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ANTIOXIDATIVE POTENTIAL OF SEAWEEDS FROM KARACHI COAST

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ABSTRACT

In this study total 14 samples of seaweeds (5 red, 4 green and 5 brown) were collected from Karachi coast for their bio screening. *Sargassum tenerrimum, Melanothamnus somalensis and Valoniopsis pachynema* showed strong antioxidant potential by DPPH assay. Phenolic contents were estimated in mg GAE/100 g of seaweeds, and most of the species did not show significant ($p<0.01$) correlation to oxidative stress. Moreover, oxidative burst assay was performed by using chemiluminescence technique, in which ethanolic extract of *Melanothamnus somalensis* was only found active for anti-inflammatory activity with $IC_{50} \pm$ SD value (13.6 ± 1.3).

KEYWORDS: Seaweeds, Antioxidant, Phenols, Anti-inflammatory activity.

INTRODUCTION

In living organisms free radicals are produced by metabolism of oxygen, they are highly reactive molecules and commonly termed as reactive oxygen species (ROS) (Czarna and Jarmuszkiewicz, 2006). Naturally these free radicals scavenges through defend system of the body but in case of depletion of antioxidants, they may involve in other unnecessary metabolic activities which may lead to several diseases like cancer, gastric cancer, alcoholic liver cirrhosis, rheumatoid arthritis, cardiovascular diseases, skin aging, diabetes and Alzheimer's disease (Bizimenyera *et al.*, 2007; Tariq *et al.*, 2015; Butt *et al.*, 2014). Free radicals can trigger various compounds of cell membrane and produce tissue damage, Antioxidants can remove these free radicals from body, by trap the free radicals and inhibit or delay their oxidation reaction (Kumpulainen and salonen, 1999; Chuhan and Chuhan, 2006; Wresdiyati and Matika, 1997).

Seaweeds are valuable natural source of antioxidants they produce some useful bio chemicals like vitamins, minerals, proteins, pigments and phenolic compounds which can remove toxic metals from body (Ali *et al.*, 1999; Mellouk *et al.*, 2017; Newman and Cragg, 2007). Many natural compounds of seaweeds have been reported for exhibiting strong antioxidant potential (Kranl *et al.*, 2005; Farasat *et al.*, 2013; Butt *et al.*, 2014; Tariq *et al.*, 2011).

Earlier, few reports have been published for antioxidant activity of seaweeds from coastal area of Pakistan. Methanolic extract of two brown seaweeds S*argassum* spp. and *Iyengaria* spp. showed significant activity for ferrous ion chelating assay (Butt *et al.*, 2014). Moreover, 15 species of red, green and brown seaweeds from coast were investigated for their antioxidant potential by Tariq *et al.* (2015). Hanif *et al.* (2016) also contributed to the antioxidant potential of red seaweeds from Karachi coast. In present study some more seaweeds are selected for exploring their antioxidant potential with relation to their phenolic content and anti-inflammatory activity.

MATERIALS AND METHODS

Ethanol extract preparation: 100 gm of each seaweeds chopped into pieces and was extracted three times with 70% ethanolic solution for a month. Extract was filtered and concentrated to dryness on a rotary vacuum evaporator (Buchi rotvapor R-200) to obtain thick extract.

DPPH -free radical scavenging activity (Antioxidant activity): Antioxidant activity of sample was performed by DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay (Afshan *et al.,* 2016). An aliquot of 200µL of extract was reacted with 800µL of 10 mM Tris-HCl buffer (PH 7.4). In the mixture 30µM DPPH (dissolved in DMSO) was mixed and vortex. Control was prepared by 1mL of aqueous ethanol with 1 mL of DPPH. The absorbance was noted at 517 nm on UV-visible spectrophotometer at 0 minute and after 30 minutes, against aqueous ethanol as blank. BHT was used as standard antioxidative drug to compare with test samples. The antioxidant activity was calculated by using the formula:

$$\text{Antioxidant activity} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total polyphenol analysis: Polyphenols were estimated by following Afshan *et al.* (2016), 100 µL aliquots were reacted with 2 mL of (2% w/v) Na_2CO_3 and incubated for 2 minutes at room temperature. Later 100 µL of 50% Folin-Ciocalteu Phenol reagent was mixed with reaction mixture and placed in dark for 30 minutes at room temperature. Absorbance of samples was noted at 720 nm on spectrophotometer. Standard curve was prepared with gallic acid for the calculation of phenolic content in µg mL^{-1}.

Oxidative burst assay using chemiluminescence technique (anti-inflammatory activity): Immunomodulation assay was designated by Helfand *et al.* (1982). Concisely, 25 µL of diluted whole blood $HBSS^{++}$ (Hanks Balanced Salt Solution, comprising of calcium chloride and magnesium chloride) [Sigma, St. Louis, USA] was mixed with 25 µL of three different concentrations of compounds (10, 50 and 200 µg/mL), individually in triplicate. Control wells contained only $HBSS^{++}$ and cells. Test was done in white half area 96 well plates [Costar, NY, and USA], incubation was performed for 15 minutes at 37^oC in the thermostat chamber of luminometer [Labsystems, Helsinki, Finland]. Afterward, 25 µL of serum opsonized zymosan (SOZ) [Fluka, Buchs, Switzerland] and 25 µL of intracellular ROS sensing probe, luminol [Research Organics, Cleveland, OH, USA] were mixed into each well, excluding blank wells (holding only $HBSS^{++}$). The intensity of the reactive oxygen species was noted by luminometer in term of relative light units (RLU)**.** Ibuprofen was used as standard with IC50 ± SD 11.2 ± 1.9.

Statistical analysis: Results were analysed by two way ANOVA, lowest significant difference (LSD) was evaluated at $p<0.001$ for DPPH assay and $p<0.01$ for phenols estimation. Duncan's multiple range test was employed to compare treatment as 'mean ± standard deviation (SD)' value by using "Statistica software".

Table 1. Total phenols, antioxidant and anti-inflammatory potential of seaweeds from Karachi coast.

Seaweeds	DPPH assay at 0 minutes %	DPPH assay at 30 minutes %	Total phenols mg GAE/100 g.	Anti-inflammatory activity* % inhibition/ IC50 ± SD
Melanothamnus somalensis	23.876 ± 6.378	26.793 ± 6.579	69.106 ± 40.213	13.6 ± 1.3
Gracillaria corticata	13.903 ± 0.625	16.523 ± 0.236	32.466 ± 29.17	Not active
Gelidium pusillum	16.376 ± 0.601	19.293 ± 2.996	40.413 ± 14.499	×
Laurencia obtusa	13.526 ± 1.170	20.633 ± 9.393	4.033 ± 1.761	Not active
Coelarthrum mullerii	13.68 ± 3.997	15.016 ± 6.385	1 ± 0	×
Sargassum tenerrimum	22.406 ± 4.346	23.523 ± 4.380	42.17 ± 14.99	Not active
Cystoseira indica	16.323 ± 1.036	18.896 ± 2.010	30.9 ± 34.73	Not active
Padina tetrastromatica	16.793 ± 1.645	20.84 ± 2.148	54.1 ± 74.39	×
Jolyna laminarioides	16.98 ± 3.370	20.066 ± 2.892	6.66 ± 2.57	×
Spatoglossum variabile	15.96 ± 1.923	14.7 ± 0.496	84.8 ± 21.04	×
Enteromorpha intestinales	13.82 ± 0.252	14.7 ± 0.496	97.92 ± 51.48	Not active
Valoniopsis pachynema	11.726 ± 0.876	28.566 ± 15.508	1.06 ± 0.11	×
Caulerpa scalpeliformis	12.076 ± 2.295	22.506 ± 11.801	55.36 ± 39.72	Not active
Codium iyengarii	15.013 ± 0.436	14.733 ± 1.005	4.36 ± 5.06	
Control	1.665 ± 0.453	1.645 ± 0.505	-	-
BHT (Standard)	70.133 ± 4.500	77.2 ± 3.3	-	-

* ×= not tested, results were showed as mean ± SD value for DPPH assay and total phenols

RESULTS AND DISCUSSION

DPPH assay: In this study results showed that all of the fourteen specimens belonging to three different phyla of seaweeds, Rhodophycota (5), Chlorophycota (4) and Phaeophycota (5), were found significantly ($p<0.001$) active in DPPH assay, it was also noticed that by increasing incubation time at 30 minutes, all of the seaweed extracts showed an increase in their antioxidant potential by donating hydrogen ion. Furthermore, *Melanothamnus somalensis*, *Sargassum tenerrimum* and *Enteromorpha intestinales* were found with highest mean ± SD value (Table 1). Similar kind of results were reported by Tariq *et al.* (2011), in which 15 species of red, green and brown seaweeds from Karachi coast, showed enhanced activity in DPPH assay by increasing incubation period. Likewise, several scientific reports showed the potent antioxidant activity of seaweed extract by DPPH assay (Farasat *et al.,* 2013; Hanif *et al.,* 2016; Moubayed *et al.,* 2017; Mellouk *et al.,* 2017).

It is considered that seaweeds produce phenolic compounds which are responsible for reduced oxidative stress (Decker 1995), and their amount may vary from species to species depending on different climatic conditions and growing stage of seaweeds (Kayalvizhi *et al.,* 2014). In current study all the selected seaweeds showed phenolic contents at various degree (Table 1), the significantly higher phenolic contents were showed by *Enteromorpha intestinales*, *Spattoglossum variabile* and *Melanothamnus somalensis* respectively. Previously it has been stated that antioxidant potential of seaweeds has a co-relation to their natural phenolic compounds (Farasat *et al.,* 2014). Although in this study most of the seaweeds from all three phyla did not show any positive correlation between phenolic compound and antioxidant potential. Similar kind

of results were also observed earlier (Tariq *et al.,* 2011), moreover it was also reported that some other bioactive component of extract may be responsible for their antioxidant potential (Heo *et al.,* 2005).

Previous reports confirmed the anti-inflammatory potential of red seaweeds (Vijayalakshmi, 2015) as the members of Rhodophycota have been found with most active bio components as compared to Chlorophycota and Phaeophycota (El-Gamal, 2010; Hanif *et al.,* 2016). Similar results observed in present study, among seven seaweeds belonging to different phyla only *Melanothamnus somalensis* showed anti-inflammatory activity in chemiluminescence assay. It also showed higher amount of phenolic compounds which may be responsible as active ingredient for its antioxidant and anti-inflammatory activity in bioassay.

This study would suggest that seaweeds from Karachi coast, can be utilized as a potential source of natural antioxidants, as seaweed extracts are enriched in polysaccharides, vitamins, minerals, proteins, phenols and other low molecular weight compounds (Farasat *et al*., 2014; Tariq *et al.*, 2015), which make them unique and have gained much attention in global market (Rizvi and Valeem, 2012).

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