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Original Research Article

In Vitro Free Radical Scavenging Activity of *Tolypiocladia Glomerulata* (C.Agardh) F.Schmitz

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Abstract: Marine algae are in exhaustible sources of chemical compounds contain bioactive compounds that plays an important role against various diseases against the aging process because they protect cells from oxidation. The present study is aimed to analyze the phenol and flavonoids content of the marine red alga *Tolypiocladia glomerulata* and their potential of free radical scavenging properties. The free radical scavenging capacity of the red alga was analyzed by inhibiting the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The maximum percentage of inhibition (61.13 ± 0.02% for DPPH and 50.35 ± 0.00 for ABTS) was observed at the concentration of 900 µg/mL. The free radicals scavenging ability of extracts followed close dependent trend. The results indicate that the red alga *T. glomerulata* have good free radical scavenging capacity with ABTS and DPPH and it could be used as a potential candidate for natural antioxidants.

Keywords: Marine algae, *Tolypiocladia glomerulata*, antioxidant, free radical, DPPH and ABTS

INTRODUCTION

Seaweeds are known to contain reactive antioxidant molecules, such as ascorbate and glutathione (GSH) when fresh, as well as secondary metabolites such as carotenoids (α and β-carotene, fucoxanthin, astaxanthin), mycosporine amino acids (mycosporine-glycine) and catechins (e.g., catechin, epigallocatechin, epigallocatechin), gallate, phlorotannins (e.g., phloroglucinol), and tocopherols (α-tocopherols) [1]. Macroalgae have received much attention as potential natural antioxidants [2-7] and there has been very limited information on antioxidant activity of macroalgae [8]. Hence, the present study was aimed to investigate the antioxidant potentials of marine red alga *T. glomerulata* by using the DPPH and ABTS.

MATERIALS AND METHODS

Materials

Sample collection and algal extract preparation

Fresh materials of *Tolypiocladia glomerulata* (C.Agardh) F.Schmitz was collected from intertidal regions of Mandapam, South East Coast of Tamilnadu, India, by the hand picking method. The freshly collected samples were thoroughly cleaned using sterilized sea water to remove the sand and salt contents. The sample was also gently brushed with a soft brush to remove attached epiphytes, other marine organisms and debris. Cleaned seaweeds were shade dried and powdered yield crude extract. The powered algal material soaked in methanol (1:20 w/v) over night, filtered and it was concentrated into crude extract

Methods

DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometric method [9]. To a methanol solution of DPPH (200 µM), 0.05 mL of the test compounds were added at different concentrations (100-900 µg/mL). An equal volume of methanol was added to the control. After 20 min, the decrease in the absorbance of the test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the following formula [10]. The experiment was repeated in triplicate.

$$Inhibition\ (\%) = \frac{(absorbance)^{517\ nm}_{control} - (absorbance)^{517\ nm}_{test}}{(Absorbance\ )^{517\ nm}_{conrol}}$$

X 100

ABTS free radical scavenging assay

Experiments were performed according to Re *et al.*,[11] with small modifications. ABTS and potassium persulfate were dissolved in distilled water to a final concentration of 7 mM and 2.45 mM respectively. These two solutions were mixed and the

mixture has allowed to stand in the dark at room temperature for 16 hrs before use in order to produce ABTS radical ($ABTS^{\bullet+}$). For the study, different concentrations (100 to 900 µg/mL) of methanol extract (0.5 mL) were added to 0.3 mL of ABTS solution and the final volume was made up with 1 mL of methanol. The absorbance was read at 745 nm and the percentage of inhibition was calculated by using the following formula [10]. The experiment was repeated in triplicate.

$$Inhibition\ (\%) = \frac{(absorbance)^{745\ nm}_{control} - (absorbance)^{745\ nm}_{test}}{(Absorbance\ )^{745\ nm}_{conrol}}$$

X 100

Total phenolic content

The total phenol content was measured using the Folin-Ciocalteau method [12]. In this method, 100µL aliquot of stock sample was mixed with 2.0 mL of 2% Na_2CO_3 and allowed to stand for 2 min at room temperature. Then 100 µL of Folin-Ciocalteau's phenol reagent was added. After incubation for 30 min at room temperature in darkness, the absorbance was read at 720nm using a spectrophotometer. The total phenolic content was calculated as gallic acid equivalent (GAE) by the following equation: T = C x V/M. T is the total phenolic content in mg/g of the extracts as GAE, C is the concentration of gallic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract in g.

Total flavonoid content

The total flavonoid content was determined according to the method described by Zhishen *et al.*, [13]. Briefly, a 250 µL of 5% $NaNO_2$ solution was added to 0.5 mL of the stock sample along with 150 µL of 10% $AlCl_3.H_2O$ solution. After 5 min, 0.5 mL of 1M NaOH solution was added and then the total volume was made up to 2.5 mL with deionized distilled water and the absorbance was read at 510 nm using a spectrophotometer. The absorbance was measured at 510 nm spectrophotometer using UV-visible Jasco V-630 instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids of polyherbal formulation were expressed as mg of quercetin equivalents/ 100 g of dry mass [14, 15].

STATISTICAL ANALYSIS

Data were obtained as the mean and standard deviation (SD) and determined using SPSS version 17.0 for windows.

RESULTS AND DISCUSSION

DPPH and ABTS are most common free radicals generators [16], so it is expected that using these two methods accurately reflects all of the antioxidants in a sample.

Table 1: *In vitro* antioxidant activity of methanol extract of *Tolypiocladia glomerulata*

S. No	Concentration (µg/mL)	Free radicals scavenging activity (inhibition %)			
		DPPH radical	ABTS	BHT	L-ascorbic acid
1	100	33.11 ± 0.15	21.33 ±0 .01	56.54 ± 0 .03	74.67 ± 0 .01
2	300	36.27 ± 0.56	23.18 ± 0.00	60.75 ± 0.02	76.15 ± 0.03
3	500	41.52 ± 0.01	30.94 ± 0.01	66.67 ± 0.02	78.66 ± 0.02
4	700	53.86 ± 0.00	35.24 ± 0.01	70.14 ± 0.03	80.34 ± 0.03
5	900	61.13 ± 0.02	50.35 ± 0.00	74.65 ± 0.02	82.61 ± 0.02
P -Value		0.000	0.000	0.000	0.000
F -Value		6.578333	5.521666	1.945555	4.742444

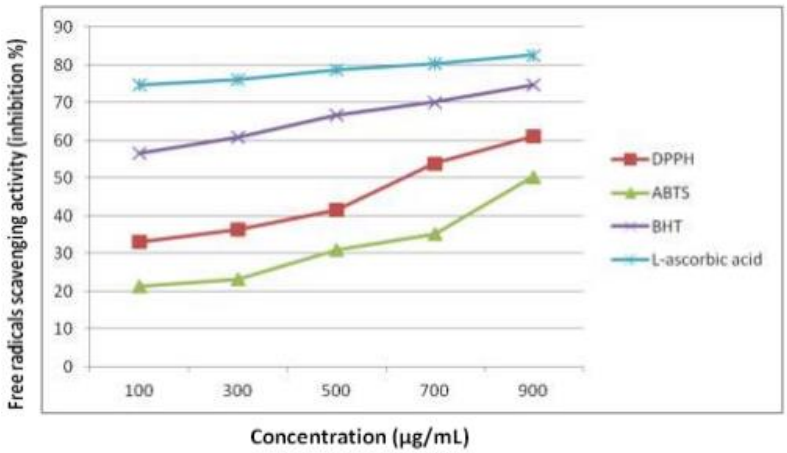

Fig-1: Comparision of free radicals (DPPH, ABTS) scavenging activity of methanol extract of *Tolypiocladia glomerulata* with standard BHT and L-ascorbic acid

In the present study, the total phenol and flavonoid content were quantified and the antioxidant activities were tested using two different *in vitro* assays such as, DPPH radical scavenging activity and ABTS radical scavenging activity. These two methods represented different mechanisms of antioxidant action.

DPPH radical scavenging assay

DPPH have been extensively used as a free radical to evaluate reducing substances such as polyphenols and flavonoids [17]. The methanol extract of marine red alga *T. glomerulata* was tested for their free radical scavenging activity against the stable free radicals of DPPH by spectrophotometrically (Table.1). A freshly prepared DPPH solution exhibits a deep purple color with absorption maximum at 517 nm. This purple color disappears when an antioxidant compound present in the sample. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them to a colorless/bleached product (i.e., 2, 2-diphenyl-1-hydrazine, a substituted analogous Hydrazine), resulting in a decrease in absorbance at 517 nm. Hence, the more rapidly the absorbance decreases, it indicates the more potent antioxidant activity of the extract.

The methanol extract of *T. glomerulata* revealed the maximum percentage of inhibition 61.13 ±0.02 against DPPH at the highest concentration of 900 µg/mL. The DPPH free radical scavenging activities of the present study was higher than the red algae *Gracilaria corticata* and *Hypnea musciformis* [18]. It was lesser than the red algae *Chondrococcus hornemannii* and *Spyridia fusiformis* [19] and *Grateloupia lithophila* [20].

T. glomerulata showed lower scavenging activity in comparison to standard antioxidants such as BHT and ascorbic acid, exhibited antioxidative potential in increased concentration. In general, the scavenging effects on the DPPH radical increased sharply with increasing concentration of all the samples and standards. The results show that this alga had the highest DPPH scavenging activity and indicates that *T. glomerulata* as a good source of natural antioxidant. Extracts of red alga from the Rhodomelaceae and Bangiaceae families have been reported to exhibit weak DPPH quenching activity when obtained using water [21, 22]. Thus, the solvent used to extract the seaweed material which had a dramatic effect on the chemical species recovered.

More recently, Murugesan *et al.,* [7], Parthiban *et al.,* [18] and Bhuvaneswari *et al.,* [19] determined that the DPPH free radical scavenging using methanol extracts of red algae were positively correlated with the total phenolic content of the methanol extracts. The present study also indicates the correlation between the total phenolic and flavonoid content and total antioxidant capacity of the methanol extract of red alga *T. glomerulata*.

ABTS radical scavenging assay

The methanol extract of marine red alga *T. glomerulata* was tested for their free radical scavenging activity against the stable free radicals of ABTS by spectrophotometrically. ABTS assay is a simple indirect method for determining the activity of natural antioxidants. In the absence of phenol, ABTS radical is rather stable, but it reacts rapidly with an H-atom donor, such as phenol, been converted into a colourless form of ABTS [21]. The ABTS radical cation-scavenging assay showed that the antioxidant activity increases with increasing concentration. The maximum scavenging effect (50.35 ± 0.00%) was shown at the highest concentration of 900 µg/mL. In the present study the ABTS free radical scavenging activities of were higher than the red alga *Grateloupia lithophila* [20]. Parthiban *et al.,* [18] reported that the strongest anti-oxidant activity in the ABTS method was achieved by the compound phenols. The results of the present study correlated with the result of Sroka and Cisowski [23] that they were confirmed the strong quench $ABTS^{\bullet+}$ radical is related with the more phenol contents of the experimental alga.

The antioxidant mechanism 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, the red seaweed *T. glomerulata* can be used for a variety of beneficial chemo-preventive effects. However, further studies on the anti oxidative components of seaweed extracts and more *in vivo* evidence are required.

Total phenol and flavonoid content

The total phenolic content of *T. glomerulata* was evaluated using the Folin-Ciocalteu method . It was observed that the methanol extract of the red alga *T. glomerulata* contained higher amount of total phenol content (0.105 ± 0.00 mg/g dry wt.) and flavonoid content (0.155 ± 0.00 mg/g dry wt), showed high antioxidant activity. (Table.2). To sum up, the DPPH and ABTS methods complement one another and give full information of anti-oxidative capacity of phenols and flavonoids.

Table 2: Total phenol and flavonoid content of *Tolypiocladia glomerulata*

S. No	Phytochemicals	mg/g dry wt
1	Phenols	0.105 ± 0.000
2	Flavonoids	0.155 ± 0.000

A number of studies focused on the biological activities of phenolic compounds, have shown that are

potential antioxidants and free radical scavengers [24-26]. Earlier reports revealed that seaweed extracts, especially polyphenols, have antioxidant activity [2, 27,28].

Phenolic compounds are generally more soluble in polar organic solvents [29]. So far, the great majority of antioxidant assays has been based on methanol as a solvent [18, 30]. Maximum antioxidant activity exhibited by higher phenolic content [31]. Several studies have demonstrated that highly significant correlation between the phenolic content and the antioxidant activity in seaweed extracts [27, 32]. The derivatives phenolic compounds produced as a secondary metabolize by the marine algae significantly contributed to their antioxidant capacity [33].

The red alga *T. glomerulata* had higher phenol content and it is in agreement with previous studies Duan *et al.*, [3], Nahas *et al.*, [34], that there was a significant correlation between antioxidant activity and phenol content of the seaweed species. The principal antioxidant components in seaweeds are thought to be phenols. Studies have demonstrated the correlation between phenol content and radical scavenging activity [2]. The presence of hydrophilic polyphenols in seaweeds such as phlorotannins, which are bi-polar in nature and mostly found in seaweeds, could function as antioxidative components and thus assist the algae to overcome oxidative stress [35].

The total flavanoid content of methanol extract was 0.155 ± 0.000 mg/g. These results indicate flavonoids extracted from seaweeds could be an important source of antioxidant molecules [36]. The capacity of flavonoids to act as antioxidants depends upon their molecular structure. It is believed that the antioxidant properties of flavonoids are a result of their ability to act as reducing agents.i.e hydrogen donors and free radical quenchers and phenolics can also act as metal chelators which prevent the catalytic function of metal in the process of initiating radicals [37]. The results of the present study revealed that there is a correlation between antioxidant activity and the phenol and flavonoid content. The potent that the antioxidant activity of the methanol extracts of red alga *T. glomerulata* can be the result of the presence study of higher concentration of total phenolic and flavonoids compounds.

CONCLUSION

From the present study, it can be concluded that the red alga *T. glomerulata* could be a potential rich source of natural antioxidants, which are known as phenols and flavonoid compounds. The present finding, help in further research in the investigation of seaweed with their solvent fraction for antioxidant activity and it also useful to utilize of the red alga *T. glomerulata* as a source food and medicine. Hence, further research is needed to analyze and isolate the active compounds responsible for the significant antioxidant activity of the red alga *T. glomerulata.*

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