Research Journal of Pharmacognosy (RJP) 1, 2014: 27-31
Received: Sep 2013
Accepted: Nov 2013

Original article

A survey of cytotoxic effects of some marine algae in the Chabahar coast of Oman Sea

M. Mosaddegh[1], B.M. Gharanjik[2], F. Naghibi[1], S. Esmaeili[1,3*] A. Pirani[1], B. Eslami Tehrani[1], B. Keramatian[1] , A. Hassanpour[1]

[1]*Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*
[2]*Offshore Waters Research Center, Chabahar, Iran.*
[3]*Department of Traditional Pharmacy, School of Traditional Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

Abstract

Iran has 1260 km of coastline that borders the Persian Gulf and the Oman Sea in the northwest Indian Ocean. Marine algae are one of the natural resources in the marine ecosystem which produce a wide range of new secondary metabolites with various biological activities that play an important role in the pharmaceutical care. In this study the cytotoxic activity of 28 marine algae of Chabahar coast was assessed against 5 cell lines including MCF-7, HepG-2, A-549, HT-29 and MDBK, through MTT assay. The methanol extract of the algae did not show cytotoxicity against any of the tested cell lines up to 100 µg/mL concentration, except for *Jania adhaerens* (IC_{50} 85.03 µg/mL) against MCF-7 cells.

Keywords: Cytotoxicity, *Jania adhaerens*, Marine algae, MTT assay

Introduction

Cancer is called to a class of diseases in which a cell or a group of cells represent uncontrolled growth and is a major cause of death worldwide and causes serious problems in human life. Many kinds of cancer therapies, including various anticancer agents, have been developed. However, they also have several problems such as serious side effects and drug resistance [1]. To resolve these difficulties, screenings of natural products as potential anticancer agents have been performed in Iran [2-4].

Human has been using marines since very old times. South East Asian countries such as China and Philippine and some of European countries have practiced a wide use of these natural sources [5]. Until now, more than 2400 marine natural products have been isolated from the algae of subtropical and tropical populations, which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation [6]. Iran has a rich resource of marines on its southern coast. The Iranian coastlines in the Persian Gulf and Oman Sea are about 1260 km. There are four coastal provinces, from west to east: Khuzestan, Bushehr, Hormozgan, and Sistan and Baluchistan. Coastal areas in the south of Iran

**Corresponding author: sesmaeili@sbmu.ac.ir*, Tel/Fax: +9821-88776027

provide excellent condition for the growth of marine algae.

Marine algae are one of the natural resources in the marine ecosystem. They contain various biologically active compounds which have been used as sources of agar, carrageenan, alginates, proteins, unsaturated fatty acids, vitamins and minerals. Hence, they are used in many industries such as pharmaceuticals, textile, human food and for treatment of some disease such as children fever, muscle and joint pains, digestive disorders and as sedatives and antibiotics [7]. Additionally, marine algae produce a wide range of new secondary metabolites with various biological activities that lead to the development of new pharmaceutical agents and play an important role in the production of pharmaceuticals [8].

Recent data has showed 153 species of marine algae from coastlines of Iranian islands and Hormozgan Province [9]. There have been only a few studies on the pharmacological effects of the marine algae in this region hence, it is necessary to conduct a comprehensive study on screening of the pharmaceutical activities of marines.

Chabahar is one of the coastline cities of Sistan and Baluchistan province which is located at 25º10'-25º21' N / 59º52'-61º3' E in southeast of Iran that consists of 300 Km coastline along Oman Sea. The present study intended to evaluate the cytotoxic activity of the marine algae in this area by MTT (Methyl Thiazol Tetrazolium) assay on 5 cell lines including MCF-7 (human breast adenocarcinoma), HepG-2 (human hepatocellular liver carcinoma), MDBK (Median- Darby bovine kidney), A-549 (non-small cell lung carcinoma) and HT-29 (human colon adenocarcinoma).

Experimental

Sample preparation

Marine algae were collected from Chabahar coast (figure 1) and identified. Sampling was accomplished in three times in different seasons from March to October. Samples were collected from rocky substrate in the middle portion of intertidal zone by bottle knife and in subtidal zone by diving. The fresh samples of the marine algae were thoroughly washed with sea water and cleaned of sand and overgrowing organisms at the site of collection, and transported to the laboratory for extraction and other examinations. The voucher specimens were deposited at the Herbarium of Traditional Medicine and Materia Medica Research Center (TMRC).

Extraction

The extracts were obtained by macerating of the dried marine algae powder in methanol for 24 h. After filtering, the extract was dried and kept at 4 °C. Extracts were dissolved in DMSO (Dimethyl Sulfoxide) and further diluted with cell culture medium. Each experiment was replicated thrice by 6 concentrations (3.125 -100 µg/mL).

Fractionation

Different solvents including petroleum ether, chloroform and methanol were used for fractionation with a similar method as extraction.

MTT assay

The selected cells were seeded: MCF-7 (6000 cells), HepG-2 (15000 cells), MDBK (5800 cells), A-549 (8500 cells) and HT-29 (4800 cells) in 96-well plates and incubated for 24 h at 37 °C in a CO_2 incubator. They were then exposed to different concentrations of the extracts and incubated for another 72 h. Cells treated with medium only served as the negative control and 5-Fluorouracil as the positive control. After incubation for 4 h with MTT solution (final concentration of 0.5 mg/ mL), the supernatant was removed and the resultant formazan crystals were dissolved in DMSO and the absorbance intensity was measured by a microplate reader at 570 nm.

Results and Discussion

Total 28 marine algae, belonging to 13 families were collected. Most of the marine plants from Chabahar coast were collected in intertidal zone. Collection in subtidal zone was carried out only selectively in some regions between 25º 21'-25º 14' N / 60º 18'- 60º 39' E that consists of 55 km. Intertidal zone consist of 7 km at 25º 17' N / 60º 39' E in rocky and sandy coasts.

The samples were screened for the cytotoxic activity against 5 cell lines including MCF-7,

HepG-2, HT-29, A-549 and MDBK by MTT assay (Table 1). 5-Fluorouracil showed cytotoxic activity against all selective cell lines (IC_{50} 0.03-56.28 µg/mL). The samples did not show cytotoxicity against any of the selected cell lines up to 100 µg/mL except for *Jania adhaerens* (IC_{50} 85.03 µg/ml) against MCF-7 cell line. Based on the data obtained, the fractions of *J. adhaerens* have also been investigated. The petroleum ether fraction exhibited no cytotoxic activity, but the chloroform fraction demonstrated cytotoxicity to HT-29, MCF-7, HepG-2, A-549 and MDBK (IC_{50}< 100 µg/mL) (Figure 2). The methanol fraction also exhibited cytotoxic activity in HT-29 (72.6 µg/mL) and MCF-7 (58.31 µg/mL) cell lines.

Marine organisms are considered as sources of biological active compounds as well as food additives. Some biological active metabolites of marine organisms possess pharmaceutical potential to cure diseases [10]. Several cytotoxic compounds such as fucoidans, laminarians, and terpenoids with anticancer, antitumor and antiproliferative properties have been reported to be abundant in algae [11] and many marine algae have been used as food in some parts of the world. In fact polysaccharides of the edible algae have attracted extensive interest due to their biological activities [12].

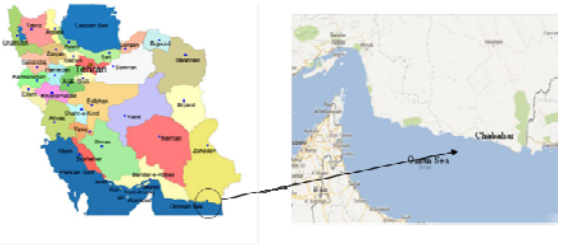

Figure 1. Map of the studied area

Table 1. Cytotoxicity of marine algal from Chabahar coast

NO	Scientific Name	Family	IC_{50} (µg/mL)				
			A-549	HepG-2	HT-29	MCF-7	MDBK
1	*Acanthofora spicifera* (Vahl) Boergessen	Rhodomelacaea	>100	>100	>100	>100	>100
2	*Champia compressa* Harvey	Champiaceae	>100	>100	>100	>100	>100
3	*Colpomenia sinuosa* (Mertens ex Roth) Derbes and Solier	Scytosiphonaceae	>100	>100	>100	>100	>100
4	*Cystoseira indica* (Thivy et Dosi) Mairh	Cystoseiraceae	>100	>100	>100	>100	>100
5	*Cystoseria myrica* (S.G. Gmelin) J. Agardh	Cystoseiraceae	>100	>100	>100	>100	>100
6	*Dictyota cervicornis* Kuetzing	Dictyotaceae	>100	>100	>100	>100	>100
7	*Gelidiella acerosa* (Forssekal) Feldmann & G. Hamel	Gelidiaceae	>100	>100	>100	>100	>100
8	*Gelidium micropterum* Kuetzing	Gelidiaceae	>100	>100	>100	>100	>100
9	*Gracilaria corticata* (J. Agardh) J.Agardh	Gracilariaceae	>100	>100	>100	>100	>100
10	*Gracilaria folifera*(Forsskal) Boergessen	Gracilariaceae	>100	>100	>100	>100	>100
11	*Hypnea boergeseni* Tanaka	Hypneaceae	>100	>100	>100	>100	>100
12	*Hypnea charoides* Lamx.	Hypneaceae	>100	>100	>100	>100	>100
13	*Hypnea valentiae* (Turner) Montagne	Hypneaceae	>100	>100	>100	>100	>100
14	*Jania adhaerens* Lamouroux	Corallinaceae	>100	>100	>100	85.03	>100
15	*Laurencia obtuse* (Huds.) Lamx.	Rhodomelaceae	>100	>100	>100	>100	>100
16	*Nizimuddinia zanardini* Schiffner	Alariaceae	>100	>100	>100	>100	>100
17	*Padina australis* Hauck	Dictyotaceae	>100	>100	>100	>100	>100
18	*Padina tetrastromatica* Hauck	Dictyotaceae	>100	>100	>100	>100	>100
19	*Sargassum glaucescens* J. Agardh	Sargassaceae	>100	>100	>100	>100	>100
20	*Sargassum ilicifolium* (Turner) C. Agardh	Sargassaceae	>100	>100	>100	>100	>100
21	*Sargassum tenerimum* J. Agardh	Sargassaceae	>100	>100	>100	>100	>100
22	*Scinaia furcelata* (Turner) J.Agardh	Galaxauraceae	>100	>100	>100	>100	>100
23	*Spatoglossum asperum* J. Agardh	Dictyotaceae	>100	>100	>100	>100	>100
24	*Spatoglossum dichotomum* Tseng et. Lu	Dictyotaceae	>100	>100	>100	>100	>100
25	*Stoechospermum marginatum* C. Agardh	Dictyotaceae	>100	>100	>100	>100	>100
26	*Ulva fasciata* Delile	Ulvaceae	>100	>100	>100	>100	>100
27	*Ulva rigida* C. Agardh	Ulvaceae	>100	>100	>100	>100	>100
28	*Valonopsis pachynema* (Mart.)Boergessen	Valoniaceae	>100	>100	>100	>100	>100

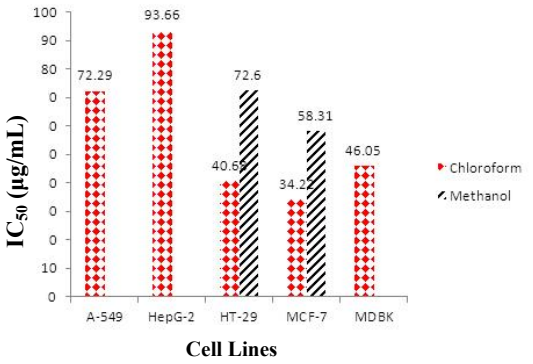

Figure 2. IC_{50} values of *Jania adhaerens* chloroform and methanol fractions in cell line

Results of recent studies on the biological and pharmaceutical activities of some marine plants which were investigated in the present study are as follows: Pillai and Varier reported that *Padina tetrastromatica* was the major source for the manufacture of alginic acid in Kerala [13]. Researchers exhibited that *Hypnea charoides* was a red alga along the coast of Japan and Korea which contained a water-soluble mucilaginous polysaccharide, used for making jelly food in Japan [14]. The red alga *Acanthophora spicijka* has been reported as a rich source of carrageenan, ecamtene and antheraxanthin, and contains plentiful supply of nutrients. Alcoholic extracts of *Ulva fasciata* and *U. lactuca* have exhibited antiviral and anti-implantation activities [15]. *U. fasciata* has been reported to produce a novel sphingosine derivative with antiviral activity [16]. *U. fasciata* is used in soups and salads, and has been reported to possess antioxidant and antibacterial activity. *Ulva* species are rich in essential nutrients and they exhibit anti-peroxidative and anti-hyperlipidaemic activities. Chloroform and methanol fractions of an ethanol extract of *Spatoglossum asperum* has showed antifungal activity against the highly destructive plant pathogens. From the crude extracts of 21 brown algae collected from the south coast of England and the West coast of Ireland, three algae extracts, have demonstrated cytotoxic activity [17].

Aqueous extracts of brown alga *Sargassum oligocystum*, gathered from Persian Gulf seashore, has showed antitumor activity against K-562 and Daudi human cancer cell lines [18]. Antitumor activity has also been observed with the macro alga *S. stenophyllum* [19]. Also, the methanol extract of *S. swartzii* collected from the Persian Gulf has demonstrated cytotoxic effect against T-47D cells (IC_{50} <100 µg/mL) [20]. In the same study the speices of *Sargassum* showed no cytotoxicity.

Several sulfated polysaccharides isolated from algae have shown antitumor, anticancer and antimetastatic activities in mice. The hydroquinone diterpene from *Cystoseira mediterraneol* has shown inhibitory effects on mitotic cell division [21]. Fractions of the alcohol extract of *C. myrica* have demonstrated four new cytotoxic hydroazulene diterpenes [22].

A new ketosteroid, 6β, 16 β-dihydroxycholest-4-en-3-one was isolated from the red alga *J. adhaerens*. This compound possessed protective antigenotoxicity effect in human peripheral blood cells [23]. Additionally, *J. adhaerens* from central Mediterranean region has been examined for the production of antibacterial, antifungal, antiviral, cytotoxic and antimitotic compounds. This species has demonstrated antifungal and antimitotic properties [24].

In the present study, the methanol extract of *J. adhaerens* has exhibited cytotoxic effects on MCF-7 and HT-29 cell lines and the chloroform was cytotoxic to all cell lines which is promising for isolation of effective constituents in future investigations.

Acknowledgements

The authors wish to thanks the Traditional Medicine and Materia Medica Research Center, Shahid Beheshti University of Medical Sciences for the grant No. 130.

References

[1] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years, *J Nat Prod*. 2007; 70(3): 461-77.

[2] Mosaddegh M, Esmaeili S, Naghibi F, Hamzeloo-Moghadam M, Haeri A, Pirani A, Moazzeni H. Ethnomedical survey and cytotoxic activity of medicinal plant extracts used in Kohgiluyeh and Boyerahmad Province in Iran. *J Herbs Spices Med Plants.* 2012; 18(3): 211-221.

[3] Sahranavard S, Naghibi F, Ghaffari S. Cytotoxic activity of extracts and pure compounds of *Bryonia aspera*. *Int J Pharm Pharmaceut Sci.* 2012; 4(3): 541-543.

[4] Hamzeloo-Moghadam M, Naghibi F, Atoofi A, Asgharian Rezaie M, Irani M, Mosaddegh M. Cytotoxic activity and apoptosis induction by gaillardin. *Z Naturforsch.* 2013; 68c (4): 108-112.

[5] Trono JR, Gavino C. *Field Guide and Atlas of the Seaweed Resources of the Philippines.* Published by book mark, 1997.

[6] Zandi K, Ahmadzadeh S, Tajbakhsh S, Rastian Z, Yousefi F, Farshadpour F, Sartavi K, Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines. *Eur Rev Med Pharmacol Sci*. 2010; 14(8): 669-73.

[7] Iwashima M, Mori J, Ting X, Matsunaga T, Hayashi K, Shinoda D, Saito H, Sanakawa U, Hayashi T. Antioxidant and antiviral activities of plastoquinones from the brown alga *Sargassum micracanthum*, and a new chromene derivative converted from the plastoquinones. *Biol Pharm Bull.* 2005; 28: 374–377.

[8] Gamal El. Biological importance of marine algae. *Saudi Pharmaceut J*. 2010; 18: 1–25.

[9] Sohrabipour J, Nejadsatari T, Assadi M, Rabei R. The marine algae of the southern coast of Iran, Persian Gulf, Lengeh area. *Iran Journ Bot*. 2004; 10: 83-93

[10] Ananthan G, Sivaperumal P, Mohamed Hussain S. Cytotoxicity of the crude extracts of Marine ascidians (Tunicata: Ascidiacea) from Tuticorin, Southeast coast of India, *Arch Appl Sci Res*. 2011; 3 (2): 139-142.

[11] Smit AJ. Medicinal and pharmaceutical uses of seaweed natural products: A review, *J Appl Phycol*. 2004; 16: 245-262.

[12] Ara J, Sultana V, Qasim R, Ehteshamul-Haque S, Ahmad VU. Biological activity of *Spatoglossum asperum*: A brown alga, *Phytother Res*. 2005; 19: 618-623.

[13] Varrier NS, Pillai KS, Studies on marine products. Part II, Optimum conditions for the large-scale extraction of alginic acid from *Sargassum*. Seaweeds of Cape Comorin. *Bull Cent Res Inst Univ Travane*. 1951; 2: 23-62.

[14] Shimahara H, Sugiyama N. A Sulfated Galactan from the Red Seaweed *Hypnea charoides*. *Biol Chem*. 1974; 38 (12): 2569-2570.

[15] Bhakuni DS, Rawat DS. *Bioactive Marine Natural Products.* New Delhi: Springer, Anamaya Publishers, 2005.

[16] Jha RK, Zi-rong X. Biomedical compounds from marine organism. *Marine Drugs*. 2004; 2: 123-146.

[17] Spavieri J, Allmendinger A, Kaiser A, Casey R, Hingley-Wilson S, Lalvani, A, Guiry M D, Blunden G, Tasdemir D. Antimycobacterial, antiprotozoal and cytotoxic potential of twenty-one brown algae (Phaeophyceae) from British and Irish waters. *Phytother Res*. 2010; 24 (11): 1724–1729.

[18] Zandi K, Tajbakhsh S, Nabipour I, Rastian Z, Yousefi F, Sharafian S, Sartavi K. In vitro antitumor activity of *Gracilaria corticata* (a red alga) against Jurkat and molt-4 human cancer cell lines. *Afr J Biotechnol*. 2010; 9(40): 6787-6790.

[19] Dias PF, Siqeira JR, Vendruscolo LF, Neiva TDJ, Gagliardi AR, Marashin M, Ribeiro-Do-Valle RM. Antiangiogenic and antitumoral properties of a polysaccharide isolated from the seaweed *Sargassum stenophyllum*. *Cancer Chemoth Pharm.* 2005; 56: 436-446.

[20] Khanavi M, Nabavi M, Sadati N , Shams Ardekani M, Sohrabipour J, Nabavi SMB, Ghaeli P, Ostad SN. Cytotoxic activity of some marine brown algae against cancer cell lines. *Biol Res*. 2010; 43: 31-37.

[21] Francisco C, Banaigs B, Valls R, Codomier L. Mediterraneol, a novel rearranged diterpenoidhydroquinon from the marine alga *Cystoseira mediterranea*. *Tetrahedron Lett.* 1985; 26: 2629-2632.

[22] Ayyada SN, Abdel-Halim OB, Shierc TW, Hoyed TR. Cytotoxic Hydroazulene Diterpenes from the Brown Alga *Cystoseira myrica. Z Naturforsch*. 2003; 58c, 33-38.

[23] Alarif WM, Ayyad SE, El-Assouli SM, Al-Lihaibi SS. Antigenotoxic ketosteroid from the red alga *Jania adhaerens*. *Nat Prod Res.* 2012; 26(9):785-91.

[24] Ballesteros E, Martin D, Uriz MJ. Biological Activity of Extracts from Some Mediterranean Macrophytes. *Bot Mar*. 1992; 35: 481-485.