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Research Article

Assessment of antifungal, antimicrobial and cytotoxic activities of marine zoanthid (Phylum Cnidaria, Class Anthozoa) extract in marine habitats of Hengam Island, Persian Gulf, Iran

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Abstract

In search for bioactive products, three zoanthid species (*Zoanthus* spp., *Palythoa tuberculosa and Palythoa mutuki)* were collected from offshore zone of Hengam Island. Three extracts of each zoanthids (methanol, dichloromethane (DCM) and n-hexane) were tested for antifungal and antibacterial activities against certified strains of bacteria (two Gram-positive: *Bacillus subtilis*, *Staphylococcus aureus* and three Gram-negative: *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*) and fungi (*Candida albicans*, *Microsporum gypseum*, *Microsporum canis*) through the disk diffusion assay. Cytotoxic activity of these extracts was evaluated against *Artemia* nauplii. The results showed that 8 extracts (88.88%) of the zoanthids were active against at least one bacterial strain and 6 extracts (66.6%) were active against at least one fungus (the activity against bacteria was moderate). Also, minimum inhibitory concentrations (MICs) of the extracts with desirable (inhibition zone more than 9mm) in the previous stage were assessed. Among the 9 zoanthids extract, 88.88% showed activity against some of the five bacteria, and 66.6% showed activity against some of the three fungi. The most active zoanthid extract against three fungi was dichloromethane extract of the *Zoanthus* ssp. that showed promising antifungal activity against *Candida albicans in vitro* models. The minimum inhibitory concentrations and LC_{50} values of dichloromethane extract of *Zoanthus* ssp were 125µg/mL and 181µg/mL, respectively. Therefore, this extract can be a candidate for candidiasis therapy. LC_{50} of DCM, crude extract of *Palythoa mutuki* was 31µg/ml, showing high toxicity. This is the first report of biological activities of marine zoanthids from an Iranian Island of Persian Gulf.

Keywords: Zoanthids, Antifungal activity, Antimicrobial activity, Cytotoxic activity, Hengam Island, Persian Gulf

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Introduction

In recent decades, the discovery of Secondary metabolites isolated from marine organisms has increased. These substances may be having well biological activities, e.g., antimicrobial, antitumor, anti-inflammatory, antiviral and antioxidant activities (Blunt *et al*., 2014). Sessile marine organisms synthesize diverse natural products with biological activities that have specific protective functions against grazing, infections caused by microorganisms and epiphytes. These natural products may also have therapeutic applications (Radjasa *et al*., 2011; Senthilkumar and Kim, 2013). Marine zoanthids belong to many genera, including *Zoanthus* and *Palythoa*, phylum Cnidaria, class Anthozoa, subclass Hexacorallia and order Zoantharia. They have a wide variety of shape, colour and size (Tsuda *et al*., 1960; Kittredge and Hughes, 1964; Kokke *et al*., 1979; Rao *et al*., 1984; Rao *et al*., 1985; Rao *et al*., 1989, Lakshmi *et al*., 2004). In this case marine invertebrates belonging to the phylum Cnidaria and order Zoantharia can produce several unique/novel molecules. Some examples are as follows, palytoxin which was isolated from *Palythoa* species in the Hawaiian islands which is one of the most potent known toxins, LD_{50}=15 mg/kg in mice (Moore and Scheuer, 1971), zoanthusterone as an ecdysteroid isolated from a *Zoanthus* species (Shigemori *et al*., 1999; Suksamrarn *et al*., 2002), zoanthamine alkaloids with effects on aggregation of human platelets isolated from *Zoanthus pulchellus* (Villar *et al*., 2003), peridininol with a promising pharmacological and biological activity (Parameswaran and Achuthankutty, 2005), prostaglandins, such as PGA2, isolated from *Palythoa kochii* stabilize microtubules in a manner similar to paclitaxel (Han *et al*., 2006), and two described cytotoxic lipidic α-amino acids (1a and 1b) from the zoanthid *Palythoa variabilis* (Wilke *et al*., 2009). Many of the reports on antimicrobial and antifungal activity of extracts of marine organisms were tested against human pathogens as potential useful drugs. Antimicrobial and antifungal activity was tested and found mainly in marine sponges and gorgonians. Little is known on the antimicrobial activity of other corals (Mayer *et al*., 2007). Only one study exists about antimicrobial activity of the marine zoanthid, *Palythoa caribaeorum* (Alencar *et al*., 2015). The aim of the current study was to characterize the antifungal and antimicrobial extracts activity of three species of order Zoantharia in Hengam Island (Persian Gulf). Antifungal and antibacterial activities of these marine zoanthids have never been reported. The present study evaluates crude extracts of three spices of Zoantharia against fungi and bacteria.

Materials and methods

Sampling of Zoantharia

Three species of zoanthids were collected by scuba diving at low tide in the Persian Gulf coasts of Hengam-Island, Hormozgan, Iran, which is

located at latitudes 26°40 55 "N and longitudes 55°52'17 "E. The zoanthid samples were cleaned and stored at -20ºC until use in extraction. The investigated species in this study are listed in Table 1 (identified by Noori Koupae *et al.*, 2014).

Extract preparation

Each zoanthid sample (100g wet weight) was chopped into small pieces, homogenized and allowed to stand in a dark chamber with a combination of methanol (2 v), dichloromethane (3 v), n-hexane (1 v) and ethyl acetate (1 v), extracted for 48 hours at room temperature and filtered (Touati *et al*., 2007). After that, each Zoanthid extract was evaporated at reduced pressure.

At first stage, methanol was added to the 1/2V of concentration. After that, 1/3 of methanol concentration was separated and the same volume of n-hexane was added to the methanol concentration, after shaking completely, n- hexane concentration was separated. In the second stage, dichloromethane was combined to the methanol concentration and again dichloromethane concentration was separated with separatory funnel. Then three type crude extracts were dried under rotary vacuum evaporator at 35ºC (Touati *et al*., 2007; Lakshmi *et al*., 2009), and screened against five human pathogen bacteria and three fungi.

Antifungal and Antimicrobial assays

Disk diffusion assay

At first, antimicrobial activity of the zoanthids extracts was assessed using the agar-disk diffusion method (Lakshmi, 1980). Antimicrobial activity was determined against certified strains of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Proteus vulgaris* ATCC 7829. Filter paper disks of 6.4 mm diameter were used. The bacterial cultures were first grown on nutrient agar plates at 37ºC for 24h to seed into the Mueller Hinton infusion agar for bacteria. Three to four discrete bacterial colonies with similar morphology were transferred into sterile distilled water and adjusted to the 0.5 McFarland turbidity standards. Inocula of the respective bacteria were seeded on Mueller Hinton agar. The sterile disks were impregnated with different extracts and then dried (1mg/disc). The sterile filter disks with 6.4 mm diameter were placed on inoculated agar medium and incubate at 37ºC for 24h. Disks of erythromycin (15µg/disc), ampicillin (10µg/disc), tetracycline (30µg/disc), gentamycin (10µg/disc) and amikacin (30µg/disc) were used as positive controls. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the disks. Solvent controls were conducted by adding 100 mL of methanol, dichloromethane, and n-hexane into the disks and placing them on the same agar plates (McClintock and Gauthier, 1992; Talaro and Talaro, 2002). All the antifungal activity was carried out against *Candida albicans* ATCC10231, *Microsporum gypseum* PTCC 5070,

and *Microsporum canis* PTCC 5069. The microorganisms were grown in sabouraud dextrose agar plates at 24ºC for 48h prior to seeding into the sabouraud dextrose agar. One or several discrete *Candia albicans*, *Microsporum canis* and *Microsporum gypseum* colonies with similar morphology were transferred in to sterile distilled water and adjusted to the 0.5 McFarland turbidity standard. The inocula of the respective fungi were seeded on sabouraud dextrose agar. The sterile disks were impregnated with different extracts and then dried (1mg/disc). The sterile filter disks with 6.4 mm diameter were placed in inoculated agar medium and incubate at 24ºC for 48h. Nystatin (100 U/disc) was used as positive control for *Candida albicans* and Clotrimazol 1% for *Microsporum canis* and *Microsporum gypseum*. The diameter (mm) of the growth inhibition halos caused by different extracts was measured. All the assays were carried out in triplicates.

Minimum inhibitory concentration (MIC) method

MIC method was used to assess the MIC of the zoanthids extracts which showed good activity (growth inhibition halos more than 9 mm) in agar disk diffusion method. To perform the classic broth dilution susceptibility test, for each organism eight tubes were chosen. Standard inocula of each organism (1.5×10^6 colony forming units/mL equal to 0.5 McFarland) were added to each tube. Nutrient broth was added as liquid medium for bacteria and sabouraud dextrose broth was added as liquid medium for fungi. The suspension of bacteria, yeast and dermatophyte fungi were adjusted in extracts to match the density of 0.5 McFarland. In every series of tubes, seventh and eighth tubes were used as control with solvents and sterile distillated water. Tubes were incubated at 37ºC for 24h for bacteria and 24ºC for 48h for yeast and fungi. Tubes were examined for turbidity, indicating growth of the microorganisms. The organisms will grow in the control tubes and in other tubes that do not contain enough antimicrobial agents to inhibit growth. The lowest concentration of the agent that inhibited growth of the organism, as detected by lack of visual turbidity was designated as the MIC (Baron and Finegold, 1990).

Minimum Bactericidal Concentration (MBC) or Minimum Fungicidal Concentration (MFC) method

The MBC or MFC was determined by sampling all macroscopically clear tubes and the first turbid tube in the series. 100 µL of sample was placed on a single antibiotic-free nutrient agar plate for bacteria and sabouraud dextrose agar plate for yeast and fungi. The MBC-determining lawned plates were incubated at 37ºC for 24h for bacteria and 24ºC for 48h for yeast and fungi. After the incubation periods, the lowest concentrations of the extract that did not produce any bacterial growth on the solid medium were regarded as the MBC or MFC values for the crude extract (Mims *et al*., 2003).

Artemia Lethality Test

The *Artemia* lethality test was conducted according to Carneiro *et al.* (2013). The *Artemia* cysts were hatched in artificial seawater at 28ºC under constant lighting and strong aeration. The cysts were incubated in a polyethylene cylindro-conical tube with 1g cysts per liter of artificial seawater. This hatching condition mimics *Artemia's* natural environment: shallow seawater. After a period of 48h, the aeration was halted, and the lighting was directed to the bottom of the hatching vessel. Based on their phototropic nature, nauplii migrate in the direction of the light to the bottom of the tube, while the unhatched cysts float. The nauplii were then collected and used for bioassays. 9 crude extracts were dissolved in artificial seawater at a concentration of 500µg/mL. The assay was performed boarding 24-well plates in which each well contained 10 *Artemia* nauplii in a final volume of 2 mL. Extract was added to the wells at final concentrations of 12.5, 25, 50, 100, 250 and 500µg/mL. The experiments were performed in triplicates, and negative control wells contained 2mL of artificial seawater with 10 *Artemia* nauplii. After 24h, dead nauplii in each well were counted. From these data, the percentage of dead nauplii at each concentration and the LC_{50} value were calculated by probit analysis as described by Finney (1971).

Results

The classification of each studied zoanthids species are noted in Table 1. The results of the agar-disk diffusion assay of the zoanthid extract (methanol, dichloromethane, and n-hexane) against five bacteria (two Gram-positive: *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and three Gram-negative: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC7829) and three fungi (*Candida albicans* ATCC10231, *Microsporum gypseum* PTCC 5070, *Microsporum canis* PTCC 5069) are listed in Table 2. Solvents did not have any effect on microorganisms. The results of five antibiotics on the bacteria nystatin on the yeast and clotrimazol 1% on the fungi are shown in Table 3.

Table 1: Classification of the marine zoanthids.

Serial No.	Order	Family	Species
Z-101	Zoantharia	Zoanthidae	*Zoanthus* spp.
Z-102	Zoantharia	Sphenopidae	*Palythoa tuberculosa*
Z-103	Zoantharia	Sphenopidae	*Palythoa mutuki*

Table 2 shows the results of *in vitro* antimicrobial activity against pathogenic bacteria and three fungi for three zoanthid species in 9 extracts. The results showed that 88.8% of the zoanthid extracts (8 from 9 extracts) presented activity against at least one bacterial strain and 66.6% were active against fungi. Against the gram-negative bacteria *Escherichia coli*

ATCC25922 four from 9 extracts (44.4%) displayed activity and three extracts (33.3%) showed good activity: methanol and n-hexane extract of *Palythoa mutuki* and n-hexane extracts of *Zoanthus* spp. Against the gram-negative bacteria *Pseudomonas aeruginosa* which was resistant to the tetracycline, ampicillin and erythromycin (antibiotics), two extracts (22.2%) showed weak and moderate activity: n-hexane extract of *Palythoa mutuki* and n-hexane extracts of *Zoanthus* spp. Against the gram-negative bacteria *Proteus vulgaris* ATCC7829, one from 9 extracts (11.1%), showed weak and moderate activity: dichloromethane extract of the *Palythoa mutuki.*

Table 2: Antimicrobial activity of different extracts from marine zoanthids.

Zoanthids	Extracts (1mg/disc)	Bacteria					Yeast	Fungi	
		B. subtilis	***S. aureus***	***Ps. aeruginosa***	***E. coli***	***P. vulgaris***	***C. albicans***	***M. canis***	***M. gypseum***
Zoanthus	M	R	9[a]	R	R	R	R	R	R
spp	H	R	10.8	7.4	9	R	R	R	12
	D	R	9.4	R	R	R	11.7	R	R
Palythoa	M	R	10	R	R	R	R	7.6	R
tuberculosa	H	R	9.7	R	8.7	R	R	R	R
	D	R	R	R	R	R	7.5	R	R
Palythoa	M	16.1	10.1	R	9	R	R	8.1	R
mutuki	H	R	R	8	9.6	R	R	R	R
	D	R	R	R	R	7.4	R	R	7.5

Table 3: Antimicrobial activity of different antibiotics, Nystatin and Clotrimazol.

Antibiotic and Antifungal	Bacteria					Yeast	Fungi	
	B. subtilis	***S. aureus***	***Ps. aeruginosa***	***E. coli***	***P. vulgaris***	***C. albicans***	***M. canis***	***M. gypseum***
Erythromycin(15µg/disc)	19.6	31	R	10.4	N/A	--	--	--
Ampicillin (10µg/disc)	8.5	32.4	R	10	11.5	--	--	--
Tetracycline (30µg/disc)	N/A	19	R	10	10.5	--	--	--
Gentamycin (10µg/disc)	N/A	-	16.7	5	21.4	--	--	--
Amikacin (30µg/disc)	10.8	28	23.4	20	22	--	--	--
Nystatin (100 U/disc)	--	--	--	--	--	23.4	12.2	10.1
Clotrimazol 1%	--	--	--	--	--	--	25.5	22

M: methanol extract; H: n-hexane extracts; D: dichloromethane extracts.

[a] Average of the microbial inhibition halos in millimeters. R: resistant. Inhibition halos in millimeters caused by five different antibiotics for bacteria and nystatin for yeast and fungi and clotrimazol for *M. canis*, *M. gypseum* as control, where (R) is resistant, (N/A) is not applicable.

Against the gram-positive bacteria *Staphylococcus aureus*, six from 9 extracts (66.6%) displayed activity and six extracts (66.6%) showed good activity: methanol, dichloromethane and n-hexane extracts of *Zoanthus* spp, methanol and n-hexane extracts of *Palythoa tuberculosa* and methanol extract of *Palythoa mutuki.* Again against the gram-positive bacteria *Bacillus subtilis* one from 9 extracts (11.1%) showed activity displayed interesting activity: methanol extract of *Palythoa mutuki.* According to Table 2, from 9 zoanthid extracts analyzed 2 extracts showed activity against yeast

Candida albicans (22.2%) and one extract (11/1%) displayed interesting activity: dichloromethane extract of *Zoanthus* spp. From 9 zoanthid extracts analyzed, four extracts showed activity against fungi *Microsporum gypseum* PTCC 5070, *Microsporum canis* PTCC 5069 (44.4%) and one extract (11/1%) displayed good activity: n-hexane extract of *Zoanthus* spp. against *Microsporum gypseum* PTCC 5070.

The results of the MICs and MBC or MFC are shown in Tables 4 and 5.

Table 4: MIC activity of the zoanthids extracts.

Microorganism	Zoanthids extracts (µg/mL)								
	***Zoanthus* spp.**			***Palythoa tuberculosa***			***Palythoa mutuki***		
	M	**H**	**D**	**M**	**H**	**D**	**M**	**H**	**D**
Erythromycin(15µg/disc)	--	--	--	--	--	--	1000	--	--
Ampicillin (10µg/disc)	1000	500	500	500	500	--	500	--	--
Tetracycline (30µg/disc)	--	1000	--	--	--	--	500	1000	--
Gentamycin (10µg/disc)	--	--	125	--	--	--	--	--	--
Amikacin (30µg/disc)	--	1000	--	--	--	--	--	--	--

M: methanol extract; D: dichloromethane extract; H: n-hexane extract.

Table 5: MBC or MFC activity of zoanthids extract.

Microorganism	Zoanthids extracts (µg/mL)								
	***Zoanthus* spp.**			***Palythoa tuberculosa***			***Palythoa mutuki***		
	M	**H**	**D**	**M**	**H**	**D**	**M**	**H**	**D**
B. subtilis	--	--	--	--	--	--	*	--	--
S. aureus	*	*	1000	*	*	--	*	--	--
E. coli	--	2000	--	--	--	--	*	*	--
Candida albicans	--	--	250	--	--	--	--	--	--
M. gypseum	--	2000	--	--	--	--	--	--	--

M: methanol extract (polar); D: dichloromethane extract (semi polar); H: n-hexane extract (non-polar), *: not determined.

The methanol, dichloromethane, n-hexane extracts at all tested concentrations exhibited different levels of lethality against *Artemia sp*. nauplii. LC_{50} values are shown in Table 6. The dichloromethane extract of *P. mutuki* was the most toxic with an LC_{50} of 31µg/mL, whereas the least toxic was the dichloromethane extract of *P. tuberculosa* with an LC_{50} of 345µg/mL. The dichloromethane extract of *Zoanthus* spp. with LC_{50} of 181 and MFC 125µg/mL was the applicable extract for *Candida albicans* therapy.

Table 6: Cytotoxic activity of the marine Zoanthids.

Zoanthids	**Crude extract**	**LC_{50} 24 h**
Zoanthus spp.	methanol	---
	n-hexane	125 µg/mL
	dichloromethane	181 µg/mL
Palythoa tuberculosa	methanol	250 µg/mL
	n-hexane	62 µg/mL
	dichloromethane	345 µg/mL
Palythoa mutuki	methanol	---
	n-hexane	58 µg/mL
	dichloromethane	31 µg/mL

Discussion

The secondary metabolites of zoanthids are unique natural products (Rocha,

2013). Antifilarial, antioxidant, hemolytic, anti-inflammatory and cytotoxic activities have been observed for various zoanthids species (Mayer *et al*., 2007; Alencar *et al*., 2015). Several alkaloids, including zoanthenol, zoanthamine, norzoanthamine, oxyzoanthamine, epioxyzoanthamine, zoanthaminone and norzoanthaminone have been isolated from a marine zoanthid (Daranas *et al*., 1999). In the present research methanol, n-hexane and dichloromethane extracts of *Zoanthus* ssp. had antibacterial activity (growth inhibition halos of more than 9 mm) against *S. aureu*s with MIC of 1000, 500 and 500µg/mL respectively. The dichloromethane extract of *Zoanthus* ssp. exhibited antifungal activity (growth inhibition halos of more than 9 mm) against *Candida albicans* with MIC of 125µg/mL and also, n-hexane extract of *Zoanthus* ssp. had antibacterial activity (growth inhibition halos of more than 9 mm) against *E. coli* and *M. gypseum* with MIC of 500 and 1000µg/mL respectively. *Palythoa tuberculosa* extracts (methanol and n-hexane) had antibacterial activity (growth inhibition halos of more than 9 mm) against *Staphylococcus aureu*s with MIC of 500µg/mL. The methanol extracts of *Palythoa mutuki* had antibacterial activity (growth inhibition halos of more than 9 mm) against *B. subtilis, S. aureu*s and *E. coli* with MIC of 1000, 500 and 500µg/mL, respectively. Also the n-hexane extract of *Palythoa mutuki* had antibacterial activity (growth inhibition halos of more than 9 mm) against *E. coli* with MIC of 1000µg/mL. There is only one study about antimicrobial activity of zoanthids that resulted in, 70% EtOH crude extract and DMC, EtOAc and Aq fractions at 100µg/mL of *Palythoa caribaeorum* which did not show any antimicrobial activity against the tested bacteria (Alencar *et al*., 2015). Previous reports suggested that variability of natural extracts and their antimicrobial activity can be affected by parameters such as species, season and micro geography, apart from extraction methods (Muricy *et al*., 1993, Mayer *et al*., 2007). The present study is the first report on antimicrobial and anti-fungal efficacy of marine zoanthids. The dichloromethane extracts of *Palythoa tuberculosa and Palythoa mutuki* did not show antibacterial activity against both gram negative and gram positive bacteria while the n-hexane and methanol extract of *Palythoa tuberculosa*, the methanol extract of *Palythoa mutuki* and n-hexane and dichloromethane extract of *Zoanthus* spp. showed highest activity against *S. aureus*. This study is the first report of compact of *S. aureus* with pointed extracts and so, there is no explanation about their mechanism. Ecologically, *S. aureus* is resistant in dry environment and in evolutionary processes it is adapted with substance that exist in its environment and its resistant genes in continues contact have highest chance for expression and diversity. It is most likely that the externality of two groups can affect the expression chance of resistant genes in *S. aureus*. Gram

negative bacteria tend to adapt to aquatic environment and have been able to adapt with marine compounds in evolutionary process. The dichloromethane extract of *Zoanthus* spp. had effective anti-fungi activity against *Candida albicans* yeast in MIC 125µg/mL (Table 4). In efficiency of defense metabolites, it is clearly demonstrated that existence of receptors have important role in their performance. Species of *Candida* genus living in ocean may be in relation to several species of zoanthids but, there is no report of presence of any species of candida in *Zoanthus* (Kutty and Philip, 2008; Pagani *et al*., 2016). Relationship between marine candida and *Palythoa* genus is reported before (Pagani *et al*., 2016) so secondary metabolites of *Palythoa* genus do not have antifungal activity against candida. Therefore, probably candida is intrinsically resistant to secondary metabolites of *Palythoa* or these secondary metabolites don't have antifungal activity. Our results confirmed this phenomenon. However, there is no relationship between candida and *Zoanthus* genus (Pagani *et al*., 2016), that most likely represents antifungal activity of *Zoanthus* metabolites against candida. Our results confirmed this also. As a result, these metabolites probably act as defense for candida.

The tested concentrations of crude extracts (methanol, dichloromethane and n hexane) in 3 zoanthid species exhibited different levels of lethality against *Artemia* sp. nauplii. LC_{50} values are shown in Table 6. The dichloromethane extract of *Palythoa mutuki* was the most toxic with an LC_{50} of 31µg/mL, whereas in dichloromethane extract of *Palythoa tuberculosa* the minimal toxic amount was observed with LC_{50} of 345µg/mL. In *Palythoa mutuki and Palythoa tuberculosa methanol*, dichloromethane and n-hexane extracts toxicity amount (LC_{50}) were lower than the MIC amount. So in vision of therapeutic they are not useful. But in *Zoanthus* spp. dichloromethane extract amount of LC_{50} was 181µg/mL and was higher than the MIC amount (125µg/mL). Therefore, this extract can be a candidate for candidiasis therapy that has been identified as prevalent fungal infection causing deaths worldwide. This study was the first report of antimicrobial and anti-fungal effects in *Zoanthus* spp. dichloromethane extract which were investigated and showed promising results. Based on findings of Pagani *et al*. (2016) and the present study, dichloromethane extract of *Zoanthus* species is effective, especially against *Candida* species, in marine and human pathogens so this *Zoanthus* secondary metabolite can have promising use in the process of developing a candidiasis drug. Usage of purified products of dichloromethane extract can have the effective activity against candidiasis. This study introduced a new source of marine organism that can be able to produce anti-fungal compounds.

Marine organisms that were collected from the Persian Gulf showed a potential to have biological activities.

This is the first anti-fungal report of the secondary metabolite of marine zoanthids that improves our knowledge about marine metabolites. Persian Gulf ecosystem has a unique environment that its creatures are not well investigated. So the investigation of its products can be useful and applicable, especially in medical cases.

There is no previous report about three type extracts (polar-non polar and semi polar) from natural zoanthids. The antimicrobial and anti-fungal activity of zoanthid has never been reported. The dichloromethane extract of *Zoanthus* ssp. had anti-fungal activity against *Candida albicans* and it could be introduced as a new source of anti-fungal compound for candidiasis disease.

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