Comparison of Antioxidant Activity and Total Phenolic Contents of some Persian Gulf Marine Algae

Sadati N (Pharm.D.)[1], Khanavi M (Ph.D.)[1, 4*], Mahrokh A (Pharm.D.)[1], Nabavi SMB (D.Sc.)[2], Sohrabipour J (D.Sc.)[3], Hadjiakhoondi A (Ph.D.)[1]

1- Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
2- Khorramshahr University of Marine Science and Technology, Khuzestan, Iran
3- Agriculture and Natural Resource Research Center of Hormozgan. Bandar Abbas, 79145 - 1577, Iran
4- Iranian Pharmacy Research Center, Faculty of Traditional Iranian Medicine, Tehran University of Medical Sciences, Tehran, Iran
*Corresponding author: Department of Pharmacognosy and Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 – 21 – 66954706, Fax: +98 – 21 – 66461178
Email: khanavim@sina.tums.ac.ir

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Abstract

Background: Marine algae, especially brown species, produce a wide range of metabolites with various biological activities.

Objective: Since marine algae are rich source of dietary fibers, minerals, proteins, vitamins and phenolic components with antioxidant effect, in addition to the rule of oxidative stress in pathogenesis of chronic diseases, the aim of this study was to investigate possible antioxidant activity and total phenolic contents of three brown algae species (*Sargassum swartzii, Cystoseira myrica, Colpomenia sinuosa*) collected from Asaloye-Niband marine protected area of the Persian Gulf.

Methods: Antioxidant activity and total phenolic contents of partitional fractions of n-Hexane, Chlorofrom ($CHCL_3$), Ethylacetate (EtOAc) and Methanolic extract of the samples were studied. Total phenolic contents was measured using Folin-Ciocalteu method while ferric reducing antioxidant power (FRAP) assay were used to study their antioxidant activity.

Results: MeOH -H_2O and chloroform fractions of *Sargassum swartzii* were found to have the highest antioxidant activity as 73.92 ± 12.3, 55.32 ± 4.8 mmol Fe^{II} per 100 g dried plant and total phenolic contents, 12.0 ± 0.5, 11.05 ± 0.64 mg gallic acid equivalents per 100 g dried plant respectively.

Discussion: There was a significant Correlation (R^2=0.999) between the antioxidant activity and total phenolic contents of MeOH-H_2O fractions obtained from total extracts of these algae. It is recommended that these algae could be potential sources of natural antioxidants.

Keywords: Antioxidant activity, Total phenolic contents, *Sargassum swartzii, Cystoseira myrica, Colpomenia sinuosa*, Persian Gulf

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Introduction

Marine macroalgae or seaweeds are rich sources of several compounds with biological effects including antioxidant activities [1]. Furthermore, phylopheophytin, fucoxantine and phlorotannins, as antioxidant compounds, were detected from brown algae [2 - 4]. Oxidative stress is one of the most common cause in pathogenesis of chronic diseases [5] and dietary antioxidant have positive role in control of degenerative disorders such as cardiovascular disease, neurological disorders, diabetes, Alzheimer's disease [6 - 8] and gastric ulcer [9]. In attempt to find useful ways for curing diseases arising from oxidative deterioration more recent reports revealed antioxidant effects of the brown algae of genus Sargassum (Sargassaceae) [10 - 15]. Bioactive compounds such as several sulfated polysaccharides from algae have inhibited oxidative stress in mice [16] and the hydroquinone diterpen from *Cystoseira mediterraneol* has been shown an inhibitory effect on mitotic cell division [17]. Also macro algae *Sargassum stenophyllum* [18] showed brilliant antitumor activity. Iran has about 1260 km of coast lines along the Persian Gulf and Oman Sea. Recent information described 153 species of marine algae from coast lines of Iranian islands and coast of Hormozgan province [19, 20]. However, only few studies have been performed about the pharmacological effects of marine algae in this region. The aim of this study is to determine the antioxidant activity and total phenolic contents of different fractions obtained from MeOH (70%) extract of three species of the brown algae collected from coastlines of the Persian Gulf in south of Iran. In addition correlation between antioxidant activity and total phenolic contents were also considered in this study.

Materials and Methods

Materials

TPTZ (2, 4, 6 tripyridyl triazine) was purchased from Fluca and other solvents and materials were provided from Merck company (Darmstadt, Germany).

Plant material

Brown algae, *Sargassum swartzii* C. Agardh (Sargassaceae), *Cystoseira myrica* (S.G.Gmelin) C. Agardh (Cystoseiraceae), *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier (Scytosiphonaceae), were collected in June 2007, from Asaluye-Niband coast of the Persian Gulf. The algae were identified by J. Sohrabipour, Agriculture and Natural Resource Research Center of Hormozgan, the voucher specimens were deposited at this center.

Extraction and fractionation of marine algae

The algae were air –dried in the shade at room temperature and ground to powder with a mortar and pestle. Fifty grams of each sample were extracted with Methanol (70%) (5×200 ml) at room temperature. The combined extracts were evaporated under vacuum. The concentrated extract was successively partitioned between MeOH–H_2O (9:1) and n- Hexane, MeOH-H_2O (8:2) and $CHCL_3$, MeOH-H_2O (1:1) and EtOAc.Removal of the solvents was resulted production of n- Hexane, $CHCL_3$, EtOAc and MeOH-H_2O fractions.

Evaluation of antioxidant activity using TPTZ

The FRAP (Ferric reducing antioxidant power assay) procedure which described by Benzie and Strain was followed [21]. The principle of this method is based on the reduction of a ferric-tripyridyl triazine

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complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 ml of a (10 mmol/L) TPTZ (2, 4, 6- tripyridyl- s- triazine) solution in 40 mmol/L HCL plus 5 ml of (20 mmol/L) $FeCl_3$ and 50 ml of (0.3 mol/L) Acetate buffer, PH 3.6 which was prepared freshly and warmed at 37˚C. Aliquots of 50 µl extract were mixed with 1.5 ml FRAP reagent and after incubation at 37˚ c for 10 min, absorbance of reaction mixture at 593 nm was measured by spectrophotometer. For construction of calibration curve, five concentrations of $FeSO_4$ $7H_2O$ (125, 250, 500, 750, 1000 µmol/L) were used and the absorbencies were measured as sample solution. The values were expressed as the concentration of antioxidants activity as a ferric reducing ability equivalent for 1 mmol/L $FeSO_4$.

Measurment of total phenolic contents

Total phenolics were determined calorimetrically using Folin-Ciocalteu reagent as described by Velioglu et al. [22] with slight modifications. The prepared extract (200 µl) was mixed with 1.5 ml of folin-ciocaltue reagent (previously diluted to ten fold with distilled water) and allowed to stand at 200°C for 5 min. A 1.5 ml sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 min at 22°C, absorbance was measured at 725 nm using a UV spectrophotometer (Pharmacia Biotech). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid (GA) standard (20-150 mg/Lit). The concentrations are expressed as milligrams of gallic acid equivalents (GA) per 100 g dry plant.

Statistical analyses

The values are reported as mean ± SD. One-way ANOVA and Tukey post-hoc multicomparison tests were used for date analysis. P-values<0.05 was considered as significant.

Results

Antioxidant activity

The results of antioxidant activity of n- Hexane, Chloroform, Ethylacetate and Hydroalcoholic fractions of *Sargassum swartzii*, *Cystoseira myrica*, and *Colpomenia sinuosa* by using FRAP (ferric reducing antioxidant power) assay expressed as FRAP value. These values represented mmol. Fe^{II} per 100 g dried plant. The antioxidant activity of the samples ranged from 0 in EtOAc fraction of *Colpomenia sinuosa* to 73.92±12.36 mmol Fe^{II} / 100 g dried plants in MeOH/W fraction of *Sargassum swartzii* (Table 1).

Total Phenolic contents

Table 2 shows total phenolic contents of n- Hexane, Chloroform, Ethylacetate and Hydroalcoholic fractions of three species of brown algae using Folin - Ciocalteu method. Total phenolic contents are expressed as mg gallic acid equivalents per 100 g dried plant.

Correlation of antioxidant activity and total phenolic contents

Figure1 shows a relationship between free radical scavenging as FRAP value and total phenolic contents of different fractions obtained from total methanolic (70%) extract of three species of brown algae.

Discussion

Natural products with antioxidant activity in the FRAP assay are electron donor and are commonly considered by the presence of radical scavenger metabolites. Brown algae especially Sargassum species exhibited higher antioxidant activity and phenolic contents than

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red algae species. According to Matanjum (2008), *Sargassum polycystum* showed antioxidant activity as 366.69 ± 11.85 µM Fe^{II} /mg dry extract in FRAP assay and total phenolic contents, 45.16±3.01 mg phloroglucinol equivalents (PEG)/g dry extract that were evaluated by using the Folin-Ciocalteu method [12]. Rastian (2007) indicated that total phenolic content in water extract of *Sargassum baveanum* was 17 ± 0.492 mg catechin equivalent (CE)/g dry plant using Folin-Ciocalteu method and antioxidant activity was high about 90% inhibition of

Table 1- Antioxidant activity (mmol Fe^{II} /100 g dried plant) of fractions obtained from three brown algae (concentration of extract used=10 mg/ml)

Algae	Fractions			
	n- Hexane	CHCL3	EtOAc	Me-OH/W
Sargassum swartzii	19.62 ± 1.9	55.32 ± 4.8*	0.56 ± 0.54	73.92 ± 12.36
Cystoseira myrica	14.80 ± 0.9	18.06 ± 3.16	7.82 ± 0.66	50.95 ± 4.33
Colpomenia sinuosa	11.13 ± 2.07	7.42 ± 1.06	0	0.59 ± 1.03

$CHCL_3$: Chloroform, EtOAc: Ethyl acetate, Me-OH/W: Hydroalcoholic
*Significant difference ($p<0.05$)

Table 2 - Total phenolic contents (mg gallic acid/100 g dried plant) of fractions obtained from three brown algae (concentration of extract used=10 mg/ml)

Algae	Fractions			
	n- Hexane	CHCL3	EtOAc	Me-OH/W
Sargassum swartzii	6.41 ± 1.43	11.05 ± 0.6*	0.81 ± 0.35	12.00 ± 0.55*
Cystoseira myrica	3.01 ± 0.00	2.97 ± 0.02	1.37 ± 0.05	10.08 ± 1.13*
Colpomenia sinuosa	4.88 ± 0.72	4.40 ± 2.30	0.92 ± 0.14	5.30 ± 0.77

$CHCL_3$: Chloroform, EtOAc: Ethyl acetate, Me-OH/W: Hydroalcoholic
*Significant difference ($P<0.05$)

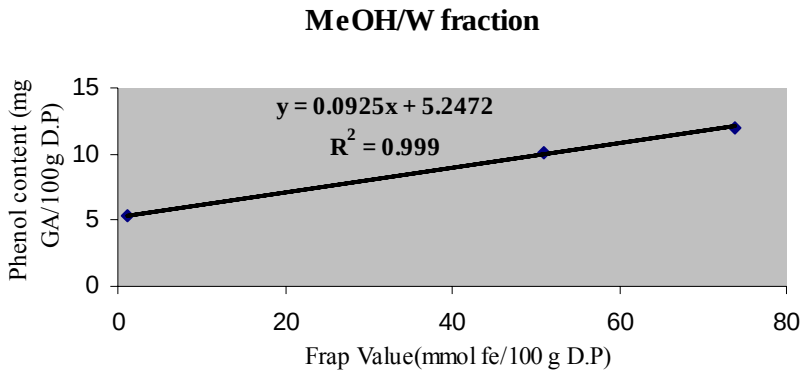

Fig. 1- Correlation between antioxidant activity and total phenolic contents of different fractions of total methanolic (70%) extract of three brown algae

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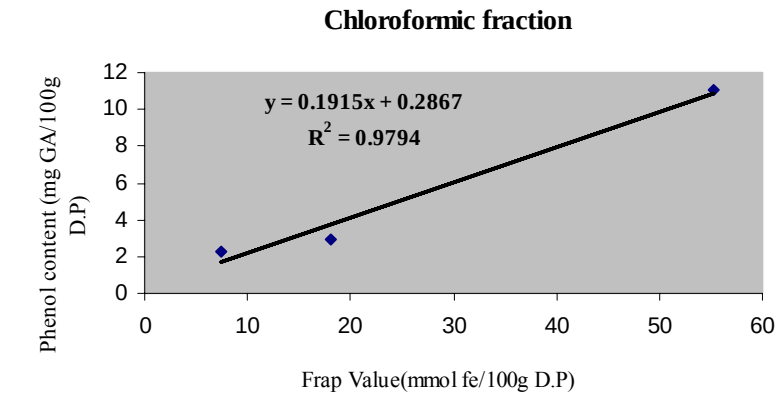

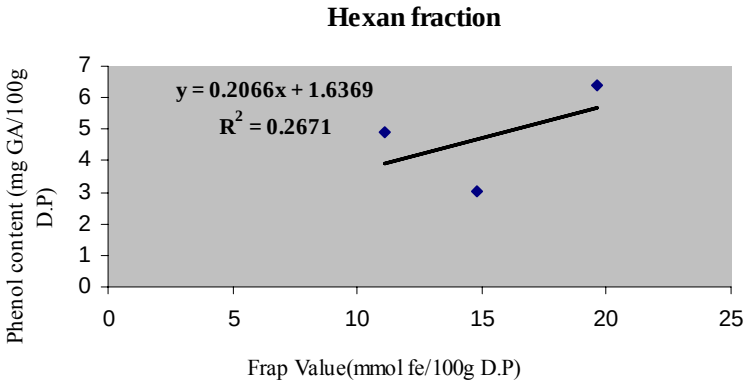

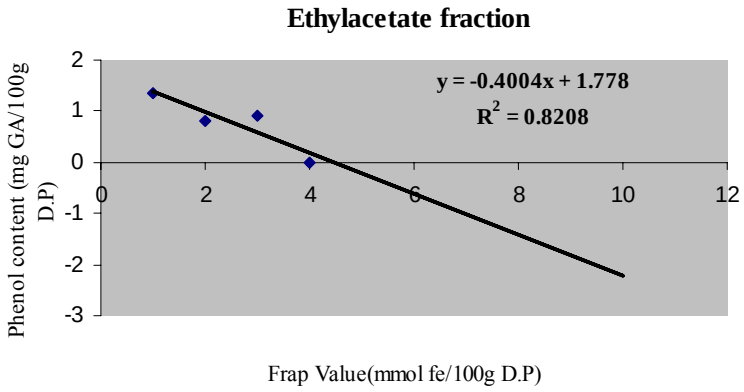

Continue Fig. 1- Correlation between antioxidant activity and total phenolic contents of different fractions of total methanolic (70%) extract of three brown algae

linoleic acid peroxidation with 7 mg dry sample/ml solvent [14]. To compare the effect of antioxidant activity and content of total phenols in different fractions of three brown algae, four different solvents were used. In this study among the three species of brown algae

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collected from the Persian Gulf, hydroalcoholic and chloroformic fractions of *S. swartzii* exhibited significantly the highest antioxidant activity ($p<0.05$), 73.92 ± 12.3, 55.32±4.8 mmol. Fe^{II} per 100 g dried plant, respectively.

Phenolic compounds that mostly found in plants, have been reported to have several biological effects including antioxidant, antiapoptosis, anti-aging, anti carcinogen [23] and have been highly considered for their important dietary roles as antioxidant and chemoprotective agents [24]. The total phenolic contents in hydroalcoholic and chloroformic fractions of *S. swartzii* 12.0 ± 0.5, 11.05 ± 0.64 mg gallic acid equivalents per 100 g dried plant and hydroalcoholic fraction of *C. myrica*, 10.08 ± 1.13 mg gallic acid equivalents per 100 g dried plant, were significantly higher than the other fractions ($p<0.05$). EtOAc fraction of three samples showed lowest antioxidant activity and total phenolic contents. There was a significant Correlation ($R^2=0.999$) ($p<0.05$) between the antioxidant activity and total phenolic contents of hydoalcoholic fractions of these algae.

Conclusion

It can be concluded that these algae especially *S. swartzii* could be potential rich source of natural antioxidants which lots of them are known as phenolic compounds. Isolation and characterization of hydroalcoholic and chloroformic fractions of *S. swartzii* and hydroalcoholic fraction of *C. myrica* as well as investigation of specific radical scavenger pathway may help to find whether the extract is valuable for more biological effects.

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