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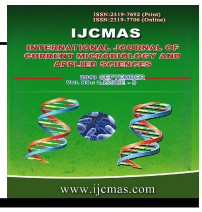

Original Research Article

Evaluation of *in vitro* antioxidant properties of some selected seaweeds from Tuticorin coast

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K e y w o r d s

Antioxidant capacity; DPPH radical scavenging activity; Hydrogen peroxide radical scavenging assay; Reducing power.

A B S T R A C T

The antioxidant potential of acetone and ethanol extract of six seaweeds collected from Tuticorin coast were determined using total antioxidant activity, total phenolic activity, DPPH radical scavenging activity, hydrogen peroxide radical scavenging assay and reducing power. The acetone and ethanolic extract of brown seaweed *Dictyota dichotoma* showed higher phenolic content than all seaweeds studied. Higher antioxidant activity was observed in acetone extract of *D. dichotoma* and *Turbinaria ornata.* Higher DPPH radical scavenging activity was also observed in the acetone extracts of *D. dichotoma* and *T. ornata*. The maximum and minimum FRAP value was observed in acetone extract of *D. dichotoma* and ethanol extract of *Enteromorpha compressa* respectively. In the present study, the extract of *D. dichotoma* was found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of seaweed extracts.

Introduction

The most important and biologically significant free radicals are the radical derivatives of oxygen called Reactive Oxygen Species (ROS). These free radicals contain two unpaired electrons in their outer shell. The most common ROS include the super oxide anion ($O_2^{\cdot-}$), the hydroxyl radical (OH.), Nitric Oxide (NO.), singlet oxygen (O_2) and hydrogen peroxide (H_2O_2). Hydroxyl radicals, though short lived, are the most damaging radicals within the body. Hydrogen peroxide is produced *in vivo* by many reactions and it can be converted to the highly damaging hydroxyl radical or catalyzed and excreted harmlessly as water. If hydrogen peroxide is not converted into water, singlet oxygen is formed. Singlet oxygen, though not a free radical, can cause further reactions and act as a catalyst for free radical formation.

Antioxidant activity is intensively focused due to the currently growing demand from the pharmaceutical industry where there is interest in anti-aging and anti carcinogenic

natural bioactive compounds, which possess health benefits (Matsukawa *et al.*, 1997). Therefore antioxidants of natural origin are much preferred (Gülçin *et al.*, 2002). The development of safe and effective antioxidants received much attention in recent time. The search for powerful but non-toxic antioxidants from natural sources, especially edible or medicinal plants, is continuing for several years. Recently, the health effects especially the suppression of active oxygen species by phytochemicals like tea; spices and herbs received much attention as natural antioxidants.

The use of seaweed as food and medicine prior to 2000BC found mention in ancient Chinese medicinal literature (Abbott 1996). Seaweeds also have a number of secondary metabolites that serve as chemical defense mechanisms against herbivores and fouling (De Nys *et al.*, 1998, De Lara-Isassi *et al.*, 2000). It is thus highly probable that algae have the potential to provide an alternative source of leads in solving many biomedical problems, including oxidative damage (Ruberto *et al.*, 2002).

Phenolic compounds play an important role in the antioxidative properties of many plant derived antioxidants (Canadanovic-Brunet *et al.*, 2006; Farombi *et al.*, 2000; Kaur and Kapoor 2001) and phenolic substances were also reported to possess a wide range of biological effects, including antioxidant, antimicrobial, anti-inflammatory and vasodilatory actions. The antioxidant effect of naturally occurring phenolic components has previously been studied in relation to the prevention of coronary diseases and cancer, as well as for age-related degenerative brain disorders (Stoclet *et al.*, 2004; Stevenson and Hurst 2007).

The Gulf of Mannar located along the South East coast of India is adorned with plenty of seaweed resources. But, seaweeds are mainly utilized for agar and algin production. Seaweeds have not found a place in the dietary habits of people in India. But, the rich nutrients and health benefits associated with the seaweeds make them the best low cost supplementary food for the people especially the poor. Hence, these studies were undertaken to investigate the biochemical composition and evaluate the antioxidant potential of the seaweeds such as *Enteromorpha compressa*, *E.intestinalis, Dictyota dichotoma, Turbinaria ornata, Gracilaria corticata* and *Hypnea musciformis* collected from the Tuticorin coast.

Materials and Methods

Collection of sample

Two green seaweeds (*Enteromorpha compressa* and *E.intestinalis),* two brown seaweeds *(Dictyota dichotoma and Turbinaria ornata) and* two red seaweeds *(Gracilaria corticata* and *Hypnea musciformis)* were collected from the Tuticorin coast. The collected seaweeds were shade dried and grounded to fine powder, then kept in air tight container and stored in a freezer until further analysis.

Antioxidant assay

Preparation of extract

Each sample weighed 10 gm was transferred into a conical flask. The seaweed powders were extracted with acetone and ethanol in a soxhlet extractor for six hours. The extracts were then

concentrated under reduced pressure and the resultant residue was stored in dark at 4ºC until further use.

Evaluation of antioxidant activity

The lyophilized seaweed extracts were dissolved in distilled water at a concentration of 10 mg ml^{-1}. The free radial scavenging activity of the seaweed extracts was evaluated using standard procedures and Gallic acid was used as the reference compound. All analysis were run in triplicates and averaged.

Total phenolic content

Phenolic contents of crude extracts were estimated by the method of Taga *et al.*, (1984). Briefly 100 µl of aliquot sample was mixed with 2.0 ml of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. After incubation, 100 µl of 50% Folin Ciocalteau's phenol reagent was added and the 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 (Phenolic content are expressed as Gallic acid equivalent per gram).

Total Antioxidant activity

Total antioxidant activity was measured following the method of Prieto *et al.*, (1999). To 7.45 ml of sulphuric acid (0.6 mM solution), 0.9942 g of sodium sulphate (28 mM solution) and 1.2359 g of ammonium molybdate (4 mM solution) were mixed together in 250 ml distilled water and labeled as Total Antioxidant Capacity (TAC) reagent. 300 µl of extract was dissolved in 3 ml of TAC reagent. Blank was maintained with distilled water replacing the TAC reagent. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid.

Reducing Power

Reducing power of different crude extract was determined by the method prescribed by Oyaizu (1986). 1.0 ml of different solvent extract containing different concentration of samples was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferric cyanide (1%). Reaction mixture was kept in a water bath at 50ºC for 20 min. After incubation, 2.5 ml of Trichloroacetic acid (10% of TCA) was added and centrifuged at 650 rpm for 10 min. From the layer, 2.5 ml solution was mixed with 2.5 ml of distilled water at 0.5 ml of ferric chloride (0.1%). Absorbance was measured at 700 nm. Increased absorbance indicates increased reducing power.

Hydrogen peroxide radical scavenging assay

The ability of seaweed extract to scavenge hydrogen peroxide was determined by following the standard procedure (Gulçin *et al.*, 2004). Hydrogen peroxide 10 mM solution was prepared in the phosphate buffer saline of 0.1 M, pH 7.4. 1 ml (0.25 mg) of the extract was rapidly mixed with 2 ml of hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV spectrophotometer against a blank (without hydrogen peroxide) after 10 min of incubation at 37ºC. The percentage of scavenging of hydrogen peroxide was calculated using the following formula

% scavenging (H_2O_2) = (A_0-A_1/A_0) X 100

A_0 - absorbance of control

A_1 – Absorbance of sample).

DPPH radical scavenging activity

The Scavenging effects of crude methanol extract and fractions were determined by the method of Yen and Chen (1995). Briefly, 2.0 ml of 0.16 mM DPPH solution (in methanol) was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbance of all the sample solutions was measured at 517 nm. The Scavenging effect (%) was calculated by using the formulae given by Duan *et al.,* (2006).

Scavenging effect (%) = [1-(A sample-A blank)/A control] × 100

A control - absorbance of the control (DPPH solution without sample)

A sample - absorbance of the test sample (DPPH solution +Test sample)

A sample blank - absorbance of the sample only (sample without DPPH solution).

Results and Discussion

Antioxidant capacity of seaweeds

There are many methods to determine 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 (Cao and Prior 1998). The Reducing power assay can react with iron (II) and SH-group containing antioxidants (Benzie and Strain 1996) and DPPH method use organic radicals (Chandrasekar *et al.,* 2006), so it is expected that using these two methods accurately reflects all of the antioxidants in a sample. In this study, antioxidant activities were tested using five different assays, total phenolic content, total antioxidant activity, DPPH radical scavenging activity, Hydrogen peroxide scavenging assay and Reducing power assay. These five methods represented different mechanisms of antioxidant action.

Total Phenolic content

The phenolic content of seaweeds was evaluated using the Folin-Ciocalteu method and the results are presented in Fig. 1. The variation in phenolic content was quite large. The acetone (16.375±0.44 mg gallic acid equivalents g^{-1}) and ethanolic extract (12.84±0.39 mg gallic acid equivalents g^{-1}) of brown seaweed *Dictyota dichotoma* showed higher phenolic content than the all other seaweeds used in this experiment. The minimum total phenolic content was noticed in ethanol extract (1.04±0.03 mg gallic acid equivalents g^{-1}) of red seaweed *Hypnea musciformis.* Phenolic compounds are commonly found in plants and have been reported to have several biological activities including antioxidant properties. A number of studies focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Rice-Evans *et al.,* 1995; Sugihara *et al.,* 1999; Kahkonen *et al.,* 1999). Earlier reports revealed that seaweed extracts, especially polyphenols, have antioxidant activity (Yan *et al.,* 1999; Lim *et al.,* 2002; Kuda *et al.,* 2005). The brown seaweed *Dictyota dichotoma* showed higher phenolic contents than all the seaweeds tested. Jimenez-Escrig *et al.,* (2001) also reported similar findings that brown seaweeds contain higher phenolic content than the red seaweeds. Earlier reports revealed that phenolic compounds are one of the most effective antioxidants in brown algae (Nagai and Yakimoto 2003).

Total Antioxidant activity

The total antioxidant activity of seaweeds was evaluated and the results are presented as mg ascorbic acid/g (Fig. 2). In phosphor molybdenum method, molybdenum VI (Mo^{6+}) is reduced to form a green phosphate/Mo^{5+} complex. Higher antioxidant activity (232.76±3.80 mg ascorbic acid g^{-1}) was observed in acetone extract of *D. dichotoma* and *T. ornata* (231.70±2.64 mg ascorbic acid g^{-1}) followed by ethanolic extract of *T. ornata* (165.42±1.34 mg ascorbic acid g^{-1}). The minimum activity was noticed in acetone extract of *E. compressa* (68.80±2.55 mg ascorbic acid g^{-1}). However, Kumaran and Karunakaran (2007) have reported total antioxidant activity in the range of 245 to 376 mg ascorbic acid equivalents g^{-1} in *Phyllanthus* species. Ye *et al.,* (2009) have noticed higher antioxidant activity (30.50 µmol $FeSo_4$ mg^{-1}) in ethanol extract of brown seaweed *Sargassum pallidum*. Ganesan *et al.,* (2008) noticed higher activity (32.01 mg ascorbic acid equivalent g^{-1}) in ethyl acetate fraction of red seaweed *A. spicifera*. It has been reported that solvents used for extraction have dramatic effect on the chemical species (Yuan *et al.,* 2005).

Reducing Power Ferric Reducing Antioxidant Power (FRAP)

In the Reducing power assay (Figs. 3 and 4), antioxidants in the sample reduce ferric (III) to ferrous (II) in a redox-linked colourimetric reaction (Li *et al.,* 2006) that involves single electron transfer. The reducing power indicates that the antioxidant compounds are electron donars and reduce the oxidized intermediate of the lipid peroxidation process, so that they can act as primary and secondary antioxidants (Yen and Chen 1995). The maximum (2.413±0.001) reducing power value was observed in acetone extracts of *D. dichotoma* and minimum (0.764±0.003) was obtained from ethanol extracts of *E. compressa.* The reducing power of the extracts increased with the increasing the concentration of extracts. Same trend has also been reported by Kumaran and Karunakaran (2007) in methanolic extracts of *Phyllanthus* species.

Hydrogen peroxide radical scavenging activity

H_2O_2 scavenging activities (% inhibition) of acetone and ethanol extracts of seaweeds are presented on Fig. 5. The effect of extracts in scavenging hydrogen peroxide radicals to prevent oxidative degradation of substrate was determined. The percent of inhibition was more than 45.08% in *D. dichotoma* followed by *T. ornata* (44.92%). Lower inhibition rate of 25.29% was observed in ethanolic extract of *E. intestinalis*.

DPPH radical scavenging assay

The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule. When a DPPH solution is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical form of DPPH is obtained with simultaneous change of the violet colour to pale yellow (Molyneux 2004). Hence DPPH has been used extensively as a free radical to evaluate reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds (Duan *et al.,* 2006).

Figure.1 Total phenolic content of selected seaweeds from Tuticorin coast

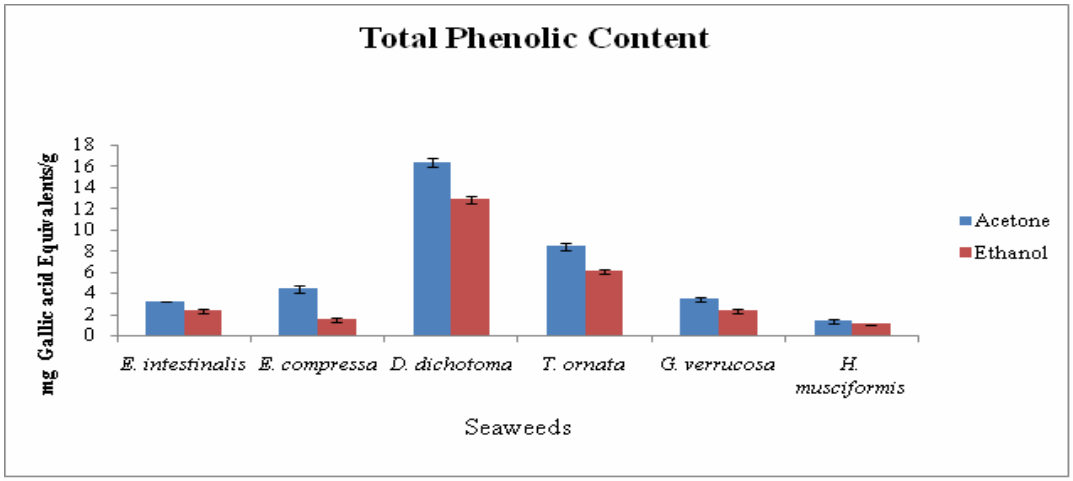


Figure. 2 Total antioxidant activity of selected seaweeds from Tuticorin coast

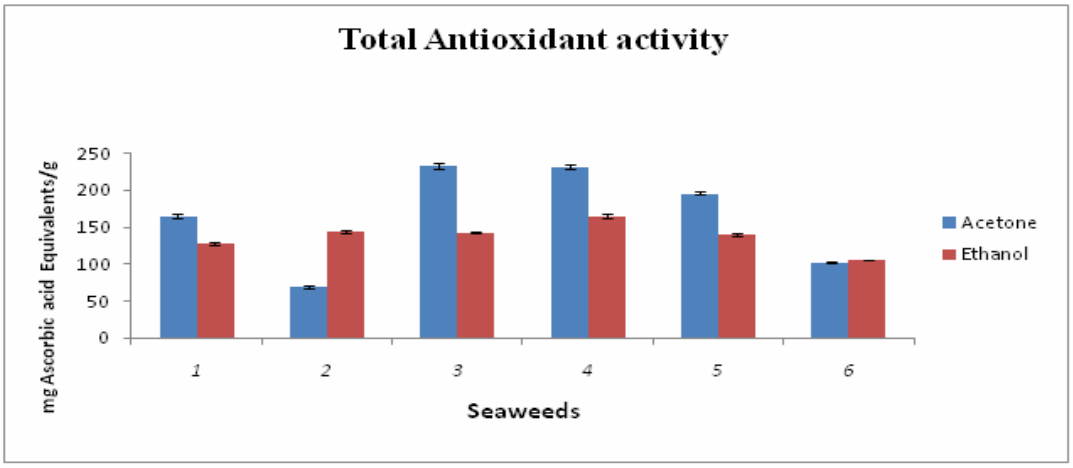


Figure.3 Reducing power of acetone extract of selected seaweeds from Tuticorin coast

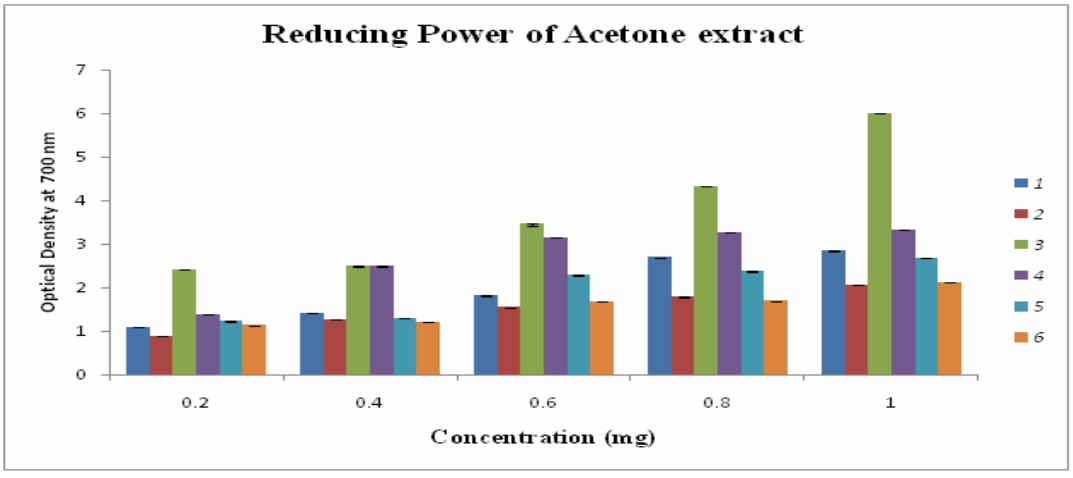


Figure.4 Reducing power of ethanol extract of selected seaweeds from Tuticorin coast

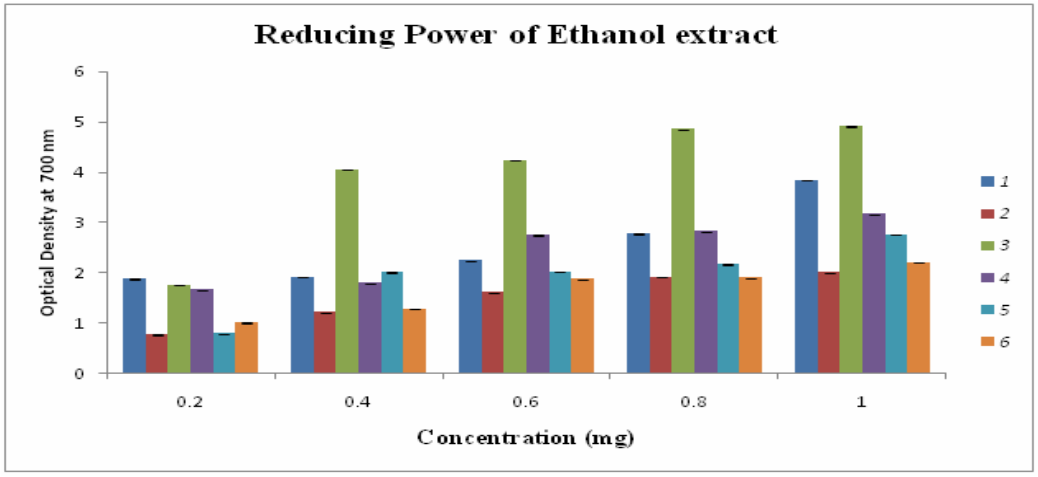


Figure.5 Hydrogen peroxide scavenging activity of selected seaweeds from Tuticorin coast

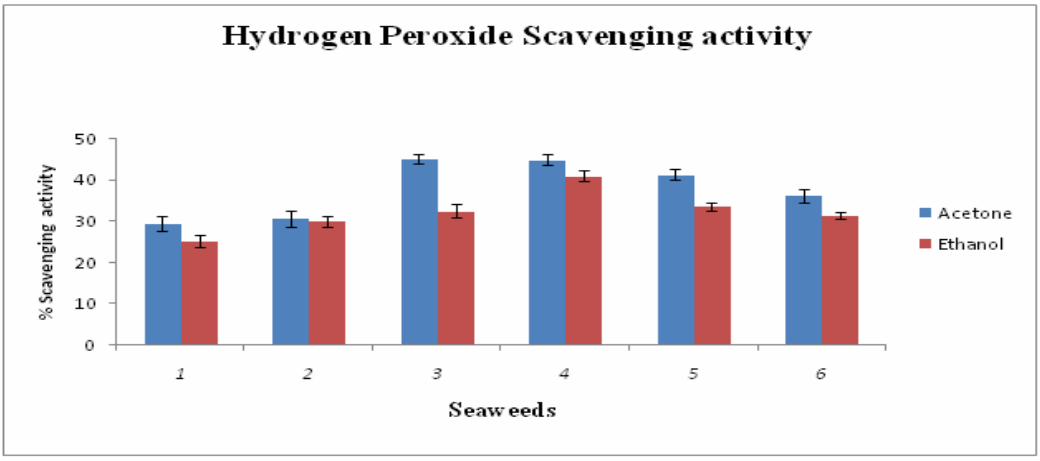
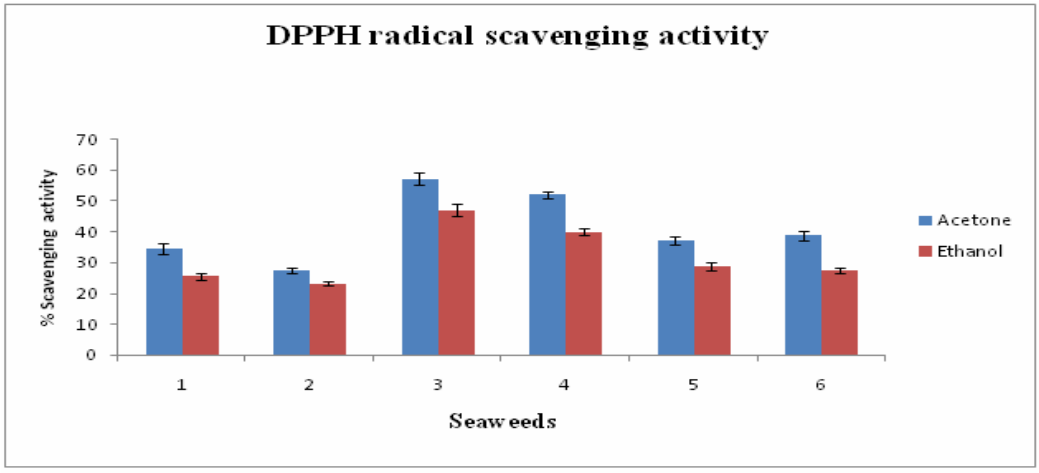


Figure.6 DPPH radical scavenging activity of selected seaweeds from Tuticorin coast



In the present study, the DPPH radical assay was evaluated and the results are presented in Fig. 6. The higher DPPH radical scavenging activity was observed in the acetone extracts of *D. dichotoma* (57.153±1.87%) and *T. ornata* (52.071±1.05%). The minimum radical scavenging activity was observed in ethanol extract of *E. compressa* (23.199±0.63%). Ganesan *et al.,* (2008) also noticed higher percentage DPPH radical scavenging activity in methanol extract of brown seaweed *T. conoides.*

In the present study, the extracts from *Dictyota dichotoma* were found to possess strong antioxidant activity. The antioxidant mechanisms of seaweed extracts may be attributed to their free radical-scavenging ability. In addition, phenolic compounds appear to be responsible for the antioxidant activity of seaweed extracts. On the basis of the results obtained, seaweeds can be used for a variety of beneficial chemo-preventive effects. However, further studies on the antioxidative components of seaweed extracts and more in vivo evidence are required.

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