

Screening for antimalarial and acetylcholinesterase inhibitory activities of some Iranian seaweeds

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Abstract

Alcoholic extracts of 8 different types of seaweeds from Iran's Persian Gulf were tested for their antimalarial and acetylcholinesterase enzyme (AChE) inhibitory activities for the first time. A modified Ellman and Ingkaninan method was used for measuring AChE inhibitory activity in which galanthamine was used as the reference. The antimalarial assay was performed using microculture radioisotope technique. Mefloquine and dihydroartemisinin were used as the standards. The extract of *Sargassum boveanum* (*Sargasseae* family) showed the highest AChE inhibitory activity (IC_{50} equals to 1 mg ml^{-1}) while *Cystoseira indica* (*Cystoseiraceae* family) exhibited the least activity (IC_{50} of 11 mg ml^{-1}). The species from Rhodophyta (*Gracilaria corticata* and *Gracilaria salicornia*) also showed moderate activities (IC_{50} 9.5, 8.7 mg ml^{-1} , respectively). All extracts were inactive in antimalarial assay.

Keywords: Acetylcholinesterase inhibitor; Antimalarial; *Sargassum*; *Cystoseira*; *Gracilaria*; *Padina*

INTRODUCTION

Alzheimer's disease (AD) is a progressive and degenerative problem in brain regions, mainly campus and neocortex that are responsible for mental functions. This disease can lead to the loss of memory, behavior abnormalities and also cognitive disturbances (1). An estimated 24 million people worldwide are suffering from dementia, which two-thirds of them live in the developing countries, with no suitable treatment, the population of people suffering from AD will be more than 80 million by 2040 (2). In the cholinergic hypothesis, serious loss of cholinergic neurotransmitter acetylcholine (ACh) in the CNS contributes significantly to the symptoms of AD (3). The inhibition of acetylcholinesterase (AChE) enzyme, which catalyzes the breakdown of ACh, plays a key role in enhancing cholinergic transmission in the brain, resulting to the treatment of AD (4).

Malaria represents the world's greatest public health problem in terms of number of people affected and levels of morbidity and mortality. The malaria protozoan parasites (*Plasmodium* spp.) are transmitted by infected female mosquitoes when feeding on blood. Parasites soon enter liver cells, and after several days of multiplication, are released into the blood stream where further cycles of asexual reproduction occur, giving rise to the clinical symptoms of malaria. This parasite has become increasingly resistant to standard antimalarials such as chloroquine and antifolates. Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria (5).

Ninety percent of the world's biomass exist in the oceans which comprising approximately half of the total global biodiversity (6,7). This wide diversity of organisms is being recognized to have a potent reservoir of secondary metabolites such as terpenes, alkaloids and

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polyphenolics which many of these compounds are halogenated. (7,8). Among marine organisms, marine algae have been identified as an under-exploited plant resources (9,10).

This group of marines can be classified into three classes based on their pigmentation, namely brown, red, and green algae, which are referred to as Phaeophyceae, Rhodophyceae, and Chlorophyceae, respectively (11). Researchers have reported that algal originated compounds exhibit various biological activities such as anticoagulant (12,13), anti-viral (14,15), antioxidant (16-18), anti-allergic (19), anti-cancer (20), anti-inflammatory (21) and anti-obesity (22-24). Furthermore, several scientific studies have provided insight into neuroprotective and antiprotozoal properties of marine algae (25). The oceans, with their unique and wide range of biodiversity, producing unusual metabolites, also emerge as a good candidate for new pharmaceutical agents.

In recent years, biological activities, nutritional value, and potential health benefits of marine algae have been intensively investigated and reviewed. This survey, however, focuses specifically on the antimalarial and AChE inhibitory activity of marine algae of Persian Gulf, Iran for the first time and looking for their potential application as future pharmaceutical candidates to be used for the treatment of AD and malaria.

MATERIALS AND METHODS

Plant material

Eight different types of seaweeds were collected from Persian Gulf, Bushehr Province in the south part of Iran during the autumn (October and November 2010). The species were *Cystoseira indica*, *Cystoseira merica* (*Cystoseiraceae* family), *Sargassum angostifolium*, *Sargassum oligocystum* and *Sargassum boveanum* (*Sargasseae* family) and *Padina australis* (*Dictyotaceae* family) which belong to Phaeophyta and also *Gracilaria corticata* and *G. salicornia* (*Gracilariaceae* family) belonging to Rhodophyta. They were transported to the laboratory within 24 h of collection, cut in to small pieces and air-dried under shade at room temperature (30°C). Voucher specimens (codes: 2660 to 2667) from all collected seaweeds were made and

deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences (Isfahan, Iran) and were identified by Agricultural and Natural Resources Research Center of Bushehr.

Extraction and isolation

The dried materials were macerated in methanol for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. The residue from the filtration was macerated in methanol again for 3 days and filtered. The filtrate was evaporated with the same procedure and combined with the extract from the first extraction.

Microplate assay for acetylcholinesterase enzyme activity

The assay for measuring AChE activity was modified from the assay described by Ellman and coworkers (26) and Ingkaninan and coworkers (27). Briefly, 125 µl of 3 mM Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB], 25 µl of 15 mM ATCI (acetylthiocholine iodide), 50 µl of phosphate buffer, and 25 µl of sample were dissolved in buffer containing not more than 10% methanol and added to the wells followed by addition of 25 µl of 0.28 U/ml AChE. The blank for such a run consists of buffer, substrate, and DTNB solutions. The microplate was then read out at 405 nm every 5 sec for 2 min by a CERES UV 900C microplate reader (Bio-Tek Instrument, USA). The velocities of the reactions were measured. Enzyme activity was calculated as a percentage of the velocities compared to that of the assay using buffer without any inhibitor. Inhibitory activity was calculated from hundred subtracted by the percentage of enzyme activity. Every experiment was performed in triplicate. Various concentrations of seaweed extracts were taken for the study. IC50 values were determined by plotting a percent inhibition versus concentration curve, in which the concentration of sample required for 50% inhibition was determined and expressed as IC50 values.

Antimalarial test

The targeted parasite was *Plasmodium falciparum* (K1 multidrug resistant strain), which was maintained in Roswell Park

Memorial Institute 1640 (RPMI 1640) medium containing 20 mM *N*-2-hydroxyethyl piperazine- *N*-2-ethanesulfonic acid (HEPES), 32 mM NaHCO₃, and 10% heat activated human serum (28). Quantitative assessment of *in vitro* antimalarial activity was determined by means of the microculture radioisotope technique based upon the method previously described by Desjardins and coworkers (29). The assay uses the uptake of [³H] hypoxanthine by parasites as an indicator of viability. Continuous *in vitro* cultures of asexual erythrocytic stages of *Plasmodium falciparum* were maintained. Extracts were tested on K1 strain. Initial concentration of the extracts was 10 µg/ml. Mefloquine and dihydroartemisinin were used as positive references. In brief, a 200 µl mixture of 1.5% of erythrocytes with 1% parasitemia at an early ring state was exposed to 25 µL of RPMI medium containing tested sample in a serial dilution (doped with DMSO, 0.1% final concentration). This was inoculated at 37°C under a 5% CO₂ atmosphere for 24 h. A 25 µl

of [³H] hypoxanthine in the RPMI medium was added to each well and the plate was incubated for additional 24 h. Detection of incorporated [³H] hypoxanthine was performed on a TopCount microplate scintillation counter. The active sample was able to cause 50% inhibition in growth and activity was reported in IC₅₀ scale.

RESULTS

Of the 8 seaweeds representing 4 different families, the most active extracts with AChE activity as shown in Table 1 were made from *S. boveanum* (IC₅₀ 1 mg ml⁻¹) and *S. oligocystum* (IC₅₀ 2.5 mg ml⁻¹) while the least active extracts were made from *C. indica* (IC₅₀ 11 mg ml⁻¹), *G. corticata* (IC₅₀ 9.5 mg ml⁻¹) and *G. salicornia* (IC₅₀ 8.7 mg ml⁻¹). The three remaining showed medium activities (Fig. 1). Galanthamine, the pure compound used as a reference, had an IC₅₀ of 0.0007 mg ml⁻¹. Mixture of Ellman's reagent, ATCI and phosphate buffer was used as negative controls.

Table 1. *In Vitro* quantitative inhibition of acetylcholinesterase by the crude extracts of different seaweeds of Persian Gulf

Seaweed	Family	Division	IC ₅₀ (mg ml ⁻¹)
<i>Cystoseira indica</i>	Cystoseiraceae	Phaeophyta	11
<i>Cystoseira merica</i>	Cystoseiraceae	Phaeophyta	6
<i>Sargassum angostifolium</i>	Sargasseae	Phaeophyta	5.4
<i>Sargassum oligocystum</i>	Sargasseae	Phaeophyta	2.5
<i>Sargassum boveanum</i>	Sargasseae	Phaeophyta	1
<i>Padina australis</i>	Dictyotaceae	Phaeophyta	6.3
<i>Gracilaria corticata</i>	Gracilariaceae	Rhodophyta	9.5
<i>Gracilaria salicornia</i>	Gracilariaceae	Rhodophyta	8.7

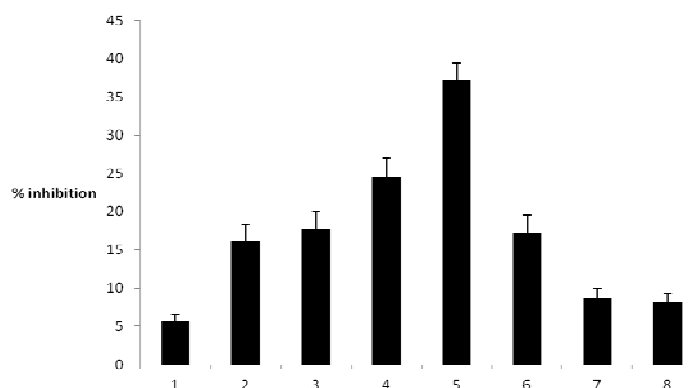


Fig 1. Percent inhibition activity of acetylcholinesterase by the crude extracts of different seaweeds (mean ± S.D. of three assays). Concentrations of samples: (100 µg/ml), 1. *Cystoseira indica*, 2. *Cystoseira merica*, 3. *Sargassum angostifolium*, 4. *Sargassum oligocystum*, 5. *Sargassum boveanum*, 6. *Padina australis*, 7. *Gracilaria corticata*, 8. *Gracilaria salicornia*

Table 2. Antimalarial activity of crude extracts of different seaweeds of Persian Gulf

Screening code	Sample code	Final concentration (µg/ml)	CPM1	CPM2	Average	Parasite growth (%)	Activity	IC50 (µg/ml)
Negative	Cell+ DMSO	0.1% DMSO	10399	10789	10594	100.0		
Positive 1	Dihydroartemisinin	5 nM	138	191	168	1.6	Active	2.17 nM
		1.67 nM	6436	6776	6897	65.1		
Positive 2	Mefloquine	0.1 µM	582	579	643	6.1	Active	0.0324 µM
		0.01 µM	768	9925	10256	10156		
V 7640	<i>Cystoseira merica</i>	10	9909	11397	10653	100.6	Inactive	
		1	11104	11414	11259	106.3		
V 7641	<i>Sargassum angustifolium</i>	10	10397	10488	10443	98.6	Inactive	
		1	10665	11007	11007	102.3		
V 7642	<i>Padina australis</i>	10	9405	10388	9897	93.4	Inactive	
		1	10445	10674	10560	99.7		
V7643	<i>Cystoseira indica</i>	10	9790	10623	10207	96.3	Inactive	
		1	10845	11079	10782	101.8		
V 7644	<i>Sargassum oligosystem</i>	10	10058	10712	10385	98.0	Inactive	
		1	11107	11265	11186	105.6		
V 7645	<i>Sargassum boveanum</i>	10	9777	10902	10340	97.6	Inactive	
		1	10825	11858	11342	107.1		
V 7646	<i>Gracilaria corticata</i>	10	9990	10815	10403	98.2	Inactive	
		1	11359	11196	11278	106.5		
V 7647	<i>Gracilaria salicornia</i>	10	11908	10636	11272	106.4	Inactive	
		1	10445	10726	10586	99.9		

With regard to the antimalarial activity (Table 2), the solution of 0.1% DMSO was used as negative control while dihydroartemisinin and mefloquine were used as the positive ones with the IC₅₀ of 2.17 nM and 0.0324 µM, respectively. The extracts could not inhibit 50% growth in the final concentration of 10 µg/ml.

DISCUSSION

Many species of marine algae have long been used in food diets in Eastern countries and more recently in Europe and America. Also various activities of this group of marines especially cytotoxic, antibacterial, antioxidant and antifungal activities have been widely screened. Only a few studies have been reported regarding the AChE inhibitory and antimalarial activities of this diverse group of organisms. Also in a study for screening the AChE inhibitory of 11 seaweeds from South Indian coastal areas, the IC₅₀s of methanolic extracts were similarly from 1 to 10 mg ml⁻¹ for most of the seaweeds although some of them showed less activity. The above mentioned study also represents that time of collection may be an important factor affecting this activity due to some changes in secondary

metabolites (30). In the present study, it was found that *Sargassum* was the most active genus among all of different genera tested. Studies have shown that the genus *Sargassum* which is a widely distributed algae that represents various activities such as antioxidants, antibacterial, immune-stimulating and also AChEI activity while *S. boveanum*, the most active species, has previously shown antioxidant, antifungal and phytotoxic effects (16,31) but it has not yet been tested for AChE inhibitory activity. Besides, major classes of compounds reported to have such an activity are the alkaloids, terpenoids, glycosides and coumarins (32). During the search for anticholinesterase compounds from marine organisms, two plastoquinones, sargaquinoic acid and sargachromenol were isolated from *S. sagamianum* (33). Both compounds unexpectedly showed moderate AChE inhibitory activity in a micromole range. These two compounds were also previously isolated from *S. serratifolium* (34). In the light of aforementioned considerations, further work is in progress to identify the compounds responsible for this activity which may exist in *S. boveanum* and *S. oligocystum*, the two species which indicated more activities.

There are several antimalarial molecules that

can efficiently integrate the panel of lead compounds isolated from marine sources with new chemical backbones and sometimes with unique functional groups (35). For instance, some seaweeds have been found to contain a powerful class of natural substances that can effectively destroy the malaria parasite. Researchers believe that bromophycolide compounds, are substances produced by the seaweeds as a chemical defense against marine fungi attacks, but they also appear to be effective against the malaria parasite (36). Although the extracts of seaweeds tested in this work were not active against malaria but work on the other seaweeds from Persian Gulf may lead to the isolation and structure elucidation of a number of exciting new pharma-cophores.

Whereas the southern coast of Iran bears a luxuriant and unique growth of seaweeds, but there are only limited publications about their pharmaceutical abilities (37). This was the first report of the selected species from Persian Gulf of Iran tested for AChE inhibitory and antimalarial activity and shows that a potential source of AChE inhibitors is certainly provided by seaweeds.

CONCLUSION

The methanolic extracts of seaweeds from Persian Gulf, Iran were screened for inhibitory activity on AChE and malaria for the first time. Of 8 seaweed extracts tested, the extracts from *Sargassum boveanum* showed the highest AChE inhibitory activity at the concentration of 0.1 mg/ml. All extracts were inactive about malaria. These seaweed extracts and their active components could emerge as natural and alternative anticholinesterase drugs or serve as starting points for synthesizing more effective AChE inhibitors.

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