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Research Article
Antioxidant Activity of Rhodophyta Algae *Polysiphonia* and *Laurencia* Collected from the Arabian Gulf

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Abstract

Background and Objective: Seaweeds are considered as a potential source of antioxidant which reduces the risk of some diseases and a good source of new drugs with lower side effect and toxicity. This study aimed to estimate the antioxidant activity of Rhodophyta algae genus *Polysiphonia* and *Laurencia* (Rhodomelaceae, Ceramiales). **Materials and Methods:** Samples were collected from different regions of the Arabian Gulf. Antioxidant activity represented by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, total flavonoid, phenolic and tannin content were quantified in ethanolic and aqueous extract of these two red algal genera. **Results:** Ethanolic and aqueous extracts of *Polysiphonia* and *Laurencia* were compared for their antioxidant properties. *Polysiphonia* showed higher flavonoid, phenolic and tannins content. While the DPPH radical scavenging activity of ethanolic and aqueous extract of *Laurencia* showed higher antioxidant activity with IC_{50} (5.64±0.68 and 6.34±0.41 mg mL^{-1}, respectively) as compared to *Polysiphonia* which has IC_{50} (9.30±0.18 and 9.57±0.46 mg mL^{-1}, respectively). **Conclusion:** These results showed that red algae are high in antioxidants activity and support its use in dietary supplements, cosmetics and food industry.

Key words: Antioxidant, free radical scavenging activity, *Laurencia*, phenol, *Polysiphonia*, rhodophyta

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Macroalgae are categorized based on their nutrient and chemical complex into three classes, green algae (*Chlorophyta*), Brown algae (*Phaeophyta*) and red algae (*Rhodophyta*). They are also considered as a potential source of antioxidant which reduces the risk of some diseases and suggested sources of new drugs with lower side effect and toxicity[1].

Free radicals such as superoxide anion (O_2), hydroxyl (OH-), hydroperoxyl (OOH-), peroxyl (ROO-) and alkoxyl (RO-) radicals are unstable atoms or molecules due to owning unpaired electrons[2]. The excessive amount of free radicals causes cell damage and various human diseases. Antioxidants have the ability to protect cells from damage as a result of free radicals by scavenging free radical and thus prevented from free radical-induced diseases[3]. Antioxidants can act through several defending mechanisms against free radicals[4]. Reduction of reactive oxygen species by donating hydrogen is one of the antioxidant defending mechanisms against free radicals[5]. Enzymes such as superoxide dismutase (SOD) glutathione peroxidase (GPx) and catalase consider as an important defense system. Reduced glutathione (GSH) is an antioxidant derived from sulfhydryl-containing amino acids and plays an important role in antioxidant metabolism.

Due to the free radical scavenging properties of polyphenols, they are considered as a potent natural antioxidant, which reduces the cancer mortality, heart disease and various other oxidative stress-related diseases as shown in the previous studies. Marine algae contain significant amounts of polyphenols such as phenolic acids, flavonoids, anthocyanidins, lignin, tannins, catechin, epicatechin, epigallocatechin and Gallic acid[4]. The aim of this study was to estimate the polyphenolic contents and antioxidant activity of two rhodophyta algae genera (*Polysiphonia* and *Laurencia*).

MATERIALS AND METHODS

Chemicals: In the present study, sodium nitrite, sodium hydroxide and Folin-ciocalteu reagent were purchased from (Winlab). Sodium carbonate, aluminium chloride and polyvinylpolypyrrolidone were purchased from (Loba Chemie). Gallic acid was purchased from (Sigma). Quercetin was purchased from Sterilin England. DPPH was purchased from (Atlantic) and ascorbic acid from (Avonchem).

Collection of algal material: The red algae strains were collected at low tide time along the coast of the Arabian Gulf of Saudi Arabia. The algal material was washed and allowed to dry in air. Finally, air dried algae were powdered and stored at room temperature.

Extract preparation: The powdered algae samples (1 g 100 mL^{-1}) were extracted successively with absolute ethanol and sterile autoclave water using a magnetic stirrer for 1 h and then soaked at 25°C for 48 h. The mixture was sonicated 5 times for 30 sec at 500 W, 25 KHz and then filtered through Whatman No.1 filter paper. The obtained filtrates were aliquoted and stored at -80°C for further studies.

Total flavonoid content: The total flavonoids content was estimated using the procedure described by Zhishen *et al.*[6]. "A total of 1 mL of plant extracts was diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 µL of aluminum chloride (10 %) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. The total flavonoids content was expressed as mg quercetin equivalent (mg QE/g) extract on a dry weight basis"[7].

Total phenolics content: The total phenolic content was estimated using the Folin-ciocalteu reagent. About 500 µL of aqueous and ethanol extracts were taken separately and it was made up to 1 mL with distilled water. Then 250 µL of diluted Folins-phenol reagent and 1.25 mL of 20% sodium carbonate (Na_2CO_3) were added. The mixture was shaken well and incubated in dark condition for 20 min. After incubation, the absorbance was measured at 735 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 0.25-10 mg L^{-1}. The total phenolic content in the plant extracts was expressed as mg of gallic acid equivalent (mg GAE/g extract)[8].

Estimation of tannins content: Tannin content was estimated by Siddhuraju and Manian method[9]. "The phenolic content of the supernatant was measured at 725 nm and expressed as the content of free phenolic on a dry matter basis. From the results, the tannin content of the extract was calculated as follows[7]:

$$\text{Tannins (mg GAE/g extract)} = \text{Total phenolics (mg GAE/g extract)} - \text{Free phenolics (mg GAE/g extract)}$$

DPPH radical scavenging activity: The ability of algae extracts to scavenge the DPPH• radicals was assessed by using the method of Blois with some modifications[10]. Then changes in the absorbance of the plant samples were measured at 517 nm. Results were compared with different concentrations of standard antioxidant ascorbic acid (0.01-0.05 mg mL^{-1}). The ability of DPPH• radical scavenging activity was calculated by using the following equation:

$$\text{DPPH}^{\bullet}\ \text{scavenging effect}\ (\text{Inhibition}\,\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts. The IC_{50} (the milligram of extract to scavenge 50% of the radicals) value was calculated using linear regression analysis[7].

Statistical analysis: All data were analyzed using Microsoft Excel and expressed as mean values±SD of triplicate. The mean values were analyzed by one-way ANOVA. Significant differences between the means of parameters were determined ($p<0.05$).

RESULTS

Total flavonoid, phenolics and tannins content: Phenolic compound such as flavonoid, phenol and tannin in ethanolic extract of polysiphonia were 176.95±0.99 mg QE g^{-1}, 19.03±0.60 mg GAE g^{-1} and 18.70±0.02 mg GAE g^{-1}, respectively, while in Laurencia were 79.43±0.89 mg QE g^{-1}, 18.99±0.99 mg GAE g^{-1} and 17.75±0.81 mg GAE g^{-1}, respectively which displayed that the ethanolic extracts of both red algae strains had higher Phenolic compound than aqueous extracts as shown in Table 1. *Polysiphonia* showed higher phenolic compound than *Laurencia* in both aqueous and ethanol extracts.

DPPH• radical scavenging activity: To evaluate the antioxidant capacity of red algae strains (*Ploysiphonia* and *Laurencia*), DPPH• radical scavenging method was applied. There is an inverse relation between IC_{50} and scavenging capacity. As the scavenging capacity increase IC_{50} decrease. From Table 2 ethanolic and aqueous extracts of *Laurencia* exhibited low IC_{50} (5.64±0.68, 6.34±0.41 mg mL^{-1}, respectively) compared with *Polysiphonia* which indicates high scavenging capacity. Both of ethanolic and aqueous extracts of *Laurencia* were significantly different compared with ethanolic and aqueous extracts of *Polysiphonia* ($p<0.05$) as shown in Fig. 1. Standard antioxidant (ascorbic acid) showed IC_{50} at 0.03±0.01 mg mL^{-1} which was significantly different compared with red algae strains ($p<0.05$).

Table 1: Total phenolics, total flavonoids and tannins contents of ethanolic and aqueous extracts of red algae genera *Ploysiphonia* and *Laurencia*

Samples	Solvent	Total flavonoid (mg QE g^{-1})	Total phenolics (mg GAE g^{-1})	Tannins (mg GAE g^{-1})
Ploysiphonia	Ethanol	176.95±0.99	19.03±0.60	18.70±0.02
	Aqueous	54.24±0.44	1.93±0.09	1.45±0.05
Laurencia	Ethanol	79.43±0.89	18.99±0.99	17.75±0.81
	Aqueous	1.97±0.94	0.83±0.08	0.78

Data were performed in triplicates and represented as Mean±SD

Table 2: Radical scavenging activity of ethanolic and aqueous extracts of red algae genera *Ploysiphonia* and *Laurencia*

Samples	Solvent	DPPH• radical scavenging activity IC_{50} (mg mL^{-1})
Ploysiphonia	Ethanol	9.30±0.18
	aqueous	9.57±0.46
Laurencia	Ethanol	5.64±0.68
	aqueous	6.34±0.41
Ascorbic acid	aqueous	0.03±0.01

Data were performed in triplicates and represented as Mean±SD. Ascorbic acid as standard antioxidant. All data were significantly different ($p<0.05$)

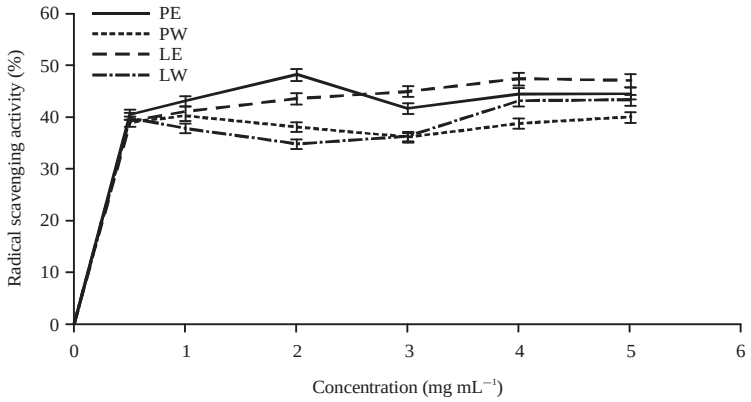

Fig. 1: Radical scavenging activity. PE: Ethanolic extract of *Polysiphonia*, PW: Aqueous extract of *Polysiphonia*, LE: Ethanolic extract of *Laurencia*, LW: Aqueous extract of *Laurencia*

DISCUSSION

Lipid peroxidation and free radical generation is a naturally occurring phenomenon in biological and food systems as a result of exposure to pro-oxidants present in the environment such as ultraviolet radiations, air pollution and cigarette smoking[11,12]. Antioxidants are a group of compounds that inhibit oxidation of other molecules thus preventing and maintaining the redox balance in the biological system[13]. Polyphenol, flavonoids and tannins compounds have gained much attention in recent years as their unique role in providing protection against the harmful effect of free radicals thus reducing the risk of several diseases such as cancer, hypertension, inflammation, alzheimer's disease and various other forms of neurological disorder[14-16].

In this study, researchers tried to explore the antioxidant potential of two rhodophyta algae genera which were collected from different coastal regions of the Arabian Gulf. The aqueous and ethanolic extracts were prepared and compared with respect to their total phenolic, flavonoid and Tannin content. Ethanolic and aqueous extracts of *Polysiphonia* showed flavonoid (176.95±0.99 and 54.24±0.44 mg QE g^{-1}, respectively), phenolic (19.03±0.60 and 1.93±0.09 mg GAE g^{-1}, respectively) and tannin content (18.70±0.02 and 1.45±0.05 mg GAE g^{-1}, respectively). While the ethanolic and aqueous extracts of *Laurencia* showed total flavonoids (79.43±0.89 and 1.97±0.94 mg QE g^{-1}, respectively), total phenolic (18.99±0.99 and 0.83±0.08 mg GAE g^{-1}, respectively) and Tannins (17.75±0.81 and 0.78 mg GAE g^{-1}, respectively) contents. These results supported other studies which suggested that organic solvents are better for extraction of total flavonoids, phenols and tannin compounds[17].

The DPPH assay is one of the most common and extensively used methods in evaluating antioxidant activity[18]. The radical scavenging activity of the two extracts was compared using their respective IC_{50} values. The IC_{50} was used to express the amount of concentration of extracts needed to scavenge 50% of the free radicals. The radical scavenging activity of both the ethanolic and aqueous extracts were shown in Table 2. The best free radical scavenging activity was exhibited in the ethanolic and aqueous extract of *Laurencia*, which showed higher antioxidant activity with IC_{50} (5.64±0.68 and 6.34±0.41 mg mL^{-1}, respectively) as compared to *Polysiphonia* which has IC_{50} (9.30±0.18 and 9.57±0.46 mg mL^{-1}, respectively).

Although the antioxidant properties of numerous genera of marine red algae, including *Colpomenia*, *Gracilaria*, *Polysiphonia* and *Laurencia* are reported[19], there are no published data on the antioxidants potential of *Polysiphonia* and *Laurencia* collected from Arabian Gulf. As exposure of marine seaweeds to higher UV radiation could result in the production of bioactive compounds such as phenols and flavonoids, the presence of high antioxidants activity in these red algae could be a result of their location in Arabian Peninsula.

CONCLUSION

The results of this study revealed that the two different red algae genera possess antioxidant activity. The result also indicates that the ethanolic extract of both red algae genera (*Polysiphonia* and *Laurencia*) exhibited higher antioxidant activity compared to aqueous extract. Finally, these red algae genera are a natural source of antioxidants due to the presence of free radical scavenging compounds such as phenol, flavonoids and Tannin. The higher antioxidant potential of these algae could be utilized in their applications in healthcare and related products as well as chemoprevention of various diseases including cancer.

SIGNIFICANCE STATEMENT

Seaweeds are thought to be a good source of antioxidants and novel phytochemicals. This study explored the antioxidant potential of red algae (*Polysiphonia* and *Laurencia*) which made them potential therapeutic agents against various diseases and their use as food and nutritional supplement. This study is significant as this is the first report on the antioxidant potential of these algae strains collected from different regions of the Arabian Gulf.

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