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In vitro antioxidant activities of selected seaweeds from Southeast coast of India

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ABSTRACT

Objective: *In vitro* antioxidant activities of three selected Indian seaweeds viz., *Halimeda tuna (H. tuna)*, *Turbinaria conoides (T. conoides)* and *Gracilaria foliifera (G. foliifera)* were evaluated. **Methods:** Total antioxidant activity, total phenolic content, and reducing power of crude methanol and diethyl ether extracts were determined. **Results:** Total phenolic content and total antioxidant activity were higher (1.231±0.173 mg GAE/g, 1.675± 0.361 mg GAE/g) in *T. conoides* respectively. Reducing power of crude methanol extract increased with concentrations of the extract. The Fourier transform-infra red spectrum analysis revealed the presence of polyphenolic signals. The seaweed extracts displayed moderate antioxidant activity compared to gallic acid standard. **Conclusions:** The seaweeds could be considered for curing diseases from oxidative deteriorations.

1. Introduction

Seaweeds or marine macro algae are the potential renewable resource in marine environment. About 6 000 species of seaweeds have been identified and are grouped into three different classes viz., green (Chlorophyceae), brown (Phaeophyceae) and red (Rhodophyceae) algae. The total global seaweed production of the world in 2004 was more than 15 million metric tonnes of which nearly 15%-20% is contributed by Indian coastal regions[1].

Seaweed harvest across Indian coast is about 100 000 metric tonnes (wet weight)[2]. Seaweeds provide for an excellent source of bioactive compounds such as carotenoids, dietary fibre, protein, essential fatty acids, vitamins and minerals[3-6]. In Asian countries, Japanese are the main consumers of seaweed with an average of 1.6 kg (dry weight) per year per capita[7]. However, in India, seaweeds are exploited mainly for the industrial production of phycocolloids such as agar-agar, alginate and carrageenan; and not as cookery item or for recovering beneficial biomolecules.

All living organisms contain complex systems of antioxidant enzymes. Some of these systems, e.g. the thioredoxin system, are conserved throughout evolution and are required for life. Antioxidants in biological systems have multiple functions, including defending against oxidative damage and participating in the major signaling pathways of cells. One major action of antioxidants in cells is to prevent damage caused by the action of reactive oxygen species. Reactive oxygen species include hydrogen peroxide (H_2O_2), the superoxide anion ($O_2^{\cdot}$), and free radicals, such as the hydroxyl radical ($-OH^{\cdot}$). These molecules are unstable and highly reactive, and can damage cells by chain reactions, such as lipid per oxidation or formation of DNA adducts that could cause cancer-promoting mutations or cell death. In order to reduce or prevent this damage, all cells invariably contain antioxidants.

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous

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factors and exogenously result in extensive oxidative damage that in turn leads to geriatric degenerative conditions, cancer and a wide range of other human diseases[8-10]. Carotenoids are the natural pigments from plant origin react rapidly with these free radicals and retard or alleviate the extent of oxidative deterioration[11].

Furthermore, antioxidants from natural sources increase the shelf-life of foods[12]. Therefore, consumption of antioxidant and addition of antioxidant in food materials protect the body as well as against these materials. Many researchers have reported various types of antioxidants in different kinds of higher plants[13-14]. More recent reports revealed seaweeds to be a rich source of antioxidant compounds[15-18].

Some active antioxidant compounds from marine algae were identified as phylopheophylin in *Eisenia bicyclis*[19], phlorotannins in *Sargassum kjellamanianum*[20] and fucoxanthinin in *Hijikia fusiformis*[21]. Several researchers have reported the antioxidant properties of both brown and red seaweeds from across the globe[22-25]. Further, there are evidence available in the literature to show the potential protective effects of seaweeds against oxidative stress in target tissues and lipid oxidation in foods[25, 26]. However, there is paucity of data on the antioxidant potential of seaweeds harvested in India. Any studies evaluating the antioxidant potential of these Indian seaweeds would enhance their utility value. Further, reports on the antioxidant properties of seaweed extracts from India are very limited. Hence, the present study was intended to investigate the antioxidant properties of three seaweeds. In addition, relationship between antioxidant activity and total phenolic content were also analysed.

2. Materials and methods

2.1. Collection of samples

Halimeda tuna (H. tuna), *Turbinaria conoides (T. conoides)* and *Gracilaria foliifera (G. foliifera)* were collected from South East Coast of India during August 2009. The samples were thoroughly washed with seawater and then freshwater to remove epiphytes and other dirt particles, followed by shade - drying for two to five days. It was then brought to the laboratory, oven dried at 70 ℃ for 4 h to obtain a constant weight and pulverized in the grinder (size 2 mm). The samples were used to determine the phenolic content, as well as for antioxidant activity with the help of analytical grade.

2.2. Preparation of extracts

The pulverized moisture free seaweed material (20 g) was extracted in 200 mL of methanol and diethyl ether in a soxhlet extractor. The extraction was repeated many times to obtain a required quantity of extract. Consequently, the extract was concentrated in a rotary evaporator at 40 ℃. The antioxidant efficacy of the extracts from *H. tuna, T. conoides* and *G. foliifera* was determined in triplicate samples.

2.3. Determination of total phenol

Phenolic contents of crude methanol and diethyl ether extract and fractions were estimated by the method of Taga MS *et al*[27]. Briefly, 100 μL aliquot of sample was mixed with 2.0 mL of 2% Na_2CO_3 and allowed to stand for 2 minutes at room temperature. After incubation, 100 μL of 50% Folin-Ciocalteau's phenol reagent was added, and then reaction mixture was mixed thoroughly and allowed to stand for 30 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer (Shimadzu, UV-160, and Japan). Phenolic contents are expressed as Gallic acid equivalent per gram (GAE/g).

2.4. Total antioxidant activity

Total antioxidant activity of methanol and diethyl ether extracts was determined according to the method of Prieto *et al*[28]. Briefly, 0.3 mL of sample was mixed with 3.0 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95 ℃ for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of gallic acid.

2.5. Reducing power

Reducing power of crude methanol and diethyl ether extract obtained from seaweeds was determined by the method prescribed by Oyaizu[29]. Briefly, 1.0 mL of methanol containing different concentration of sample was mixed with 2.5 mL of Phosphate buffer (0.2 M, pH 6.6) and 2.5 mL Potassium ferricyanide (1%). Reaction mixture was incubated at 50 ℃ for 20 min. After incubation, 2.5 mL of Trichloroacetic 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%). Absorbance of all the sample solutions was measured at 700 nm. Increased absorbance indicated an increased reducing power.

2.6. FT-IR spectrometry (Fourier transform -Infra red spectrum analysis)

The seaweed extract samples (10 mg) was mixed with 100 mg of dried Potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectrometrically (Bio-Rad FTIR-40 model. USA). The

frequencies of different components present in active sample were analyzed.

3. Results

The Total phenolic content (TPC) of *T. conoides*, *G. foliifera* and *H. tuna* along with the standard gallic acid is shown in Figure 1. *T. conoides* exhibited higher activity than *G. foliifera* and *H. tuna*. All the activities were however; relatively lower than that of standard compound. *T. conoides* showed higher TPC of 1.231±0.173 mg GAE/g in methanol extract (Figure 1), when compared to diethyl ether extract 1.190 ± 0.006 mg GAE/g in diethyl ether extract.

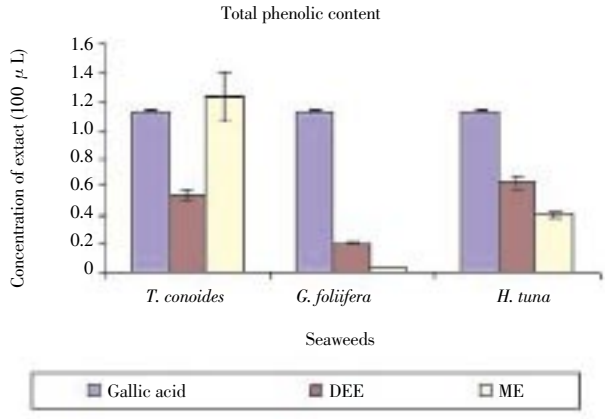

Figure 1. Total phenolic content.

The antioxidant activity of *T. conoides, G. foliifera* and *H. tuna*, along with standard Gallic acid as shown in Figure 2. Among the three seaweeds *T. conoides* exhibited higher radical scavenging activity when compared to *G. foliifera* and *H. tuna*. All the activities were however; relatively lower than that of standard compound. The methanolic extract of *T. conoides* showed higher antioxidant potential as 1.235 ±0.027 mg GAE/g (Figure 2) than 1.251±0.039 mg GE/g in diethyl ether extract of *G. foliifera*.

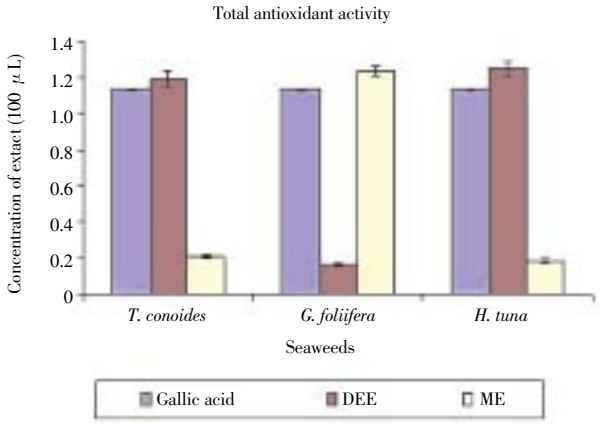

Figure 2. Total antioxidant activity.
DEE-diethyl ether, ME-methanol

In case of, the antioxidant activity were investigated as a function of reducing power.

Concentration dependency of antioxidant activity was investigated as a function of reducing power as shown in Figure 3. The reducing capacity of various concentrations of *T. conoides*, *G. foliifera* and *H. tuna* extracts behaved in a dose dependent manner (0.2 to1.0 mg/mL). Similar to the total antioxidant activity (TAA), *T. conoides* extract showed better reducing power than *G. foliifera* and *H. tuna*.

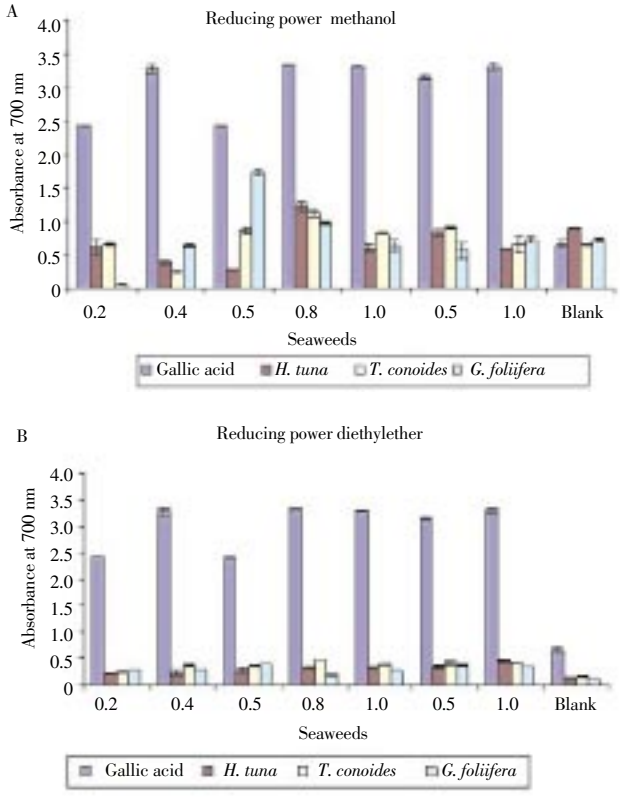

Figure 3. Reducing power

Among the three seaweeds, *T. conoides* exhibited higher radical scavenging activity when compared to *G. foliifera* and *H. tuna*. However, the antioxidant activities were lower than that of standard. The higher antioxidant activity of brown alga, *T. conoides* extract alone chosen for characterization studies. To understand the role of secondary metabolites present in the *T. conoides*, the screened seaweed samples were subjected for FT-IR analysis.

In FT-IR analysis, the chemical composition of methanol and diethyl ether extract of *T. conoides* was observed to be similar. The characteristic absorption of sulfate and carboxyl groups were identified in the FT-IR spectra (Figure 4).The peaks at 537-3396 and 3419 cm^{-1} were caused by the stretching vibration of O-H of strong relative strength in axial position, the stretching vibration of C-H of strong and C=O stretch mode respectively. Signals at 3440-536 cm-1 corresponded to stretching vibration of O-H.

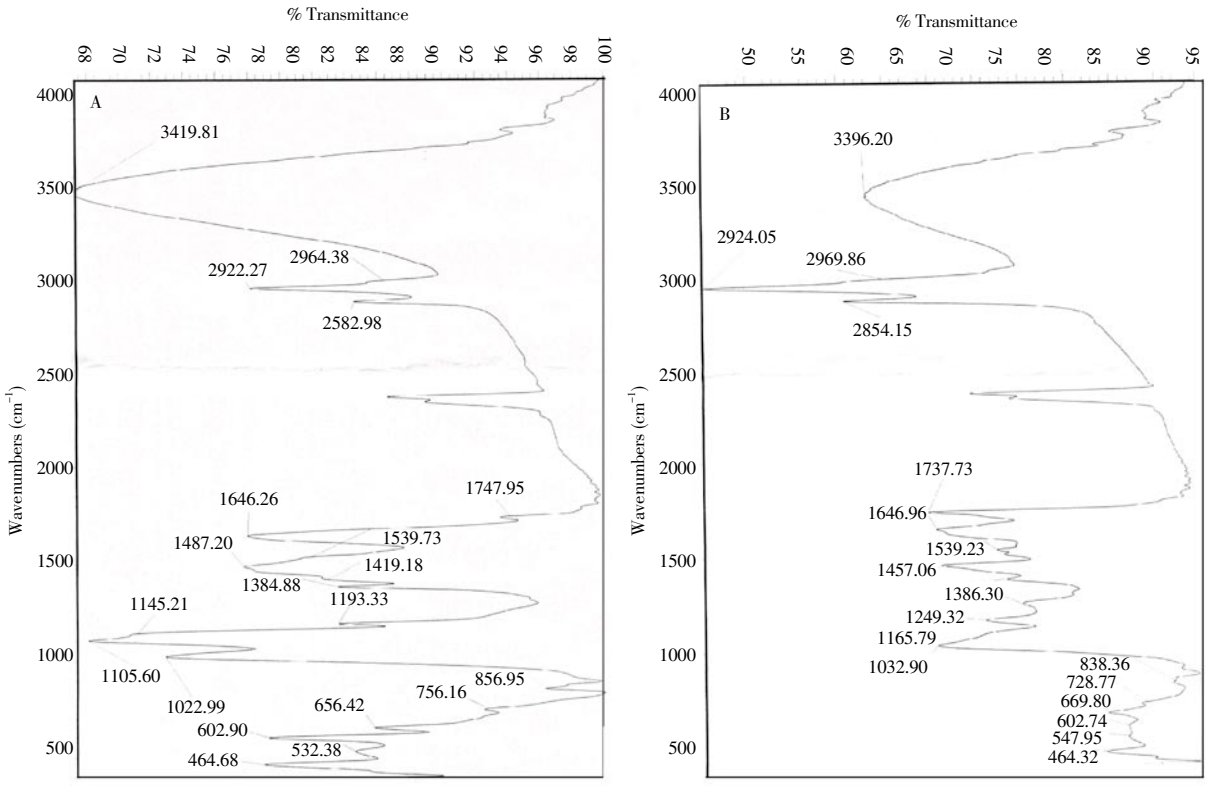

Figure 4. FT-IR Spectra of *T. conoides*.
A: Diethyl ether extract
B: methanol extract

4. Discussion

Phenolic compounds are commonly found in plants, reportedly having several biological activities including antioxidant properties. Earlier reports have revealed that marine seaweed extracts, especially polyphenols, have antioxidant activity[21, 16, 17.]. The major active compounds in different seaweed extracts have been reported to be phlorotannins and fucoxanthin[20-21]. Duan *et al*.,[15] recorded high phenolic content (73.7 GE/g) in ethyl acetate soluble fraction of red alga, *Polysiphonia urceolata*. Phenolic compounds are regarded for their important dietary roles as antioxidants and chemo preventive agents[30]. Previous reports suggested that there is a direct relationship between the antioxidant activity and the total phenolic content in some herbs, vegetables, and fruits[31]. However, there are also some reports that found no such relationship[32]. In the present study, we found that most of the marine algae contained phenolic constituents in various proportions and showed antioxidant activity to various degrees.

A number of studies have been focused on the biological activities of phenolic compounds, which are potential antioxidants and free-radical scavengers[33]. Brown algae generally contain higher amounts of polyphenols than red and green algae. Phenolic compounds are generally more soluble in polar solvents. The effective extractants recommended are aqueous mixtures of methanol, ethanol and acetone[34].

Phlorotannins[35], is bipolar in nature, and mostly found in brown seaweeds[36], such as *T. conoides*, the phlorotannin function as a antioxidant component due to the presence of multiple phenolic groups which could assist the algae to overcome oxidative stress as well as play a putative adaptive role in defense against grazers, such as marine herbivores[37] due to their plasticity characters. The presence of antioxidative phlorotannins in *T. conoides* could cause to have higher TPC compared to the other two species; on the other hand, both *T. conoides* and *H. tuna* had fairly low TPC. According to Fayaz Metal[38] *G. foliifera* contains ascorbic acid and polyphenols, which are hydrophilic.

Published reports on the total antioxidant activities of seaweed extracts are not available. However, a total antioxidant activity of 245-376 mg ascorbic acid/g extract has been reported in higher plant extracts[39]. Higher activity in fractions may be due to the interferences of other compounds present in crude methanol extracts; and, it has also been reported that solvents used for extraction have dramatic effect on the chemical species[40-41].

The antioxidant activity is system -dependent. Moreover, it depends on the method adopted and the lipid system used as substrate[42]. In addition, the fact that phenolic content of seaweeds was not significantly different ($P>0.05$) barring *T. conoides* indicating the role of other compounds in antioxidant activity of seaweeds. It has been earlier reported that antioxidative properties are of phlorotannins and fucoxanthin[21]. The activity cannot be attributed to pigments fucoxanthin carotenoids alone[43].

Concentration dependency of antioxidant activity was

investigated as a function of reducing power as this gave a general view of reductones present in the sample. Reducing power increased with increasing concentrations in all the samples. Same trend has also been reported by Altena *et al*[37] in methanol extracts of higher plants. All concentrations exhibited the OD value <1.0. Similar findings have also been reported by Kuda *et al*[16]. This property is associated with the presence of reductones that are reported to be terminators of free radical chain reaction[44].

In Ferric Reducing Antioxidant Power (FRAP), the antioxidant activity was determined based on the ability of the antioxidant components in the samples to reduce Ferric (III) to Ferrous (II) in a redox -linked colourimetric reaction[45] that involves single electron transfer. The phlorotanins which are usually present in brown seaweeds are strong chelators of heavy metals, which are believed to be responsible for the chelating ability of *P. antillarum*. *K. alvarezzi* also have the same trend as *P. antillarum*, but its chelating ability at the same concentrations is lower[46].

Ganesan *et al*.,[47] evaluated the *in vitro* antioxidant activities of methanolic extract and it solvent fractions obtained from selected Indian red seweeds. Ethyl acetate fraction of *Acanthophora spicifera* exhibited higher total antioxidant activity (32.01 mg ascorbic acid equivalent /g extract) among all the fractions. Higher phenolic content (16.26 mg gallic aicd equivalent /g extract) was noticed in petroleum ether fraction of *Gracilaria edulis*. Reducing power of crude methanol extract increased with increasing concentration of the extract. Reducing power and hydrazyl radical scavenging activity of *Eucheuma kappaphycus* were higher compared to standard antioxidant (α -tocopherol).

Tao Wang *et al*.,[48] 2009 screened the potential antioxidant activities of water and 70% acetone extracts from ten species of Icelandic seaweeds was performed using three antioxidant assays. Significant difference were observed both in total phenolic contents (TPC) and antioxidant activities of extract from various species evaluated using 2,2- Diphenyl -1- picrylhydrazyl (DPPH) , Oxygen radical absorbance capacity (ORAC) and Ferrous ion chelating ability assays. Acetone extracts from three fucoid species had the highest TPC and consequently exhibited the strongest radical scavenging activities. High correlation was found between TPC of seaweed extracts and their scavenging against DPPH and peroxyl radical, indicating an important role of algal polysaccharide as chain -breaking antioxidants. However, water extracts generally had higher ferrous chelating activity than 70% acetone extracts and no correlation was found with this TPC, suggesting other components such as polysaccharides, proteins or peptides in the extracts.

Patricia Matanjun *et al*.,[49] reported that the antioxidant activity of eight edible species of Malaysian North Borneo seaweeds obtained from Sabah waters (Kudat, Tanjung Aru and Semporna) consisting of three red seaweeds (*Eucheuma cottonii, Eucheuma spinosum* and *Halymenia durvillaei*), two green seaweeds (*Caulerpa lentillifera* and *Caulerpa racemosa*) and three brown seaweeds (*Dictyota dichotoma, Sargassum polycystum* and *Padina* sp.) were determined. Methanol and diethyl ether were used as extraction solvent. The antioxidant activities were determined by two methods, TEAC (trolox equivalent antioxidant capacity) and FRAP (ferric reducing antioxidant power) assays. The total phenolic content of the extract was determined according to the Folin-Ciocalteu method and results were expressed as phloroglucinol equivalents. The methanolic extracts of green seaweeds, *Caulerpa lentillifera* and *Caulerpa racemosa*, and the brown seaweed, *Sargassum polycystum* showed better radical-scavenging and reducing power ability, and higher phenolic content than the other seaweeds. The TEAC and FRAP assays showed positive and significantly high correlation (R^2=0.89). There was a strong correlation (R^2=0.96) between the reducing power and the total phenolic content of the seaweeds methanolic dry extracts. In our study the brown alga, *T. conoides* exhibited higher activity than *G. foliifera* and *H. tuna*. because of the reason brown alga contains more phlorotannin components are present.

Mayalen Zubia *et al*.,[50] demonstrated the extracts from 48 marine macroalgae species (17 Chlorophyta, 8 Phaeophyta and 23 Rhodophyta) from the coasts of Yucatan and Quintana Roo (Mexico) were evaluated for antioxidant activity. The antioxidant activity was measured with the DPPH (2,2-diphenyl-1-picrylhydrasyl) method, and the phenolic content of each extract were also evaluated. All species exhibited a DPPH radical scavenging activity, and three species (*Avrainvillea longicaulis, Chondria baileyana* and *Lobophora variegata*) demonstrated great antioxidant potential with very low oxidation index EC50 (1.44±0.01, 2.84±0.07 and 0.32±0.01 mg/mL, respectively), significantly equivalent to EC50 of some commercial antioxidants such as α -tocopherol, ascorbic acid, BHA and BHT. Moreover, extracts of the most active species exhibited reducing activities, superoxide anion radical scavenging and inhibition of lipid peroxidation.

Lekameera *et al*.,[51] reported the brown alga, Colpomenia sinuosa was subjected to DPPH (1,1-diphenyl-2-picryl hydrazyl), nitric oxide radical, hydrogen peroxide, scavenging of ABTS radical and total antioxidant capacity (TAC) inhibitory assays to assess its antioxidant property. The dimethyl sulphoxide (DMSO) and methanol extracts showed highest antioxidant activity in DPPH (96.56% and 88.57%), nitric oxide radical [(81.2±4.1)% and (76.69±3.1) %], hydrogen peroxide [(70.7±3.5)% and (56.6±2.0)%] and ABTS [(76.8±3.8)% and (74.0±2.8)%]. The moderate activity was observed in total antioxidant capacity (60.3±4.3 and 57.0 ±2.0) assay. The activity was higher and comparable to that of commercial antioxidants butylated hydroxyl anisole (BHA) [(87.38±1.32)%] and butylated hydroxy tolune (BHT) (56.05±0.19%) at 2 mg/mL concentration. The present study methanolic extract of *T. conoides* showed higher antioxidant potential as (1.235±0.027) mg GAE/g. The results proved that, the methanolic extract of brown alga is convey the antioxidant compounds.

Chewa *et al*.,[52] determined the Total phenolic content (TPC) and antioxidant activity (AOA) of 50% aqueous methanol extracts of the marine algae, *Padina antillarum, Caulerpa racemosa* and *Kappaphycus alvarezzi* were studied. TPC was measured using Folin-Ciocalteu method while 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP), ferrous ion chelating

(FIC) assay and beta carotene bleaching (BCB) assay were used to study their AOA.

P. antillarum was found to have the highest TPC, 24307208 mg gallic acid equivalents (GAE) per 100 g dried sample and ascorbic acid equivalent antioxidant capacity (AEAC), 1140785 mg AA/100 g. *C. racemosa* and *K. alvarezzi* displayed lower TPC and AEAC. *Caulerpa racemosa* had 144722 mg GAE/100 g dried sample of TPC and 14.372.0 mg AA/100 g of AEAC,while *K. alvarezzi* had 115735 mg/100 g dried sample of TPC and 37.8716.8 mg AA/100 g of AEAC. In addition, *Panaelus antillarum* displayed the highest reducing power, 15.772.6 mg GAE/g and highest chelating ability. *Caulerpa racemosa* and *K. alvarezzi* exhibited lower reducing power, 0.73770.423 mg GAE/g and 0.56170.269mg GAE/g, and lower chelating ability. However, the AOA of these three seaweeds as assessed by BCB assay were equally high.

The FT-IR spectra of methanol and diethyl ether extractions of *T. conoides* exhibited similar characteristic absorption bands at 2924, 1737,1646,1537,1165 and 602 cm^{-1}. The band at 860 cm^{-1} is ascribed to β -configuration of glycosidic linkage[53]; the bands at 1165 cm^{-1} correspond to the glycosidic linkage vibration of C-O-C and C-O-H. In addition, the signals at 1646 and 1537 cm^{-1} are due to the asymmetric and symmetric stretch vibration of -COO- of uronic acid[54-56] and 838 cm^{-1} is due to the characteristic absorption of mannuronic acid[57].The band at 1255 cm^{-1} may be the stretch vibration of S=O.

The characteristic absorption of sulfate and carboxyl groups were identified in the FT-IR spectra. The peaks at 537 - 3396 and 3419cm^{-1} are caused by the stretching vibration of O-H of strong relative strength in axial position, the stretching vibration of C-H of strong and C=O stretch mode respectively. Signals at 3440-536 cm^{-1} correspond to stretching vibration of O-H.

Seaweeds can be utilized as a source of natural antioxidant compounds as their crude extracts exhibit antioxidant activity. The results indicate that methanolic extract exhibit higher antioxidant activities when compared to diethyl ether extract. Further research to identify, isolate and to characterize the specific chemical which is responsible for higher antioxidant activity. Bioactive compounds found in seaweeds await a major breakthrough for a variety of food - medical application as they have the potential for applications of such compounds as natural antioxidant in different food and pharmaceutical products.

Conflict of interest statement

We declare that we have no conflict of interest.

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