Research Article



Investigating biological characteristics of two jellyfish (Rhopilema nomadica and Chrysaora hysoscella) venoms on human fibroblast

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Abstract

This paper aims to investigate the biological and cytotoxic characteristics of venoms extracted from Rhopilema nomadica and Chrysaora hysoscella jellyfish collected from the Persian Gulf. The cytotoxic effect of the venoms is surveyed on human fibroblast skin cells (HU02) using the The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Moreover, the protein molecular weight was determined and the toxicity test (LD50) of venoms were explored on BALB/c mice. The R. nomadica venom possessed a higher protein concentration with the lowest molecular weight protein via SDS-PAGE (12.5 %) along with more peaks obtained by HPLC. In addition, the results of both LD50 and MTT are affected by the venom characteristics. Besides, intravenously and intraperitoneally LD50 were 1.26 and 2.35 for C. hysesella and 0.65 IV and 1.6 IP for R. nomadica, respectively, suggesting that R. nomadica venom was more lethal. The results of the MTT assay on Hu02 fibroblast cells for 24 h revealed that R. nomadica had more lethal effects on skin cells compared to C. hysoscella. Escherichia coli, whereas methicillin-resistant Staphylococcus aureus had no antibacterial activities in the presence of both R. nomadica and C. hysoscella venoms. Meanwhile, the venoms had antibacterial effects on Pseudomonas aeruginosa and S. aureus, which were still weak compared to the other reported species. Overall, R. nomadica venom was more lethal in the case of mice and human skin cells and likely with more symptoms in prey. Finally, considering the relatively high viability of different bacteria in diverse dilutions of venom, it seems that victims not only should face dermal injuries and possible scars caused by direct stings but also the presence of venomous protein on the dermal tissue may provide a cultivation medium for different kind of bacteria.

Keywords: Bacterial viability rate, Bradford assay, Human fibroblast skin cells [HU02], Jellyfish venom, MTT assay

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Introduction

investigations Major marine on organisms have been focused on bioactive compounds for various purposes, including the discovery of novel drugs, nutritional supplements, applications for industrial and biotechnology (Urbarova et al., 2012). In recent years, attention has been paid to natural products derived from the sea, because they are natural. compatible with the human body and the environment, and less harmful than synthetic products (Gulhan and Selamoglu, 2016). Venomous marine organisms are potentially an important source of pharmacological tools with promising biological activities. One of the ways to use marine products is to manage resources and control pollution in order to increase the production efficiency of products (Selamoglu, 2021).

The Persian Gulf with high and extensive temperature fluctuations and naturally elevated salinity is a hostile environment for marine organisms (Sheppard et al., 1992). This sea accommodates numerous venomous marine organisms including jellyfish such as Rhopilema sp. (Jafari et al., 2019), Chrysaora hysoscella (Gharibi et 2016), Cassiopea Andromeda (Mirshamsi et al., 2016), sea anemone, Stichodactyla haddoni (Moghadasi et al., 2018), some gastropods (Mohebbi and Nabipour, 2021); green scat, Scatophagus argus (Ghafari et al., 2013), black spot stone fish, Synanceia sp. (Fathi et al., 2020), and sea snake, Hydrophis cyanocinctus (Calvete et al.,

2012; Mohebi and Nabipour 2016). The Persian Gulf, represented as a semienclosed sea with harsh environmental conditions, provides an opportunity to form a variety of venoms compounds with diverse structures, biological activities, toxicity, clinical symptoms, pharmacological and aspects (Moghadasi et al., 2018; Ballesteros et al., 2022; Nabipour et al., 2015; Avci et al., 2023). For example, the venom of the sea snake, H. cyanocinctus, contains a combination of organic and inorganic bioactive substances such as protease and non-enzymatic proteins that provide a wide range of medicinal activities. Note that high value of LD₅₀ is comsidered in these poisons to kill mice (Calvete et al., 2012; Mohebi and Nabipor, 2016). Venomous organisms can play a very important role in identifying, developing and treating various diseases (Kakoolaki et al., 2013). These effects depend on the type of toxic substance or organism used and also on the water quality and exposure time (Orun et al., 2005).

The venom of the sea anemone, S. haddoni, from the Persian Gulf has analgesic activities, proteolytic characteristics, edematogenic activity, inflammation properties, and tissue necrosis effects (Moghadasi et al., 2018). For instance, the green scat, S. argus in the Persian Gulf produces venom with toxic activities, a protein structure with 7-250 kDa, hemolytic activity on human red blood cells, phospholipase C activity, and edematous activity (Ghafari et al., 2013). The black spot stone fish, Synanceia sp., is the most dangerous venomous organism in the Persian Gulf that contains venom with LD₅₀ equal to 185 and a protein percentage of 2.272 mg/mL (Fathi et al., 2020). The statistical reports describe that scorpionfish and jellyfish are the main marine organisms responsible for severe injuries in humans in Iran due to inhabiting coastal waters (Nabipour et al., 2015; Dehghan Madiseh et al., 2017; Zeng et al., 2016; Mi et al., 2022). Jellyfish with the largest known medusa in the Cnidaria phylum are the most abundant species in the Persian Gulf and Gulf of Oman in all seasons. Therefore, incidence with jellyfish pose more threats to human health than other venomous marine organisms (Ghafari et al., 2023). Nowadays, attention to social and economic well-being has been paid attention to, along with reducing environmental hazards, increasing the health of the environment, and reducing its pollution (Mesut, 2021). Also, the estimation of the risks caused by toxic organisms on non-target organisms such as fish is a significant sign of the greatest impact on the economic trade of the sea (Orun and Erdogan, 2014). Fish is a rich nutrient in the human diet and plays an important role in industry and commerce (Selamoglu, 2021). Therefore, toxic organisms and toxic environmental pollution in the seas and oceans can directly or indirectly affect nutritional cycle of humans through the effect on fish tissues, as well as the effect on fresh water and its different ecosystems (Selamoglu, 2018).

Globally, jellyfish create environmental risks, economic crises, and serious

impacts on human health with about 150 million stings annually (Purcell et al., 2007; CIESM, 2011; Montgomery et al., 2016; Ballesteros et al., 2022). Jellyfish have a gelatinous bell and trailing tentacles equipped with stinging epithelial cells named cnidocysts (Cegolon et al., 2013; Helm, 2018) which are specialized to inject venom for capturing prey and defense (Lassen et al., 2011).

If contact happens, death seldom however, local happens, systemic reactions such as itching, pain, rash, terrible poisoning, muscle spasm, nausea, and vomiting are common (Öztürk and İşinibilir, 2010; Gharibi et al., 2016). Clinically, the main factor is the extent of the venom put into the blood. In recent decades, jellyfish sparked interest among venom researchers around the world and also in the Persian Gulf and the Gulf of Oman. but the diversity of venom structures and their functional mechanisms remain unknown. Generally, jellyfish venom has a complex mixture of organic and inorganic compounds, including peptides and proteins with variable degrees of complexity (Currie, 2003; Fenner, 2014). In some jellyfish-like Crambionella orsini which have venom with a protein structure and protein bands between 11 and 250 kDa, the LD₅₀ was reported to be 0.5 mL (Saki et al., 2017). The anticancer properties have been identified for the venom of the jellyfish Cassiopea andromeda, which the crude venom significantly decreases mitochondrial succinate dehydrogenase activity and induces

apoptosis in tumoral mitochondria (Mirshamsi et al., 2016). Among different jellyfish species, Rhopilema nomadica and Chrysaora hysoscella belonging to class Scyphozoa bloom in several parts of the world, and are abundant in warm waters including the northern coastal waters of the Persian Gulf (Siokou-Frangou et al., 2006; Mariottini and Pane, 2010; Gharibi et al., 2016; Jafari et al., 2019). The hemolytic effects of venom from the jellyfish Rhopilema sp. with protein bands below 100 kDa were investigated in humans, mice, and sheep cells (Jafari et al., 2019). Protein bands of C. hysoscella were in the range of 72-250 kDa, with lower toxicity than other jellyfish species (Gharibi et al., 2016). Here, it is worthwhile to mention that the venoms extracted from jellyfish are an available source of potential pharmaceuticals. Moreover, swimmers, fishermen, and researchers are always at risk of jellyfish venom, which causes some immediate and delayed immune reactions (Buecher et al., 2001; Fathi et al., 2020). However, the causative allergens are mostly unknown. Unfortunately, although many excellent studies have recently been conducted to identify the characteristics and effects of venoms extracted from jellyfish, there has been little effort committed to exploring these issues in the Persian Gulf.

Therefore, this paper aims to compare the biological and cytotoxic characteristics of venoms extracted from the nomad jellyfish *Rhopilema* nomadica and the compass jellyfish

Chrysaora hysoscella, collected from the Persian Gulf. In doing so, the quantity and characteristics of the protein structure of the venoms from these species were investigated. the nomad jellyfish is a jellyfish that cause injury to human (Buecher et al., 2001; Benov and and Al-Ibraheem. 2002: Mariottini and Pane, 2014). It is an epipelagic-neritic and planktotrophic jellyfish, native to the West Indian Ocean, including the Red Sea, and the Persian Gulf. Generally, its umbrella-shaped body is about 30 to 50 cm in diameter (Feng et al., 2010). Its envenomation almost emerges via immediate pain and redness, although its skin reactions may be postponed (Bloom et al., 1988). The compass jellyfish is reported as a nearly low-risk jellyfish and its venom has adverse effects on skin cells (Del Negro et al., 1992).

It occurs abundantly in oceanic coastal water such as the Persian Gulf (Morandini and Marques, 2010). The compass jellyfish is 6 to 30 cm in length and is composed of a yellowish medusa associated with brown lines on the bell surface (Buecher et al., 2001; Houghton et al., 2007). Rhopilema nomadica and Chrysaora hysoscella species were caught with fishing nets from the coastal around Qeshm Island waters Bushehr Province, Iran, respectively, from July to September 2019 (Fig. 1B and D). After the biometry, the tentacles specimens of were removed immediately stored and on ice until delivery to the laboratory where kept at -20°C. The laboratory analyses were conducted Venomous Animal Laboratory of Razi

Vaccine and Serum Institute, Karaj, Iran.

Materials and methods

Biological sampling and venom extraction

The employed methods were protein assaying by Bradford test, exploring protein bands by electrophoresis to obtain the differences in peptide structure of venoms, and confirming the results of protein assaying by HPLC and LC Mass. In this regard, the venom pH, and its antibacterial properties were determined using MIC test and growth inhibition halo. Using the MTT test, the cytotoxic effects of the venoms were investigated on human skin fibroblast cells [HU02]. The toxicity test (LD50) of venom was explored on the animal

model (Balb/c mouse) (Gundogdu et al., 2021; Mamur et al., 2020; José Palmieri et al., 2021). Several methods have been suggested to extract the intracellular proteins, and one of the most common is the rapid freeze-thaw method, which induces the lysis of prokaryotic and eukaryotic cells (Benov and Ibraheem, 2002). Here, the protocol proposed by Bloom et al. (1988) was employed with some modifications. The containers of tentacle matrices were placed in a freeze-drier. The consecutive freeze-thaw with liquid nitrogen was employed to break down the wall of nematocysts as well as to release the venom. The obtained material was centrifuged to extract the supernatant solution of the venom at 13,000 ×g at 4°C for 20 min (Feng et al., 2010).

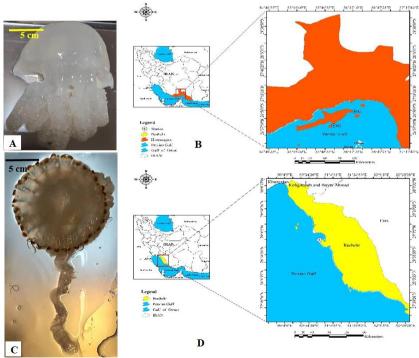


Figure 1: Jellyfish species and their distribution in the Persian Gulf. A. Rhopilema nomadica and B. its distribution in Qeshm Island near-shore water. C. Chrysaora hysoscella and D. its distribution in Bushehr province near-shore water.

Protein assay

The Bradford protein assay with bovine serum albumin (BSA) solution at 595

nm was employed to measure the protein concentration of venom after preparing 0.25 mg/µL of BSA solution (Bradford,

1976). Different concentrations of the solution were prepared with double-distilled water to achieve the final volume of $800 \mu L$. Then, the obtained solution was vortexed for a few seconds, and its optical density (OD) was read under a spectrophotometer after 5 min. The protein concentration diagram was plotted in Excel.

Electrophoresis

The molecular weight of proteins obtained from crude venom was measured by SDS-PAGE following Laemmli (1970). According to the silver nitrate method, 12% polyacrylamide gel was used to isolate protein bands based

on their molecular weight (Chevallet *et al.*, 2006).

Toxicity test (LD₅₀)

To determine the toxicity (LD₅₀), the prepared venom was injected into 10 BALB/c mice 20 g, both intravenously (IV) and intra-peritoneally (IP) for 24 h. This experiment was repeated on three groups, and the average data was investigated. For the control group, 20 g saline solvent was injected into BALB/c mice. LD₅₀ was measured by the Spearman-Karber method (Hamilton *et al.*, 1977) using Eq. (1):

$$LD_{50} = 100\% \text{ lethal log } \pm \frac{\text{Logarithm coefficient}}{\text{Number of samples per dose}} [\Sigma \text{ Number of dead} - n/2] = A \& B \qquad (1)$$

In this method, a double answer was calculated by the antilogarithm in Eq. (1) of which the received number in the dose range was chosen as the correct answer.

High-performance liquid chromatography (HPLC)

HPLC was employed to isolate and identify the proteins of venom in an advanced manner (Gharibi *et al.*, 2016). For this purpose, 100 μ g of the crude venom was dissolved in 5 μ L of distilled water. Then, the insoluble residue was centrifuged at 13,000 \times g for 15 min at 4°C to become removable. Meanwhile, to separate different parts of the venom obtained by HPLC and UV detector, 0.12 μ L of crude venom of *R. nomadica* as well as 0.11 μ L of crude venom of *C. hysoscella* were passed through a 0.22-

um membrane filter and were injected into vials along with 988 and 989 µL of HPLC water for the previous and latter ones, respectively. Afterward, 20 µL of the extract was analyzed by HPLC. The flow rate in the C18 column was 1 mL per minute [5 μ m, 250 mm \times 4.6 mm]. The HPLC and detector specifications were Water2695 separations Module Waters996 Photodiode Detector. In the next step, 20 µL of filtered venom solution was injected into the HPLC WATERS2695 to analyze in the analytical column C18 by the gradient method, in which different percentages of Solution A containing 0.1% TFA, HPLC water, Solution B containing 0.1% TFA, and acetonitrile were combined. This process was performed at 280 nm via a flow rate of 1

mL/m for 60 min. Because the column used in HPLC is column 18 which is a polar column, if the constituents of the sample are polar, they will be removed later due to greater interaction with the stationary phase. So the compounds that are later in the graph, have changed and are more polar.

Cytotoxicity and MTT assay

Human skin fibroblast cells (HU02) were provided by the Iranian Biological Resources Center. Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), and trypsin were purchased from Gibco-BRL (US) while gentamicin, phosphate-buffered saline, and MTT powder [3-[4,5-dimethylthiazol-2-yl]-2,5-

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide] were prepared from Sigma-Aldrich (St Louis, MO, US). To prepare 10 mL of complete culture medium, a mixture of 8.9 mL of DMEM powder, 1 mL of FBS, 0.1 mL of L-glutamine (100X), and 2 µL of gentamicin were cultured in a 25-mL flask and then incubated under 5% CO2 at 37°C. After the second passage by checking the viability and proliferation of cells, the subsequent experiments were implemented. Cells were cultured

on a 96-chamber plate at a concentration of 5000 cells per chamber and further incubated for 24 hours to allow the cells to adhere to the plate.

Then, normal skin cells (Hu02) were exposed to the venom of both jellyfish under graduated concentrations for 24 h. Then an MTT test was performed. The crude venom was also aliquoted for 1/2 and 1/4 solutions. Then they were treated with 1, 3, 7, and 10 µg per µL of crude venom at concentrations of complete, 1/2, and 1/4. The cytotoxicity and viability of normal human skin fibroblast cells were measured by MTT assay, (Freimoser et al., 1999), which was later performed within 24 hours. The culture medium was discharged into the microplates and then rinsed with PBS buffer, and added to the serum-free culture medium (100 µL). In the next step, 10 µL of MTT solution was added to each chamber and then incubated for 2 hours. The supernatant solution was rinsed with a PBS buffer. Next, the values were read by an ELISA microplate reader at 570 nm after adding 10 µL of DMSO solution to each chamber. The viability rate was calculated using Eq. (2):

$$Viability\ rate = \frac{Mean\ OD\ of\ Test\ Group}{Mean\ OD\ of\ Control\ Group} \times 100 \tag{2}$$

The pH of crude venom was also measured by a pH meter (WPA CD510) at room temperature.

Antibacterial properties and growth inhibition halo of venom

To determine the antibacterial properties of venoms, a 0.5 McFarland suspension was prepared at 600 nm. The chamber wells were classified into a series of

dilutions under three replicates apiece. The **MIC** (Minimum **Inhibitory** Concentration) tests of the venoms were performed (Kowalska-Krochmal et al., 2021) on four bacteria, including Pseudomonas aeruginosa (gramnegative). Staphylococcus aureus (gram-positive), methicillin-resistant Staphylococcus aureus (MRSA, grampositive), and Escherichia coli (E. coli, gram-negative). All bacteria belonged to American Type Culture Collection (ATCC). Then, dilutions of 1.2, 1.4, 1.8, 1.16, 1.32, 1.64, and 1.128 were prepared under three replicates to be used for MIC testing of laboratory bacteria at 37°C.

After importing the data into Excel, the MIC was determined based on the dilution series of each bacteria. To evaluate the growth inhibitory halo, the chambers on the agar plate were filled with the venom of the studied jellyfish. A 0.5 McFarland suspension was prepared from the tested bacteria and further cultured on the surface of the plate, where the compartments were placed on the agar plate. In addition,

pure and dilute extracts of venom compounds were added to the chambers. Since bacteria under the antimicrobial properties cannot grow on the edges of the chambers containing this compound, a clear area is formed on the edges of the chambers, called the growth inhibitory halo. It should be mentioned that for the fidelity of results, each assay was undertaken in triple replicates.

Results

Protein assay and electrophoresis

According to the concentration of bovine serum albumin protein as well as the obtained formula, the concentration of toxin protein measured by UV spectrophotometer in the range of 595 nm 0.898 mg/mL, and 0.948 mg/mL was estimated by BSA for the species of C.hysoscella and R. nomadica respectively (Fig. 2). Moreover, the molecular weight of proteins was determined by electrophoresis and SDSpage gel at 13, 21, 25, 33, 53, 98, 120, 170, and 190 kDa for R. nomadica as well as 21, 25, 35, 38, 40, 53, 100, 120, and 190 kDa for C. hysoscella (Fig. 3).

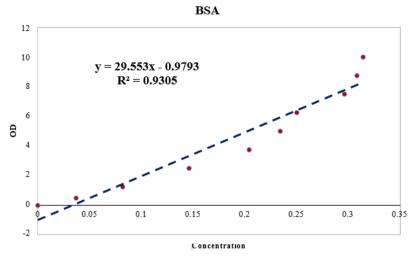


Figure 2: Protein profile of toxin concentration measured concerning the bovine serum albumin protein concentration.

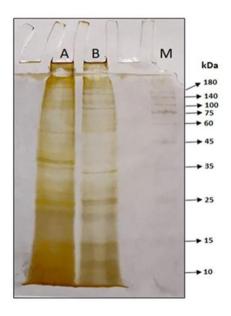


Figure 3: Electrophoresis with silver nitrate staining. A. Rhopilema nomadica, B. Chrysaora hysoscella, and M. Protein marker.

A large number of protein bands indicate the presence of a variety of proteins with different molecular weights. Note that as the number of protein bands with a molecular weight of fewer than 100 kDa is considerable, it seems that venom is mostly made up of lighter protein compounds. In a previous study, some proteins associated with molecular weights of 72 and 250 kDa were

reported for C. hysoscella, caught from the Persian Gulf (Gharibi et al., 2016). Jafari et al. (2019) reported the molecular weights of 45, 65, and 95 kDa for venom proteins in R. nomadica caught from the Persian Gulf. Likewise, Gusmani et al. (1997) reported proteins with molecular weights of 16, 50, 91, 148, and 186 kDa in R. nomadica. The variation in recognized protein bands could be related to some differences in the number of nematocytes, venom component variants based on the or life cycle seasonal changes, differences in the methods of extracting venom, and even the peptides diversity (Gharibi et al., 2016).

Toxicity test (LD_{50})

By IV injection of *C. hysoscella* venom, the LD₅₀ value was 25.1 µgr/mouse and 1.26 mg/kg mouse body weight. Moreover, intraperitoneally (IP) injection of 52 µg/mouse and 2.35 mg/kg body weight was observed. In the case of *R. nomadica* intravenously (IV) injection, the LD₅₀ value was 12.9

 μ gr/mouse and 0.65 mg/kg body weight. However, IP injection was reported as 32.5 μ gr/mouse and 1.6 mg/kg body weight. These results confirmed that by decreasing the LD₅₀, the lethality of the

venom is increased (as reported in Table 1). More importantly, in the case of both species, injecting a lower volume of crude venom using the IV method, i.e. injecting intravenously was more lethal.

Table 1: LD_{50} was determined by Intravenously (IV) and intraperitoneally (IP) venom injection during 24 hours and calculated after the Sperman-Karber method.

No	Species		μg/mouse	mg/kg body weight		
		IV	IP	IV	IP	
1	Chrysaora hysoscella	25.1	52	1.26	2.35	
2	Rhopilema nomadica	12.9	32.5	0.65	1.6	

In the case of both IV and IP injection methods, the result revealed that the LD₅₀ for R. nomadica was lower than that for C. hysoscella. This suggests that R. nomadica venom is more lethal and even brief contact with human skin can be harmful. In the study by Jafari and Zargan (2019), IP injection of R. nomadica venom in the mice yielded LD₅₀ of about 0.45 ug/kg which was lower than the results of the present study. Compared to the other jellyfish, like Carybdea alata, LD₅₀ was in the range of 5 - 25 µg/kg (Lee et al., 2015), the LD₅₀ of both examined species was nearly low whereas they might cause more injuries than death terms of contact. The difference in calculated LD₅₀ could be related to the differences in the size of the jellyfish, the sampling location of the studied species, the method by which the venoms were stored before injection, the method of injection, and venom variations (Gharibi et al., 2016; Jafari et al., 2019).

The crud venom of *R. nomadica* had a pH of 6.45, whereas *C. hysoscella* had a pH of 6.6 at the laboratory temperature indicating that the venom of *R*.

nomadica was slightly more acidic than the venom of *C. hysoscella*. As such, the venom of *R. nomadica* could leave a more lethal effect on human skin cells.

HPLC and Liquid Chromatography Mass (LC Mass)

Chromatography analysis of C. hysoscella venom divided the C_{18} column into seven sections. Adsorption was recorded at 280 nm and main peaks were recorded at 3, 6, 7, 9, 15.3, 27, and 48 min (Figs. 4A, B). Chromatography analysis of R. nomadica venom divided the C_{18} column into eight sections and the main peaks were recorded at 3.5, 5, 6.1, 7, 9, 15, 48.8, and 54 min. (Figs. 4C and D).

The results of HPLC and LC Mass indicate seven and eight peaks per 60 min for *C. hysoscella* venom and *R. nomadica* venom, respectively. Besides, the venom for *R. nomadica* with more peaks indicates more components than that of *C. hysoscella* venom. Gharibi *et al.* (2016) showed 15 sections in HPLC analysis and the main peaks of 8.62 and 11.23 min for *C. hysoscella* venom.

Furthermore, Ras et al. (2020) found five peaks of C. hysoscella proteins identified by FPLC and HLPC from the column determined, which were related hemolytic activity, proteolytic activity, and phospholipase A. The same results for R. nomadica were expressed by Gusmani et al. (1997) who found 5 main peaks related to hemolytic activities, proteolytic activity, phospholipase A.

MTT test

In the MTT test, venom toxicity on Hu02 fibroblast cells for 24 h in crude form and 1/2 and 1/4 diluted concentrations indicated that the R. nomadica venom (10 and 7 μ g/ μ L of crude venom) had more capability to destroy the skin cells. On the other hand, in the case of C. hysoscella, venom toxicity on Hu02 fibroblast cells was recorded in 10 ug/uL of crude venom. The highest percentage of cell viability was recorded in 1/4 diluted concentration of the venoms in both species, with a higher rate in R. nomadica. The results revealed that although the highest volume of crude R. nomadica venom (10 µg/µL) had the highest mortality, mortality significantly reduced at 1/4 dilution (Fig. 4; Tables 2 and 3).

Therefore, washing the damaged skin with fresh water can be an effective way to reduce cell damage. Moreover, an adverse relationship was found between the volume of the venom and the viability of the cells. This can be related

to the different protein structures or different kinds of venoms associated with various types of nematocytes. In this regard, three types of nematocytes have been reported in *C. hysoscella*. However, there is a gap in knowledge about their toxicity. While robust skin cell destruction has been previously reported for *R. nomadica* venom (Mariottini and Pane, 2010), little information is available on *C. hysoscella* toxicity (Del Negro *et al.*, 1992; Parodi, 2009). Parodi (2009) reported 55% mortality for the *C. hysoscella* venom.

Here, it is worthwhile to mention that the obtained results are in accordance with those obtained by Mariotini *et al.* (2014) in which they argued that *C. hysocella* was almost harmless while *R. nomadica* was relatively toxic. Likewise, Lee *et al.* (2015) investigated the effect of *R. nomadica* venom on various types of cancer cells and using an MTT assay demonstrated that jellyfish venom could strongly inhibit the growth of HepG2 cancer cells.

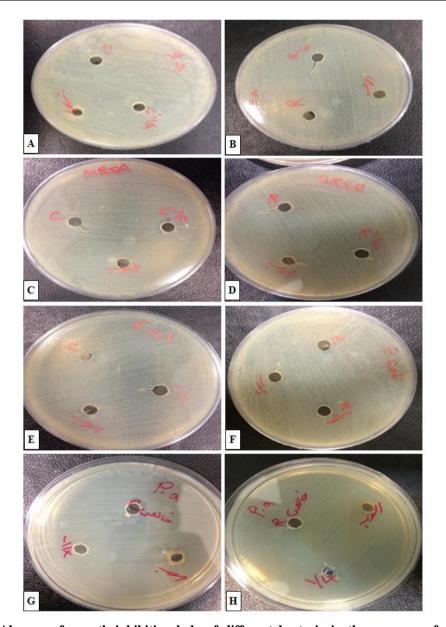


Figure 4: Absence of growth inhibition halo of different bacteria in the presence of venom. A. Interaction of Staphylococcus aureus (SA) and Chrysaora hysoscella, venom, B. SA and Rhopilema nomadica venom; C. C. hysoscella venom and methicillin-resistant Staphylococcus aureus (MRSA), D. R. nomadica venom and MRSA; E. Escherichia coli (E. coli) and C. hysoscella venom and F. E. coli and R. nomadica venom; G. C. hysoscella venom and Pseudomonas aeruginosa (Pa) H. R. nomadica venom and Pa.

Table 2: Viability of skin fibroblast cells with the effect of Chrysaora hysoscella venom.

Concentration µg/µL	C1	С3	C7	C10	C1/2 1	C1/2 3	C1/2 7	C1/2 10	C1/4 1	C1/4 3	C1/4 7	C1/4 10
Viability%	61	45	20	11	55	44	39	35	61	55	47	42

Table 3: Viability of skin fibroblast cells with the effect of Rhopilema nomadica venom.

Concentration µg/µL	R1	R3	R7	R10	R1/2 1	R1/2 3	R1/2 7	R1/2 10	R1/4 1	R1/4 3	R1/4 7	R1/4 10
Viability%	64	39	10	6	61	50	42	36	86	75	64	61

Antibacterial properties and growth inhibition halo of venom

Comparing the venoms of two jellyfish species with the same concentrations, showed that when a more lethal venom was added to the bacterial suspension, the percentage of bacterial survival decreased. As a result, the OD of the bacterial suspension was further reduced when exposed to the lethal toxin. The lack of visible inhibitory growth halo around the discs indicates the absence of antimicrobial activity. In the control group, the bacteria survived and there were no harmful substances. Their viability was optimal and their OD was measured as the constant OD of the control groups. In the experimental group, when the venom triggered the growth of bacteria, the survival of the bacteria was higher than that of the control group (100%) (Eq2). Regarding the effect of C. hysoscella venom, the toxin had a variable but stronger effect on Pa. The most antibacterial effect was observed on the 1/2 dilution of the venom on Pa, which killed about 48% of the bacteria. No constant adverse trend was observed and the antibacterial effects were noticeable at concentrations of 1/64 and 1/128, more than 1.4 to 1/32 of the venom concentration. The effects of the toxin on E. coli were neutral as in the control group in concentrations of 1/64 and 1/128, and in other dilutions that caused more growth of the bacteria. The highest effect of C. hysocella toxin on Sa was observed at 1/4 concentration. As for other concentrations, the effect of the venom was almost neutral. In the case of MRSA, similar to E. coli, at

lower concentrations, the effect was and almost neutral. higher concentrations, it promoted bacterial growth (Fig. 5A). Pa had different behaviors in the presence of R. nomadica venom. The venom caused bacterial growth in the concentrations of 1/2 to 1/8 while a weak antibacterial effect was observed at 1/16 to 1/128 concentrations (~ 20 %). The venom raised E. coli growth in 1/2 to 1/16 concentrations and was almost neutral in the rest of the concentrations. The venom of R. nomadica almost neutrally affected Sa bacteria in 1/2 to 1/16 and caused negligible growth. At the concentrations of 1/32- 1/128, a slight antibacterial activity was observed against Sa (~ 13 %). MRSA bacterial growths were increased in the presence of 1/2 to 1/32 venom concentrations and their growth was slightly dropped at 1/64 to 1/128, which was about the neutral rate. Compared to the control group, OD is a very accurate method for any bacteria that has an optical absorbance given by Eq. (2) (Fig. 5B).

The antibacterial effects of the venoms were investigated by the viability of 2 Gram-positive bacteria (MRSA and Sa) and 2 Gram-negative bacteria (*P. aeruginosa* and *E. coli*) against jellyfish venom. The results varied depending on the bacteria and the dilutions applied. The viability of MRSA bacteria in dilutions of 1/2, 1/4, 1/8, 1/16, and 1/32 against *C. hysoscella* toxin was similar to *E. coli* and was higher compared to the control samples. While in 1/64 and 1/128 dilutions, the survival rate was almost equal to the

control group. The highest survival rate was for MRSA at 1.8 dilution and for *S. aureus* at 1/16 dilution. Instead of antibacterial properties, it seems that the protein substances of this venom cause more growth of MRSA and *E. coli* bacteria. In general, almost no

antibacterial properties were observed for these venoms. Bacteria grew around and on the edge of the wells and there was no clear halo. Therefore, their venom had almost no antimicrobial activity (Fig. 4).

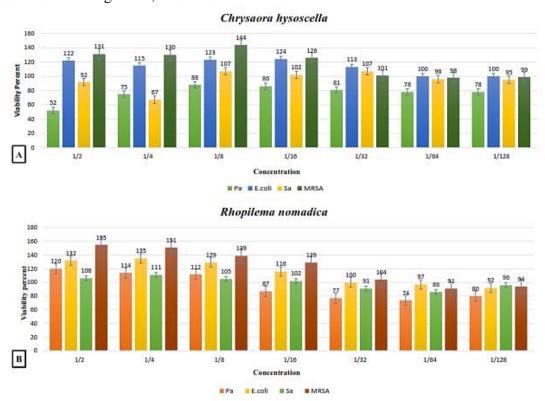


Figure 5: Effect of *Chrysaora hysoscella* (A) and *Rhopilema nomadica* (B) venoms on four studied bacterial species, Pseudomonas aeruginosa: Pa, Escherichia coli: E. coli, Staphylococcus aureus: Sa, methicillin-resistant Staphylococcus aureus: MRSA.

In the case of *S. aureus*, a relatively reliable antibacterial effect, and the only lethal dose, were observed in 1/4 of the diluted *C. hysoscella* venom (67% bacterial survival). In the remaining dilutions, survival was almost identical to the control group. Likewise, in all dilutions, the viability of *P. aeruginosa* decreased and signs of the antibacterial effect of *C. hysoscella* venom were observed. The highest antibacterial effects were observed in 1/2 dilution with 52% survival of bacteria.

In the case of *R. nomadica*, the growth of MRSA and *E. coli* was observed in dilutions of 1/2, 1/4, 1/8, 1/16, and 1/32. At 1/32 dilution of *E. coli* and 1/64 and 1/128 dilutions of both bacteria, viability was almost equal to the control group. The result that at 1/32 dilution of *E. coli* and 1/64 and 1/128 dilutions of both bacteria, viability is almost equal to the control group indicates that *R. nomadica* venom contains proteins that provide a suitable environment for the growth of bacteria. The highest growth rates in

MRSA and *E. coli* were at 1/2 and 1/4 dilutions, respectively. The reaction of *S. aureus* against *R. nomadica* venom was nearly neutral with no explicit antibacterial effect. The only dilution which showed a slight antibacterial effect was 1/64 [86% bacterial survival].

For Р. aeruginosa, significant bacterial growth was observed in dilutions about 1/2, 1/4, and 1/8, while further dropped in dilutions about 1/16, 1/32, 1/64, and 1/128 where there were some antibacterial effects. The most effective antibacterial dilution was 1/64 (viability: 74%). Given no clear halo around the wells, the results confirmed that the venom had no antimicrobial properties. Considering the relatively high viability of different bacteria in diverse dilutions of venom, it seems that victims not only should face dermal injuries and possible scars caused by direct stings but also the presence of venomous protein on the dermal tissue may provide a cultivation medium for different kind of bacteria. Despite our observations, Bhosale et al. (2002) demonstrated that jellyfish Cassiopeia sp. exhibited significant activity against Bacillus pumilus and Pseudomonas vesicularis. Likewise, Liu et al. (2011) inhibitory activity found against pathogenic bacteria including MRSA 3089 in the venom of jellyfish Nemopilema nomurai. They further argued that the venom of different species of jellyfish may have diverse reactions against variants of bacteria.

Research on venomix and their poisoning mechanism can facilitate the possibility of obtaining some suitable, cheap, and low-risk medicines of marine origin in the future, and in addition, it can contribute to therapeutic methods and lead to the improvement of food and cosmetic industries (Mariottini and Pane, 2014; Riccio et al., 2022). Moreover, cytotoxicological studies are the basis for developing an antivenom to treat or reduce the effects of marine organisms' stings. In this way, there are some problems in studying the effect of jellyfish stings such as catching the animal, determining the sensitivity of the victim to the venom due to temperature and the properties of the venom protein, and the problem of separating and purification of the toxin from the unnecessary parts nematocytes (Lassen et al., 2011; Ghaffari et al., 2013). Accordingly, this paper investigates the characteristics of the venom of R. nomadica and C. hysoscella from the Persian Gulf by employing conventional methods.

The large number of found protein bands in the venoms indicates the presence of a variety of proteins with different molecular weights. Meanwhile, a significant number of protein bands with molecular weights less than 100 kDa indicate that the venoms are mainly composed of lighter protein compounds. The reported molecular weights were in the range of venom of C. hysoscella, caught from the Persian Gulf, in which there were some proteins associated with molecular weights of 72 and 250 kDa (Gharibi et al., 2016). Jafari et al. (2019) also studied the venom of R. nomadica and further reported the molecular weights for the obtained proteins about 45, 65, and 95 kDa. Gusmani *et al.* (1997) reported proteins with molecular weights of 16, 50, 91, 148, and 186 kDa in *R. nomadica*. The differences in identified protein bands probably reflect differences in some issues such as the number of nematocytes, variations in the venom components based on the seasonal or life cycle changes, methods of extracting venoms, and even the peptides diversity (Gharibi *et al.*, 2016).

LD₅₀ for *C. hysoscella* and *R.* nomadica crude venoms were obtained at 2.35 mg/kg and 1.6 mg/kg of mice body weight, respectively. In the case of both species, injecting a lower volume of crude venom using the IV method (i.e., intravenously injecting) was more lethal. LD₅₀ for R. nomadica in both IV and IP methods was lower than that for C. hysoscella. This suggests that R. nomadica venom is more lethal, and even brief contact with human skin can be harmful. In the study by Jafari and Zargan (2019), LD₅₀ for R. nomadica through the IP method being injected into mice was about 0.45 µg/kg which was lower than in the present study. In contrast to the other jellyfish, such as Carybdea alata, which its LD50 was in the range of 5 - 25 μ g/kg (Lee *et al.*, 2015), the LD_{50} of both examined species in the present study was nearly low, and in case of contact, and they could cause more injuries than death. The differences in calculated LD₅₀ could be related to the size of the jellyfish, the difference in sampling location of the same species, the way the venoms were handled and stored before injection, the method of injecting, and the diverse

characteristics of the venoms from different species (Gharibi *et al.*, 2016; Jafari *et al.*, 2019)

The number of peaks recorded for the venoms of C. hysoscella and R. nomadica were seven and eight peaks per 60 min, respectively. Since the number of HPLC columns was 18 which is a polar column, they would be removed more slowly due to greater interaction with the stationary phase if the constituents of the sample were polar. Therefore, the compounds that were later illustrated in the diagram could be changed and became more polar. The venom of R. nomadica had more peaks and was composed of more compounds than that of C. hysoscella. Gharibi et al. (2016) found 15 fractions in HPLC analysis in which the main peaks were 8.62 and 11.23 min for C. hysoscella venom. Ross et al. (2020) also found five peaks in C. hysoscella venom proteins and attributed them to hemolytic activity, proteolytic activity, and phospholipase A.

The same results were reported for R. nomadica by Gusmani et al. (1997), where there were five main peaks related hemolytic activity, proteolytic activity, and phospholipase A. The results of the MTT test, exploring the venom toxicity (crude venom and 1/2 and 1/4 diluted concentrations) on Hu02 fibroblast cells for 24 h indicated that R. nomadica venom (10 and 7 µg/µL of crude venom) had more ability to destruct the skin cells compare to C. hysoscella (10 µg/µL of crude venom). expected As was 1/4 diluted concentration of the venoms from both

species had the highest percentage of cell viability which was higher in R. nomadica. However, the results showed that in the case of the highest volume of crude venom (10 µg/µL), R. nomadica resulted in more mortality, and the mortality decreased dramatically in 1/4 dilution. Therefore washing the injured skin with freshwater could be an effective method to decrease cell There was an inverse damage. relationship between the volume of the venom and the viability of the cells might be related to the differences in protein structures, and different types of venoms belonging to various types of nematocytes.

Fish is a rich nutrient in the human diet and plays an important role in industry and commerce (Selamoglu, 2021). Therefore, toxic organisms and toxic environmental pollution in the seas and oceans can directly or indirectly affect the nutritional cycle of humans through the effect on fish tissues, as well as the effect on fresh water and its different ecosystems (Selamoglu, 2018).

Furthermore, three different types of nematocytes have been identified in *C. hysoscella*, while there is no definite knowledge of their toxicity. Besides, the strong skin cell destructs has been reported earlier by the venom of *R. nomadica* (Mariottini and Pane, 2010), little data are available about the toxicity of *C. hysoscella* (Del Negro *et al.*, 1992; Parodi, 2009). Parodi (2009) explored the toxicity of *C. hysoscella* and further demonstrated that its crude venom could cause 55% mortality. Similar to our findings, Mariottini and Pane (2014)

reported that C. hysoscella was nearly harmless while R. nomadica was relatively toxic. Likewise, Lee et al. (2015) treated different types of cancer cells using R. nomadica venom and performed an MTT test to indicate that jellyfish venom could strongly inhibit the growth of HepG2 cancer cells. The results of the present study revealed that the crud venom of R. nomadica had a pH of 6.45 whereas C.hysoscella had a pH of 6.6 at laboratory temperature. This suggests that R. nomadica venom is slightly more acidic than that of C. hysoscella. This could be one of the reasons underlying the highly lethal effects of R. nomadica venom on human skin cells.

The results of exploring the antibacterial effects of the venoms were diverse depending on the bacteria and the applied dilutions. MRSA bacterial viability was similar to E. coli in dilutions 1/2, 1/4, 1/8,1/16, and 1/32 against venom C. hysoscella and was more compared to the control samples. In contrast, in the dilutions of 1/64 and 1/128, the viability was nearly equal to those of the control groups. Survival was the highest for MRSA in dilution of 1/8 and for S. aureus in dilution of 1/16 suggesting that the protein substances of C. hysoscella venom could cause more growth of MRSA and E. coli rather than possessing antibacterial properties.

In the case of *S. aureus*, a relatively reliable antibacterial effect, the only lethal dose, was observed in 1/4 of the venom of *C. hysoscella* dilution (67% survival of bacteria). In the remaining dilutions, the viability was

approximately similar to the control groups. In all series of dilutions, the viability of *P. aeruginosa* was reduced and the signs of antibacterial effect were observed against *C. hysoscella*. The highest level of antibacterial effects was observed in 1/2 dilution with 52% survival of bacteria.

Discussion

Regarding *R. nomadica*, MRSA and *E. coli* growth were observed in dilutions of 1/2, 1/4, 1/8, 1/16, and 1/32. In 1/32 dilution of *E. coli* and 1/1 64 and 1/128 of both bacteria, viability was nearly equal to the control groups. This suggests that *R. nomadica* venom possesses proteins that provide a suitable environment for bacterial growth. The highest rate of growth in MRSA and *E. coli* was related to the dilutions of 1/2 and 1/4, respectively.

The reaction of S. aureus against R. nomadica venom was nearly neutral meaning that R. nomadica venom had no clear antibacterial effect. The only dilution that showed slight antibacterial effect was 1/64 [86% bacterial survival]. Regarding Ρ. bacterial growth aeruginosa, significantly observed in the dilutions of 1/2, 1/4, and 1/8, while dropped in the dilutions of 1/16, 1/32, 1/64, and 1/128 where some antibacterial effects were recorded. The most effective antibacterial dilution was 1/64 (viability: 74%). Since there was no clear halo around the wells, the results confirmed that the venom had no antimicrobial properties. Considering the relatively high viability of different bacteria in

diverse dilutions of venom, it seems that victims not only should face dermal injuries and possible scars caused by direct stings but also the presence of venomous protein on the dermal tissue may provide a cultivation medium for different kind of bacteria.

Despite our observations, Bhosale et showed that al.(2002)jellyfish Cassiopeia sp. exhibited significant activity against Bacillus pumilus and Pseudomonas vesicularis. Likewise, Liu et al. (2011) argued that compounds of jellyfish Nemopilema nomurai had inhibitory activity against pathogenic bacteria including MRSA 3089. It was assumed that the venom of different species of jellyfish may have diverse reactions against variants of bacteria. Tinta et al. (2012) explored the impact of jellyfish substrate on bacterial community phylotype selection and further argued that jellyfish biomass was bioavailable to associated and freeliving bacteria, and could serve as an important microbial microenvironment.

In this paper, the characteristics of the venoms in two jellyfish species were investigated including *R. nomadica* and *C.hysoscella* in the Persian Gulf. The results revealed significant differences in the characteristics of the venoms. Overall, the measured toxin protein concentration by UV spectrophotometer was in the range of 595 nm which was more in *R. nomadica* than *C. hysoscella*. In electrophoresis, the number of identified protein bands was the same in both species. The molecular weight of most of the bands was more or less the

same but in two cases, the difference was meaningful.

The first identified protein for R. nomadica had a high molecular weight. The difference in the protein concentration, protein molecular weight, and pH affected the LD₅₀ and MTT results. In IV and IP injection methods, the IV method was more lethal due to direct release into the bloodstream followed by a faster spread through the entire body. In both injection methods, R. nomadica venom possessed more lethality. A logical and expected MTT trend was observed such that increasing venom dilution resulted in greater lethality.

As it was expected, the lethality of R. nomadica venom on skin cells was more significant. The bactericidal effects of venoms were controversial unfounded. The venom of both species not only was weak in killing bacteria but also in some cases raised their growth. This could increase the risk of infection after being bitten by the jellyfish, therefore even though the venom is not mortal, the possible post-infection could be dangerous or even fatal for the prey. However, the victim is challenged by the effects of the venom including hemolysis and phospholipase A, the immune system is under pressure due to the growth of bacteria on the wounds.

In conclusion, our results revealed some unique aspects of the venom of jellyfish species. It is hoped that jellyfish, as abundant animals in marine ecosystems, can be exploited as a research resource in the field of advanced pharmaceutical research. In this regard, more accurate data collection by HPLC and LC Mass profiles is suggested for further research.

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References

Avci, H., Gunduz Altintas, H. and Yildiz, Y., 2023. Effects of chalcone derived compounds on cell cycle and migration capability of human breast and lung cancer cells. *Biology Bulletin of the Russian Academy of Sciences*, 5, 11-22. DOI:10.1134/S1062359022603159

- Ballesteros, A., Trullas, C., Jourdan, E. and Gili, J.M., 2022. Inhibition of Nematocyst discharge from *Pelagia noctiluca* (Cnidaria: Scyphozoa)—prevention measures against jellyfish stings. *Marine Drugs*, 9, 571. DOI:10.3390/md20090571
- Benov, L. and Al-Ibraheem, J., 2002.

 Disrupting Escherichia coli: a comparison of methods. BMB Reports, 35, 428-431.

 DOI:10.5483/bmbrep.2002.35.4.428
- Bhosale, S.H., Nagle, V.L. and Jagtap, T.G., 2002. Antifouling potential of some marine organisms from India against species of *Bacillus* and *Pseudomonas*. *Marine Biotechnology*, 4, 111-118.
 DOI:10.1007/s10126-001-0087-1
- Bloom. D.A., Burnett, J.W. and Alderslade. P., 1988. **Partial** purification of box iellyfish nematocyst (Chironex *fleckeri*) venom isolated at the beachside. Toxin, 36,1075-1085. DOI:10.1016/s0041-0101(98)00096-8
- **Bradford, M.M., 1976.** A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.

DOI:10.1006/abio.1976.9999

Buecher, E., Sparks, C., Brierley, A., Boyer, H. and Gibbons, M., 2001. Biometry and size distribution of *Chrysaora hysoscella* (Cnidaria, Scyphozoa) and *Aequorea aequorea* (Cnidaria, Hydrozoa) off Namibia with some notes on their parasite

- Hyperia medusarum. Journal of Plankton Research, 23, 1073-1080. DOI:10.1093/plankt/23.10.1073
- Calvete, J.J., Ghezellou, P., Paiva, O., Matainaho, T., Ghassempour, A., Goudarzi, H. and Williams, D.J., **2012.** Snake venomics of two poorly known hydrophiinae: comparative proteomics of the venoms terrestrial **Toxicocalamus** longissimus and marine Hydrophis cyanocinctus. Journal of Proteomics, 75, 4091-4101. DOI:10.1016/j.jprot.2012.05.026
- Cegolon, L., Heymann, W.C., Lange, J.H. and Mastrangelo, G., 2013.

 Jellyfish stings and their management: a review. *Marine Drugs*, 11, 523-550.

 DOI:10.3390/md11020523
- Chevallet, M., Luche, S. and Rabilloud, T., 2006. Silver staining of proteins in polyacrylamide gels. *Nature Protocols*, 1, 1852-1858. DOI:10.1038/nprot.2006.288
- **CIESM, 2011.** Gelatinous plankton outbreaks: theory and practice. *CIESM work Series*, 14, 112.
- **Currie, B.J., 2003.** Marine antivenoms: antivenoms. *Journal of Clinical Toxicology*, 41, 301-308. DOI: 10.1081/clt-120021115
- Dehghan Madiseh, S., Koochaknejad,
 E., Mousavi Dehmourdi, L.,
 Zarshenas, A. and Mayahi, M.,
 2017. Jellyfish of Khuzestan coastal waters and their impact on fish larvae populations. *Iranian Journal of Fisheries Sciences*, 16(1), 422-430.
 DOI: 10.22092/IJFS.2018.114667

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- Del Negro, P., Kokelj, F., Tubaro, A. and Della Loggia, R., 1992. Chrysaora hysoscella in the Gulf of Trieste: presence, evolution and cutaneous toxicity in man. *Marine Coastal Eutrophication*, 2, 427-430. DOI:10.1016/b978-0-444-89990-3.50039-0
- **Fathi, A., Forouzan, A. and Jafari, H., 2020.** Extraction, study and determination of LD50 and protein percentage of Persian Gulf black spot stone fish venom–Khuzestan. *Journal of Marine Biology*, 12(1), 109-118.
- Feng, J., Yu, H., Li, C., Xing, R., Liu, S., Wang, L. and Li, P., 2010. Isolation and characterization of lethal proteins in nematocyst venom of the jellyfish *Cyanea nozakii* Kishinouye. *Toxin*, 55, 118-125. DOI: 10.1016/j.toxicon.2009.07.008
- **Fenner, P.J., 2014.** Venomous marine animals. *South Pacific Underwater Medicine Society*, 34, 196-202.
- Freimoser, F.M., Jakob, C.A., Aebi, M. and Tuor, U., 1999. The MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay is a fast and reliable method for colorimetric determination of fungal cell densities. *Applied and Environmental Microbiology*, 65, 3727. DOI:10.1128/AEM.65.8.3727-3729.1999
- Ghafari, S.M., Jamili, S., Bagheri, K.P., Ardakani, E.M., Fatemi, M.R., Shahbazzadeh, F. and Shahbazzadeh, D., 2013. The first report on some toxic effects of green scat, *Scatophagus argus* an Iranian

- Persian Gulf venomous fish. *Toxin*, 66, 82-87. DOI:10.1016/j.toxicon.2013.01.025
- Ghafari, S., Jamili, S., Shahbazzadeh, D. and Pooshang Bagheri, K., 2023. Research Article: The first report on isolation of a new highly hemolytic toxin, scatotoxin, from *Scatophagus argus* venomous bonny spines. *International Journal of Fertility and Sterility*, 22(3), 631-642. http://jifro.ir/article-1-3857-en.html
- Gharibi, S., Nabipour, I., Kim, E., Ghafari, S.M., Hoseiny, S.M., Kamyab, M. and Seyedian, R., 2016. Characterization and pharmacological activities of jellyfish, Chrysaora hysoscella captured in Bushehr Port Iran. Iranian **Journal** of Toxicology, 10, 7-10. DOI:10.29252/arakmu.10.5.7
- Gulhan, F. and Selamoglu, Z., 2016. Comparison of the effects of propolis and pollen extracts in the same concentrations on some biochemical and hematological parameters in rainbow (Oncorhynchus trout mykiss). Journal of Survey in*Fisheries* Sciences, 3(1), 1-8. DOI:10.17762/sfs.v3i1.90
- Gundogdu, G., Dodurga, Y., Cetin, M., Secme, M. and Cicek, B., 2021. The cytotoxic and genotoxic effects of daidzein on MIA PaCa-2 human pancreatic carcinoma cells and HT-29 human colon cancer cells. *Drug and Chemical Toxicology*, 43(6), 581-7.
 - DOI:10.1080/01480545.2018.15278 49

- Gusmani, L., Avian, M., Galil, B., Patriarca, P. and Rottini, G., 1997. Biologically active polypeptides in the venom of the jellyfish. *Toxicon*, 35, 637-648.
- Hamilton, M.A., Russo, R.C. and Thurston, R.V., 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environmental Science & 714-719. Technology, 11(7), DOI:10.1016/S0041-0101(96)00182-1
- **Helm, R.R., 2018.** Evolution and development of scyphozoan jellyfish. *Biology Revision*, 93, 1228-1250. DOI:10.1111/brv.12393
- Houghton, J.D.R., Doyle, T.K., Davenport, J., Lilley M.K, Wilson, R.P. and Hays, G.C., 2007. Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusae (Cnidaria: Scyphozoa). *Hydrob*, 589, 1-13. DOI:10.1007/s10750-007-0572-2
- Jafari, H., Zargan, J. and Tamadoni Jahromi, S., 2019. Extraction of the venom of *Rhopilema nomadica* from the Persian Gulf coast and the investigation of its hemolytical activity. *Yafteh*, 21, 421-431.
- Jafari, H., Honari, H., Zargan, J. and Jahromi, S.T., 2019. Identification and hemolytic activity of jellyfish (*Rhopilema* sp., Scyphozoa: Rhizostomeae) venom from the Persian Gulf and Oman Sea. *Journal of Biodiversity*, 20, 1228-1232. DOI:10.13057/biodiv/d200440

- José Palmieri, M., Ribeiro Barroso, A., Fonseca Andrade-Vieira, L., Monteiro, M.C., Martins Soares, A., Souza Cesar, P.H., Aparecida Braga, M., Cardoso Trento, M.V., Marcussi, S. and Chamma Davide, L., 2021. Polybia occidentalis and Polybia fastidiosa venom: a cytogenotoxic approach of effects on human and vegetal cells. Drug and Chemical Toxicology, 44(6), 566-74. DOI:10.1080/01480545.2019.16313
- Kakoolaki, S., Talas, Z.S., Cakir, O., Ciftci, O. and Ozdemir, I., 2013. Role of propolis on oxidative stress in fish brain. *Basic and Clinical Neuroscience*, 4(2), 153-8.
- **Kowalska-Krochmal, B. and Ruth, D.W., 2021.** The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, 10(2), 165. DOI:10.3390/pathogens100201 65
- **Laemmli, U.K., 1970.** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 5259, 680-685. DOI:10.1038/227680a0
- Lassen, S., Helmholz, H., Ruhnau, C. and Prange, A., 2011. A novel proteinaceous cytotoxin from the northern Scyphozoa *Cyanea capillata* (L.) with structural homology to cubozoan haemolysins. *Toxin*, 57, 721–729. DOI:10.1016/j.toxicon.2011.02.004
- Lee, H., Kwon, Y.C. and Kim, E., 2015. Jellyfish venom and toxins: a

- review. *Marine* and *Freshwater Toxins*, 1, 1-14.
- DOI:10.1007/978-94-007-6650-1_26-1
- Liu, J., Li, F., Kim, E.L., Li, J.L., Hong, J., Bae, K.S. and Jung, J.H., 2011. Antibacterial polyketides from the jellyfish-derived fungus *Paecilomyces variotii*. *Journal of Natural Products*, 74, 1826-1829. DOI:10.1021/np200350b
- Mamur, S., Ünal, F., Yılmaz, S., Erikel, E. and Yüzbaşıoğlu, D., 2020. Evaluation of the cytotoxic and genotoxic effects of mycotoxin fusaric acid. *Drug and Chemical Toxicology*, 43(2), 149-57. DOI:10.1080/01480545.2018.14997
- Mariottini, G.L. and Pane, L., 2010.

 Mediterranean jellyfish venoms: A review on scyphomedusae. *Marine Drugs*, 8, 1122-1152.

 DOI:10.3390/md8041122
- Mariottini, G.L. and Pane, L., 2014. Cytotoxic and cytolytic enidarian venoms. A review on health implications and possible therapeutic applications. *Toxins*, 6, 108-151. DOI:10.3390/toxins6010108
- Mesut, S., 2021. Blue economy and blue Ocean strategy. *Journal of Ecology & Natural Resources*, 5, 000263. DOI:10.23880/jenr-16000263
- Mi, T., Zhao, S. and Qiu, M., 2022.

 Using LA-ICP-MS to analysis elemental composition of statoliths of Scyphozoan jellyfish. *Acta Oceanologica Sinica*, 41, 81–87.

 DOI:10.1007/s13131-022-2034-0
- Mirshamsi, M.R., Omranipour, R., Vazirizadeh, A., Fakhri, A.,

- Zangeneh, F., Mohebbi, G.H. and Pourahmad, J., 2016. Persian Gulf Jellyfish (Cassiopea andromeda) venom fractions induce selective injury and cytochrome c release in mitochondria obtained from breast adenocarcinoma patients. Asian Journal Pacific ofCancer 18. 277-283. Prevention. DOI:10.22034/APJCP.2017.18.1.27 7
- Moghadasi, Z., Jamili, S., Shahbazadeh, D. and Bagheri, K.P., 2018. Toxicity and potential pharmacological activities in the Persian Gulf venomous sea anemone, Stichodactyla haddoni. Iranian Journal of Pharmaceutical Research, 17, 940-950.
- Mohebbi, G.H. and Nabipour, I., 2021. Toxinology of Marine Venomous Snails. *Iranian South Medical Journal*, 24, 505-581. DOI:10.52547/ismj.24.5.505
- Mohebi, S. and Nabipour, R., 2016.
 Toxicology of sea snakes, a systematic review. *Iranian South Medical Journal*, 19, 662-703.
 DOI:10.18869/acadpub.ismj.19.4.66
- Montgomery, L., Seys, J. and Mees, J., 2016. To Pee or Not to Pee: A Review on Envenomation and Treatment in European Jellyfish Species. *Marine Drugs*, 14, 127-134. DOI:10.3390/md14070127
- Morandini, A.C. and Marques, A.C., 2010. Revision of the genus Chrysaora Péron & Lesuer, 1810 (Cnidaria: Scyphozoa). *Zootaxa*,

- 2464, 1–97. DOI:10.11646/zootaxa.2464.1.1
- Nabipour, I., Khoshdel, **A.R.**. Tashakori A.R., Golaghaei, Beheshti, A. and Afshari, R., 2015. Clinico-epidemiologic study marine envenomations and injuries in south Iran. Persian Gulf coasts. Asia Pacific **Journal** of Medical Toxicology, 4. 112-115. DOI:10.22038/apjmt.2015.5983
- Orun, I., Ates, B., Selamoglu, Z., Yazlak, H., Ozturk, E. and Yilmaz, İ., 2005. Effects of various sodium selenite concentrations on some biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss). Fresenius Environmental Bulletin, 14(1), 18-22. DOI:10.1007/s10695-007-9146-5
- Orun, I. and Erdogan, K., 2014. Role of propolis on biochemical and hematological parameters of Oncorhynchus mykiss exposed to cypermethrin. *Journal of Survey in Fisheries Sciences*, 1(1), 21-35. DOI:DOI:10.17762/sfs.v1i1.36
- Öztürk, B. and İşinibilir, M., 2010. An alien jellyfish Rhopilema nomadica and its impacts to the Eastern Mediterranean part of Turkey Yabancı denizanası Rhopilema nomadica ve Türkiye'nin doğu Akdeniz sahillerine etkileri. *Journal of the Black Sea / Mediterranean Environment*, 16, 149-156.
- **Parodi, A., 2009.** Cytotoxic effect on human keratinocytes of crude extracts from planktonic. *Journal of Biological Research*, 82, 11-18. DOI:10.4081/jbr.2009.4734

- Purcell, J.E., Uye, S.I. and Lo, W.T., 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Marine Ecology Progress Series*, 350, 153-174. DOI:10.3354/meps07093
- Ras, V., 2020. There are three species of *Chrysaora* (Scyphozoa: Discomedusae) in the Benguela upwelling ecosystem, not two. *Zootaxa*, 4778, 401-438. DOI:10.11646/zootaxa.4778.3.1
- Riccio, G., Martinez, K.A., Martín, J., Reyes, F., D'Ambra, I. and Lauritano, C., 2022. Jellyfish as an alternative source of bioactive antiproliferative compounds. *Marine Drugs*, 20, 350. DOI:10.3390/md20060350
- Saki, N., Nikpour, Y., Taghavi Moghadam, A. and Ghanemi, K., 2017. Extraction and neutralization nematocyst venom of *Crambionella orsini* jellyfish [whit] using of chelating Na-EDTA. *Journal of Marine Sciences and Techniques*, 16(2), 103-113. (In Persian)
- **Selamoglu, M., 2021.** Importance of the cold chain logistics in the marketing process of aquatic products: An update study. *Journal of Survey in Fisheries Sciences*, 8(1), 25-29. DOI:10.18331/SFS2021.8.1.2
- Selamoglu, M., 2021. The effects of the Ports and water transportation on the Aquatic ecosystem. *Open Access Journal of Biomedical Science*, 10(1), 2021.

DOI:10.46718/JBGSR.2021.10.0002

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- **Z.**, 2018. Selamoglu, Selenium compounds for fish health: An update. Journal ofSurvey in **Fisheries** Sciences, 4(2),1-4. DOI:10.18331/SFS2018.4.2.1
- Sheppard, C.R.C., Price, A. and Roberts, C., 1992. Marine ecology of the Arabian Region: Patterns and processes in extreme tropical environments. Academic Press, UK. 359 P.
- Siokou-Frangou, I., Sarantakos, K. and Christou, E.D., 2006. First record of the scyphomedusa *Rhopilema nomadica* Galil, 1990 (Cnidaria: Scyphozoa: Rhizostomeae) in Greece. *Aquatic Invasions*, 1, 194-195. DOI:10.3391/ai.2006.1.3.17
- Tinta, T., Kogovšek, T., Malej, A. and Turk, V., 2012. Jellyfish modulate

- bacterial dynamic and community structure. *PloS One*, 7(6), e39274. DOI: 10.1371/journal.pone.0039274
- Urbarova, I., Karlsen, B., Okkenhaug, S., Seternes, O., Johansen, S. and Emblem, A., 2012. Digital marine bioprospecting: Mining new neurotoxin drug candidates from the transcriptomes of cold-water sea anemones. *Marine Drugs*, 10, 2265–2279. DOI:10.3390/md10102265
- Zeng, Y., Huang, X. and Huang, B., 2016. Relationship between bacteria and phytoplankton during the giant jellyfish Nemopilema nomurai bloom in an oligotrophic temperate marine ecosystem. *Acta Oceanologica Sinica*, 35, 107–113. DOI:10.1007/s13131-016-0894-x