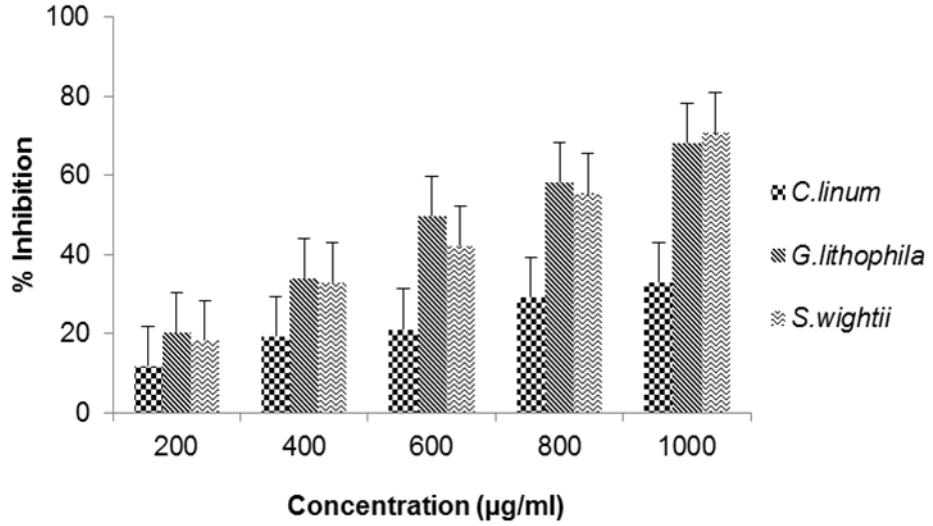

B

Fig. 4: H_2O_2 radical scavenging activity of (A) Ethanol extract and (B) Acetone extract of seaweeds

ABTS Radical Scavenging Assay

ABTS assay is a simple indirect method for deter mining 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 [41]. The ABTS radical cation-scavenging assay performed showed that the antioxidant activity increases with increase in the concentration. The maximum scavenging effect was shown by the ethanol extract of *S.wightii* (76.8±0.71 % inhibition) (Figure-7A) and acetone extract showed 75.45±0.48 % inhibition. (Figure-7B). *G.lithophila* also showed good scavenging activity as that of *S.wightii*. The lowest inhibition was shown by *C.linum* (15.2±0.73%). Similar report was given, where brown algae *C. sinuosa* showed a good inhibition of 76.8% and 74% % with the DMSO and methanol extracts [34].

DISCUSSION

There are many methods to deter mine antioxidant capacity. These methods differ in terms of their assay principles and experimental conditions; consequently, in different methods antioxidants in particular have varying contributions to total antioxidant potential [42]. In the present study the brown seaweed *S.wightii* extract showed a good radical scavenging activity and a higher phenolic content. Likewise earlier report demonstrated that the brown algae showed maximum antioxidant activity exhibited higher phenolic content [43]. The reducing power increased with increasing concentration in all the samples [23]. Same trend has also been reported in methanolic extracts of higher plants [44]. All concentrations exhibited the OD value <1.0 [8]. The methanolic extracts of the brown seaweeds were able to reduce Fe^{3+} to Fe^{2+} in a

concentration-dependent manner as a function of reducing power as reported earlier [35].

Several studies have demonstrated a highly significant correlation between the phenolic content and the antioxidant activity in seaweed extracts [11] [37]. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic compounds [45]. The phenolic compounds produced by the marine algae significantly contributed to their antioxidant capacity compared to red and green seaweed [46]. The crude extract from brown seaweeds showed higher peroxyl radical scavenging activity compared to red seaweeds [17]. The antioxidant activity was found to be higher in the *S.wightii* (brown algae) which had the higher phenolic content and it is in agreement with previous studies [9] [47], that there was a significant correlation between antioxidant activity and phenolic content of the seaweed species.

There are not many reports on comparative evaluation of antioxidant activity of seaweeds belonging to different groups. The high antioxidant activities of extracts from brown seaweeds are comparable to that of commercial antioxidants [48]. The extracts from fresh seaweeds shows higher antioxidant activity compared to extracts from dry seaweeds and red seaweeds possess higher activity than brown seaweeds [49]. The aqueous extract from brown seaweeds possesses stronger antioxidant activity compared to ethanol extract [8]. However, in the present study it is observed that antioxidant substances present in the seaweeds are non-polar in nature as organic solvent fractions, particularly hexane and ethyl acetate fractions exhibit higher antioxidant activity. The present study carried out showed that the green algae *C.linum* showed a very low antioxidant activities, like wise reports show that the green algae possessed low antioxidant activity [42]. The principal antioxidant components in seaweeds are thought to be polyphenols. Studies have demonstrated the correlation between polyphenol content and radical scavenging activity [8]. The presence of hydrophilic polyphenols in seaweeds such as phlorotannins, which are bi-polar in nature, and mostly found in brown seaweeds, could function as antioxidative components and thus assist the algae to overcome oxidative stress [50].

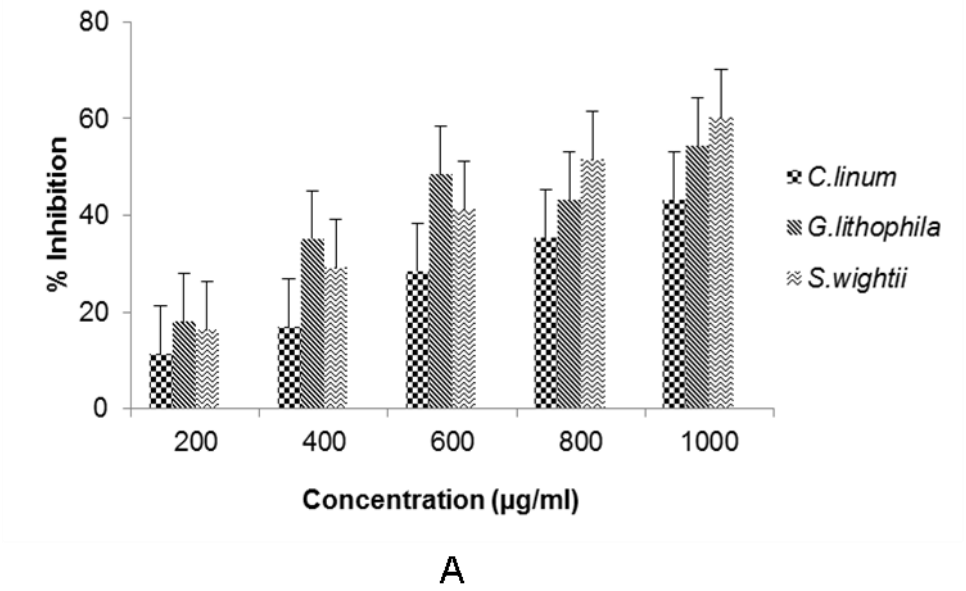

Fig. 5: Nitric oxide inhibition assay of (A) Ethanol extract and (B) Acetone extract of seaweeds

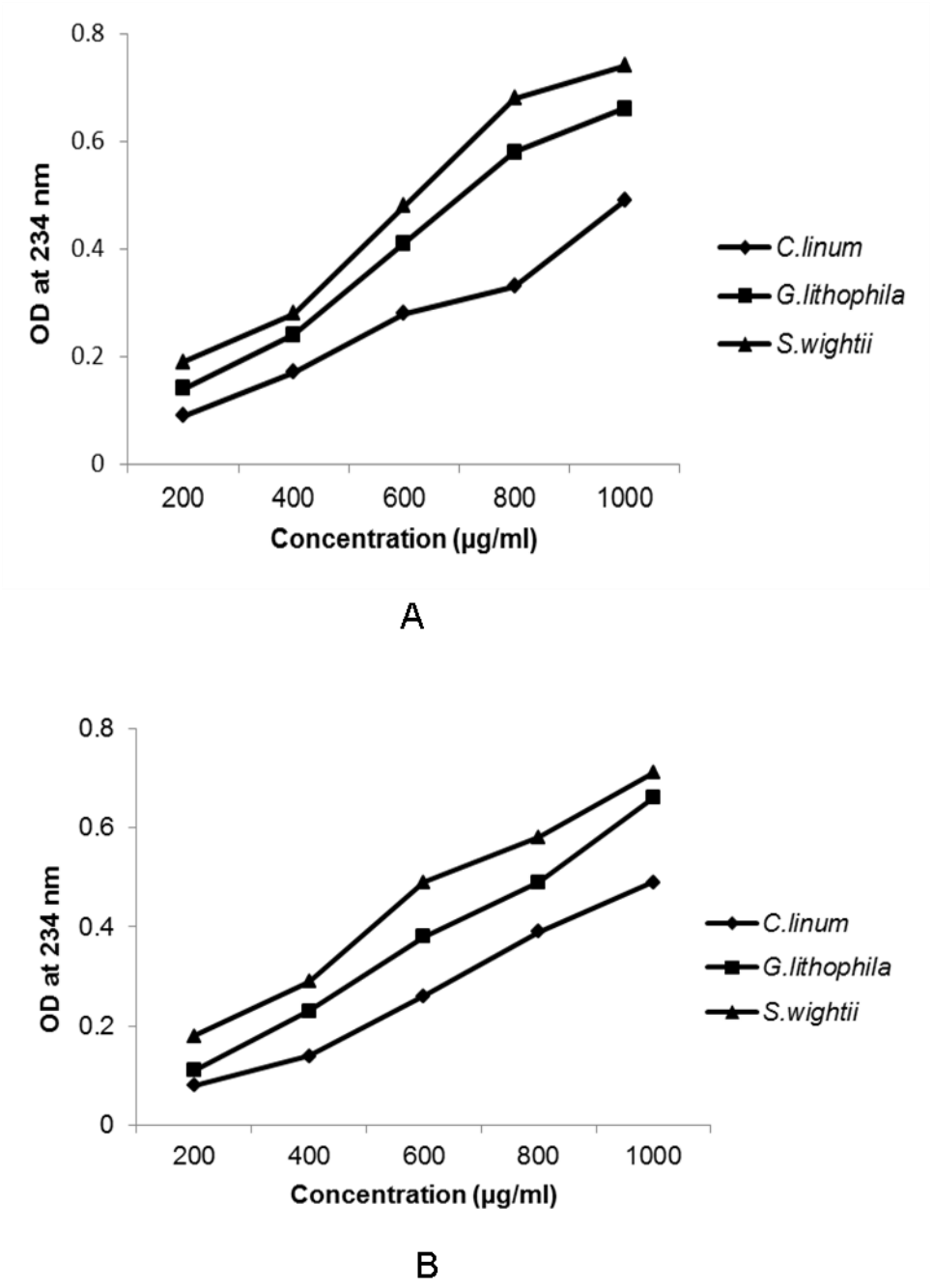

Fig. 6: Lipoxygenase activity of (A) Ethanol extract and (B) Acetone extract of seaweeds

The water-soluble natural antioxidants from another seaweed *Sargassum thunbergii* exhibited the DPPH free radical scavenging activities, and the scavenging activity of the radicals increased with increasing concentrations of the extract [7] The genus *Sargassum* has been studied extensively showing high antioxidant potential *in vitro* [8] [11] [15] [48] and in vivo [51] [52]. Compared to green and brown algae *Sargassum* possessed a good DPPH scavenging capacity [50]. The brown algae *Sargassum boveanum* exhibited noticeable scavenging effects in DPPH free radical scavenging assay. The radical scavenging activity (RSA) was about 94% [18]. The brown algae *Sargassum mangarevense* showed a scavenging activity of (84% inhibition of the DPPH [50]. The DPPH radical scavenging activity of methanol extracts of brown algae *Sargassum marginatum* showed high activity [35].A novel chromene Mojabanchromanol isolated from *Sargassum siliquastrum* showed 96.07% of radical scavenging activity against DPPH molecule [53].

Sargassum sp. shows the OH- radical scavenging activity compared to red and brown algae [50]. These polyphenols are known to be strong chelators of heavy metals [54] and thus are believed to be related to such effective OH- scavenging ability of *Sargassum* sp. in the study. Acidic polysaccharides from *Sargassum fusiforme* showed a dose dependent hydroxyl radical scavenging activity in a dose dependent manner which correlates with the present study [55]. The hydroxyl radical scavenging activity by the brown algae *Sargassum marginatum* was found to be higher [23] that gives similar results in the study with *Sargassum*. Similarly 47% inhibition in enzymatic extract of *Sargassum fulvellum* and concluded that enzymatic extracts of seaweed possessed little effect on scavenging the hydroxyl radical [15]. The seaweed extracts were effective in scavenging hydroxyl radicals to prevent degradation of Deoxyribose substrate in a dose dependent manner elaborates the fact that these seaweed extracts are able to prevent Deoxyribose damage

associated with the direct binding of iron to Deoxyribose and the subsequent attack by hydroxyl radicals generated via the Fenton reaction [10]. A good DPPH scavenging activity of the polysaccharides extracted from the brown seaweed *Sargassum pallidum* was also studied [21]. The DPPH free radicals scavenging activity in some brown seaweed methanol extracts exceeded 50%, the most active seaweed species being *Hijiki fusiformis* (65%), followed by *Undaria pinnatifida* (51.1%) and *S. fulvellum* (36.3%) [3]. The brown algae *L. variegata* showed maximum inhibition in the DPPH radical scavenging assay [50]. The brown algae *F. vesiculosus* exhibited maximum DPPH radical scavenging activity compared to the red algae [56]. A comparison study on the antioxidant activity of red and brown algae showed that the scavenging activity of all samples on the DPPH radical was found to be strongly dependent on concentration. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards to a certain extent and then slowly increased. The difference in the DPPH radical scavenging activity of each sample in different extracts implies that the extracting solvent used would affect the radical scavenging potency. This may be due to the different polarities of each antioxidant compound group present in the seaweeds [57]. It was observed that the extracts containing high levels of TPC were also potent DPPH radical scavenger, suggesting that algal polyphenols may be the principal constituents responsible for the antiradical properties of the extracts [55]; similarly in this study *S.wightii* possessed a high DPPH scavenging assay and Total phenolic content. A novel hydroxyl radical scavenger was explored from the brown algae *Sargassum myriocystem* that showed good, Total Antioxidant, DPPH radical scavenging and deoxyribose radical scavenging activity [58].

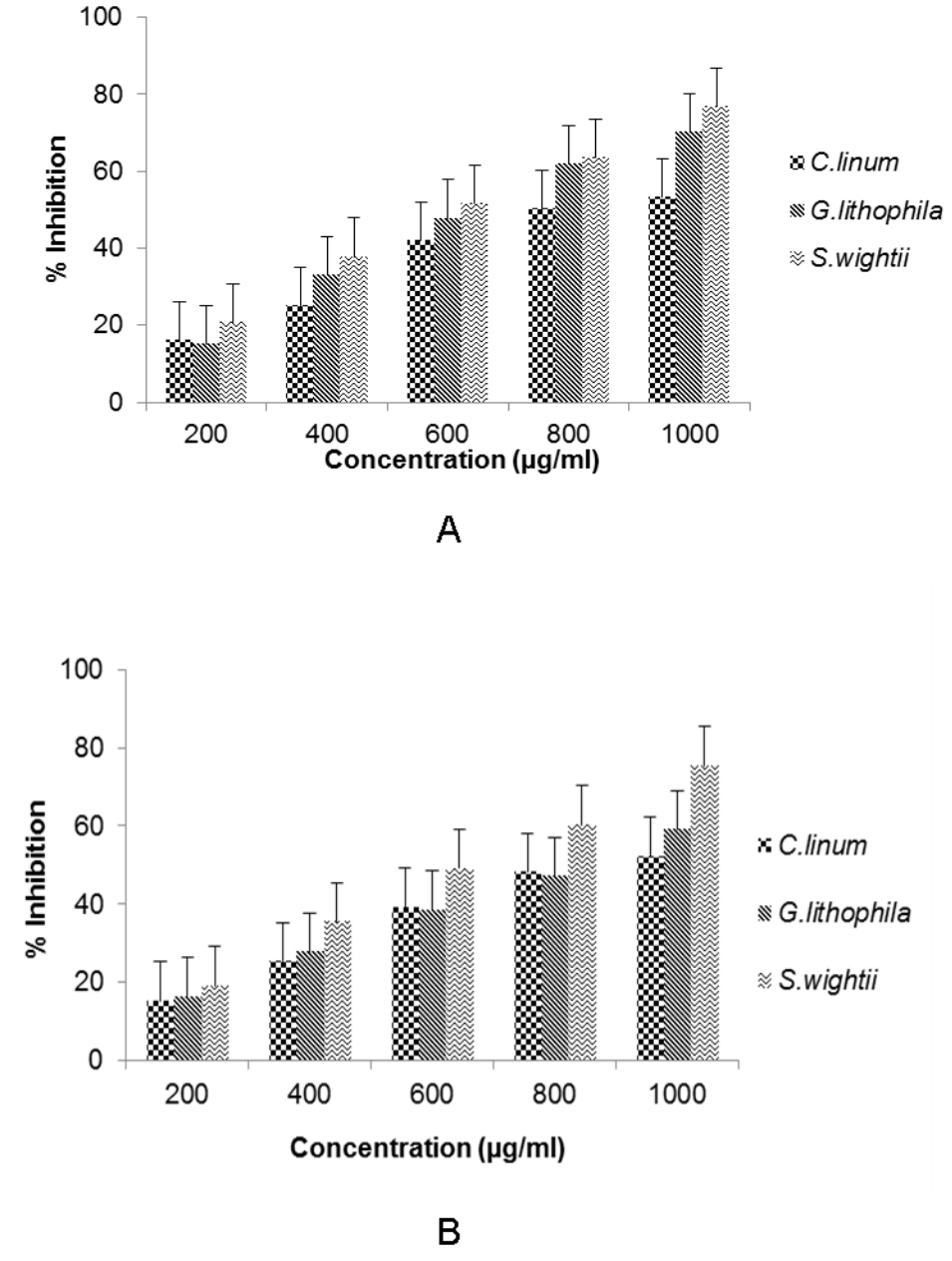

Fig. 7: ABTS radical cation scavenging assay of (A) Ethanol extract and (B) Acetone extract of seaweeds

In the present study the brown seaweed *S.wightii* showed the highest activity in ABTS radical scavenging assay compared to green and red seaweed. The crude extract and its fractions from brown seaweeds, *T. conoides* and *P. tetrastomatica* showed higher ABTS radical scavenging activity compared to other four red seaweeds [17] The brown algae *Padina minor* showed 98% inhibition of the ABTS molecule [59]. The results of the present study indicate that the Ethanol extracts from brown seaweed exhibited higher ABTS radical activity compared to red seaweed that correlates with the study [17]. However, the limitations of ABTS assay, such as the capability of a sample to react with ABTS radical rather than to inhibit the oxidative process and slow reaction of many phenolics [41] necessitate compatible evaluation of antioxidant activity using other assays as well. The antioxidant activity of extracts is strongly dependent on the types of solvent used due to compounds with different polarity exhibiting differing rates of antioxidant potential [57]. The ethanol extract was found to be having the maximum antioxidant activity. This revealed the fact that the extracts obtained from polar solvents like ethanol was found to be having the maximum antioxidant activity in comparison with the one obtained from non-polar solvents [60]. For most seaweed species, 70% aqueous acetone was more efficient to extract polyphenolic compounds compared to water [55]. Phenolic compounds are generally more soluble in polar organic solvents than in water. The effective extractants recommended are aqueous mixtures of methanol, ethanol and acetone [61]. 70% aqueous acetone (v/v) was found to be the most efficient solvent.

Ethanol extract of *S.wightii* showed a good antioxidant activity in almost all the assays compared to the red and green algae, hence it can be confirmed that the *Sargassum* species posses good antioxidant property. Similar finding was reported that the *Sargassum siliquastrum* methanol extract demonstrated an antioxidant effect since the hydroxyl group and aromatic ring of the antioxidant components in the *Sargassum siliquastrum* functioned as nonpolar chain-breaking antioxidants [6]. Studies on different species Sargassum Viz, *Sargassum flvelum, Sargassum horneri, Sargassum coreanum, Sargassum thunbergii* and reported that it posses strong antioxidant activity, H_2O_2 radical scavenging activity and reducing power [15]. In addition, screening of antioxidant activity in methanol extract of three *Sargassum* species (*S. horneri, S. macrocarpum & S. siliquastrum*), which exhibited the greatest antioxidant activities. The scavenging activity was increased with increasing the extract concentrations (50 & 100 µg mL-1), the maximum value was obtained by brown *S. dentifolium* (82 & 86%) compared to green and red algae [40]. Additionally, reports show that the phenol moiety of the chromene derivative separated from *Sargassum micracanthum* reacts with radicals and demonstrates antioxidant effects by stabilizing phenoxyl radicals [51], which coincides with earlier report [6]. It has been earlier reported that some major active compounds from brown seaweed that have antioxidative properties are phlorotannins and fucoxanthin [3].

CONCLUSION

In the present study the acetone and ethanol extracts of seaweeds at varying concentrations were shown as a potential reducing agent, hydrogen peroxide, nitric oxide scavengers, ABTS, DPPH, Deoxyribose radical scavengers. 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 ethanol extract showed a good result when compared to the acetone extract. 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.

ACKNOWLEDGEMENT

The authors are thankful to the Chancellor and Dean, SBST, VIT University for providing the necessary facilities to carry out the research work.

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