Original Research Article

Assessment of *In Vitro* Antioxidant Potential of the Polyphenols and the Sulphated Polysaccharides fractions of *Ulva lactuca* and *Turbinaria ornata*

ABSTRACT

Aims: To assess the *in vitro* antioxidant potential of the polyphenols and the sulphated polysaccharides fractions from green marine alga *Ulva lactuca* and brown marine alga *Turbinaria ornata*.

Study design: In the present work, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), lipid peroxidation, hydroxyl radical and hydrogen peroxide scavenging activities were used for the evaluation of the antioxidant potential of the polyphenols and the sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata*.

Place and Duration of Study: Department of Biochemistry, Adhiparasakthi College of Arts and Science (Autonomous), G.B. Nagar, Kalavai – 632 506, Tamil Nadu, India.

Methodology: The polyphenols and the sulphated polysaccharides fractions were extracted from green marine alga *Ulva lactuca* and brown marine alga *Turbinaria ornata*, and were assayed for *in vitro* antioxidant potential using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), lipid peroxidation, hydroxyl raidcal and hydrogen peroxide scavenging activities.

Results: Both the polyphenols and the sulphated polysaccharides fractions were extracted from green marine alga *Ulva lactuca* and brown marine alga *Turbinaria ornata*, showed good *in vitro* antioxidant potential with the 1,1-diphenyl-2-picryl-hydrazyl (DPPH), lipid peroxidation, hydroxyl radical and hydrogen peroxide scavenging activities. The IC_{50} values ranged from 0.185 mg/ml to 0.430 mg/ml.

Conclusion: Both the polyphenols and the sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* are shown to be capable of scavenging a wide range of synthetic and naturally occurring free radicals, and they could be utilized as good natural source of antioxidants and possible potential food supplements in pharmaceutical industry. These data may contribute to a rational basis for the use of antioxidant-rich marine algal fractions in the therapy of diseases related to oxidative stress.

Keywords: Ulva lactuca, Turbinaria ornata, Marine algae, Antioxidant potential, DPPH, lipid peroxidation, hydroxyl radical, hydrogen peroxide scavenging.

1. INTRODUCTION

There is increasing interest in the study of the antioxidant actions of plant and marine algal phenolic compounds due to various evidences that have shown that the consumption of

Comment [WU1]: Make an abstract according to the abstract format in a scientific journal, omit the words » Aims, Study design, Methodology, Conclusion
Abstract writing directly in one paragraph by making a summary of the background, objective, research methods, research results, conclusion and recommendation

Comment [WU2]: DELETE

Comment [WU3]: DELETE

Comment [WU4]: DELETE

Comment [WU5]: DELETE

Comment [WU6]: DELETE

Comment [WU7]: Add.. **Its Percentage Inhibition (in %)**

Comment [WU8]: DELETE

Comment [WU9]:
It is better change these statement to explain directly in summary the research results fromTable 1-4 rather than explain indirectly results in which it was not carried out in this research

Comment [WU10]: **Sort the words in the keywords in alphabetical order for the initial letters of each word in keywords; and the first letter of the first word is capitalized, while the next initial letter is lowercase**
The order:
DPPH, *hydrogen peroxide scavenging, hydroxyl radical,*
lipid peroxidation, Turbinaria ornata, Ulva lactuca,

Comment [WU11]: DELETE:too much key words max 6 words

Comment [WU12]: **Use references if possible, the year 2012 and above is 50% or more**

these compounds contributes to the protection from a number of ailments. In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases [1]. Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [2]. One of the mechanisms by which antioxidants bring about their action is by scavenging free radicals [3]. Hence it is important to assess the scavenging ability of the marine algal fractions.

Phenolic compounds, including phenols and flavonoids, are secondary metabolites that have an important role in the maintenance of the human body. Marine algal polyphenols have been shown to 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. Phenols are very important marine algal constituents because of their scavenging ability due to their hydroxyl groups [4]. It was reported that phenolic compounds are associated with antioxidant activity and play an important role in stabilizing lipid peroxidation [5]. A highly positive relationship between total phenols and antioxidant activity has been observed in many marine algal species [6, 7]. The phenolic compounds may contribute directly to the antioxidative action [4]. Marine algae contain a variety of natural antioxidative compounds specially polyphenolic compounds.

In recent years, a broad series of polysaccharides from edible marine algae have emerged as an important class of bioactive natural products, possessing many properties of pharmacological relevance [8]. The sulphated polysaccharides are widespread in nature, occurring in a great variety of marine organisms. Marine algae provide us a large source of bioactive compounds such as polysaccharides, minerals, vitamins and non-caloric dietary fibers [9] More recent reports revealed marine algae to be a rich source of antioxidant compounds [6, 10].

Ulva lactuca or Sea lettuce is a green marine alga and belongs to the phylum *Chlorophyta*, described by Linnaeus in the Baltic Sea in the seventeenth century [11]. *Ulva lactuca* is rich in flavonoids [12]. It has been shown to contain the phenolic acids – gallic acid, protocatechuic acid, gentisic acid, *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, syringic acid, caffeic acid, salicylic acid and coumaric acid [13]. It is important to mention also that ulvan, a major sulphated polysaccharide (SP) found in the cell wall of green algae, composed mainly of rhamnose, glucuronic acid, iduronic acid, xylose, glucose, sulphate with small amounts of iduronic acid and traces of galactose and represents 8-29% of the algal dry weight [14]. The polyphenols fraction from *Ulva lactuca* has been shown to possess hepatoprotective [15] and cardioprotective [16] activities.

Turbinaria ornata is a tropical brown marine alga of the order Fucales native to coral reef ecosystems of the South Pacific. Fucoidans were detected in *T. ornata*, collected from the Maldives [17]. Glucosamine, a sulphated fucan-like polysaccharide with amino sugar was isolated from the ethanolic extract of *T. ornata* from coasts of Tahiti [18]. The methanol extract of *T. ornata* collected from Gulf of Mannar (India), was detected to have phenolic content [19].

Therefore an attempt was made to study the antioxidant potential of the polyphenols and sulphated polysaccharides fractions from green marine alga *Ulva lactuca* and brown marine alga *Turbinaria ornata*. In the present work, DPPH, lipid peroxidation, hydroxyl radical and hydrogen peroxide scavenging activities were successfully used for the evaluation on the

antioxidant activity of the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata*.

2. MATERIAL AND METHODS

2.1 Chemicals

5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB or Ellman's reagent) and thiobarbituric acid (TBA) were obtained from Hi-media Laboratories, Mumbai. 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and butylated hydroxy toluene (BHT) were purchased from Sigma, St. Louis, MO, USA. The rest of the chemicals and biochemicals utilized were obtained from local firms and were of analytical grade. Water was distilled prior to use.

2.2 Collection and Extraction of Polyphenols and Sulphated Polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata*

Fresh and healthy specimens of *Ulva lactuca*, belonging to Ulvaceae family (Chlorophyceae class) and *Turbinaria ornata*, belonging to Sagassaceae family (Phaeophyceae class) were collected from the intertidal regions of the Mandapam coast of Gulf of Mannar. The collected samples were cleaned well with the seawater until unnecessary impurities, adhering sand particles, extraneous matter like epiphytes, pebbles and shells were removed and they were brought to the laboratory in sterile plastic bags containing sea water in order to prevent evaporation. Then they were washed thoroughly with tap water and distilled water to remove the surface salty materials. They were air dried for 1 week and later ground in an electric mixer. The powdered samples were subsequently stored in the refrigerator for future use.

2.2.1 Extraction of Polyphenols fraction from *Ulva lactuca* and *Turbinaria ornata*

The powdered samples were extracted with 80% ethanol for 24 h under continuous shake at 20 °C. The extracts were then concentrated in a rotary evaporator under reduced pressure at 40 °C [20]. The solid mass obtained was dried and stored at 4 °C. When required it was dissolved in distilled water to required concentration.

2.2.2 Extraction of Sulphated Polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata*

The powdered samples were depigmented with acetone for 24 h and then the sulphated polysaccharides fraction was extracted by an optimized single-step extraction procedure [21]. The depigmented sample was extracted in 0.03 M HCl with continuous stirring at 200 rpm for 4 h at 90 °C water bath. The suspended sample was filtered, and the extract was precipitated using 60% ethanol, the precipitate collected after centrifugation at 10,000 rpm for 10 min, and the resulting pellet was dried. This dried pellet constituted the sulphated polysaccharides fraction and was stored at 4 °C. When required it was dissolved in distilled water to required concentration.

2.3 Determination of Antioxidant Potential of the Polyphenols fraction and the Sulphated Polysaccharides fraction of *Ulva lactuca* and *Turbinaria ornata*

The *in vitro* methods are based on inhibition. Samples are added to a free radical-generating system, inhibition of the free radical action is measured and this inhibition is related to antioxidant activity of the sample. Methods vary greatly as to the generated radical, the reproducibility of the generation process and the end point that is used for the determination. Even though *in vitro* methods provide a useful indication of antioxidant activities, data

obtained from *in vitro* methods are difficult to apply to biological systems and do not necessarily predict a similar *in vivo* antioxidant activity. All the methods developed have strengths and limitations and hence a single measurement of antioxidant capacity usually is not sufficient. A number of different methods may be necessary to adequately assess *in vitro* antioxidant activity of a specific compound or a biological fluid. In the present study, all the test samples – ULPP (*Ulva lactuca* polyphenols fraction), TOPP (*Turbinaria ornata* polyphenols fraction), ULSP (*Ulva lactuca* sulphated polysaccharides fraction) and TOSP (*Turbinaria ornata* sulphated polysaccharides fraction) and the standards (BHT - butylated hydroxy toluene, or ascorbic acid) were, therefore, tested for their *in vitro* antioxidant potential using four standard methods *viz.,* DPPH free radical scavenging activity, inhibition of lipid peroxidation activity in liver homogenate, hydroxyl radical scavenging activity and hydrogen peroxide scavenging activity. The final concentration of the test samples and standard solutions used were 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml. The absorbance was measured against the corresponding control solution. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})] \times 100}{A_{control}}$$

IC_{50} values (the concentration of the sample to scavenge 50% of the free radicals) were determined by linear regression.

Comment [WU13]:
Give no 1) at the end of this formula
Formula..... 1)

2.3.1 Determination of DPPH Free Radical Scavenging Activity

The scavenging ability of the inherent antioxidants of the polyphenols fraction and sulphated polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata* towards the relatively stable free radical DPPH was determined according to the method adapted from Shimada *et al.* [22]. Various concentrations of sample dissolved in (1.5 ml, to give a final concentration of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/ml) were mixed with 3 ml of 0.2 mM DPPH solution. Methanol served as the blank and DPPH in methanol without plant extracts served as positive control. The mixture was shaken vigorously and allowed to stand for 40 min, and the absorbance was measured at 517 nm using a Shimadzu UV-1601 UV-Visible spectrophotometer. Butylated hydroxy toluene (BHT) was used as the standard. All the measurements were done in triplicates. The percentage of inhibition was calculated according to the formula:

$$\text{Radical scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})] \times 100}{A_{control}}$$

IC_{50} values (the concentration of the sample to scavenge 50% of the free radicals) were determined by linear regression.

Comment [WU14]:
Give no 2) at the end of this formula
Formula.....
……..2)

2.3.2 Inhibition of *in vitro* Lipid Peroxidation Activity in Liver Homogenate

Goat liver homogenate was used for the induction of lipid peroxidation, mediated by ferrous sulphate ($FeSO_4$) as a pro-oxidant and the efficiency of the polyphenols fraction and sulphated polysaccharides fraction inhibiting the *in vitro* lipid peroxidation was studied as per the method of Okhawa *et al.* [23]. The measurement of thiobarbituric acid reactive substances was done spectrophotometrically at 535 nm in the experimental mixture. A 5% goat liver homogenate was prepared in cold TBS and 50 µl of it was used in the assay. The sample was homogenized in 1 ml of cold TBS. Aliquots of 50 µl of it were used in the assay. Ferrous sulphate at a final concentration of 10 µmoles was added to the assay medium to induce oxidation. The final volumes in the test tubes were made up to 500 µl with cold TBS. Controls were prepared for each sample, containing the respective plant extract (50 µl), liver homogenate (50 µl) and TBS to make up the final volume to 500 µl). Pro-oxidant was not added to the control tube.

A blank containing only ferrous sulphate and TBS (but not the sample and liver homogenate) was prepared and the volume was made up to 500 µl. An assay medium corresponding to 100% oxidant was prepared by adding all the other constituents except the plant extract and the volume was made up to 500 µl with cold TBS. The experimental medium corresponding to auto-oxidation contained only the liver homogenate and TBS to make the final volume to 500 µl. All the tubes were incubated at 37 °C for one hour. Following the incubation period, 500 µl of 70% alcohol was added to all the tubes to stop the reaction. One ml of 10% TBA was added to all the tubes, followed by boiling in hot water bath for 20 min. After cooling to room temperature, the tubes were centrifuged. To the clear supernatants collected in test tubes, 500 µl of acetone was added and the TBARS was measured at 535 nm using a Shimadzu UV-1601 UV-Visible spectrophotometer. Butylated hydroxy toluene (BHT) was used as the standard. The percentage of inhibition was calculated according to the formula:

$$\text{Radical scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})] \times 100}{A_{control}}$$

IC_{50} values were determined by linear regression.

Comment [WU15]:
Give no 3) at the end of this formula
Formula.....
……..3)

2.3.3 Hydroxyl Radical Scavenging Activity

The DNA damage induced *in vitro* by hydrogen peroxide in the presence and the absence of the polyphenols fraction and sulphated polysaccharides fraction was quantified as thiobarbituric acid reactive substances spectrophotometrically as per the procedure given by Kunchandy and Rao [24]. The reaction mixture contained in a final volume of 0.98 ml, 2.8 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 1 mM H_2O_2, 0.1 mM ascorbate and 20 mM buffer. 20 µl of the sample was added, so that the final volume was 1.0 ml. The reaction mixture was incubated at 37 °C for 1 h. Deoxyribose degradation was measured as TBARS by adding 0.5 ml of TBA and 0.5 ml of HCl and boiling in a water bath for 20 min. It was then allowed to cool and the absorbance was measured at 532 nm using a Shimadzu UV-1601 UV-Visible spectrophotometer. Butylated hydroxy toluene (BHT) was used as standard. The percentage of inhibition of deoxyriboe degradation was calculated according to the formula:

$$\text{Radical scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})] \times 100}{A_{control}}$$

IC_{50} values were determined by linear regression.

Comment [WU16]:
Give no 4) at the end of this formula
Formula.....
……..4)

2.3.4 Hydrogen Peroxide Scavenging Activity

The ability of the polyphenols fraction and sulphated polysaccharides fraction to scavenge hydrogen peroxide was determined according to Ruch *et al.* [25]. The sample at the concentration of up to 1 mg/ml was added to 0.6 ml of 40 mM H_2O_2 solution. The total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm using a Shimadzu UV-1601 UV-Visible spectrophotometer. The blank solution contained phosphate buffer without H_2O_2. Ascorbic acid was used as the standard. The percentage of H_2O_2 scavenging of plant extracts was calculated according to the formula:

$$\text{Radical scavenging activity (\%)} = \frac{[(A_{control} - A_{sample})] \times 100}{A_{control}}$$

IC_{50} values were determined by linear regression.

Comment [WU17]:
Give no 5) at the end of this formula
Formula.....
……..5)

3. RESULTS AND DISCUSSION

Comment [WU18]:
Use references if possible in the discussion with references in 2012 and above by 50% more

3.1 Antioxidant Potential of Polyphenols Fraction and the Sulphated Polysaccharides fraction of *Ulva lactuca* and *Turbinaria ornata*

3.1.1 DPPH Free Radical Scavenging Activity

DPPH is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid autooxidation. Being a stable free radical, DPPH is regularly used to determine radical scavenging activity of natural compounds. The DPPH is a stable free radical, which has been accepted as a tool for estimating free radical scavenging activity of the natural extracts [26]. The radical scavenging activity in the presence of a hydrogen-donating antioxidant can be monitored by a decrease in the absorbance of DPPH solution. This method is used worldwide in the quantification of free radical scavenging activity .The reaction is based on the drop of colour intensity when the odd electron of the nitrogen atom in $DPPH^{\bullet}$ is reduced by receiving a hydrogen atom from antioxidant compounds. $DPPH^{\bullet}$ is known as a stable free radical but is sensitive to light, oxygen, pH change and the type of solvent used [27]. The free radical quenching potential of the polyphenols and sulphated polysaccharides fractions was studied through its bleaching ability of the stable radical DPPH.

Comment [WU19]:
Paragraphs that describe supporting data results from Table 1 based on related references are placed under Table 1

The DPPH free radical scavenging effects of the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* and the standard butylated hydroxy toluene (BHT) are given in Table 1. The fractions had significant scavenging effects on the DPPH radical. The positive DPPH test suggests that the samples are free radical scavengers. The IC_{50} values of the extracts in the DPPH free radical scavenging assay are also given in the Table 1. The present investigation has shown that both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* exhibited significant DPPH scavenging activity (100 % inhibition for polyphenols fraction and 86.93 – 95.23 % inhibition for sulphated polysaccharides) when compared with the highest concentration of standard butylated hydroxy toluene (BHT). The results are indicative of the hydrogen donating ability of the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata,* since the effects of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability [28], suggesting that the polyphenols and sulphated polysaccharides fractions are good antioxidants with radical scavenging activity.

Comment [WU20]:
Paragraphs that describe Table 1 are placed in the first paragraph above Table 1

It has been suggested that phenolic compounds and sulphated polysaccharides may contribute directly to the antioxidative action [29]. These results indicate a strong relationship between total phenolic and sulphated polysaccharides contents and radical scavenging activity, suggesting that phenolic compounds and sulphated polysaccharides are responsible for the antioxidative properties of *Ulva lactuca* and *Turbinaria ornata* Thus, the therapeutic properties of the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* may be possibly attributed to the phenolic compounds and sulphated polysaccharides present.

Comment [WU21]:
Paragraphs that describe supporting data results from Table 1 based on related references are placed under Table 1

Table 1. DPPH Free Radical Scavenging Assay of Polyphenols fraction and Sulphated Polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata*

Concentration (mg/ml)	Percentage Inhibition (in %)				
	Standard	Polyphenols fraction		Sulphated Polysaccharides fraction	
	BHT	ULPP	TOPP	ULSP	TOSP
0.0625	18.93 ± 0.95	16.63 ± 0.67	14.60 ± 0.74	10.77 ± 0.44	7.83 ± 0.34
0.125	39.53 ± 1.82	36.60 ± 1.66	34.00 ± 1.71	31.23 ± 1.53	28.00 ± 1.33
0.25	62.15 ± 3.08	59.53 ± 2.95	56.30 ± 2.54	51.07 ± 2.26	47.37 ± 2.26
0.5	87.70 ±	84.13 ±	81.07 ±	72.47 ±	67.37 ±

1.0	4.55 100.0 ± 0.00	4.15 100.0 ± 0.00	4.15 100.0 ± 0.00	3.67 95.23 ± 4.66	3.17 86.93 ± 4.36
IC_{50} (mg/ml)	**0.171**	**0.185**	**0.199**	**0.233**	**0.273**

Values are expressed as mean ± SD (n=3)

3.1.2 Inhibition of *in vitro* Lipid Peroxidation Activity in liver homogenate

Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus reduce the level of oxidative stress and slow or prevent the development of complications associated with oxidative stress [30]. Metal chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation [31].

The TBARS levels (as % inhibition) and the IC_{50} values obtained in the lipid peroxidation studies with the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* are presented in Table 2. The present investigation has shown that both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* exhibited significant lipid peroxidation activity (90.37 – 93.95 % inhibition for polyphenols fraction and 73.90 – 78.73 % inhibition for sulphated polysaccharides) when compared with the highest concentration of standard butylated hydroxy toluene (BHT).

The polyphenols and sulphated polysaccharides fractions had significant lipid peroxidation inhibitory effects. These results indicate that the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* exhibit the ability to inhibit lipid peroxidation, suggesting that the fractions are good antioxidants with radical scavenging activity.

Comment [WU22]: Paragraphs that describe supporting data results from Table 2 based on related references are placed under Table 2

Comment [WU23]: The paragraphs that describe Table 2 are placed in the first paragraph above Table 2

Comment [WU24]: The continuation paragraph that describes Table 2 is placed in the continuation paragraph after the initial paragraph above Table 2

Table 2. Lipid Peroxidation Assay of Polyphenols fraction and Sulphated Polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata*

Concentration (mg/ml)	Percentage Inhibition (in %)				
	Standard	Polyphenols fraction		Sulphated Polysaccharides fraction	
	BHT	ULPP	TOPP	ULSP	TOSP
0.0625	22.13 ± 1.01	16.43 ± 0.76	11.53 ± 0.47	5.87 ± 0.31	3.23 ± 0.14
0.125	39.33 ± 1.78	32.70 ± 1.55	28.93 ± 1.14	21.37 ± 1.06	18.53 ± 1.33
0.25	57.47 ± 2.80	51.03 ± 2.59	45.73 ± 2.19	39.33 ± 1.94	34.77 ± 1.62
0.5	78.43 ± 3.57	71.07 ± 3.51	66.33 ± 3.16	57.47 ± 2.69	51.97 ± 2.48
1.0	100.0 ± 0.00	93.95 ± 4.59	90.37 ± 4.17	78.43 ± 3.91	73.90 ± 3.40
IC_{50} (mg/ml)	**0.179**	**0.224**	**0.262**	**0.360**	**0.430**

Values are expressed as mean ± SD (n=3)

3.1.3 Hydroxyl Radical Scavenging Activity

The hydroxyl radicals are produced by incubating ferric-EDTA with ascorbic acid and hydrogen peroxide (H_2O_2) at pH 7.4, and reacting with 2-deoxy-2-ribose to generate a malondialdehyde (MDA)-like product. This compound forms a pink chromogen upon heating with TBA at low pH [32]. The hydroxyl radical is very active and has a very short life *in vivo*. However, it is very harmful to organism. Therefore, the removal of hydroxyl radical is important for antioxidant defense in cell or food systems. For hydroxyl radical, there were two types of antioxidation mechanism; one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated. In the former, the antioxidant activity may ligate to the metal ions which react with H_2O_2 to give the metal complexes. The metal complexes thus formed cannot further react with H_2O_2 to give hydroxyl radicals [33].

The hydroxyl radical scavenging activity (as % inhibition) and the IC_{50} values obtained in the hydroxyl radical scavenging activity with the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* are presented in Table 3. The present investigation has shown that both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* exhibited significant hydroxyl radical scavenging activity (92.13 – 96.50 % inhibition for polyphenols fraction and 84.60 – 88.63 % inhibition for sulphated polysaccharides) when compared with the highest concentration of standard butylated hydroxy toluene (BHT).

According to an earlier report, sulphated polysaccharides from algae had hydroxyl radical scavenging effect [34]. Obviously, the effects of scavenging hydroxyl radicals were in a concentration-dependent manner. ULPP, TOPP, ULSP and TOSP showed excellent radical scavenging performances. All the data in the hydroxyl radical assay indicated that the antioxidant activities of all the samples were related to their ability to scavenge hydroxyl radical. These results indicate that the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata*, exhibit the ability to scavenge hydroxyl radicals, suggesting that the extracts are good antioxidants with radical scavenging activity.

Comment [WU25]: Paragraphs that describe supporting data results in Table 3 based on related references are placed under Table 3

Comment [WU26]: The paragraph describing Table 3 is placed in the first paragraph above Table 3

Comment [WU27]: Further paragraph that describe supporting data results from Table 3 based on related references are placed under Table 3

Table 3. Hydroxyl Radical Scavenging Activity of Polyphenols fraction and Sulphated Polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata*

Concentration (mg/ml)	Percentage Inhibition (in %)				
	Standard	Polyphenols fraction		Sulphated Polysaccharides fraction	
	BHT	ULPP	TOPP	ULSP	TOSP
0.0625	20.97 ± 1.09	18.87 ± 0.81	17.30 ± 0.77	13.63 ± 0.64	9.43 ± 0.54
0.125	38.63 ± 1.64	36.03 ± 1.44	33.05 ± 1.42	29.53 ± 1.55	26.57 ± 1.34
0.25	60.50 ± 2.95	57.00 ± 2.93	53.67 ± 2.31	48.60 ± 2.14	45.27 ± 1.97
0.5	81.47 ± 3.85	77.97 ± 3.72	74.67 ± 3.53	69.40 ± 3.56	65.73 ± 3.43
1.0	100.0 ± 0.00	96.50 ± 4.35	92.13 ± 4.29	88.63 ± 4.64	84.60 ± 3.54
IC_{50} (mg/ml)	**0.174**	**0.194**	**0.215**	**0.251**	**0.286**

Values are expressed as mean ± SD (n=3)

3.1.4 Hydrogen Peroxide Scavenging Activity

Scavenging of H_2O_2 by the natural drugs may be attributed to their phenolics or other antioxidants, which donate electron to H_2O_2, thus reducing it to water. The measurement of H_2O_2 scavenging activity is one of the useful methods of determining the ability of antioxidants to decrease the level of pro-oxidants such as H_2O_2 [35]. Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly; once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of many of its toxic effects [36].

The H_2O_2 scavenging activity (as % inhibition) and IC_{50} values obtained in the H_2O_2 scavenging activity with the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* are presented in Table 4. The present investigation has shown that both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* exhibited significant H_2O_2 scavenging activity (91.47 – 83.73 % inhibition for polyphenols fraction and 68.73 – 75.50 % inhibition for sulphated polysaccharides) when compared with the highest concentration of standard ascorbic acid.

Comment [WU28]:
Paragraph that describe supporting data results in Table 4 based on related references is placed under Table 4

Comment [WU29]:
The paragraph describing Table 4 is placed in the first paragraph above Table 4

Table 4. Hydrogen Peroxide Scavenging Assay of Polyphenols fraction and Sulphated Polysaccharides fraction from *Ulva lactuca* and *Turbinaria ornata*

Concentration (mg/ml)	Percentage Inhibition (in %)				
	Standard	Polyphenols fraction		Sulphated Polysaccharides fraction	
	Asc Acid	ULPP	TOPP	ULSP	TOSP
0.0625	18.97 ± 0.75	17.23 ± 0.95	16.27 ± 0.72	14.07 ± 0.63	13.43 ± 0.48
0.125	40.27 ± 1.63	37.43 ± 1.76	34.77 ± 1.55	30.03 ± 1.58	27.33 ± 1.37
0.25	62.07 ± 2.93	57.20 ± 2.99	53.23 ± 2.58	47.23 ± 2.29	42.67 ± 2.03
0.5	82.13 ± 4.08	74.87 ± 3.52	69.20 ± 3.43	62.87 ± 3.52	56.40 ± 2.61
1.0	100.0 ± 0.00	91.47 ± 4.19	83.73 ± 4.28	75.50 ± 3.54	68.73 ± 3.38
IC_{50} (mg/ml)	**0.174**	**0.203**	**0.236**	**0.300**	**0.372**

Values are expressed as mean ± SD (n=3)

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate and phenyl propanoid pathways in plants. Phenolics are antioxidants with redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They have also metal chelation properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and antiinflammatory action [37]. Several reports have shown a close relationship between total phenolic content and high antioxidant activity, and many researchers have demonstrated that phenolic compounds are one of the most effective antioxidants in marine algae [38, 39].

Several works have demonstrated that the presence of sulphate groups in marine algal polysaccharides is responsible for numerous types of biological activities, such as antioxidant activities [40]. It has been shown that crude sulphated polysaccharides have the ability to scavenge superoxide, hydroxyl and hypochlorous acid radicals directly *in vitro* and to inhibit the damage induced by excess free radicals [41]. It has been reported that sulphate content had a significant effect on superoxide radical and hydroxyl radical scavenging effects

[42]. The antioxidant mechanisms of sulphated polysaccharides might be attributed to strong hydrogen donating ability, a metal chelating ability, and their effectiveness as scavengers of superoxide and free radicals [43].

4. CONCLUSION

On the basis of the results in this study, it can be concluded that both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* are capable of scavenging a wide range of synthetic and naturally occurring free radicals. It is evident from the present study that the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* could be utilized as good natural source of antioxidants and possible food supplements in pharmaceutical industry. These data may contribute to a rational basis for the use of antioxidant-rich marine algal fractions in the therapy of diseases related to oxidative stress. The finding of the current study appear useful for further research aiming to isolate, identify and characterize the specific antioxidant compounds in both the polyphenols and sulphated polysaccharides fractions from *Ulva lactuca* and *Turbinaria ornata* for their industrial and pharmaceutical applications

Comment [WU30]: Create a conclusion that also shows a summary of research results quantitatively in addition to a summary of research results qualitatively

Comment [WU31]: Explaemore in summary the capabilityin quantitaively

Comment [WU32]: It is better change these statement to explain directly in summary the research results fromTable 1-4 rather than explain indirectly results in which it was not carried out in this research

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

Comment [WU33]: Of the 43 references, only 7 references (red colours) are in 2012 and above, if possible use references from 2012 and above for above 50%

1. Halliwell B, Gutteridge JM. Free Radicals in Biology and Medicine. Oxford: Oxford University Press; 1998.
2. Yazdanparast R, Ardestani A. *In vitro* antioxidant and free radical scavenging activity of *Cyperus rotundus*. J Med Food. 2007;10:667-74. DOI: 10.1089/jmf.2006.090 PMID: 18158839
3. Dastmalchi K, Dorman HJD, Kosar M, Hiltunen R. Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L) extract. Food Sci Tech, 2007;40:239-48. DOI: 10.1007/s13197-017-2765-7. PMC: 5602985.
4. Wang T, Jonsdottir R, Olafsdottir G. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. 2009;116: 240-48. DOI: 10.1016/j.foodchem.2009.02.041.
5. Yuan VY, Bone ED, Carrington FM. Antioxidant activity of Dulse (*Palmaria palmate*) extract evaluated *in vitro*. Food Chem. 2005;91:485-94. DOI: 10.1016/j.foodchem.2004.04.039.
6. Duan XJ, Zhang WW, Li XM, Wang BG. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chem. 2006; 95:37-43. DOI: 10.1016/j.foodchem.2004.12.015.

7. Kaur IP, Geetha T. Screening methods for antioxidants – A Review. Mini Rev Med Chem. 2006;6:305-12. DOI: 10.2174/138955706776073448. PMID: 16515469.
8. Shanmugam M, Mody KH. Heparinoid-active sulphated polysaccharides from marine algae as potential blood anticoagulant agents. Curr Sci. 2000;79(12):1672-83.
9. Dawczynski C, Schubert R, Jahreis G. Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem. 2007;103:891-99. DOI: 10.1016/j.foodchem.2006.09.041.
10. Kuda T, Tsunekawa M, Goto H, Araki Y. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. J Food Comp Anal. 2005;18:625-33.
11. Wichard T, Charrier B, Mineur F, Bothwell JH, Clerck OD, Coates JC. The green seaweed *Ulva*: A model system to study morphogenesis. Front Plant Sci. 2015;6:72–82. DOI: 10.3389/fpls.2015.00072.
12. Meenakshi S, Gnanambigai DM, Mozhi ST, Arumugam M, Balasubramanian T. Total flavanoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram coast. Global J Pharmacol. 2009;3(2):59–62.
13. Fernando IP, Shanura MK, Kwang-Tae S, Yoonhwa J, You-Jin J. Antioxidant Activity of Marine Algal Polyphenolic Compounds: A Mechanistic Approach J Med Food. 2016;19(7): 1–14. DOI: 10.1089/jmf.2016.3706.
14. Wijesekara I, Pangestuti R, Kim SK. Biological activities and potential health benefits of sulphated polysaccharides derived from marine algae. Carbohyd Polym. 2011;84: 14-21. DOI: 10.1016/j.carbpol.2010.10.062.
15. Ravindran NT, Mohamed Sadiq A. Pharmacological activity of *Ulva lactuca* polyphenols fraction: Hepatoprotective and antioxidant activities against paracetamol-induced liver damage in rats. Asian J Pharm Clin Res. 2019;12(4):55-8, DOI:10.22159/ajpcr.2019.v12i4.31214.
16. Ravindran NT, Mohamed Sadiq A. Pharmacological activity of *Ulva lactuca* polyphenols fraction: Cardioprotective and antioxidant activities against isoproterenol-induced myocardial infarction in rats. Asian J Pharm Clin Res. 2019;12(9):133–6. DOI: 10.22159/ajpcr.2019.v12i9.34629.
17. Jayawardena TU, Fernando IPS, Lee WW, Sanjeewa KKA, Kim H-S, Lee D-S, Jeon Y-J. Isolation and purification of fucoidan fraction in *Turbinaria ornata* from the Maldives; inflammation inhibitory potential under LPS stimulated conditions in *in-vitro* and *in-vivo* models. Int J Biol Macromol. 2019;131:614–23. DOI: 10.1016/j.ijbiomac.2019.03.105.
18. Deslandes E, Pondaven P, Auperin T, Roussakis CC, Guezennec J, Stiger V, Payri C. Preliminary study of the *in vitro* antiproliferative effect of a hydroethanolic extract from the subtropical seaweed *Turbinaria ornata* (Turner) J. Agardh on a human non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). J Appl Phycol. 2000;12(3/5):257–62. DOI: 10.1023/A:1008114831862.
19. Vijayabaskar P, Shiyamala V. Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar biosphere reserve. Adv Biol Res. 2011;5:99–102.
20. Heo SJ, Cha SH, Lee KW, Jeon YJ. Antioxidant activities of red algae from Jeju island. Algae. 2006; 21:149–56.
21. Ale MT, Mikkelsen JD, Meyer AS. Designed optimization of a single-step extraction of fucose-containing sulfated polysaccharides from *Sargassum* sp. J Appl Phycol. 2012;24:715-23. DOI:10.1007/s10811-011-9690-3.
22. Shimada K, Fujikawa K, Yahara N, Nakamura T. Antioxidative properties of xanthan on the autooxidaion of soybean oil in cyclodextrin emulsion. J Agric Food Chem. 1992;40:945-8. DOI: 10.1021/jf00018a005.

23. Okhawa H, Oishi N, Yagi K. Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem. 1979;95:351-8. DOI: 10.1016/0003-2697(79)90738-3. PMID: 36810.
24. Kunchandy E, Rao MNA. Oxygen radical scavenging activity of curcumin. Int J Pharmaceu. 1990;58:237-40. DOI: 10.1016/0378-5173(90)90201-E.
25. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989;10:1003-8. DOI: 10.1093/carcin/10.6.1003. PMID: 2470525.
26. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excels* L. leaf Extracts. Food Chem. 2008;110:571-83. DOI: 10.1016/j.foodchem.2008.02.037.
27. Ozelick O, Lee JH, Min DB. Effect of light, oxygen and pH on the absorbance of 2,2-diphenyl-picrylhydrazyl. J Food Sci. 2003;68:487-90. DOI: 10.1111/j.1365-2621.2003.tb05699.x.
28. Conforti F, Loizzi MR, Statti GA, Menichini F. Comparative radical scavenging and antidiabetic activities of methanolic extract and fractions from *Achillea ligustica*. Biol Pharm Bull. 2005;28:1791-4. DOI: 10.1248/bpb.28.1791. PMID: 16141563.
29. Hatano T, Edamatsu R, Hiramatsu M, Mori A, Fujita Y, Yashuara D. Effects of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 1989;37:2016-21. DOI: 10.1248/cpb.38.1224. PMID: 2393948.
30. Wu XJ, Hansen C. Antioxidant capacity, phenolic content, polysaccharide content of *Lentinus edodes* grown in Whey permeate based submerged culture. J Food Sci. 2008;73:434-38. DOI: 10.1111/j.1750-3841.2007.00595.x. PMID: 18211355
31. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Haring Jyur (*Chrysenthemum morifolium* Ramat). Lebensmittel-Wissebchaft & Technologie, 1999;32:269-77. DOI:10.1006/FSTL.1999.0548.
32. Halliwell B, Gutteridge JMC, Aruoma OI. The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. Anal Biochem. 1987;165:215-9. DOI: 10.1016/0003-2697(87)90222-3. PMID: 3120621.
33. Ueda J, Saito N, Shimazu Y, Ozawa T. Oxidative DNA strand scission induced by copper (II)-complexes and ascorbic acid. Arch Biochem Biophys. 1996;333:377-84.
34. Costa LS, Fidelis GP, Cordeiro SL, Oliveira RM, Sabry DA, Camara RBG, *et al.* Biological activities of sulfated polysaccharies from tropical seaweeds. Biomed. Pharmacother. 2010;64:21-8. DOI: 10.1016/j.biopha.2009.03.005. PMID: 19766438.
35. Pazdzioch-Czochra, M, Zawadzka AM. Spectroflurometric determination of hydrogen peroxide scavenging activity. Anal Chim Acta. 2002;452(2):177-84. DOI:10.1016/S0003-2670(01)01455-6.
36. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharm Exp Ther. 1993:264(1):11-6.
37. Eghdami A, Sadeghi F. Determination of total phenolic and flavonoids contents in methanolic and aqueous extract of *Achillea millefolium*. Org Chem J. 2010;2:81-4.
38. Luo HY, Wang B, Yu CG, Qu YL, Su CL. Evaluation of antioxidant activities of five selected brown seaweeds from China. J Med Plants Res. 2010;4:2557-65.
39. Zakaria NA, Ibrahim D, Sulaiman SF, Supardy A. Assessment of antioxidant activity, total phenolic content and *in-vitro* toxicity of Malaysian red seaweed, *Acanthophora spicifera*. J Chem Pharm Res. 2011;3:182-91.
40. Wang J, Zhang Q, Zhang Z, Zhang J, Li P. Synthesized phosphorylated and aminated derivatives of fucoidan and their potential antioxidant activity *in vitro*. Int J Biol Macromol. 2009;44(2):170-4.

41. Arivuselvan N, Radhiga M, Anantharaman P. *In vitro* Antioxidant and Anticoagulant Activities of Sulphated Polysaccharides from Brown Seaweed (*Turbinaria ornata*) (Turner) J.Agardh. Asian J Pharm Biol Res. 2011;1(3):232-9.
42. Xu RJ, Ye H, Sun Y, Tu YY, Zeng XX. Preparation, preliminary characterization, antioxidant, hepatoproetective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). Food Chem Toxicol. 2012;50:2473-80.
43. Wang JL, Guo HY, Zhang J, Wang XF, Zhao BT, Yao J, *et al*. Sulfated modification, characterization and structure-antioxidant relationships of *Artemisia sphaerocephala* polysaccharides. Carbohydr Polym. 2010;81:897-905. DOI: 10.1016/j.carbpol.2010.04.002.