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Antioxidant properties of seaweed polyphenol from *Turbinaria ornata* (Turner) J. Agardh, 1848

Vijayabaskar. P*, Shiyamala. V

Post Graduate Research Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi 626 124, Tamilnadu, India.

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ABSTRACT

Objective: To explore the antioxidant activity of seaweed polyphenol from *Turbinaria ornate (T. ornate)*. **Methods:** The brown seaweed, *T. ornata* was collected from Mandapam coastal region of Gulf of Mannar. It was cleaned from epiphytes, washed, shade dried and powdered. Algal extraction was carried out using methanol. The total phenol content of *T. ornata* was estimated by Folin-Ciocalteau method and its antioxidant activity was determined by free radical scavenging assays such as DPPH, Superoxide anion, Hydroxyl and Nitric oxide radical assay. **Results:** *T. ornata* exhibited (43.72±1.63) mgGAE/g extract of phenol content and better scavenging activity of DPPH [(84.27 ± 2.17)%], Superoxide anion [(61.86 ± 2.03)%], and Hydroxyl radical [(70.12 ± 2.03)%]. **Conclusions:** The presence of phenolic compounds were detected by TLC and FT-IR analysis, which revealed that the antioxidant activity might be due to the high polyphenol content of *T. ornata* with respect to their free radical scavenging properties.

1. Introduction

There is ample evidence that reactive oxygen species (ROS) generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer [1]. Reactive oxygen species (ROS) such as superoxide radical ($O2^{-}$), hydroxyl radical ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$) and nitric oxide radical ($NO^{\cdot}$) attack biological molecules such as lipids, proteins, enzymes, DNA and RNA, leading to cell or tissue injury. ROS induce peroxidation of lipids (polyunsaturated fatty acids) generating secondary oxidants like heptanol and hexanal which contributes to oxidative rancidity, deteriorating the flavor of the food. These not only cause a loss in food quality but are also believed to be associated with carcinogenesis, mutagenesis, arthritis, diabetes, inflammation, cancer and genotoxicity [2]. To overcome these problems a wide range of synthetic antioxidants (butylatedhydroxytoluene (BHT), butylatedhydroxyanisole (BHA), propyl gallate (PG) and butylatedhydroquinone) have been used as food preservatives. However, these synthetic antioxidants have side effects such as liver damage and are suspected to be mutagenic and neurotoxic. Hence, most consumers prefer additive-free foods or a safer approach like the utilization of more effective antioxidants of natural origin [3, 4]. Recently, various phytochemicals like polyphenols, which are widely distributed in plants, have been reported to act as free radical scavengers[5]. Marine plants like seaweeds also contain high amount of polyphenols.

Seaweed constitutes a commercially important renewable resource. *Sargassum, Padina, Dictyota* and *Gracilaria* sps can be used as fertilizers, food additives and animal feed[6]. Although seaweeds possess wide application in food and in the pharmaceutical industry, the antioxidant activities of many types of seaweed in the South Indian coastal area are still unexplored. The main objective of the present study is to evaluate the antioxidant activity of *T. ornata*, brown seaweed obtained from Mandapam, Gulf of Mannar, India.

2. Materials and methods

2.1. Collection area

*Corresponding author: Dr. P. Vijayabaskar, Assistant Professor, Post Graduate Research Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi -626 124, Tamil Nadu, India.
Tel: +91 9994019069
Fax No: 04562 254970,
E.mail: baski_bos@yahoo.co.in, heparinbaski@gmail.com

The brown algae, *Turbinaria ornata* (Fucales/phaeophyta) (*T. ornata*) was collected from the intertidal region of Mandapam coast (Lat. 09 ° 17′N; Long 79 ° 07′E) of Gulf of Mannar, South east coast of India. Gulf of Mannar is a marine biosphere which harbor biodiversity of global significance and unique for coral reef, seaweed and sea grass ecosystems.

2.2. Collection and processing of marine algae

T. ornata grows by attaching to coral substratum. Algal sample was cleaned of epiphytes and extraneous matter and necrotic parts were removed. Plants were washed with seawater and then in fresh water. The seaweeds were transported to the laboratory in sterile polythene bags at 20 ℃ temperature.

2.3. Polyphenol extraction from seaweed

In the laboratory, algal sample was rinsed with sterile distilled water, shade dried, cut into small pieces and powdered in a mixer grinder. It was stored in air-tight polypropylene container at room temperature. 100 g of *T. ornata* (powder) was extracted with 500 mL of methanol (2:1) in a Soxhlet extractor for 6 h. The extraction was repeated twice. The total extract was filtered and the obtained filtrate (crude extract) was concentrated under reduced pressure to dryness. The concentrated crude extract served as the seaweed polyphenol for further analysis.

2.4. Estimation of total phenolic content

Phenolic contents of crude methanolic extracts were estimated by the method of Senevirathene *et al.*, [7]. 100 μL crude sample was mixed with 2 mL of 2% sodium carbonate and allowed to stand for 2 minutes at room temperature in the dark. Absorbance of all the sample solutions was measured at 720 nm using spectrophotometer. Gallic acid was used as a standard and a calibration curve was prepared with a range of concentration from 10 to 200 mg/L. Phenolic content was expressed as gallic acid equivalent per gram (GAE/g) of extract.

2.5. Identification of phenolic compounds by TLC

Thin-layer chromatography (TLC) was performed on a silica gel plate. An aliquot of each sample was spotted on the silica gel plate with a developing solvent system of chloroform/methanol (10:1, v/v). The spots were visualized by spraying the plates with spraying solution (1% potassium ferric cyanide in water and 1% ferric chloride in water) and the visualized under UV [8].

2.6. FT – IR spectrophotometer analysis

Infrared spectra (IR) were also used to identify the phenolic compounds. Seaweed extracts along with the standard gallic acid were tested using SHIMADZU- FT-IR instrument. One milligram of dry sample was mixed with 100 mg of dry potassium bromide (KBr) and then compressed to prepare salt-disc (3 mm diameter). These discs were analyzed under Fourier transform IR-Spectrophotometer. The absorption was read between 400 and 4 000 cm^{-1}.

2.7. Evaluation of antioxidant activity of seaweed polyphenol

The seaweed extract was dissolved in methanol. The residue in the methanol was subjected to the following *in vitro* assays to assess the antioxidant potential. The free radical 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.

2.8. Determination of total antioxidant capacity by the phosphomolybdenum method

The antioxidant activity of the seaweed extract was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*, [9]. The assay is based on the reduction of Mo(VI) - Mo(V) by the extract and subsequent formation of a green phosphate / Mo(V) complex at acid pH. 2 mL extract was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). In the case of the blank, methanol was used in place of sample. The tubes containing the reaction solution were capped and incubated in water bath at 95 ℃ for 90 min. After the samples were cooled to room temperature, the absorbance of the solution was measured at 635 nm using a spectrophotometer against blank. The antioxidant activity was expressed as an equivalent of gallic acid (mg GA/g dried extract). All the measurements were measured in triplicates.

2.9. Reducing power

Reducing power of the test extracts was determined based on the ability of antioxidants to form coloured complex with potassium ferric cyanide, trichloroacetic acid and ferric chloride. The reducing power of the extracts was determined according to the method of Katalinic [10]. 0.75ml of extracts at various concentrations (50, 100, 250, 500 and 1 000 μg) was mixed with 0.75 mL phosphate buffer (pH 6.6) and 0.75 mL of 1% potassium ferric cyanide. The mixture was incubated at 50℃ for 20 minutes. 0.75 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3 000 g for 10minutes. 1.5ml of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride. After an incubation of 10 minutes the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Gallic acid was used as the reference. All the measurements were measured in

triplicates.

2.10. Free radical scavenging activity of seaweed polyphenol

2.10.1. DPPH radical scavenging activity

The free radical scavenging activity of the seaweed extracts was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) following the method of Blois [11]. This method is based on the reduction of stable DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical antioxidants in a methanolic solution. In the presence of antioxidants the purple colour of the DPPH radical solution changes to a bright yellow and the intensity of this can be monitored spectrophotometrically. Used as a reagent, DPPH evidently offers a convenient and accurate method for the titration of oxidizable groups of natural or synthetic antioxidants. 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of seaweed extracts at different concentrations (50, 100, 250, 500 and 1 000 μg). After 10 minutes, absorbance was measured at 517 nm. All the measurements were measured in triplicates. The percentage scavenging was calculated as follows.

Radical scavenging activity (%) = $[(A_0-A_1/A_0) \times 100]$

Where A_0= Absorbance of control; A_1= Absorbance of sample

2.10.2. ABTS radical scavenging assay

The free radical scavenging activity was also determined by ABTS (2,2′azino bis (3-ethylbenzothiazoline-6-sulphonicacid) diammonium salt) radical cation decolourization assay [12]. ABTS was generated by mixing 5 mL of 7 mM ABTS with 88 μL of 140 mM potassium persulfate under darkness at room temperature for 16 hours. The solution was diluted with 50% ethanol and the absorbance at 734 nm was measured. The ABTS radical cation scavenging activity was assessed by mixing 5ml ABTS solution (absorbance of 0.7 ± 0.05) with 0.1 mL seaweed extract (50, 100, 250, 500 and 1 000 μg). The final absorbance was measured at 743 nm with spectrophotometer. The percentage of scavenging was calculated by the following formula,

% of scavenging = $((A_o-A_1) / A_o) \times 100$

Where Ao is absorbance of control and A1 is absorbance of sample.

2.10.3. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compounds for hydroxyl radical generated by Fe^{3+}-Ascorbate EDTA H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao [13]. The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation. The reaction mixture contained in a final volume of 1.0mL, 100 μL of 2-deoxy-2-ribose (28 mM in potassium phosphate-potassium hydroxide buffer, pH 7.4), 500 μL solutions of various concentrations of extracts (50, 100, 250, 500 and 1 000 μg) and standard in KH_2PO_4-KOH buffer (20mM, pH 7.4), 200 μL of 1.04mM ethylene diamine tetra acetic acid and 200 μL of 200 μM Ferric chloride, 100 μL of 10 mM hydrogen peroxide and 100 μL of 1.0mM ascorbic acid was incubated at 37 ℃ for 1 hour. The free radical damage imposed on the substrate, deoxyribose was measured as TBARS by the method of Yuan *et al.* [14]. 1.0 mL of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) were added to the test tubes and were incubated at 100 ℃ for 30 minutes. After cooling, absorbance was measured at 535 nm against control containing deoxyribose and buffer. The percentage scavenging was determined by the comparing the result of the test compound and control using the formula,

Radical scavenging activity (%) = $[(A_0-A_1/A_0) \times 100]$

Where A_0= Absorbance of control; A_1= Absorbance of sample

2.10.4. Superoxide anion radical scavenging assay

Measurement of superoxide anion scavenging activity of the extracts was done based on the method described by the slightly modified method of Nishimiki *et al.* [15]. About 1 mL of nitro blue tetrazolium (NBT) solution (156 μM NBT in 100mM phosphate buffer, pH 7.4), 1 mL of NADH solution (468 μM in 100mM phosphate buffer, pH 7.4) and 0.1 mL of sample at various concentrations (50, 100, 250, 500 and 1 000 μg in distilled water) were mixed and the reaction was started by adding 100 μL of phenazine methosulphate (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4). The reaction mixture was incubated at 25 ℃ for 5 minutes, and the absorbance at 560nm was measured against blank samples. The percentage scavenging value was determined as follows.

Radical scavenging activity (%) = $[(A0-A1/A0) \times 100]$

Where A_0 = Absorbance of control; A_1 = Absorbance of sample

2.10.5. Nitric oxide radical scavenging assay

Nitric oxide radicals generated from sodium nitroprusside solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Griess reaction [16]. 2 mL of sodium nitroprusside (10 mm) was mixed with 1 mL of the test extracts (50, 100, 250, 500 and 1000 μg) in phosphate buffer (pH 7.4). The mixture was incubated at 25 ℃ for 150 minutes. To 0.5 mL of the incubated solution, 1 mL of sulphanilic acid reagent (0.33% sulphanilamide in 20% acetic acid) was added and allowed to stand for 5 minutes for completing diazotization. 1 mL of 0.1% napthyl ethylene diamine dihydrochloride was added and incubated at room temperature for 30 minutes. Absorbance was read at 540 nm and percentage scavenging was calculated as follows.

Radical scavenging activity (%) = $[(A_0-A_1/A_0) \times 100]$

Where A_0 = Absorbance of control; A_1 = Absorbance of sample

2.11. Characterization of crude seaweed polyphenol by GC-MS

GC-MS analysis was perfomed using a varian CP-gas chromatography. The injector port was heated to 220 ℃. The injections were perfomed in splitless mode. The carrier gas was Helium C-60, at a constant flow of 1 mL/min. the oven temperature was set at 40 ℃ for 1min, then increasing 2 ℃/min to 220 ℃ and held for 30 minutes.

Compounds were identified by comparing the retention times of the chromatographic peaks with those of authentic compounds analyzed under the same conditions.

3. Result

3.1. Total phenol content

The total phenolic content of the seaweed extract was measured spectrophotometrically by the Folin-Ciocalteau method. The results were expressed as gallic acid equivalents (GAE). Turbinaria ornata was found to have the phenolic content of (43.72 ± 1.63) mg GAE/g extract.

3.2. Thin layer chromatography (TLC)

The phenolic compounds present in *T. ornata* was tentatively detected by TLC. After spraying with the solution composed of 1% potassium ferric cyanide and 1% ferric chloride, the appearance of blue colour spot in the TLC chromatogram indicated the presence of phenolic compounds (Figure 1).

3.3. FT-IR analysis

The FT-IR analysis of the seaweed extract was done and the functional groups associated were determined. The FT-IR spectrum of the samples was obtained and the effective peaks were compared with that of the standard gallic acid. The FT-IR spectrum of the standard gallic acid contain ten major peaks at the range of 3365.90, 3282.90, 3064.99, 2654.14, 1703.20, 1618.33, 1541.18, 1448.59, 1099.46 and 1026.16 cm^{-1}: whereas the FT-IR spectrum of the *T. ornata* also recorded the same number of peaks lying between 1026.16, 3414.12, 1028.09 and 3394.83 cm^{-1} respectively. The absorption peaks observed for hydroxyl groups (around 3300-3500 cm^{-1}) and aromatic ring (around 1450-1470 cm^{-1} and 2850-2960 cm^{-1}) in the spectra of *T. ornata* also suggested the presence of phenolic compounds.

Figure 1. TLC analysis of *T. ornate* polyphenol.

3.4. Antioxidant properties of seaweed polyphenols

3.4.1. Total antioxidant activity

The total antioxidant capacity of *T. ornata* was measured by phosphomolybdenum method. The antioxidant activities increase with increasing concentration of the sample. At the concentration of 1 000 μg/mL, the crude extract of *T. ornata* exhibited higher antioxidant activity [(88.17 ± 2.45) %] as compared with the standard, gallic acid (Figure 2).

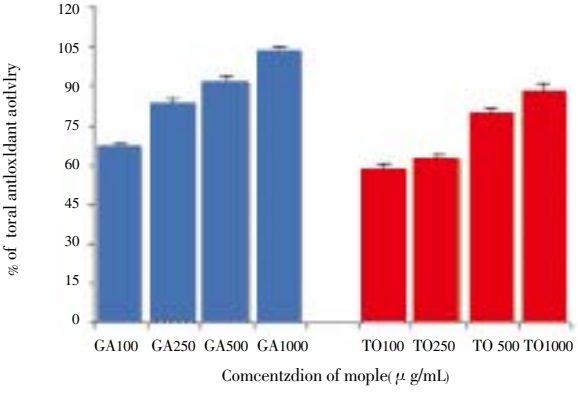

Figure 2. Total antioxidant activity of *T. ornata* (TO) extract compared with standard gallic acid (GA).

3.4.2. Reducing power

The reducing power of *T. ornata* was compared with the standard gallic acid. The reducing power increases with the increasing concentration. The reducing power of the samples was shown in Figure 3 and it was found to be in the following order: Gallic acid [(0.29 ± 0.01) - (0.91 ± 0.09)], *T. ornata* [(0.2

± 0.04) − (0.72 ± 0.07)].

3.4.3. DPPH radical scavenging activity

The effect of seaweed extracts and standard on DPPH radical was compared and shown in Figure 4. The scavenging effect increases with the concentration of standard and samples. At 1 000 μg/mL concentration, *T. ornata* possessed (84.27±2.17)% scavenging activity on DPPH. All the concentration of *T. ornata* showed higher activity than the standard gallic acid.

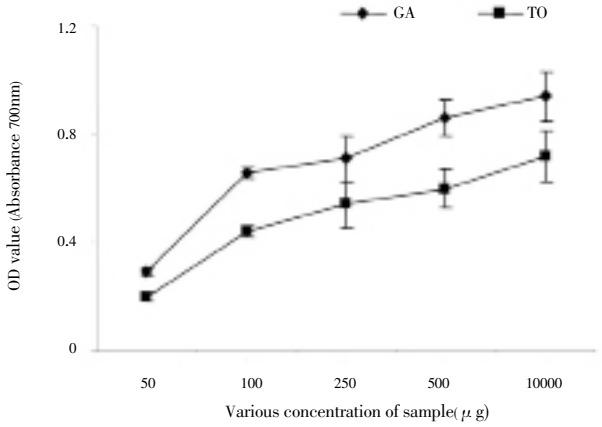

Figure 3. Reducing power of *T. ornate* (TO) extract compared with standard gallic acid (GA).

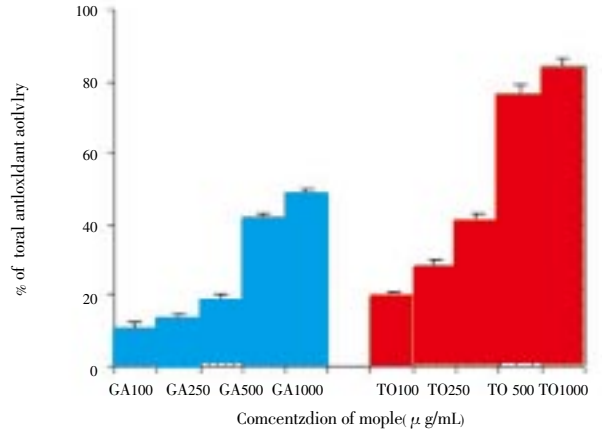

Figure 4. DPPH scavenging activity of *T. ornata* (TO) extract compared with standard gallic acid (GA).

3.4.4. ABTS cation scavenging activity

The effect of seaweed extracts and standard on ABTS cation was compared and shown in Figure 5. The scavenging effect increases with the concentration of standard and samples. At 1 000 μg/mL concentration, *T. ornata* possessed (72.58± 3.45)% scavenging activity on ABTS. All the concentration of *T. ornata* showed higher activity than the standard gallic acid.

3.4.5. Hydroxyl scavenging activity

The scavenging effect of OH. was investigated using the Fenton reaction and the results shown as the % inhibition rate in Figure 6. *T. ornata* exhibited the inhibition of about (70.12 ± 2.03)%, but this is lower than the standard gallic acid (1 000 μg/mL) whose inhibition is (44.92 ± 1.97)%.

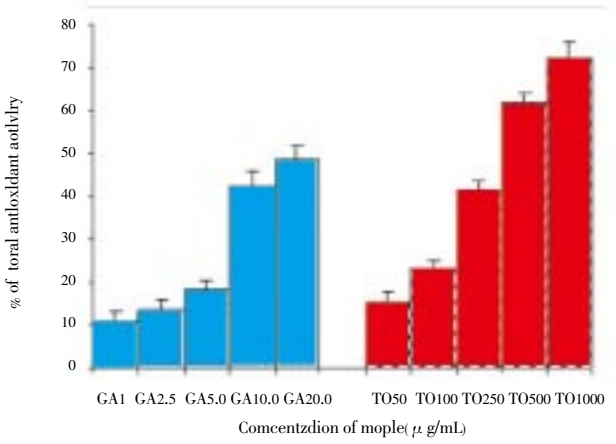

Figure 5. ABTS scavenging activity of *T. ornata* (TO) extract compared with standard gallic acid (GA).

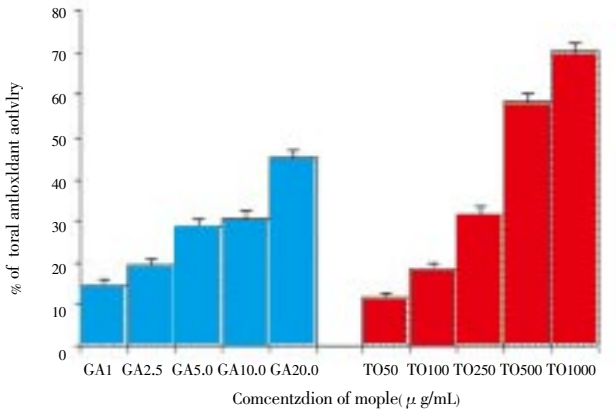

Figure 6. Hydroxyl scavenging activity *T. ornata* (TO) extract compared with standard gallic acid (GA).

3.4.6. Superoxide anion radical scavenging activity

The seaweed extracts were subjected to be superoxide scavenging assay and the results were shown in Figure 7. It indicates that *T. ornata* (1 000 μg/mL) exhibited the maximum Superoxide scavenging activity of (61.86 ± 1.27) % which is significantly higher than the standard gallic acid whose scavenging effect is (52.63 ± 1.15)%.

3.4.7. Nitric oxide scavenging activity

Suppression of NO. release may be attributed to a direct NO. scavenging effect as the seaweed extracts decreased the amount of nitrite generated from the decomposition of sodium nitroprusside in vitro as shown in Figure 8. The results show that *T. ornata* had scavenging activity of (39.8± 2.52)% higher than the standard gallic acid (33.65 ± 2.07) %.

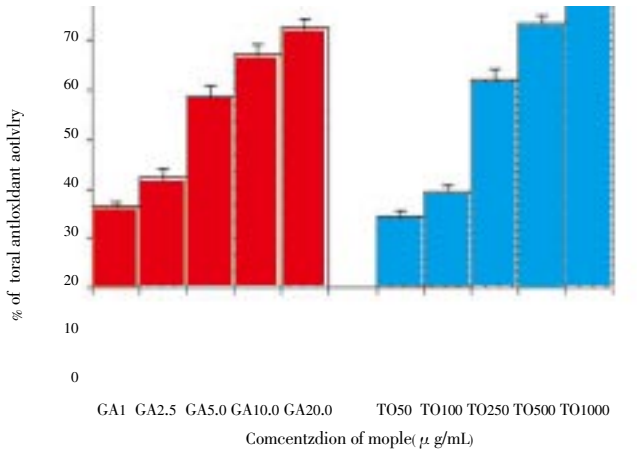

Figure 7. Superoxide scavenging activity of *T. ornata* (TO) extract compared with standard gallic acid (GA).

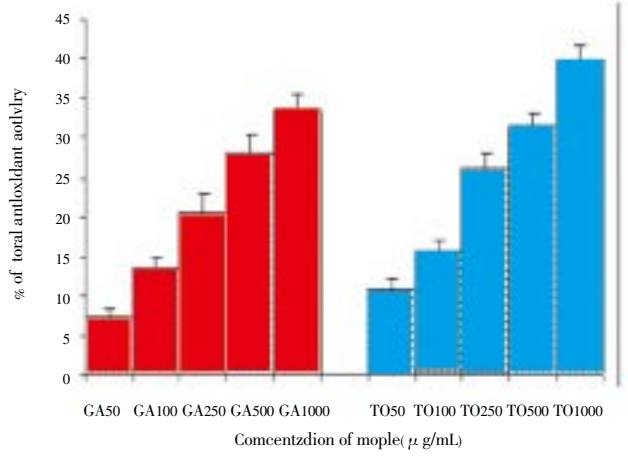

Figure 8. Nitric oxide scavenging activity of *T. ornata* (TO) extract compared with standard gallic acid (GA).

3.5. Characterization of crude seaweed polyphenol by GC-MS analysis

T. ornata was reported to have two compounds which was identified using a combination of a mass-spectral database and retention data. The analytical Polyphenols, Phenol, 2-[(1-phenylethyl)thio] (40.5%), metomidate (31.8%), were observed in *T. ornata*.

4. Discussion

The antioxidant activitiy of several naturally occurring compounds have been known for decades. Recently, many types of seaweed have been considered as source of reactive oxygen species inhibitors. They can be used as food additives and can also provide protection against tissue oxidation [17].

The present investigation has also proved that seaweed Polyphenols (*T. ornata*) possess antioxidant activity to scavenge free radicals. Dietary natural antioxidants are reported to help in preventing aging and other diseases. There are some evidences that seaweeds contain compounds with a relatively high antioxidant and antiproliferative activity. Seaweeds are low in fat but contain vitamins and bioactive compounds like terpenoids, sulfated polysaccharides and polyphenolic compounds, the latter being a potential natural antioxidant not found in land plants [18].

Antioxidant compounds scavenge free radicals such as peroxide, hydro peroxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress related diseases [19]. Many synthetic antioxidants have shown toxic and mutagenic effects, which have shifted attention towards naturally occurring antioxidants. A great number of naturally occurring substances like seaweeds have been recognized to have antioxidant abilities [20].

Total phenol content by Folin Ciocalteau Reagent and in vitro antioxidant capacity assays, such as the DPPH, ABTS and free radical scavenging assays (which were used in this study), represent convenient methods for the identification of potential sources of antioxidant compounds [21]. As already mentioned, seaweed polyphenols have significant potential health benefits; they may protect cell constituents against oxidative damage and therefore limit the risk of various degenerative diseases associated to oxidative stress such as cancer, cardiovascular disease and osteoporosis. However, the value of in vitro antioxidant capacity assays for assessing the health-related implications of a food extract has been limited for a number of reasons, mainly due to the lack of standardization amongst these methods, the changes in the antioxidant activity of Polyphenols. Nevertheless, this does not exclude antioxidant properties from being one of the key parameters in determining their biological effects [22].

The total phenolic content of *T. ornata* was of 43.72 ± 1.63 mg gallic acid equivalents/g extract. The presence of phenolic compounds in the seaweed extracts *T. ornata* was tentatively detected by TLC and IR spectral analysis. Seaweed phenolics are effective free radical scavengers and antioxidants. Phenolic compounds are commonly found in the edible brown, green and red seaweeds in which the antioxidative property has been correlated to their phenolic content [23].

Phenols are very important seaweed constituents because of their scavenging ability due to their hydroxyl groups [24]. It was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [25]. A highly positive relationship between total phenols and antioxidant activity has been observed in many seaweed species [4,26-28]. The phenolic compounds may contribute directly to the antioxidative action [29]. Seaweed contains a variety of natural antioxidative compounds specially polyphenolic antioxidative compounds. In the

present study, the seaweed was shade dried before use. The carotenoids such as fucoxanthin present in brown seaweeds had been shown to possess antioxidative properties in lower levels in shade dried samples than in fresh freeze dried samples [30]. Hence, the observation of potential scavenging activity in the seaweed could be linked especially in the presence of polyphenolics rather than to carotenoids.

Antioxidant potential of methanolic extracts of Turbinaria ornata was estimated using potassium ferric cyanide reduction method. The yellow colour of the test solution changes to various shades of green and blue, depending upon the reducing power of extracts. The presence of reductants causes the reduction of Fe^{3+}/ Ferric complex to ferrous form. Therefore the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700nm [31]. The reducing ability of a compound greatly depends on the presence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom [29].

The reducing capacity of *T. ornata* is a significant indicator of its potential antioxidant activity. Same trend has also been reported by Kumaran and Karunakaran [32], Kumar Chandini *et al*., [33] in methanolic extracts of higher plants, brown seaweeds from India. Total antioxidant activity of *T. ornata* phosphomolybdenum method. The results showed that *T. ornata* have the antioxidant activity of (88.17 ± 1.39) %. Oxidative damage within the cellular systems is a multi step process involving free radical chain initiation and propagation steps [34]. One of the mechanisms by which antioxidants bring about their action is by scavenging free radicals [35]. Hence it is important to assess the scavenging ability of the brown seaweed extracts.

In the present work, $DPPH^{\cdot}$, $ABTS^{+}$, OH^{-}, $NO^{\cdot}$, $O2^{-}$ free radical assay systems were successfully used for the evaluation on the antioxidant activity of the crude extracts derived from Turbinaria ornata. 1, 1- Diphenyl-2-picrylhydrazyl (DPPH) is stable nitrogen centered free radical which can be effectively scavenged by antioxidants [36, 37]. Hence it has been widely used to test the ability of compounds as free radical scavengers or hydrogen donors [38] and to evaluate the antioxidant activity of plant extracts relative to other methods [22].

The present investigation has shown that both the seaweed extracts being *T. ornata* which exhibited significantly higher DPPH scavenging activity [(84 ± 1.78) % inhibition] when compared with the highest concentration of standard gallic acid [(43.39 ± 1.31) % inhibition]. The result is indicative of the hydrogen donating ability of *T. ornata*. Since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [39].

The results show that Turbinaria ornata has superoxide scavenging activity which can be of potential health interest as it may be effective in reducing the level of $O_2^{\cdot-}$ which is elevated during oxidative stress in the body. $O_2^{\cdot-}$ mediated oxidative stress is believed to be involved in the pathogenesis of cardiovascular disorders, diabetes mellitus, acute respiratory distress syndrome, neurodegenerative disorders like Alzheimer's and Parkinson's diseases. NO radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with $O_2^{\cdot-}$ radicals to form peroxynitrite, which damages biomolecules like proteins, lipids and nucleic acids [40]. 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.

The methanolic extract of Turbinaria ornata [(39.8 ± 2.52) % inhibition] was higher than the standard gallic acid (33 ± 2.07) % inhibition]. The present result suggest that Turbinaria ornata might be potent and novel therapeutic agents for scavenging of $NO^{\cdot-}$ and the regulation of pathological conditions caused by excessive generation of $NO^{\cdot-}$ and its oxidation product peroxynitrite. The cell damaging action of hydroxyl radical is well known as it is the strongest among free radicals and it has short half life [41]. *T. ornata* exhibited the greatest scavenging effect of $-OH^{\cdot}$ among the seaweeds but less than the standard gallic acid. $-OH^{\cdot}$ is known to be capable of abstracting hydrogen peroxide from membranes and they bring about lipid peroxidation [42]. It is thus anticipated that *T. ornata* would show antioxidant effects against lipid peroxidation on biomembranes and would scavenge $-OH^{\cdot}$ radicals at the stages of initiation and termination.

Phenol, 2-[(1-phenylethyl)thio] represent a diverse group of pigment, widely distributed in nature. They serve as accessory pigments to harvest light for photosynthesis [43]. Moreover these types of pigments can give rise to rich in polyphenol compounds. Norisoprenoids resulting from the oxidative cleavage of carotenoids are signals in algae development, serve as antifungal and antibacterial agents and contribute to their flavor and arouma [43]. Some other Polyphenols such as ionone, dihyro actinidiode, 2, 3-Epoxy ionone and carotene have been reported as the most common marine seaweed norisoprenoids [44].

Our observation leads to the conclusion that polyphenolic compounds could be taken into account for the strong free radical scavenging activity, which is in agreement with previous reports. Gulcin *et al*., [45] reported excellent correlation for medicinal plants when antioxidant activity was compared with total polyphenolic content. Hung and Yen [46] and Siddhuraju *et al*., [47] also suggested that polyphenolic content could be related to the antioxidant activities. Patricia [48] and Xianggun *et al*., [49] found that phenolics made a major contribution to the total antioxidant activity in seaweeds.

Nagai and Yukimoto [50] revealed that phenolic compounds are one of the most effective antioxidants in brown algae. It has been earlier reported that some major active compounds from brown seaweed that have antioxidative properties are phlorotannins and fucoxanthin. Also, antioxidative

activities of brown seaweeds cannot be attributed to their characteristic pigment (fucoxanthin) or any other carotenoids alone [30].

It is believed that the antioxidant properties of phenolics are a result of their ability to act as reducing agents, hydrogen donors and free radical quenchers and phenolics may act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals [19]. It is possible that the antioxidant activity of *T. ornate* can be the result of their high concentration of phenolic compounds.

On the basis of results in this study, it can be concluded that the methanolic extracts of *T. ornata* is capable of scavenging a wide range of synthetic and naturally occurring free radicals. It is evident from the present study that the Methanolic extracts of *T. ornata* could be utilized as a good natural source of antioxidants and a possible food supplement or as an antimicrobial agent in pharmaceutical industry. However the active components responsible for the antimicrobial activities need to be evaluated. The data may contribute to a rational basis for the use of antioxidant rich marine algal extracts in the therapy of diseases related to oxidative stress. In addition, the results indicate that phenolic compounds might be major contributors to the antioxidant activities of *T. ornata*. The finding of the current report appear useful for further research aiming to isolate, identify and characterize the specific phenolic compounds in Turbinaria ornata for its industrial and pharmaceutical applications.

Conflict of interest statement

We declare that we have no conflict of interest.

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