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Study the antimicrobial activity of six marine sponges and three parts of sea anemone on *Candida albicans*

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ABSTRACT

Objective: To evaluate the antifungal and inhibitory activity of six different species of marine sponges and one species of sea anemone that were collected from the Persian Gulf on the growth of *Candida albicans* (*C. albicans*).

Methods: Sea anemone and six different sponges were gathered from the Persian Gulf and extracted by methanol macerated with dichloromethane solvents. The activity of each extracts against *C. albicans* was determined by paper disc diffusion and agar well diffusion methods. Also, minimum inhibitory concentration and minimal bactericidal concentration of each extract were determined.

Results: The finding of current research confirmed that all sponge extracts had sufficient inhibitory effect against *C. albicans* but the extracts of sponge type 2 and 5 had the best inhibitory effect on *C. albicans* and their zones of inhibition were 45 mm and 38 mm, respectively. The tentacle of sea anemone had the best inhibitory effect against *C. albicans* compared to other part of the body and its zone of inhibition was 41 mm. Besides, the sponge type 5 extracts had the best minimum inhibitory concentration and minimal bactericidal concentration values with 6.25 and 12.5 mg/mL, respectively.

Conclusions: It could be concluded that the crude extracts of six different sponges and sea anemone have high potential to produce broad spectral antifungal activity with minimal concentration against different pathogenic fungi.

1. Introduction

Ocean is known as the mother of life and many believed in the grounds that primitive forms of life from the "primordial soup" have already begun. This area has a huge variety of sea creatures that are diverse in terms of physiology and adaptation. As they grow in different climatic conditions, these microorganisms are spread with a certain adjustment mechanisms, some of which can be useful for self-defense and thus perhaps the compatibility of different ways is beneficial for human[1].

Oceans of our planet are responsible for many biological activities. A number of biologically active compounds with varying degrees of action, such as anti-tumor, anti-cancer, anti-microtubule, anti-proliferative, cytotoxic, photoprotective, as well as antibiotic

and antifouling properties, have been isolated to date from marine sources[2].

In the oceans, the main lifestyle of bacteroidetes is assumed to be attachment to particles and degradation of polymers[3]. Since the mankind nature from the beginning has been contributing considerably to drug discovery for human beings by providing remedial treatments. Marine ecosystems are approximately 75% of the earth's surface. Marine natural products play an important role in biomedical research and drug development, or directly as a medicine or inspired by biological structures to make chemical drugs[4].

Blunt *et al.* listed that in marine environment, 37% sponges, 21% coelenterates and 18% microorganisms are the main sources of biomedical compounds, followed by 9% algae, 6% echinoderms, 6% tunicates, 2% mollusks and 1% bryozoans, *etc.*[5]. Sponges are ordinary, multicellular, acaulescent animals with no true tissue layers or limb, feed on bacteria[6] and are constantly exposed to large populations of microbes in the water, including opportunistic pathogens and microorganisms in the sediment. Despite ongoing risks and lack of complex physical structure and cellular immune

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mechanisms in more advanced organisms used to combat pathogenic bacteria, sponges are highly successful members of the benthos and suffer few obvious bacterial infections[7]. A variety of biologically active constituents have been isolated from various species of sponges[8].

The first report of antimicrobial activity of sponge extracts was by Newbold *et al.*[7]. Sponges have the ability to capture bacteria in the surrounding and digest the food up to 77% enzyme. Bioactive substances produced by the sponges themselves are beneficial for digestion of food, so that the obtained bioactive courses vary according to the eating habits of each type of sponge[9]. To date, over 5000 different combinations of about 500 species from sponges were isolated[10].

Marine invertebrate, mainly sedentary sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs. Among the sea creatures, anthozoans, important animals in the environment, need to protect themselves against death or deactivate the consequences of microbial attack by parasites. Nevertheless, careful analysis was performed in *in vitro* related bacteria and cellular basis of the antibacterial activity has remained largely unexplored[11].

More recently, cytolytic toxins of sea anemone had attracted much attention to researchers, because they exhibit anti-tumor, anti-parasitic, anti-microbial, dermonecrotic and other types of biological activities due to the powerful membranolytic action. Sea anemones have tentacles surrounding an opening central mouth and used to grab and transfer food items such as crustaceans, mollusks and small fish to their mouth. The nematocysts present on the edges of the tentacles can expel specific toxins[12]. *Candida* species are one of the most usual fungal pathogens and the causative agents of superficial and invasive candidiasis. Overwhelming majority of *Candida* infections are mucosal manifesting as vaginal or oral candidiasis. In total, about 40% of infections each year constitute by them. High rate of *Candida* proliferation is also associated with some gut diseases containing Crohn's disease and ulcerative colitis, which can decrease fungal burdens and alleviate disease intensity[13].

The aim of this study is to evaluate the antifungal and inhibitory activity of six different species of marine sponges and one species of sea anemone that were gathered from the Persian Gulf on the growth of *Candida albicans* (*C. albicans*).

2. Materials and methods

2.1. Collection of marine sponge and sea anemone

Some marine sponges (six different species) were gathered from intertidal zones of the Gheshm Island located at the Persian Gulf in November 2015. Three repetition were collected for each marine sponge species. Specimens of sea anemone were captured at the Hormoz Island in the southeast coast of the Persian Gulf. They were transported alive in sea water to our laboratory and maintained in the culture tank for extraction.

2.2. Preparation of sponge and sea anemone extracts

The following protocols were used for getting the extract of freeze-dried samples of six sponges (100 g). First, a polar and unipolar solution with dichloromethane (DCM): methanol (MeOH) (1:1 v/v) was used. Each sponge was immersed into this solution for 84 h. Then, the obtained extract was filtrate and concentrated. Finally, 75 mL distilled water and 75 mL hexane were added to each extract to dissolve polar and unipolar fraction of each extract.

For extraction of sea anemones antimicrobial compounds, this protocol was used. First, sea anemones were converted into small parts and a portion of these parts (150 g) was submerged into methanol solvent. Three parts of sea anemone were used for extraction, including body, tentacles and total body (mixed). All extracts from sea anemones were stored at cold temperature (-20°C). Then, each extract passed through Whatman No. 1 filter paper. The filtrate was placed into incubator at 45°C to remove the solvent. The concentrated extracts were applied for antimicrobial activity against *C. albicans*.

2.3. Disk diffusion method

Antifungal activity of the methanolic extracts of sea anemone and DCM: MeOH extract of six sponges were assayed by standard disc diffusion method. Frozen culture of *C. albicans* was activated in potato dextrose broth medium at 37°C for 24 h. The opacity of *C. albicans* culture reached to 10^8 CFU/mL compared to the McFarland turbidometer. One milliliter of this inoculum was transferred into Mueller-Hinton agar plates by spread plate method using sterile cotton swab and allowed to stay for 60 s. Then, 0.15 mg/mL concentration of each extract prepared from sterile filter paper discs (6 mm) was placed into each of these concentrations for 1 h. The disc was put for 30 min at room temperature and transferred to the medium. Disc solvent-free extract was used as positive control. The zone of inhibition (ZOI) of each disc that contained marine extracts was calculated in millimeter and the measurements were performed in triplicate for each marine extract[14].

2.4. Assay of direct diffusion of extract into agar well

The dissemination of each extract directly into agar well was performed to detect the antifungal activity. The overnight culture of *C. albicans* was grown by spread method on potato dextrose agar (PDA) plates. Some wells (8 mm diameter and about 1.5 cm wide) were created in each PDA plate by sterile blade. The extract of each marine animal (sponge and sea anemone) was provided at 1 mg/mL concentration. About 40 μL of provided concentrations of sponge and sea anemone solvent extracts were inoculated by sterile syringe into the constructed wells and remained to diffuse at 30°C for 2 h. Also, inoculums without marine animal extracts were

designed as negative control. The PDA plates stayed at 34 °C for 24 h. ZOI was measured. These experiments were carried out three times and average of obtained values was reported as ZOI[15].

2.5. Characterization of the minimal inhibitory concentration (MIC), and minimal fungicidal concentration (MBC) of each extract

MICs were carried out by serial dilution method using 96-well microtiter plates. The different sponges and sea anemone extracts viz. DCM: MeOH and methanol were taken with 1 mg/mL and serial dilutions of the extracts with potato dextrose broth medium with respective inoculum were used. The microplates were incubated for 24 h at 34 °C, respectively. The lowest concentrations without visible growth at the binocular microscope were recorded as MICs. The MBC was characterized by spreading 50 µL on PDA plate from the sample showing no visible growth and it was further incubated for 18 h at 37 °C[16].

3. Results

3.1. Assessment of the antifungal activities of different sponge extracts and three parts of sea anemone extracts against *C. albicans*

The ZOIs for DCM: MeOH extracts of six tested sponges and methanolic extracts of three parts of sea anemone methanolic extracts that were assayed by disc diffusion and agar well diffusion methods were shown in Table 1. As shown in this table, all sponge extracts had sufficient inhibitory effect against *C. albicans* but the extracts of sponge type 2 and 5 had the best inhibitory effect against *C. albicans* and their ZOIs were 45 mm and 38 mm, respectively. ZOI of the lowest antifungal effect between these six sponges related to sponge type 3 was 18 mm. Also, the ZOI in agar well diffusion was higher than that in disc diffusion method in all tested sponges. The results of antimicrobial activity of three parts of sea anemone showed that the tentacles of this marine animal had the best inhibitory effect against *C. albicans* compared to other parts of the body and its ZOI was 41 mm (Table 1).

Table 1

The inhibitory effect of three parts of sea anemone and six sponges species extracts against *C. albicans* that were assayed by disc diffusion and agar well plate methods.

Type of marine animal	<i>C. albicans</i> (ZOI in mm)		Solvent control
	Disk diffusion test	Agar well plate test	
Sponge type 1	11.0 ± 1.1	25.0 ± 0.7	-
Sponge type 2	20.0 ± 0.9	45.0 ± 0.1	-
Sponge type 3	8.0 ± 0.6	18.0 ± 0.6	-
Sponge type 4	17.0 ± 0.7	35.0 ± 0.4	-
Sponge type 5	18.0 ± 1.3	38.0 ± 0.9	-
Sponge type 6	17.0 ± 0.8	28.0 ± 0.9	-
Sea anemone (body)	15.0 ± 0.3	24.0 ± 0.3	-
Sea anemone (tentacles)	23.0 ± 1.2	41.0 ± 0.5	-
Sea anemone (mixed)	13.0 ± 0.8	17.0 ± 0.5	-

3.2. MIC and MBC values of six sponges extracts and three parts of sea anemone extracts against *C. albicans*

The results of MIC and MBC values of the six sponge extracts and three parts of sea anemone extracts were illustrated in Table 2. As presented in this table, approximately all sponge extracts exhibited inhibitory effect in lower concentration. However, the sponge type 5 extracts had the best MIC and MBC values (6.25 and 12.50 mg/mL, respectively). Also, between three parts extracts of sea anemone, the lowest MIC and MBC values belonging to the tentacles of sea anemone were 5.67 and 15.43. Considering that these extracts were used in broth medium of MIC test, and the lower concentration (0.156–2.5 mg/mL) was used in preparing disks and inhibited *C. albicans*, it can be concluded that the inhibitory efficiency of these extracts in broth medium was more than solid medium.

Table 2

The MIC and MBC values of three parts of sea anemone and six sponges species extracts against *C. albicans* (mg/mL).

Type of marine animal	MIC and MBC of extracts	
	MIC	MBC
Sponge type 1	12.50	25.00
Sponge type 2	12.50	25.00
Sponge type 3	50.00	75.00
Sponge type 4	12.50	25.00
Sponge type 5	6.25	12.50
Sponge type 6	18.70	34.50
Sea anemone (body)	34.12	54.13
Sea anemone (tentacles)	5.67	15.43
Sea anemone (mixed)	54.16	74.18

4. Discussion

Marine organisms can produce bioactive materials with surprising actions. For example, one of the important activities is antimicrobial action against different pathogens like fungi, bacteria and virus. The marine sponges have little autoprotection, but some mechanisms are determined, such as the ability to deter predators, inhibit pathogenic microbes and discourage the formation of a bacterial biofilm.

Some researchers confirmed that the bioactive compounds from sponge can play a major role in protecting against pathogens. Sea anemone contains a variety of bioactive compounds including some toxins that are known to possess potent hemolytic properties. Also, recently, researchers have found some defense mechanisms in sea anemone against environmental pathogens.

In this study, six different species of sponge and one species of sea anemone were collected from the Persian Gulf. Marine invertebrates have high diversity in the Persian Gulf. Until now, there is still a little work to study the bioactive compounds of marine invertebrates in the Persian Gulf. These marine animals can produce many bioactive materials with various activities like antimicrobial, antifungal and antitumor.

Collected sponges were extracted by polar and nonpolar solvents. Since the sponges had different compounds with various structures, the combination polar (MeOH) and nonpolar (DCM) solvents were used. However, in the case of sea anemone, only one polar solvent

(MeOH) was used. In order to study the antifungal activities of sea anemone, three parts of this marine animal were used. This experiment was done to better understand which section of sea anemone has high antimicrobial activity.

The finding of the present study confirmed the antifungal activity of sea anemone and different sponges. And the antifungal activities of these six sponges are significantly different. For example, sponge type 3 and 5 have the highest activity compared to all collected sponges. This finding can be related to various chemical compounds that may be found in different sponge species.

Three parts of sea anemone showed different inhibitory effect against *C. albicans*. The results of current research established that tentacles of sea anemone had better inhibitory effect than other parts of sea anemone. Also, when the whole body of this marine invertebrate was extracted, the antifungal activity dramatically decreased. This finding can be interpreted that the amoebocytes of sea anemone were located at tentacles and produced bioactive compounds, thus the antifungal activity will be increased. Both sponges and sea anemone extracts had the maximum values for MIC in the range of 6.55–12.5 mg/mL. Since these extracts are generally a mixture of active and non-active compounds, higher MICs are expected.

The novelty of this research is that this is the first study to carry out on marine invertebrates of the Persian Gulf. Also, this is the first report on antifungal activity of marine sponges and sea anemone of the Persian Gulf. However, some researchers reported antibacterial activity of marine invertebrate worldwide. Some of these researchers were described in this section [12]. The antimicrobial properties of sea anemone, *Stichodactyla mertensii* collected from Mandapam coast in India were studied. Their results confirmed that the species of sea anemone has the highest inhibitory activity against *Aspergillus niger* and low inhibition against *C. albicans*. The conducted research on the bioactivity of antibacterial protein fractions isolated used a polar solvent of some sponge species on the island of Barang Lompo, Indonesia. They concluded that maximum inhibitory activity belongs to *Escherichia coli* and *Staphylococcus aureus*. They proposed that bioactive proteins from sponges may be used as a base for new antibacterial drugs, especially against *Escherichia coli* [9].

From the above discussion, it could be concluded that the crude extracts of the six different sponges and sea anemone revealed the fact that they have higher potential to produce broad spectral antifungal activity with minimal concentration against a wide range of human fungal pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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