Bactericidal and fungicidal activities of different crude extracts of *Gelliodes carnosa* (sponge, Persian Gulf)

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Abstract

Marine sponges which are known to own multiple functional properties have created significant interest among the researchers due to their biological activities and impending application in different industries .The aim of this study was to obtained bioactive components of sponges. *Gelliodes carnosa* sponge was collected from Nay Band Bay (Persian Gulf waters) and antimicrobial activities of crude extracts were explored by calculation of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) in media supplement with different concentrations of extract solutions. Seven extracts of sponge with solvents of different polarity (E1: Ethanol, E2: Methanol, E3: Acetone, E4: Ethyl acetate, E5: Chloroform, E6: Mixed (Ethanol: Ethyl acetate: Methanol 1:2:1), E7: Distilled water) were evaluated through disc diffusion assay. Different extracts were inhibited the growth of bacteria (70%) more frequently compared to fungal strains (26%). Gram-negative bacteria were more sensitive (72%) to many extract compared to Gram-positive bacteria (65%). Considerable antibacterial activity was exhibited by E6 against *Bacillus subtilis* (MIC: 203 µg/ml), *Klebsiella pneumonia* (MIC: 203 µg/ml), *Escherichia coli* (MIC: 407 µg/ml) and *Fusarium solani* (MIC: 500 µg/ml). Strong antifungal activity was obtained by E4 against *Fusarium* sp.$_2$, *Fusarium* sp.$_1$, *F. solani* and *Saprolegnia parasitica* (MIC: 500µg/ml). This is the first report of antimicrobial and antifungal activities of *G. carnosa* extracts.

Key words: Marine sponges, *Gelliodes carnosa,* Secondary metabolites, Persian Gulf, Iran

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Introduction

The oceans are the source of large groups of biological compounds that are mainly accumulated in invertebrates such as sponges (Ramasamy and Murugan, 2005). Among marine organisms, sessile invertebrates especially sponges are considered as interesting target to screen antimicrobial substances for many reasons (Touati *et al.,* 2007; Sonia *et al.,* 2008). So far approximately 7000 natural products have been isolated from marine organisms, 33% of these compounds are extracted from sponges (Galeano and Martinez, 2007; Periyasamy *et al.,* 2012; Amade *et al*., 1982). Since there has been a growing interest on biological activity of marine sponges, metabolites isolated from different sponges are extensively reviewed by several authors. Southern Reefs of Persian Gulf has a high density of shallow water sponges, which can be the source of new antimicrobial agents. *G. carnosa* belongs to Phylum Porifera, Class Demospongia, Order Haplosclerida and Family Niphatidae (Fig. 1). The sponge class Demospongia is known as a source of different types of secondary metabolites among marine invertebrates (Newbold *et al*., 1999; Selvin and Lipton, 2004). Nonetheless, there are many studies done on screening and isolation of biological compounds from *G. carnosa*. In this study antimicrobial and antifungal activities of crude extracts from *G. carnosa* were described.

Figure 1: *Gelliodes carnosa* from the Persian Gulf.

Materials and Methods

Sampling, preparation and identification of sponge

Fresh samples of *G. carnosa* were collected from Nay Band Bay (between latitudes 27° 9′ and 27° 28′ N and longitudes 52° 27′ and 52° 52′ E) in the Persian Gulf (Fig. 2). Samples were kept in sealed plastic packs in ice boxes and after freezing at -20 °C transferred to laboratory. Taxonomic identification was carried out according to standard method (Hooper and Museum, 2000).

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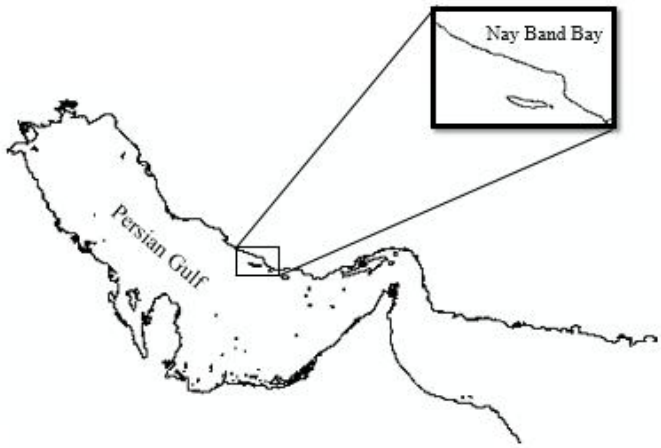

Figure 2: Nay Band Bay, Bushehr, Persian Gulf (Iranian coastal waters).

Preparation of crude extracts

10 g of sponge was cut into small pieces, homogenized and extracted with different solvents: ethanol (3×150 ml), methanol (3×150 ml), acetone (3×150 ml), ethyl acetate (3×150 ml), chloroform (3×150 ml), mixed solvents (ethanol: ethyl acetate: methanol 1:2:1) (3×150 ml) and Distilled water (3×150 ml). Each extraction was developed by mechanical shaking at room temperature. The extracts were filtered with Whatman filtere paper No. 1 and concentrated with rotary evaporator (IKA-Werke©) (McClintock and Gauthier, 1992; Sionov *et al.,* 2005; Sepi *et al*., 2010;).

Testing on microorganisms

The obtained extracts were tested on two gram-positive bacteria (*Staphylococcus aureus* PTCC 1189, *Bacillus subtilis* PTCC 1156), five gramnegative bacteria (*Escherichia coli* PTCC 1763, *Pseudomonas aeruginosa* PTCC 1310, *Proteus mirabilis* PTCC 1076, *Serratia marcescens* and *Klebsiella pneumonia*) and six pathogenic fungi (*Candida albicans* PTCC 5027, *Aspergillus niger* PTCC 5223, *F. solani* PTCC 5248, *Fusarium sp.1, Fusarium sp.2, S. parasitica* and *Saprolegnia sp*.). These microorganisms were obtained from Iranian Research Organization for Science and Technology (IROST).

Antimicrobial assay

Antimicrobial assay was carried out in vitro by disc diffusion technique (McCaffrey and Endean, 1985; Selvin and Lipton, 2004). Whatman filter paper discs No. 1 with 6mm diameter (Padtan Teb Co., Iran) were impregnated with known amounts of test samples of the extracts and positive control contained Amoxicillin (25µg/disc) for bacteria and Nystatin (30µg/disc) for fungi (Padtan Teb Co., Iran). In each extract disc of different solvents were used as negative control. The entire assay was carried out in triplicate.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)

MIC were determined by the following procedures. Between 0.2- 4 mg/ml of the

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extract concentrations of *G. carnosa* were introduced into the test tubes. Standard inoculum of each organism (10^6cell/ml) was added to each tube. Nutrient Broth (Merck©, Germany) was added as liquid medium for bacteria and Potato Dextrose Broth (Merck©, Germany) was added as liquid medium for fungi. Tubes were incubated for 24 h at 37°C for bacteria and for 48 h at 24°C for fungi. The lowest concentration of the extracts that inhibits growth of the organisms is designated the MIC (Natarajan *et al*., 2010; Ananthan *et al.,* 2011). After culturing the test organisms separately, the broth was inoculated on to freshly prepared agar plates to assay the bactericidal effects. The cultures inoculated were similar to the above mentioned cultures. The lowest concentration in which there was no bacterial and fungal growth regarded as the MBC value (Chellaram *et al*., 2009; Elayaraha, *et al*., 2010;).

Statistical Analysis

SPSS 19 statistical software program was used for all the analyses. The Kolmogorov-Smirnov test results revealed that the considered variables did not have a normal distribution. Nevertheless, the logarithm transformed data of the variable was used in a two-way analysis of variance (ANOVA). The two way ANOVA was used to study the anti-bacterial and anti-fungal effects of different extracts.

Results

Results showed that the antifungal response was generally weak in comparison with antibacterial activity. Gram-negative bacteria were more sensitive (72%) to many extracts compared to Gram-positive bacteria (65%). Negative control did not shown any effect on microorganisms. Maximum inhibition zone (17.5 mm) was observed against *B. subtilis* in the mixed extracts (E6) and minimum inhibition zone (7.5) was observed against *E. coli* (E5). Among bacteria *S. marcescens* and *P. aeruginosa* presented maximum and minimum resistance against all of the extracts, respectively. No inhibitory effect was observed on fungal strain, except in mixed (E6) and ethyl acetate extracts (E4).

Table 1: Inhibition of bacterial growth by different crude extracts of sponge *G. carnosa* (Mean±SD).

Extract form	E1	E2	E3	E4	E5	E6	E7	A
Staphylococcus aureus	R	13.5±0.5	10.5±0.86	11±0.2/51	R	14.5±.03	R	17
Bacillus subtilis	12±0.65	13.5±1.47	9.5±0.5	9.5±0.86	R	17.5±1.87	R	14
Escherichia coli	8±1.22	11±0.5	R	11.5±0.5	7.5±0.5	16.5±0.7	R	18
Pseudomonas aeruginosa	10±0.95	8.5±0.3	8±1.32	11.5±1.32	8±0.4	12.5±0.7	R	13
Klebsiella pneumonia	R	8.5±0.62	7.5±1.04	9±0.7	9.5±0.5	17±1.8	R	16
Proteus mirabilis	7.5±0.7	12±0.7	10±1.32	8.5±1.04	13±0.34	12±1.05	R	15
Serratia marcescens	R	8.5±0.2	R	8±0.2	R	13±0.5	R	11

E1: ethanol, E2: methanol, E3: acetone, E4: ethyl acetate, E5: chloroform, E6 (Mixed): (ethanol: ethyl acetate: methanol 1:2:1), E7: distilled water, A: Amoxicillin. R: Resistant
Average of the bacterial inhibition halos in millimeters.
The zones diameter with discs is 6mm

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Fusarium sp.2 with halos of 25 mm for ethyl acetate extract (E4) (MIC= 500, MBC= 1000 µg/ml) and 9.5 mm for mixed extracts (E6) had maximum and minimum inhibition zone. The range of MIC and MBC varied between 203-2800 µg/ml and between 404-4000 µg/ml, respectively.

Table 2: Inhibition of fungal growth by different crude extracts of sponge *G. carnosa* (Mean±SD).

	E1	E2	E3	E4	E5	E6	E7	N
Candida albicans	R	R	R	14±0.86	R	12±1.5	R	20
Aspergillus niger	R	R	R	15±0.5	R	10.5±1.32	R	10.5
Saprolegnia parasitica	R	R	R	16±1.25	R	12±1	R	15.8
Fusarium solani	R	R	R	22±3.6	R	15±1.3	R	16
Fusarium sp.1	R	R	R	16±2.29	R	R	R	18
Fusarium sp.2	R	R	R	25±1	R	9.5±0.86	R	17
Saprolegnia sp.	R	R	R	14±1.73	R	11±1.32	R	18

E1: ethanol, E2: methanol, E3: acetone, E4: ethyl acetate, E5: chloroform, E6 (Mixed): (ethanol: ethyl acetate: methanol 1:2:1), E7: distilled water, N: Nystatin. R: Resistant

Average of the bacterial inhibition halos in millimeters

The zones diameter with discs is 6mm

Table 3: MIC and MBC values (µg/ml) of crude extracts of *G. carnosa* on test bacteria.

E		E1	E2	E3	E4	E5	E6
Staphylococcus aureus	MIC	-	453	1400	1400	-	407
*(*PTCC 1189)	MBC	-	906	2800	2800	-	815
Bacillus subtilis	MIC	1137	453	2800	2800	-	203
(PTCC 1156)	MBC	2275	906	2800	2800	-	404
Escherichia coli	MIC	-	906	-	-	-	407
(PTCC 1763)	MBC	-	1812	-	-	-	815
Pseudomonas aeruginosa (PTCC	MIC	1137	-	2800	2800	-	1631
1310)	MBC	2275	-	2800	2800	-	3262
Proteus mirabilis	MIC	-	906	1400	1400	1000	1631
(PTCC 1076)	MBC	-	1812	2800	2800	2000	3262
Serratia marcescens	MIC	-	-	-	-	-	1631
	MBC	-	-	-	-	-	3262
Klebsiella pneumonia	MIC	-	-	-	-	2000	203
	MBC	-	-	-	-	4000	407

E1: ethanol, E2: methanol, E3: acetone, E4: ethyl acetate, E5: chloroform, E6: (ethanol: ethyl acetate: methanol 1:2:1),

- : no activity

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Table 4: MIC and MBC values (µg/ml) of different extracts of *G. carnosa* on test fungi

Fungi/Extract forms		E1	E2	E3	E4	E5	E6
Candida albicans	MIC	-	-	-	500	-	815
	MBC	-	-	-	1000	-	1631
Aspergillus niger	MIC	-	-	-	500	-	1631
	MBC	-	-	-	1000	-	3262
Saprolegnia parasitica	MIC	-	-	-	500	-	815
	MBC	-	-	-	1000	-	1631
Fusarium solani	MIC	-	-	-	500	-	407
	MBC	-	-	-	1000	-	815
Fusarium sp.1	MIC	-	-	-	500	-	1631
	MBC	-	-	-	1000	-	3262
Fusarium sp.2	MIC	-	-	-	500	-	-
	MBC	-	-	-	1000	-	-
Saprolegnia sp.	MIC	-	-	-	500	-	815
	MBC	-	-	-	1000	-	1631

E1: ethanol, E2: methanol, E3: acetone, E4: ethyl acetate, E5: chloroform, E6: (ethanol: ethyl acetate: methanol 1:2:1), - : no activity

Discussion

The largest numbers of secondary metabolites among marine organisms are isolated from sponges and have been the primary source of biologically active molecules (Belarbi *et al*., 2003). Authors recorded chemical compounds which are active against microorganisms from a variety of marine sponges (Sonia *et al*., 2008). Previous studies on marine sponges and their secondary metabolites displayed various levels of biological activities (Bulter, 2004; Tadesse *et al*., 2008). A number of sponges have been reported to possess antimicrobial activity (Bergquist and Bedford, 1978; McClintock and Gauthier, 1992; Concepcion *et al*., 1994; Purushottama *et al*., 2009; Kristina *et al*., 2010; Darah *et al*., 2011; Ravikumar *et al.,* 2011). Secondary metabolites of sponges play important role as defenses against some biotic challenges (Newbold *et al.,* 1999). Nevertheless in the present investigation crude extracts of *G. carnosa* were screened against some pathogenic bacteria and fungi that showed a higher degree of inhibition confined to mixed (E_6) and methanol (E_2) extracts. Ramasamy *et al*., (2005) used six solvents for crude extracts of two bivalves that their activity concord with our results with the exception of results of the bioactivity of water extracts. In the study conducted by Touati *et al*. (2007) antimicrobial activity of extracts was according to solvent polarity. Research report of Galeano and Martinez (2007) showed good activity of methanol and chloroform extracts, while hexane extract had weak activity against bacteria. Safaeian *et al.,* (2009) reported small differences between activity of polar (methanol) and semi polar (ethyl acetate) extracts. One of the first and most important matters in the antimicrobial study is reports of weak antifungal effects (Qaralleh *et al*., 2010; Sionov *et al.,* 2005; Chellaram *et al*., 2009). Weak antifungal activity could be due to the low therapeutic efficiency on strong wall structure of the fungal cells that consist of chitin, α and β-glucan (Concepcion *et al.,* 1994). This matter confirmed with result of (Amade *et*

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al., 1982; McCaffrey and Endean, 1985; McClintock and Gauthier, 1992) they reported that sponge extraction against *A. niger* and *C. albicans* have weak activity. Also Touati *et al*. (2007) reported that ethyl acetate extract of sponge showed weak antifungal activity.

In our study gram negative bacteria were more sensitive (72%) to crude extracts compared to gram positive bacteria (65%) being similar to other studies (Amade *et al*., 1982; Bergquist and Bedford, 1985; McCaffrey and Endean, 1985; McClintock and Gauthier, 1992). But also previous reports showed gram positive bacteria were more sensitive compared to gram negative bacteria (Muricy *et al*., 1993;Tadesse *et al*., 2008). Safaeian *et al*. (2009) showed that sponge extracts have equal effect on gram negative and gram positive bacteria. Smaller susceptibility of gram negative bacteria against the extracts could be due to the presence of an outer membrane surrounding the cell walls, which can hinder the access of active compounds through its lipopolysaccharide layer, proteins and phospholipids that serve as outer membrane barrier (Pangan *et al*., 2008; Lakshmi *et al*., 2010). The differences between the present study and other researches were due to species, environmental and ecological differences (Touati *et al*., 2007), extracting capacity of the solvents and extract compounds, chemical concentration and composition among species (Periyasamy *et al.,* 2012). It has been noticed that there may be the bioactive compounds that lose their bioactivity once mixed, which is called antagonistic effect. It is possible that the crude extracts did not show good bioactivity as a result of antagonistic effect (McClintock and Gauthier, 1992; Xue *et al*., 2004). The obtained MIC (MIC=203-1361 µg/ml and MBC= 404-3262 µg/ml) was better than some of the earlier studies (Abigail *et al*., 2007 (800 µg/ml); Darah *et al*., 2011 (500 µg/ml); Natarajan *et al*., 2010 (700 µg/ml). But in some studies (Chellaram *et al*., 2009; Elayaraja *et al*., 2010; Qaralleh *et al.,* 2010; Lakshmi *et al*., 2010; Ananthan et al., 2011) the amount of MIC and MBC were better compared to the obtained results of this study.

The results of this study showed that the marine sponge, *G. carnosa*, has the studied activity using in vitro model system. Persian Gulf is a potential source of a great variety of marine sponges worthy of further research. Furthermore this study is the first report of antimicrobial and antifungal activities of crude extracts of a Persian Gulf sponge.

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