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Biological activity of two red algae, *Gracilaria salicornia* and *Hypnea flagelliformis* from Persian Gulf.

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ABSTRACT

Among marine organisms, algae are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated. A number of these compounds possess biological activity. In this study, we aim to evaluate the cytotoxic, antibacterial and antifungal activity of two red algae, *Gracilaria salicornia* and *Hypnea flagelliformis*, collected from Persian Gulf. Ethyl acetate extracts of both algae showed a potent cytotoxic effect against *Artemia salina* nauplii (LC_{50} = 3 and 4 $\mu g.ml^{-1}$, respectively). Aqueous methanol (50%) extracts were also effective. None of the methanol and aqueous methanol extracts of the algae showed antifungal and antibacterial activity against *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger* by the Broth-dilution method. Only the ethyl acetate extracts exhibited antibacterial activity (MIC = 2 $\mu g.ml^{-1}$) on *S. aureus*. In conclusion, *G. salicornia* and *H. flagelliformis* could be a promising source of cytotoxic components.

Keywords: antibacterial, antifungal, cytotoxic, *Gracilaria salicornia, Hypnea flagelliformis.*

INTRODUCTION

The Persian Gulf, which is a shallow marginal sea of the Indian Ocean, bordered on the west by the Arabian Precambrian shield and on the east by the Persian Tertiary Fold Mountains. The Gulf is a tidal sea of the arid- tropical climate. Among marine organisms, algae are a large and diverse group of organisms from which a wide range of secondary metabolites have been isolated. A number of these compounds possess biological activity such as toxicity, antibacterial, antifungal, antiviral, anti-tumor and other specific activities (1–3).

Recently, an antitumor active agar-type polysaccharide of *Gracilaria dominguensis* has been isolated and characterized (4). Dichloromethane and methanol extracts from the red alga *Hypnea musciformis* exhibited PPE elastase inhibition and a diketosteroid was responsible for this activity (5). Also the biochemical characterization of a new lectin isolated from the Brazilian red alga *H. cervicornis* is reported (6).

A literature review show that the Persian Gulf was a relatively unknown sea until the second Gulf war of 1990-91. Some of the important algae from Persian Gulf are *Enteromorpha clathrata, Gracilaria salicornia, Hypnea flagelliformis* and *Padina australis.* So far, there has been only one paper on the antiviral activity of a brown alga (*Cystoseira myrica*) from the Persian Gulf against the *Herpes simplex* virus type 1 (7). Here, we focused on *Gracilaria*

salicornia and *Hypnea flageliformis* as two red algae. *G. salicornia* is widespread throughout the warm Indian and Pacific Oceans and varies in color from a bright yellow at the tips to orange, green or brown at the base (8). *Hypnea* has been irregularly harvested as a source for carrageenan but does not seem to have established a regular market. It has been cultivated successfully (e.g. in India) and has been harvested as beach-drift in Senegal (9).

In this study, we aim to evaluate the toxicity, antibacterial and antifungal activities of two red algae, *Gracilaria salicornia* and *Hypnea flagelliformis*, collected from Persian Gulf for the first time.

MATERIALS AND METHODS

Algae material

Red algae were collected from northern areas of Persian Gulf in July (2007) and identified as *Gracilaria salicorni* and *Hypnea flagelliformis*.

Extractions of the marine algae

Marine algae were dried carefully and reduced to small pieces, followed by extraction with ethyl acetate, methanol and water-methanol (50%) successively by percolation (72 h for each solvent) at room temperature. Then, the solvents evaporated under reduced pressure to obtain the concentrated extracts and dried under vacuum in order to give dried powder of the extracts.

Evaluation of antibacterial and antifungal activities

The antibacterial and antifungal activities of the algae extracts were assessed against *Staphylococcus aureus* (ATCC 29737), *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 14053) and *Aspergillus niger* (ATCC 16404) by the Broth-dilution method based on National Committee for Clinical Laboratory Standards (10, 11). The lowest concentration at which no growth was observed, recorded as MIC. Culture media with different concentrations of Gentamycin and Fluconazole were used as positive controls and DMSO (4 µl) was used as a negative control. All experiments were performed in triplicates.

Brine Shrimp Lethality Assay (BSA)

The method described by Mongelli *et al.* was adopted to study the cytotoxic activity of the compounds (12). Water life brand brine shrimp (*Artemia salina*) eggs were purchased from the Shilat Center (Tehran). The eggs were hatched in a flask containing 300ml artificial seawater made by dissolving distilled water. The flask was well aerated with the aid of an air pump, and kept in a water bath at 29–30 °C. A bright light was left on. The nauplii hatched within 48 h. The extracts and pure compounds were dissolved in normal saline. Different concentrations were obtained by serial dilution. Solution of each concentration (500 µl) was transferred into clean 24 wells plates via a pipette, and aerated seawater having 10–20 nauplii (500 µl) was added. A check count was performed, and the number alive noted after 24 h. The mortality end point of the bioassay was determined as the absence of controlled forward motion during 30 seconds of observation. The controls used were seawater and berberine hydrochloride (LC_{50} =26 $\mu g.ml^{-1}$). Lethality percentage was determined and LC_{50} calculated based on Probit Analysis with 95% of confidence interval (13).

RESULTS AND DISCUSSION

The results of the evaluation of toxic activity for the two red algae, *G. salicornia* and *H. flagelliformis*, collected from Persian Gulf are summarized in tables 1 and 2. The results showed that the ethyl acetate extract of *G. salicornia* indicated a potent toxic effect against the *A. salina* larvae (LC_{50} = 3 $\mu g.ml^{-1}$). The ethyl acetate extract of *H. flagelliformis* was also very effective (LC_{50} = 4 $\mu g.ml^{-1}$) compared to positive control (LC_{50} = 26 $\mu g.ml^{-1}$). As it has been shown in tables 1 and 2, the aqueous methanol (50%) extracts of both algae were effective on *A. salina* larvae. Recently, cytotoxic compounds (gracilarioside and gracilamides) were isolated from *G. asiatica* to the human A375-S2 melanoma cell line (14). *Gracilaria salicornia* might be a leading source of cytotoxic components because the previous results consistent with the correlation between cytotoxicity and brine shrimp lethality in plant extracts (15). The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological and biological activities in plant extracts also it appears that BSA is predictive of cytotoxicity and pesticidal activity (15, 16, 17).

But, none of the methanol and aqueous methanol extracts of the red algae represented antifungal and antibacterial activity against *S. aureus, E. coli, C. albicans* and *A. niger* by the Broth-dilution method. Only the ethyl acetate extracts represented a moderate antibacterial activity (MIC = 2 $mg.ml^{-1}$) on *S. aureus*. In fact, the high polarity extracts of *G. salicornia* and *H. flagelliformis* (methanol and aqueous methanol) were not effective on the tested bacteria and fungi. Where as the lesser polarity (ethyl acetate) extracts showed moderate activity. This fact is in agreement with previous authors (18) which reported that considerable antibacterial activity were only observed for the dichloromethane extracts of *Gracilaria* and other

Table 1. Toxicity effects of Gracilaria salicornia on Artemia salina larvae.

	Concentration (µg.ml^{-1})					CI %95[b]	
Algae Extract	10	100	500	1000	LC50	Lower	Upper
AcOEt	73[a]	89	100	100	3	1	9
MeOH	16	19	30	50	1349	444	4099
MeOH 50%	77	42	3	5	46	32	65

[a]mortality percentage of larva.
[b]CI = confidence interval.

Table 2. Toxicity effects of Hypnea flagelliformis on Artemia salina larvae.

Algae Extract	Concentration (µg.ml^{-1})					CI %95[b]	
	10	100	500	1000	LC50	Lower	Upper
AcOEt	64[a]	83	90	98	4	1	15
MeOH	69	55	58	52	2995	78	114536
MeOH 50%	87	70	75	83	42	31	56

[a]mortality percentage of larva.
[b]CI = confidence interval.

seaweeds. Therefore, the compounds responsible for the antimicrobial activity are at least partly lipophilic (18).

Recently, the crude aqueous extract of *G. salicornia* has been examined against HSV-2 in cell culture (19). The extract showed antiviral activity against HSV-2 not only before attachment and entry of virus to the Vero cells, but also on post attachment stages of virus replication (7, 20). The methanolic extract of *H. musciformis* exhibited strong antibacterial activity against the gram positive and seven gram negative bacteria, the former appeared to be more sensitive than the latter (21).

CONCLUSION

Ethyl acetate extracts of *G. salicornia* and *H. flagelliformis* (Rhodophyceae) were effective against the *Artemia salina* nauplii and could be a promising source of bioactive components. The mentioned extracts also showed a moderate antibacterial (on *S. aureus*). More investigation needs to evaluate the non polar extracts (such as chloroform and hexane) in order to find the more effective sources of red algae.

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