

## Cytotoxic Screening of Marine Organisms from Persian Gulf

### Abstract

**Background:** Marine organisms produce a variety of compounds with pharmacological activities, including anticancer effects. They contain several secondary metabolites with interesting biological activities. This study attempted to find cytotoxicity of Hexane, Dichloromethane and Butanol partitions of *Holothuria leucospilota* and *Echinometra mathaei*. **Materials and Methods:** *H. leucospilota* and *E. mathaei* were collected from Persian Gulf. The animals were extracted by maceration with methanol-ethyl acetate (1:1). The *H. leucospilota* extract was partitioned by Kupchan method to hexane, dichloromethane, butanol, and water partitions. The cytotoxic activity of the extracts was investigated against HeLa (cervical cancer) and human umbilical vein endothelial cells cell lines by mitochondrial tetrazolium test assay after 72 h. **Results:** The cell survivals of HeLa cell were decreased by increasing the concentration of extracts. A significant reduction in cell viability at the doses of 30 (µg/ml) of dichloromethane (DCM) partition, 0.3, 3, and 30 (µg/ml) of ButOH partitions of sea cucumber, and 0.5 (µg/ml) of *E. mathaei* was observed. The median growth inhibitory concentration value of Hex, DCM, ButOH, and water partitions were 0.301, 0.21, 2.29, and 0.229 µg/ml, respectively. **Conclusion:** This study reveals that different partitions of *H. leucospilota* and total extract of *E. mathaei* have cytotoxic activity against cancer cell lines. More study is necessary to find the active metabolites in the more active partitions.

**Keywords:** Cancer, cytotoxic, echinometra, holothuria, Persian Gulf

### Introduction

Cancer is a complicated disorder and one of the most causes of mortality worldwide, particularly in developed societies.<sup>[1]</sup> The number of patients catching this invasive disease is increasing with a rapid tilt. Fourteen million new clinical cases were reported only in the year 2010 that is going to increase to 22 million during the next two decades.<sup>[2]</sup>

Despite different studies in the field of cancer and especially in discovering of new drugs, there is not any effective and safe treatment for cancer. Hence, discovering new chemotherapeutic agents, especially from nature is a great wish for researchers. During the last two decades, about 50% of the drugs entered to the market were directly or indirectly derived from natural organisms. The marine litter is a unique resource of biologically active compounds with characteristic structural and chemical features.<sup>[3]</sup> Different organisms under the water such as seaweeds, sponges, corals, fungi, and ascidians have been analyzed for their biologic potentials, and also,

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active ingredients and several bioactive metabolites have been isolated from these marine resources showing different fields of bioactivities, such as anticancer, antibiotic, antiviral, antioxidant, anti-inflammatory, and antimalarial activities.<sup>[4-6]</sup> The dramatic marine pipeline of increasing the number of clinical and preclinical anticancer agents has matured during the last decade by the intense efforts of marine researchers in this field. Biological activities of these organisms are due to their metabolites such as terpenoid compounds, alkaloids, polyketides, peptides, shikimic acid derivatives, sugars, and steroids. In addition, the presence of halogen atoms such as chlorine and bromine is the main difference of these secondary metabolites.<sup>[7,8]</sup>

Novel structures and also novel mechanisms of action of marine metabolisms have led to new methods for treating cancer particularly for patients with solid tumors of the lung, breast, colon, or prostate.<sup>[9]</sup> Hence, an increasing search is needed for screening of marine organisms, especially in the field of cytotoxicity. In fact, the desire for this evolution has been stimulated by a great

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Golnaz Vaseghi,  
Farideh Hajakbari<sup>1</sup>,  
Shabnam Sajjadi<sup>2</sup>,  
Nasim Dana<sup>3</sup>,  
Ahmad Ghasemi<sup>3</sup>,  
Afsaneh Yegdaneh<sup>1</sup>

From the Isfahan  
Cardiovascular Research  
Center, Cardiovascular Research  
Institute, Isfahan University of  
Medical Sciences, <sup>1</sup>Department  
of Pharmacognosy, School of  
Pharmacy and Pharmaceutical  
Sciences and Isfahan Pharmacy  
Student Research Committee,  
Isfahan University of Medical  
Sciences, <sup>3</sup>Applied Physiology  
Research Center, Cardiovascular  
Research Institute, Isfahan  
University of Medical Sciences  
Isfahan, Isfahan, <sup>2</sup>Faculty of  
Pharmacy, Shahid Sadoughi  
University of Medical Sciences,  
Yazd, Iran

**Address for correspondence:**  
Dr. Afsaneh Yegdaneh,  
Department of Pharmacognosy,  
School of Pharmacy and  
Pharmaceutical Sciences  
and Isfahan Pharmaceutical  
Research Centre, Isfahan  
University of Medical Sciences  
Isfahan, Isfahan, Iran.  
E-mail: yekdaneh@pharm.mui.  
ac.ir

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demand to develop more active and less toxic therapies for treatment of cancer.<sup>[10]</sup> It can generally be concluded that contemporary screening of marine natural products in the field of cancer seems necessary.<sup>[11]</sup> Iran has coastal lines about 1260 km along the Persian Gulf and the Oman Sea.<sup>[12]</sup> Despite the existence of a great extent of marine organisms in this region, there are only a few studies on the biological activities, especially cytotoxic screening of these interesting organisms. The lack of study in this area may result from difficulties of collection and identification of marine animals. In this study, we have analyzed different fractions of *Holothuria leucospilota* and *Echinometra mathaei* from Persian Gulf for their cytotoxic activities.

## Materials and Methods

*H. leucospilota* and *E. mathaei* were collected from Bushehr, a southwest coastline of Persian Gulf in autumn 2013. Identification of both organisms was carried out kindly by Khoramshahr marine science and Technology University.

### Preparation of the extracts

The animals (about 1.4 kg wet weight of *H. leucospilota* and 800 mg wet weight of *E. mathaei*) were cut into pieces, dried by freeze drier, and extracted four times with EtOAc-MeOH (1:1). The solvent was evaporated by rotary evaporator. Ten mg of *E. mathaei* extract was used for cytotoxic test. The total extract was partitioned to hexane, dichloromethane, butanol, and water by Kupchan partitioning method. First of all, the extract was partitioned between hexane and MeOH 90% and hexane and after that dichloromethane and MeOH 80%. Finally, MeOH solvent was removed completely, and the remaining water was partitioned with ButOH. The partitions were subjected to cytotoxic test.<sup>[13]</sup>

### Cytotoxic test

After providing the partitions for cytotoxic screening, two cell lines, HeLa and human umbilical vein endothelial cell (HUVEC), were used. At the first, three cell lines were growing in the DMEM cell culture medium which supplemented with 10% fetal bovine serum. Penicillin and streptomycin were added to the media. All cell lines were cultured at 37°C in air/carbon dioxide (95:5) atmosphere.

At the second, different concentration of all partitions was tested for each cell line. Samples were dissolved in dimethyl sulfoxide (DMSO) and further diluted with cell culture medium. The final concentration used was 1% of total volume of medium in all treatments, including the control group.

For mitochondrial tetrazolium test (MTT) assay,  $1 \times 10^5$  cells/well were plated into 96 well plates. The cells were incubated for 24 h to proliferate and reach their exponential phase of growth. The incubation time for each cell line was determined twice as long as the doubling time

of each cell line. After that, 20  $\mu$ l from each partition was added to the media.<sup>[14]</sup>

After 72 h of incubation, 30  $\mu$ l of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reagent (5 mg/ml) in phosphate-buffered serum was added to each well. The plates were incubated for 4 h at 37°C. After incubation time, the medium was removed, and 100  $\mu$ l cell culture grade DMSO was added. The formazan salts were analyzed by a microplate reader at 540 nm.

Cell viability was determined as a percentage of untreated cells (control value), and cytotoxicity value was calculated as IC50 (the concentration of a drug that is required for 50% inhibition) of the reagents comparing with control.

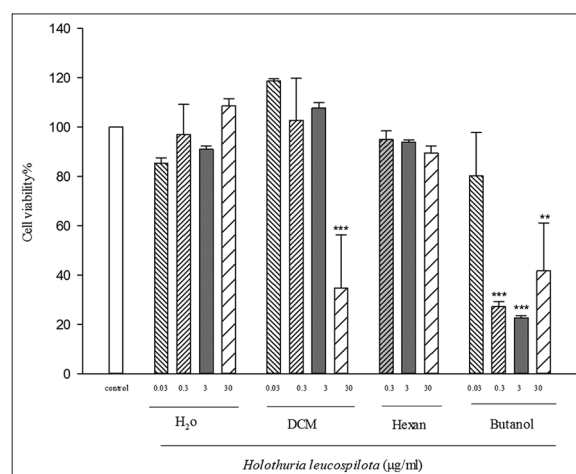
## Results

The cytotoxic activities of various extracts of *H. leucospilota* were analyzed against HeLa cancerous cell line [Figure 1] and a normal cell line, HUVEC [Figure 2] by the MTT assay. The multiple concentration 0.03, 0.3, 3, 30  $\mu$ g/ml of hexane, dichloromethane (DCM), butanol, and water extracts from *H. leucospilota* were used.

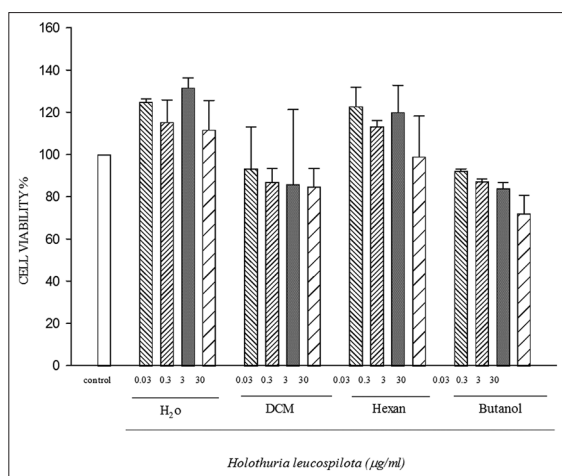
Figure 1 shows the MTT test results for different *H. leucospilota* extract partitions with different concentrations on the HUVEC cell line after 72 h. We observed a significant reduction in cell viability at the doses of 30 ( $\mu$ g/ml) of DCM partition, 0.3, 3, and 30 ( $\mu$ g/ml) of ButOH partitions.

The results for different concentrations from *H. leucospilota* partitions on HeLa cell line, after 72 h, were shown in Figure 2.

The results for different concentrations from *E. mathaei* extract on HeLa cell line, after 72 h, were shown in Figure 3.



**Figure 1: Cytotoxic activity of *Holothuria leucospilota* partitions on human umbilical vein endothelial cell line. Data represent the means  $\pm$  standard error of the mean separate experiments (significant as compared to control \*\*\* $P < 0.05$ ). All tests were repeated three times. significant as compared to control \*\* $P < 0.05$**



**Figure 2: Cytotoxic activity of *Holothuria leucospilota* partitions on HeLa cell line. Data represent the means  $\pm$  standard error of the mean separate experiments. All tests were repeated three times**

## Discussion

Currently, isolating bioactive compounds from natural food sources for producing pharmaceutical agents has become the focus of much attention. Secondary active metabolites in marine organisms such as algae, corals, sponges, mollusks, phytoplanktons, tunicates, echinoderms, and bacteria have shown various applications in pharmaceutical industries.<sup>[15,16]</sup>

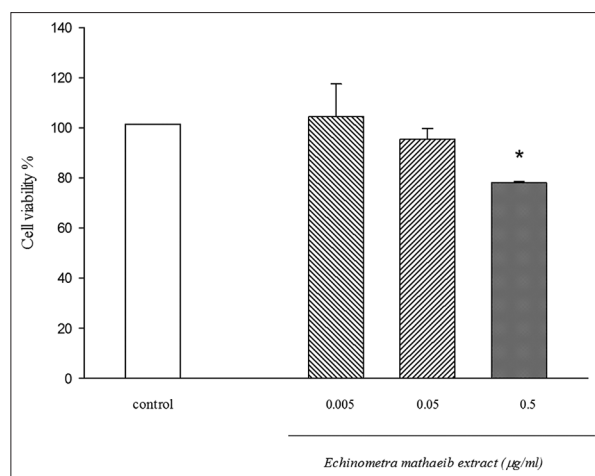
The high number of medicinal compounds extracted from terrestrial herbs, structural diversity and uniqueness in marine organisms and lack of documentation about marine invertebrates have all stimulated more researches for drug discovery from marine sources.<sup>[17]</sup>

Isolation and purification of Dolastatins from the mollusk *Dolabella auricularia* or Halicondrins from marine Japanese sponge *Halichondria aokadai* has a wide effect in marine anticancer field.<sup>[18]</sup>

Similar to antitumor activity of marine invertebrates, echinoderms containing novel natural structures with a wide range of biological activities have provided a new vision in the development of new therapeutic anticancer agents.<sup>[19]</sup>

Holothurians are one of the most important organisms of Echinodermata that have been used for their anti-inflammatory and antidisease characteristics and also for treating different ailments in the south East Asia countries such as Korea, Japan, Indonesia, and China.<sup>[20,21]</sup> They are nutrient-rich invertebrates, similar to cucumber with a leathery skin and gelatinous body.<sup>[22]</sup>

Biological activities such as antioxidant, antiproliferative, anti-inflammatory, anticancer, anticoagulant, antifungal, and antibacterial activities of the secondary metabolites isolated from marine organisms, mainly from star-fish and sea cucumbers, have been examined in several



**Figure 3: Cytotoxic activity of *Echinometra mathaei* extract on HeLa cell line. Data represent the means  $\pm$  standard error of the mean separate experiments (significant as compared to control \* $P < 0.05$ ). All tests were repeated three times. significant as compared to control \* $P < 0.05$**

researches previously.<sup>[20,21]</sup> Several biological and chemical studies carried out on various species of sea cucumbers indicated these marine organisms contain bioactive secondary metabolites, especially triterpene glycosides with cytotoxic activity. Triterpene glycosides isolated from *Pseudocolochirus violaceus* sea cucumber showed significant cytotoxic activity against MKN-45 and HCT-116 cell lines;<sup>[23]</sup> triterpene glycosides isolated from *Mensamaria intercedens* exhibited high cytotoxic activity in methanolic partition.<sup>[24]</sup>

Antiproliferative potential of three Malaysian sea cucumber species, *H. scabra*, *H. leucospilota* and *Stichopus chloronotus*, was analyzed and revealed that tested cell lines were much more sensitive to the water extract of *H. leucospilota*.<sup>[25]</sup> Evaluating the anticancer activity of the starfish *Acanthaster planci* along with Tamoxifen in human breast cancer cells indicated that the sea star extract (IC<sub>50</sub> = 15.6 µg/ml) was active against cancer cells. A few studies have been reported about the Persian Gulf Echinodermata and their biological effects.<sup>[26]</sup> In a study by Andersson *et al.*, the antibacterial and cytotoxic effect of saponin compounds of brittle star on leukemia cells was investigated.<sup>[27]</sup> Prabhu and Bragadeeswaran evaluated hemolytic and cytotoxic properties of *Ophiocnemis marmorata*<sup>[28]</sup> which is consistent with cytotoxic properties of *E. mathaei* extract in this study. In accordance with these results, our study revealed that butanol and dichloromethane extracts of *H. leucospilota* and crude extract of *E. mathaei* conducted cytotoxic effect on HeLa (cervical cancer) growth cells, demonstrating anticancer potential of echinoderms.

## Conclusion

The study reveals that various partitions of *H. leucospilota* and also the total extract of *E. mathaei* have toxic activity against HeLa cell line. More research is necessary to isolate and identify the active compounds in the active partitions.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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