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TWO ALGAE FROM OMAN SEA: THE *IN VITRO* BIOLOGICAL EFFECTS

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ABSTRACT: Algae are extensively used in cosmetics, supplements and pharmaceutical industries. Researches support their hypotensive, cytotoxic, anti-inflammatory and many other biologic effects. In the present study, the cytotoxic, antioxidant and acetylcholinesterase inhibitory activities of two brown algae, *Padina tetrastromatica* and *P. australis*, have been evaluated. Total extract was prepared using methanol 80%. The extracts were evaluated for antioxidant and acetylcholinesterase inhibitory activity and cytotoxic properties against A-549, HT-29, HepG-2 and MCF-7 cell lines through DPPH, Ellman and MTT assays, respectively. The results revealed that *P. australis* with the IC_{50} value of 163.9µg/mL demonstrated stronger antioxidant activity compared to *P. tetrastromatica* (IC_{50} 259.1µg/mL) in DPPH assay. Neither of the total extracts showed cytotoxic activity up to the maximum amount examined (100µg/mL). The total extracts of *P. australis* and *P. tetrastromatica* in concentration of 300µg/mL presented 15.6% and 23.7% enzyme inhibition in Ellman assay, respectively. It was concluded that the two algae have shown weak antioxidant and acetylcholinesterase inhibitory activity and their further use in prevention and treatment of diseases needs more investigations especially in the field of isolation of active compounds.

INTRODUCTION: There are a large number of creatures living in the oceans of which only 200,000 invertebrate species have been described [1]. Many compounds with biological activities have been isolated from marine species which have shown to be promising in the primary steps of hard to manage diseases; thus more attention has been focused to research for pharmaceuticals with marine origin. Algae comprise various species; from the bacteria-sized coccoid taxa to the world's greatest protists, including diatoms, giant kelps, red, green, and brown algae.

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The brown alga possesses chlorophyll C and kind of special xanthophyll due to which they are called brown algae [2, 3]. They are found in rocky coasts around the world and prefer cold areas [3]. *Padina australias* Hauck and *P. tetrastramonia* Hauck (Dictyotaceae family) are two known brown algae. Due to the rising interest for finding new sources of medications with natural origin and because of the increasing focus to the products with marine origin, the above two brown algae which are found at the coasts of the Oman Sea have been investigated for some biological effects in the present study.

MATERIALS AND METHODS:

Plant Material: *Padina australias* Hauck and *P. tetrastramonia* Hauck were collected from Oman Sea in 2009. Their scientific names were verified by Dr. Bairam Gharanjik, Offshore Waters Research Center, Chabahar, Iran.

Voucher specimens TMRC-3513 and TMRC-3510 were assigned to the herbarium samples, respectively and they were kept at the Herbarium of Traditional Medicine and Materia Medica Research Center, Tehran, Iran for future reference.

Chemicals and Reagents: Acetylthiocholine iodide (ATCI), acetyl-cholinesterase enzyme (AChE) from bovine erythrocytes, 5, 5′-dithiobis [2-nitrobenzoic acid] (DTNB), [3-(4, 5-dimethyl thiazol-2-yl)-2, 4-diphenyl tetrazolium bromide] (MTT) and 2, 2-diphenyl-1-picryl hydrazyl (DPPH) were provided from Sigma (Germany). All solvents were purchased from Merck (Germany). The mediums and material used in MTT assay were provided from Sigma.

Cell Lines: The cancerous cell lines to be used in the cytotoxicity evaluation assay were provided from Pasture Institute, Tehran, Iran. A-549 (non-small cell lung carcinoma), HT-29 (human colorectal adenocarcinoma, HepG-2 (human heap-tocellular carcinoma) and MCF-7 (human breast adenocarcinoma) cells were cultured in suitable medium and seeded in 96-well plates, each well containing 8×103, 5×103, 15×103 and 8×103 for A-549, HT-29, HepG-2 and MCF-7 cells, respectively. A-549 and HepG-2 cells were cultured in RPMI-1640 medium containing 5% FBS; while MCF-7 and HT-29 cells were maintained in DMEM medium enriched with 5 and 10% FBS, respectively. All media contained 1% penicillin-streptomycin and the cells were kept in humidified atmosphere with 5% CO_2.

Extraction: The algae were air dried and ground. Twenty mg of each alga powder was then extracted with 80% methanol through maceration. After 24h, the filtrate was removed and the residues of the algae were macerated again with 80% methanol. The process continued thrice. The filterate were combined and concentrated.

MTT Assay: The ability of the live cells to reduce MTT, [3-(4, 5-dimethylthiazol- 2-yl)-2, 4 diphenyl-tetrazolium bromide] to its formazan salt was measured by exposing the cell lines to different concentrations of the extracts (3.125, 6.25, 12.5, 25, 50, 100µg/mL in DMSO 1% obtained by serial dilutions) for 72 hr MTT solution (final concentration 0.5mg/mL) was added to each well after removing the media containing the extract. The resulting formazan crystals were dissolved in DMSO 4 hr later. The absorbance was recorded with an ELISA reader at 570nm. The viability was measured according to the following equation:

$$\text{Viability (\%)} = A_s / A_c \times 100$$

Where A_s is the absorbance of wells with sample and A_c is the absorbance of wells in absence of sample. IC_{50} was calculated according to the data plotted with Microsoft Excel [4 - 6].

Ellman Assay: The acetyl cholinesterase inhibitory activity of algae extracts was assessed with Ellman assay. Briefly, 25µL of sample (concentration 3mg/mL of each extract) in methanol was added to each well of 96-well plates. 1250µL of 3mM DTNB, 25µL of 15mM ATCI and 50µL of phosphate buffer (pH 8) were also added to each well. The absorbance was recorded every 13 sec for 65 sec (405nm) with a TECAN microplate reader. Twenty five µL of 0.22U/mL AChE enzyme was added afterwards and the absorbance was measured every 13 sec for 104 sec. By plotting the absorbance *vs* time, enzyme activity was determined by comparing the rates for the sample to the blank (methanol). Donepezil was used as the positive control [7, 8].

DPPH Assay: The antioxidant property of the algae extracts was measured using DPPH radicals. In case DPPH radicals meet a hydrogen donator substance, they will lose their colour and the absorbance (at 520nm) would decrease. The DPPH methanol solution (2mL, 100µM) was added to 2mL of different concentrations of the extracts followed by strong shaking. The mixture was then left at room temperature (30 min). The absorbance was measured at 517nm and the antioxidant capacity was calculated using the following equation. IC_{50} was calculated accordingly.

Scavenging capacity % = 100 - [(ABS of sample-ABS of blank) ×100/ABS of control]

The blank, negative and positive controls were 2mL of methanol along with 2mL of the extract solution, 2mL DPPH solution with 2mL methanol and Vitamin C, respectively [9, 10].

RESULTS AND DISCUSSION: The methanol extracts of *P. australis* and *P. tetrastromatica* did not show cytotoxic activity on A-549, HT-29, HepG-2 and MCF-7 cells upto the concentration of 100µg/mL. The antioxidant and AChEI activities have been presented in **Table 1**. Each experiment was done in triplicate.

TABLE 1: THE ANTIOXIDANT AND ACHEI ACTIVITY OF *PADINA AUSTRALIS* AND *PADINA TETRASTROMATICA* TOTAL EXTRACTS

Alga	IC_{50} in DPPH assay (µg/mL)	AChE inhibition (%)
Padina australis	163.9 ± 13.2	15.6 ± 1.4
Padina tetrastromatica	259.1 ± 16.2	23.7 ± 0.6
Vitamin C	2.14 ± 0.21	-
Donepezil	-	0.003 µg/mL(IC50)

As mentioned earlier, algae have recently become the center of attention in biological researches. *P. australis* and *P. tetrastromatica* were of no exception and some biological investigations from different regions of the word have been conducted on these seaweeds in recent years.

The methanol extracts of *Padina australis* collected from the Pramuka Island, Indonesia, has gone through antioxidant assay and has shown the IC_{50} of 267.1 ppm in DPPH assay [11]; while different fraction of this alga from the east coastal regions of Penyabong, Malaysia, have demonstrated the range of 1.63 – 130.14µg/mL for IC_{50}; the value has increased according to the polarity of the solvents [12]. However, Dotulong *et al.*, observed almost no activity from hexane, ethyl acetate and water fractions of *Padina australis* from North Sulawesi Nain Islandin DPPH assay [13]. In another study from Indonesia, *P. australis* ethanol and aceton extracts were evaluated in DPPH assay but the IC_{50} values were rather high compared to other mentioned studies [14].

The IC_{50} values of 0.276 ± 0.01 and 0.649 ± 0.03mg/mL have been reported for the dichloromethane and methanol fractions of *Padina australis* from Malaysia, as reported by Gany *et al.*, [15]. The free radical scavenging ability of *Padina australis* methanol extract was found to be 163.9 ± 13.2(µg/mL) in our study. The classified information in **Table 2** show that our results are somehow in accordance with most studies though others were not able to record the antioxidant activity or they recorded rather low values. It should be noted that different methods of extraction and different geographical conditions might also be the cause of these differences.

TABLE 2: RESULTS OF DPPH ASSAY OF PADINA AUSTRALIS FROM DIFFERENT GEOGRAPHICAL LOCATIONS

Samples	DPPH values	Country of brown algae collection
Methanol extracts	267.1 ppm	Indonesia [11]
Fractions with different polarity	1.63 –130.14 µg/mL	Malaysia [12]
Fractions with different polarity	Almost no activity	Indonesia [13]
Ethanol fraction	1.3 mg of sample; 3mg of sample	Indonesia [14]
Acetone fraction		
Dichloromethane fraction	0.276±0.01 mg/mL;	Malaysia [15]
Methanol fraction	0.649±0.03 mg/mL	
Methanol extract	0.164±0.013 mg/mL	Iran

Cytotoxic studies on the same species collected from Malaysia, have led to the isolation of fucoxanthin which has shown to be cytotoxic to human lung cancer cells (H1299) with IC_{50} value of 2.45mM [16]. In our current study, no cytotoxic activity was observed to the maximum tested concentration (100µg/mL). As fucoxanthin from the species has shown cytotoxicity in the study of Jaswir *et al.*, it could be assumed that higher concentrations of the extract might be able to induce toxicity in the tested cell lines of our study.

In previous studies for finding AChE enzyme inhibitory activity in natural sources, the methanol extract of *Padina australis* collected from Coasts of Persian Gulf has not shown much inhibition of the enzyme (IC_{50} 6.3mg/mL) in a study conducted by Ghnannadi *et al.*, [17]; whereas the dichloromethane fraction of the alga was reported to inhibit the acetylcholinestrase enzyme with IC_{50} value of 0.149 ± 0.046mg/mL; while this value was > 0.2 mg/mL for inhibiting butyrylcholinesterase. IC_{50} value was > 0.2 mg/mL for the methanol fraction in inhibition of both enzymes in the same study [15]. Another study reported that the n-butanol fraction of the *Padina australis* collected from Malaysia, showed IC_{50} value of 1.54 ± 0.045mg/mL for inhibiting AChE enzyme12. In our present study, *Padina australis* methanol extract could inhibit the

AChE enzyme (15.6% ± 1.4) at the concentration of 300µg/mL. Comparing the present result with previous data, it could be suggested that our result are similar to previously published data except for the study of Ghannadi *et al.,* [17] (**Table 3**).

TABLE 3: RESULTS OF ACHEI EVALUATION OF *PADINA AUSTRALIS* FROM DIFFERENT GEOGRAPHICAL LOCATIONS

Samples	AChE inhibition	Country of brown algae collection
Methanol extract	IC_{50} 6.3 mg/mL	Iran [17]
dichloromethane fraction	IC_{50} 0.149±0.046 mg/mL	Malaysia [15]
n-butanol fraction	IC_{50} 1.54±0.045 mg/mL	Malaysia [12]
Methanol extract	15.6%±1.4 inhibition at 300 µg/mL	Iran

As for *P. tetrastromatica*, several studies have been carried out to define its biological effects. In a study conducted by Sarojini *et al.,* the ethanol extract of the alga collected from coasts of India, was able to scavenge DPPH radicals with the IC_{50} of 574µg/mL [18]. In another study, fucoidan was isolated from the ethanol extract of *P. tetrastromatica* which demonstrated 66.34% inhibition of DPPH radicals at the concentration of 1mg/mL [19]. Also, with aqueous extract of *P. tetrastromatica*, at the concentrations of 0.3-0.6 mg/mL, the DPPH radicals were inhibited in the range of 13.04 ± 1.14 to 55.85 ± 56% growing as the concentration increased [20]; while methanol extract of samples from India demonstrated IC_{50} of 0.61 ± 0.005mg/mL in DPPH assay. The ethyl acetate fraction of *P. tetrastromatica* from Malaysia gave the IC_{50} 171.67 ± 2.89µg/mL which is a bit lower compared to the above mentioned studies [21, 22]. The results presented in **Table 4** show that *P. tetrastromatica* from Iran and Malaysia has shown stronger activity for scavenging DPPH radicals. Considering that all other studies were conducted on the Indian alga, it comes in to the mind that different localities might have made some changes in the chemicals responsible for the observed effects.

Padina tetrastromatica has also been evaluated for cytotoxic activity in a few studies. Its methanol derived extract has been found to be active against MCF-7 cells (IC_{50} 125 ± 2.03µg/mL) during a study in Malaysia [23]; while in the same study that was mentioned for *P. australis* from Chahbahar, again no toxicity was observed. Since our study used limited extract concentrations (up to 100µg/mL), it could be proposed that higher concentrations might lead to observation of cytotoxicity. There is not much data about the AChEI activity of *P. tetrastromatica* in literature; however the methanol extract showed 23.7 ± 0.6 inhibition of the enzyme in our study at the concentration of 300µg/mL.

TABLE 4: RESULTS OF DPPH ASSAY OF *P. TETRASTROMATICA* FROM DIFFERENT GEOGRAPHICAL LOCATIONS

Samples	DPPH	Country of brown algae collection
Ethanol Extract	IC_{50} of 574µg/mL	India [13]
Ethanol Extract	66.34% inhibition at 1mg/mL	India [19]
Aqueous Extract	13.04±1.14 to 55.85±56 inhibition % at 0.3-0.6mg/mL	India [20]
Methanol Extract	0.61±0.005mg/mL	India [21]
Ethyl Acetate Fraction	171.67±2.89µg/mL	Malaysia [22]
Methanol Extract	259.1±16.2	Iran

CONCLUSION: Taking all into consideration, it is concluded that both alga do not seem to be potent in presenting antioxidant, acetylcholinestrase inhibitory or cytotoxic activity. The overall results of the present study along with the previously published data show that high amounts of the extracts are needed for presenting biological activities. As an alternative, it is suggested that instead of increasing the concentrations, one should focus on isolating the compounds that might be responsible for the biological activities. By isolating and purifying the compounds, stronger activity of potent compounds might be observed; these compounds would then be promising for continuing studies for finding medications of marine origin.

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DECLARATION OF INTEREST: The authors declare that there is no conflict of interest.

REFERENCES:

1. National Research Council (US) Committee on the Ocean's Role in Human Health: From Monsoons to Microbes: Understanding the Ocean's Role in Human Health. National Academies Press 1999.
2. Dorrell RG and Smit AG: Do red and green make brown? Perspectives on plastid acquisitions within chromal-veolates. h. Eukaryotic Cell 2011; 10: 856-868.
3. Bhattachary D and Medlin L: Algal phylogeny and the origin of land plants, Plant Physiology 1998; 116: 9-15.
4. Hamzeloo-Moghadam M, Firouzi J, Saeidnia S, Hajimehdipoor H, Jamili S, Rustaiyan A and Gohari AR: ڕytotoxichydroperoxy sterol from the brown alga, Nizamuddiniazanardinii. Daru Journal of Pharmaceutical Sciences 2013; 21:1-4.
5. Hamzeloo-Moghadam M, Naghibi F, Atoofi A, Asgharian-Rezaie M, Irani M and Mosaddegh M: Cytotoxic activity and apoptosis induction by gaillardin. Zeitschrift für Naturforschung 2013; 68: 108-112.
6. Esmaeili S, Irani M, Moazzeni Zehan H, Keramatian B, Tavakoli Harandi Z and Hamzeloo-Moghadam M: Cytotoxic activity of some ethnic medicinal plants from southwest of Iran. Research Journal of Pharmacognosy 2016; 3: 43-47.
7. Hajimehdipoor H, Mosaddegh M, Naghibi F, Haeri A and Hamzeloo-Moghadam: Natural sesquiterpen lactones as acetylcholinesterase inhibitors. Anais da Academia Brasileira de Ciências 2014; 86: 801-805.
8. Hajimehdipoor H, Ara L, Moazzeni H and Esmaeili S: Evaluating the antioxidant and acetylcholinesterase inhibitory activities of some plants from Kohgiluyehva Boyerahmad province, Iran. Research Journal of Pharma-cognosy 2016; 3: 1-7.
9. Hajimehdipoor H, Gohari AR, Ajani Y and Saeidnia S: Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts. Research Journal of Pharmacognosy 2014; 1: 21-25.
10. Hajimehdipoor H, Naghibi F, Bandidarian A, Moazzeni Zehan H, Pirani A and Hamzeloo-Moghadam M: Evaluating the antioxidant and acetylcholinesterase inhibitory activity of three *Centaurea* species. Research Journal of Pharmacognosy 2014; 1: 33-37.
11. Santoso J, Podungge F and Sumaryanto H: Chemical composition and antioxidant activity of tropical brown algae *Padina australis* from Pramuka Island, District of Seribu Island, Indonesia. JurnalIlmudan Teknologi Kelautan Tropis 2013; 5: 287-297.
12. Murugan AC, Vallal D, Karim R, Govindan N, Yusoff MBM and Rahman M: *In vitro* antiradical and neuroprotective activity of polyphenolic extract from marine algae *Padina australis*. Journal of Chemical and Pharmaceutical Research 2015; 7: 355-362.
13. Dotulong V, Widjanarko SB and Mamahit LP: The content of total phenols and antioxidant activity three types sea algae taken at the North Sulawesi waters. Food Science and Quality Management 2013; 17: 40-46.
14. Gunji S, Santoso J, Yoshie-Stark Y and Suzuki T: Effects of extracts from tropical seaweeds on DPPH radicals and Caco-2 Cells treated with hydrogen peroxide. Food Science and Technology Research 2007; 13: 275-279.
15. Gany SA, Tan SC and Gan SY: Antioxidative, anticholinesterase and anti-neuroinflammatory properties of Malaysian brown and green seaweeds. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering 2014; 8.
16. Jaswir I, Noviendri D, Hamzah M. Taher SM and Miyashita K: Isolation of fucoxanthin and fatty acids analysis of *Padina australis* and cytotoxic effect of fucoxanthin on human lung cancer (H1299) cell lines. African Journal of Biotechnology 2011; 10: 18855-18862.
17. Ghannadi A, Plubrukarn A, Zandi K, Sartavi K and Yegdaneh A: Screening for antimalarial and acetyl-cholinesterase inhibitory activities of some Iranian seaweeds. Research in Pharmaceutical Sciences 2013; 8: 113-118.
18. Sarojini Y, Sujatha B and Lakshminarayana K: Total phenol content and antioxidant activities of ethanolic extracts of two marine brown macroalgae. International Journal Current Life Science 2013; 8: 43-49.
19. Raghu G, JeyaShakila R, Jeyasekaran G, Padmavathy P and Veeresh B: Antioxidative properties of fucoidan extracted from brown seaweed, *Padina tetrastromatica* available along Gulf of Mannar Coast. Journal of Experimental Zoology, India 2016; 19: 723-730.
20. Praveen NK and Chakraborty K: Antioxidant and anti-inflammatory potential of the aqueous extract and polysaccharide fraction from brown marine macroalgae *Padina* sp. From Gulf of Mannar of Peninsular India. Journal of Coastal Life Medicine 2013; 1: 39-49.
21. Vinayak RC, Sabu AS and Chatterji A: Bio-prospecting of a few brown seaweeds for their cytotoxic and antioxidant activities. E-Complementary and alternative Medicine 2010; 1-9.
22. Chia YY, Kanthimathi MS, Khoo KS, Rajarajeswaran J, Cheng HM and Yap WS: Antioxidant and cytotoxic activities of three species of tropical seaweeds. BMC Complementary and Alternative Medicine 2015; 15: 339.
23. Chia YY, Kanthimathi MS, Rajarajeswarana J, Khoo KS and Cheng HM: Antioxidant, antiproliferative, genotoxic and cytoprotective effects of the methanolic extract of *Padina tetrastromatica* on human breast adenocarcinoma and embryonic fibroblast cell lines. Frontiers in Life Science 2015; 8: 411-418.

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