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ORIGINAL RESEARCH

The antibacterial and antibiofilm activity of sea anemone (*Stichodactyla haddoni*) against antibiotic-resistant bacteria and characterization of bioactive metabolites

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Abstract Sea anemones produce many biologically active compounds including neurotoxins, pore-forming toxins, phospholipases and proteinase inhibitors. The Persian Gulf is an unexplored environment and maybe a rich source of marine natural products. The aim of this study is screening and identification of bioactive metabolites from *Stichodactyla haddoni* (Haddon's sea anemone) collected at the Persian Gulf. The crude extract of the sea anemone (tentacle, disc and total body) was obtained by methanol solvent. The antibacterial assays were carried out by the disc diffusion method. The antibiofilm activity (biofilm formation, biofilm destruction and reduction of metabolic activity) of the sea anemone extracts was evaluated by microtiter plate method. The bioactive compounds were identified by GC–MS analysis. Data showed that the best antibacterial effect (relate to *P. aeruginosa*) is obtained from extracts of "total body" section. Values of minimum inhibitory concentration and minimum bactericidal concentration show that the maximum antibacterial activity takes place at 10–20 mg/ml concentration. Three parts of sea anemone exhibit different inhibition against biofilm of bacteria, in particular, inhibition of biofilm observed by the tentacle, disc and total body against *P. aeruginosa*, *K. pneumonia* and *A. baumannii*, respectively. Biofilm of *P. aeruginosa* was the most sensitive and the biofilm of *B. cereus* was the most resistant structure between all pathogenic bacteria. The best reduction in the metabolic activity was observed in *P. aeruginosa* and *K. pneumonia* among tested bacteria. Aliphatic compounds were predominant bioactive metabolites in this sea anemone. The marine animal and especially sea anemone produce useful bioactive compounds that can be used to prevent bacterial biofilm; application of bioactive materials, reported in this study, can be proposed for future studies.

Keywords Antimicrobial activity · Biofilm · *Stichodactyla haddoni* · Bioactive metabolites · Aliphatic compounds

Introduction

The marine environment is an exceptional reservoir of natural products, many of which exhibit structural features, not found in terrestrial natural products. Marine invertebrates, especially sedentary sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs (Prakash et al. 2007; Mohammadi et al. 2019).

Cnidaria is simple animals with radial symmetry that contain two layers of cells, ectoderm, and endoderm. Mesoglea, a non-cellular matrix, is present between the two layers. Cnidaria feeding success relies on the

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presence of the specialized poisonous cell, the nematocysts. These organisms have specialized subcellular organelles called cnidae with several structures and functions. Cnidae can be classified into three types: nematocysts, spirocysts, and ptychocysts. Nematocysts deliver the venom through the skin, whereas spirocysts are adhesive and ptychocysts are involved in protection (Frazão et al. 2012; Masoumipour et al. 2018).

The Cnidaria is a large, diverse, and ecologically important phylum. It includes about 9400 species, of which 68% are members of the class Anthozoa. Many anthozoans reproduce through both asexual and sexual means (Schlesinger et al. 2010). In common with all animals, anthozoans need to protect themselves against the lethal or debilitating consequences of microbial or parasitic invasion (Hutton and Smith 1996).

The microbiome of certain marine invertebrates may represent a remarkable proportion of the holobiont biomass, with anthozoan cnidarians being no exception and hosting abundant and diverse communities of bacteria. Certain species able to secrete mucus may reach microbial concentrations up to 1000-fold higher than those observed in seawater. While microbial communities associated with tropical reef-building corals are already starting to be unraveled, those colonizing other groups of anthozoans are still largely unknown (Rocha et al. 2014).

Sea anemones, like other coelenterates, produce many biologically active polypeptides and proteins, including neurotoxins, pore-forming toxins (or cytolysins), phospholipases and proteinase inhibitors (Thangaraj and Bragadeeswaran 2012; Sepehri et al. 2016). Therefore, they have evolved the ability to synthesize toxic compounds obtained from marine microorganisms. These compounds help them deter predators, keep a competitor at bay or paralyze their prey (Ghosh et al. 2011). Substances exhibit a wide diversity of biological activities such as hemolysis, cytotoxicity, cardiotropic activity, membrane depolarization and the block of potassium channels (Gunasundari et al. 2013; Hamayeli et al. 2016).

Sea anemone shows a very good symbiotic partnership with the marine ornamental fishes, especially with clowns. Some of these fish species possess considerable resistance to the sea anemone but appear to be mainly protected by a mucous coat which prevents discharge of the nematocyst (Mohsenipour and Hassanshahian 2015).

As a consequence of increasing demand for the biodiversity in the screening programs seeking therapeutic drugs from natural products, there is now a greater interest in marine organism. There is a copious number of works pertaining to the antibacterial agents from marine bacteria, microalgae, seaweeds, sponges, mollusks and ascidians (John et al. 2015; Mashhadi et al. 2016).

These organisms can attach to living and non-living surfaces like medical devices which include urinary, venous, and arterial catheters, shunts, heart valves and tubes (Prasanna and Doble 2008). According to a publication by the National Institutes of Health, more than 80% of all infections involve biofilms (Afreenish et al. 2011; Masoumipour et al. 2018).

Microbial adhesion to surfaces and the consequent biofilm formation have been documented in many different environments. Biofilms constitute a protected mode of growth that allows microorganisms to survival in hostile environments, being their physiology and behavior significantly different from their planktonic counterparts. They are difficult to eradicate due to their resistant phenotype (Simões et al. 2010).

There are several advantages for microorganisms to form biofilms. They provide enclosed surface space which is occupied and can provide a degree of stability in the growth environment. They might have catalytic functions through the localizing cells in close proximity. Microbial biofilms have been associated with a lot of persistent infections which respond poorly to antibiotic therapy and can withstand host immune response (Atray and Atray 2015). Microbial resistance is a natural biological response of microbes to selective pressure, such as weather conditions, food, oxygen or water availability, or the presence of an antimicrobial drug (Soares et al. 2012). The familiar mechanisms of antibiotic resistance, such as efflux pumps, modifying enzymes, and target mutations, do not seem to be responsible for the protection of bacteria in a biofilm (Stewart and Costerton 2001).

Correcting a resistance problem, then, requires both improved management of antibiotic use and restoration of the environmental bacteria susceptible to these drugs (Odonkor and Addo 2011).

Sea anemones are evolved with rich sources of bioactive metabolites, which could be used for novel antimicrobial drugs, many of which exhibit structural features, not found in terrestrial natural products. Some natural products were extracted from marine organisms, but less than 1% has been examined so far for pharmacological activity (Subramanian et al. 2011). The Persian Gulf is an unexplored environment and maybe a rich source of marine natural products. In this optic, there are few reports on the bioactivity of the

Iranian *Stichodactyla haddoni* (Haddon's sea anemone). The aim of this investigation is screening and identification of bioactive metabolites from *S. haddoni* that collected at the Persian Gulf. Also, the antibacterial and antibiofilm activity of this sea anemone extracts against some pathogenic bacteria were evaluated.

Materials and methods

Sea anemone collection and identification

The sea anemone organisms were collected, in November 2015, at Gheshm Islands (N 26.994086, E 56.194300, Persian Gulf, Iran). All organisms were gathered from a depth of 1–5 m. Organisms have transported alive in seawater to a laboratory and maintained at 4 °C in a refrigerator before extraction. The characterization of an exemplar of the sea anemone was identified according to the protocol of Raghunathan et al. (2014). Identification revealed that organisms collected are Stichodactyla haddoni (Haddon's sea anemone), a species of sea anemone belonging to the Stichodactylidae family native from the Indo-Pacific area. The macroscopic image of this sea anemone is shown in Fig. (1).

Extraction of bioactive metabolites from sea anemone

Collected of S. *haddoni* were separated into two sections: tentacle and disc. Also, total body (mixture of whole body of this marine animal) was extracted. For extraction of biomolecules, separate sections (of sea anemones) were freeze-dried. Each part of sea anemone was immersed into a polar solution with methanol (MeOH) for 48 h. Then, the obtained extracts were filtrated and concentrated. Each extract passed through Whatman No. 1 filter paper. The filtrates were placed into an incubator at 40 ± 1 °C for 24 h to remove residual of a solvent. The concentrated extracts were applied for antimicrobial activity against pathogenic bacteria (Eash-Loucks and Fautin 2012).

Bacteria

Six antibiotic-resistant pathogenic bacteria were used in this study: *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 1611, *Bacillus cereus* ATCC 1298, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 1189. All bacterial strains, used in this study, were obtained from American Type Culture Collection, USA.

Disk diffusion method

Antibacterial activity of the extract (of three sections of sea anemone) was tested by the standard disc diffusion method. The turbidity of bacteria culture reached 0.5 Mac-Farland standards (10^8 CFU mL^{-1}); then, one

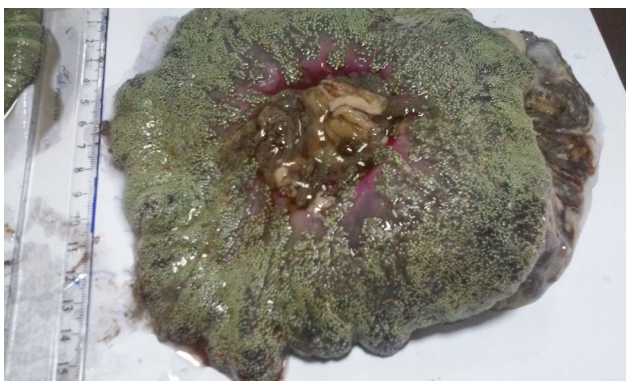

Fig. 1 Macroscopic pictures of sea anemone *S. haddoni* studied in this research

milliliter of this inoculum was transferred into Mueller-Hinton Agar (MHA) plates by a spread plate method using a sterile cotton swab and allowed to stay for 60 s. A concentration of each extract (0.15 mg mL^{-1}) was placed in a sterile filter paper discs (∅, 6 mm); samples were placed in plates for 1 h. After incubation, the discs were put (30 min) at room temperature and transferred to the medium. Disc solvent-free extract was used as a negative control. The zone of inhibition (ZOI) of each disc (contained sea anemone's extracts) was calculated in millimeter and the measurements were performed in triplicate (Mohsenipour and Hassanshahian 2016, 2002; Boyanova et al. 2005).

Determination of MIC and MBC of sea anemone extract

Minimal Inhibitory Concentration (MIC) was determined by serial dilution method using 96-well microtiter plates. The different sea anemone's extracts were taken (1 mg/mL) and serial dilutions of the extracts were prepared with Mueller-Hinton broth medium. The microplates were incubated for 24 h at 37 °C. The lowest concentrations without visible growth at the binocular microscope were recorded as MIC. The Minimal Bactericidal Concentration (MBC) was characterized by spreading 50 µL on MHA plate from the sample showing no visible growth; plates were incubated for 18 h at 37 °C (Silveira et al. 2009; Jabra-Rizk et al. 2006; Rosenberg and Rosenberg 1981).

Inhibition of biofilm formation

Biofilm formation in polystyrene microtiter plates was assayed as described by O'Toole and Kolter with some modifications (O'Toole and Kolter 1998). Three different extract concentrations (25, 12.5 and 6.25 mg/ml) were pipetted (100 µl) into the wells of the microtiter plates. Then, an overnight culture of each bacterial species was diluted 1:100 with fresh TSB and 100 µl of these inoculums was added to each well. Thereafter, microtiter plates were incubated for 24 h at 37 °C. Three control wells were maintained for each test. These include wells containing extract and growth medium (extract control); the wells containing the growth medium and inoculum and the wells containing only the growth medium (Saeidi et al. 2015).

The attached biofilm mass was quantified using crystal violet staining. After incubation, the media were aspirated and non-adherent cells were removed by washing the wells three times with sterile phosphate buffer saline (PBS). To fix the adherent cells, 150 µl of methanol 96% was added to each well for 15 min. The microtiter plates were then stained with 200 µl of crystal violet 1% (Merck, Germany) for 20 min, excess stain rinsed off with running tap water. The plates were air dried and the CV bound to adherent cells was solubilized with 160 µl 33% glacial acetic acid per well. The absorbance of each well was monitored with a microtiter plate reader (ELX-800, Biotec, India) at 630 nm. Percent inhibition of biofilm formation was calculated using the ratio between the values of OD_{630nm} wells with and without the extracts.

$$\%\,\text{inhibition} = (\text{OD negative control} - \text{OD media control}) - (\text{OD test} - \text{OD extract control})/(\text{OD negative control} - \text{OD media control}) \times 100$$

Disruption of an on established biofilm

Disruption of established biofilm structures was measured as described by Sandasi with some modifications (Sandasi et al. 2008). Biofilms were established in the microtiter plates by growing 100 µl of the standard bacterial culture (OD_{600nm} = 0.2) for 24 h at 37 °C. After incubation, the medium was aspirated and the planktonic cells were removed by washing the biofilms three times with sterile PBS 1x. Thereafter, three different concentrations (25, 12.5 and 6.25 mg mL^{-1}) of extracts were added to each well and plates were incubated at 37 °C for 24 h. The control wells were the same as those described above. The percentages of biofilm eradication in the presence of different concentrations of extracts were calculated according to the formula as described earlier.

The efficiency of sea anemone extracts on metabolic activity of biofilm

Pre-formed biofilms were washed twice with PBS 1x. Three different concentrations (25, 12.5 and 6.25 mg mL^{-1}) of *S. haddoni* extracts were added to microplates; these were incubated for 24 h at 37 °C. After incubation, 50 µl of a Triphenyl Tetrazolium Chloride (TTC; Merck, Germany) solution was added at the samples in the study. Microplates as prepared were incubated (in the dark) at 37 °C for 3 h. TTC reduction was also measured with a microplate reader at 490 nm. The percentages of biofilm metabolic activity reduction in the presence of different concentrations of extracts were calculated using the formula as described earlier (Lazarova et al. 1994; Sadeghian et al. 2012).

Gas chromatography–mass spectrometry (GC–MS) analysis of *S. haddoni* extracts

The *S. haddoni* extracts were analyzed by GC–MS for determination of bioactive metabolites. The GC–MS analysis was performed on Varian Saturn 2000 GC–MS (Agilent Technology). Specifications detectors are Mass range from 10 to 650 amu. An HP-5MS column of 60-m length, 0.25-mm diameter, and 0.25-µm film thickness was used. The oven was programmed from a primary temperature 70 °C (hold for 2 min) to the terminal temperature 300 °C at the rate of 10 (35.0 min). The final temperature hold-up time was 10 min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 2800 °C. The EI source was operated at 2300 °C and the quadruple temperature was 5000 °C. The MS was scanned from 1 to 3000 *m/z*. One microliter of the sample was injected in split mode at a split ratio of 40. WILEY library search was used for compound identifications (Rani Juneius and Selvin 2012).

Statistical analysis

Differences for individual parameters between control and treated groups were tested with Duncan's test by analysis of variance (ANOVA) using SPSS Version 16.0 for Windows. Differences were considered significant if the P value was less than 0.01, 0.05 and 0.001. All experiments were performed in triplicate and repeated three times.

Results

Inhibitory effects of three extracts of sea anemone against planktonic forms of bacteria

The zone of inhibition (ZOI) for metabolic extracts of three parts (tentacle, disc and total body) of sea anemone's extracts is shown in Table 1, also the MIC and MBC values of these extracts are illustrated in Table 1. According to this table, the best antibacterial effect of sea anemone extracts relates to *P. aeruginosa*. Between three parts of sea anemone, the optimum inhibitory effect belongs to the total body. The MIC and MBC values show that the maximum antibacterial activity takes place at 10–20 mg/ml concentration (Table 1).

Antibiofilm properties of *S. haddoni* extracts

The antibiofilm activity of *S. haddoni* extracts against six pathogenic bacteria was assayed by two methods include biofilm inhibition and biofilm destruction. The results for biofilm inhibition are presented in Fig. (2a–c). This figure revealed that three parts of sea anemone exhibited different inhibition against biofilm of bacteria. As, three parts of sea anemone (tentacle, disc and total body) had the most effective anti-biofilm activity against *P. aeruginosa*, *K. pneumoniae* and *A. baumannii* respectively.

The results for biofilm destruction are shown in Fig. (3a–c). For the destruction of the biofilm structures, the biofilm of *P. aeruginosa* was the most sensitive and the biofilm of *B. cereus* was the resistant structure between all tested pathogenic bacteria.

Table 1 The antimicrobial effect of *S. haddoni* extracts against six planktonic bacteria

	S. *haddoni* extracts	Bacterial strains					
		Pseudomonas aeruginosa	*Acinetobacter baumannii*	*Bacillus cereus*	*Klebsiella pneumoniae*	*Escherichia coli*	*Staphylococcus aureus*
Disk diffusion (∅, mm)	Tentacle	12 ± 0.3 SD	11 ± 0.3 SD	8 ± 0.6 SD	10 ± 0.5 SD	8 ± 0.2 SD	14 ± 0.6 SD
	Disc	11 ± 0.5 SD	9 ± 0.3 SD	10 ± 0.2 SD	11 ± 0.3 SD	10 ± 0.5 SD	0
	Total	14 ± 0.2 SD	12 ± 0.4 SD	11 ± 0.5 SD	12 ± 0.2 SD	10 ± 0.3 SD	12 ± 0.2 SD
MIC (mg mL^{-1})	Tentacle	20	20	40	10	40	10
	Disc	20	10	20	20	20	40
	Total	20	20	20	20	10	20
MBC (mg mL^{-1})	Tentacle	80	80	80	40	80	40
	Disc	80	40	40	80	40	80
	Total	40	40	80	80	40	80

The efficiency of *S. haddoni* extract on biofilm metabolic activity

Metabolic activity of bacteria in biofilm structure that was treated with a total of the disc and tentacle of *S. haddoni* extract had considerably decreased. The results are depicted in Fig. (4). As shown in this figure, the best reduction in the metabolic activity was seen in *P. aeruginosa* and *K. pneumonia* among the tested bacteria. However, *S. aureus* and *A. baumannii* had a low reduction in metabolic activity compared to other bacteria (Fig. 4).

Statistical analysis

The effect of bacteria genus and different concentration of sea anemone extracts on biofilm formation and destruction was analyzed statistically by Duncan's test. The results are presented in Table 2. This table confirmed that for biofilm inhibition and destruction, *S. haddonni* extract was significant at ($p < 0.05$ and $p < 0.01$, respectively).

The chemical composition of *S. haddoni* extract

The bioactive compounds that exist in the crude extract of *S. haddoni* were determined by GC–MS. The results are presented in Table 3. Table 3 confirmed that the major compounds in the *S. haddoni* extract can be classified into three groups: aliphatics, alicyclic and aromatics. Aliphatic compounds were predominant between these materials (Table 3).

Discussion

A small number of marine microbes, plants and animals have already yielded more than 16,000 novel compounds with hundreds of new compounds still being discovered every year (Raghunathan et al. 2014; Ghosh et al. 2011). All sea anemone produce venom which is delivered by the specialized sting in organelles, known as nematocysts, located on body surfaces and in high concentration on tentacles (Bragadeeswaran et al. 2011; Khoddami et al. 2018).

The antimicrobial activity of the sea anemone was studied by some researchers in the world; Williams et al. (2007) evaluated antimicrobial activity and associated bacteria from benthic sea anemone *S. haddoni* against some pathogens. They concluded that the hexane tissue extract of the sea anemone showed optimum sensitivity (24 mm) against the fish bacterial pathogen *Aeromonas hydrophila* than the other chosen pathogens. Comparatively, the tissue extracts showed promising antimicrobial sensitivity than the cell-free extracts of associated bacteria, and hence, the tissue samples from the sea anemone *S. haddoni* are recommended for further exploration of novel antimicrobial drugs than the associated bacteria (Prakash et al. 2007).

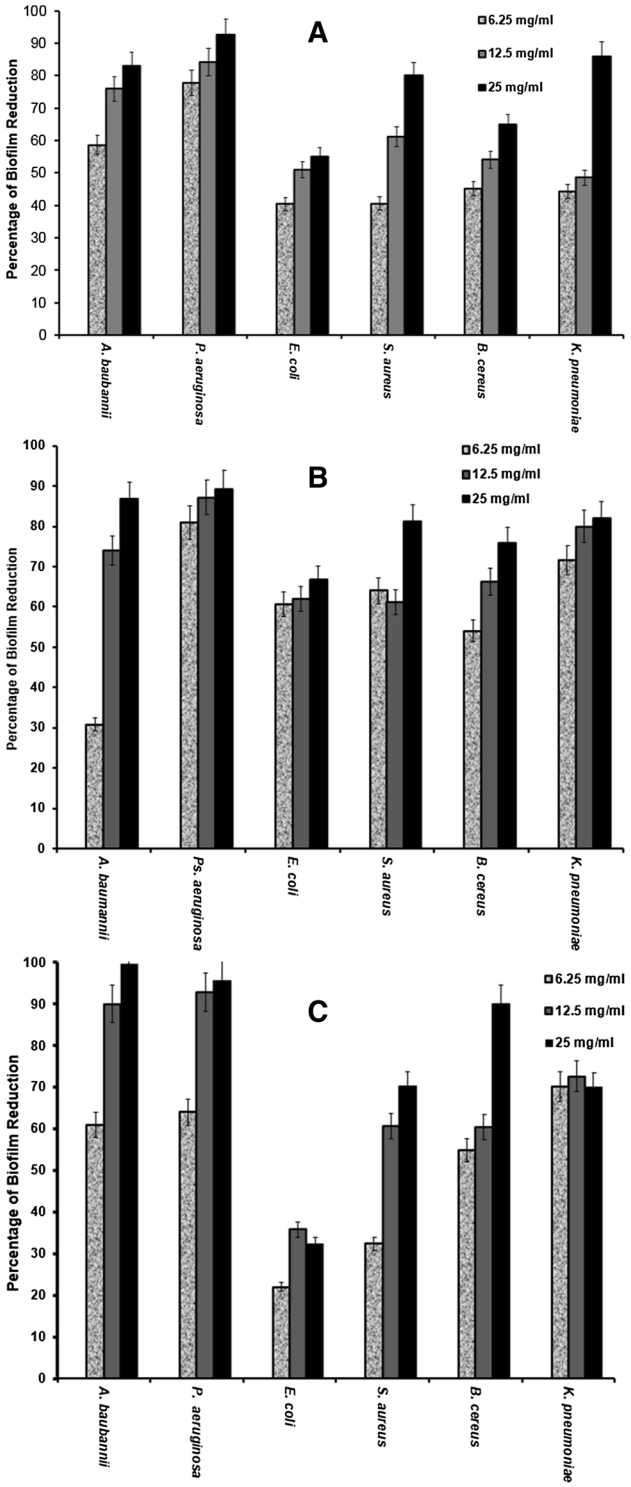

Fig. 2 Percentage reduction of biofilm formation for test bacteria treated with different concentrations of *S. haddoni* extracts from tentacle (**a**), disc (**b**) and total (**c**) for 24 h

Thangaraj et al. (2011) collected two sea anemone species from the coast of India and studied the antimicrobial activity of them. Their results showed that the antibacterial and antifungal activities were predominant in the crude extract of the *Stichodactyla mertensii* and *Stichodactyla gigantean*. The butanol and acetone extract of *S. mertensii* showed roughly 8 mm zone of inhibition against *E. coli* and *Proteus mirabilis* in the methanolic extract (Thangaraj et al. 2011).

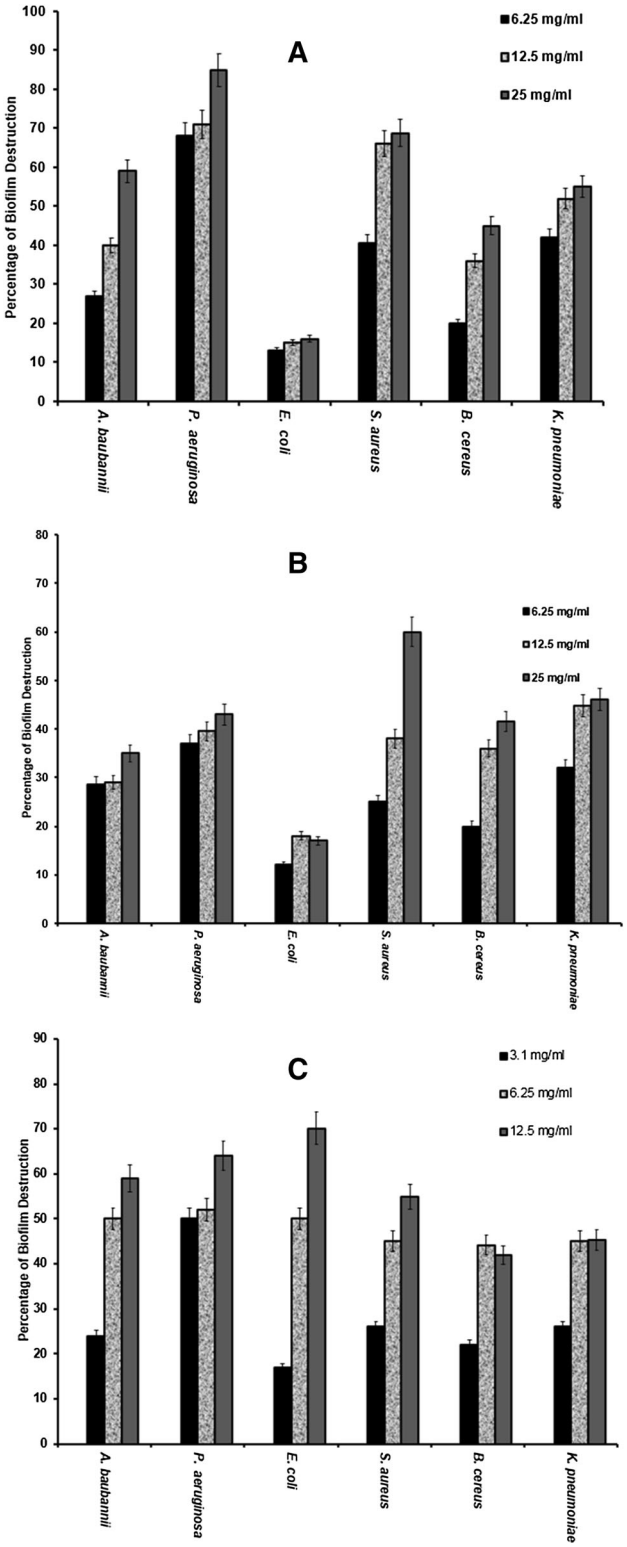

Fig. 3 Percentage disruption of biofilm for test bacteria treated with different concentrations of *S. haddoni* extracts tentacle (**a**), disc (**b**) and total (**c**) for 24 h

Gunasundari et al. (2013) screened and isolated the antibiotic compounds from the mucus of sea anemone *Heteractis magnifica*. Also, they evaluated the antimicrobial activity of this antibiotic against fish pathogens.

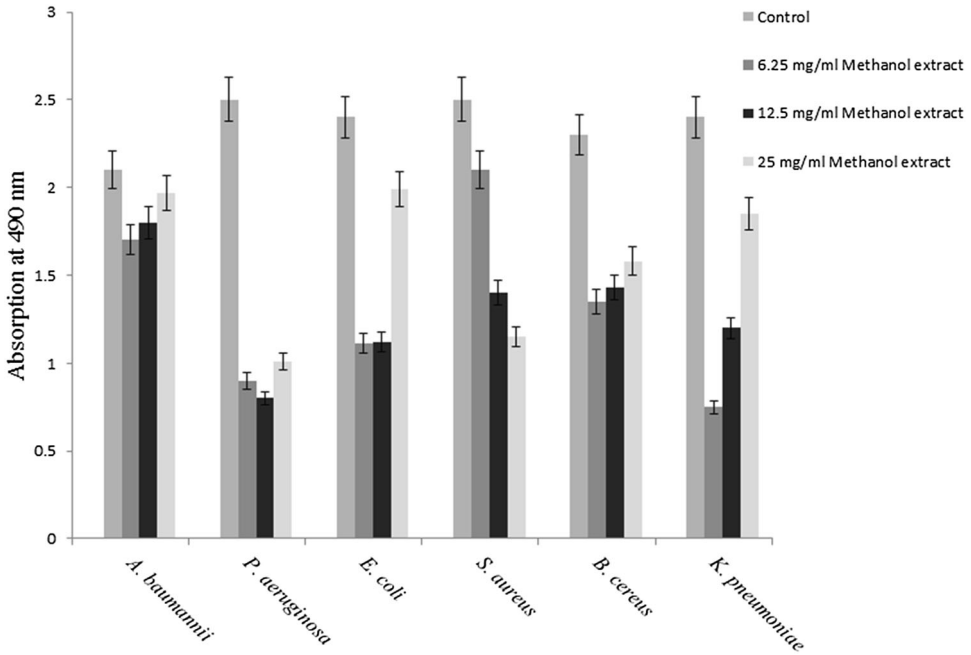

Fig. 4 Efficiency of *S. haddoni* extracts on biofilm metabolic activity (inhibition of dehydrogenase enzyme)

Table 2 Statistical analysis of the results by Duncan's test

S. haddoni extracts	Variables	Biofilm formation			Biofilm destruction		
		Df	Ms	Sig.	*Df*	Ms	Sig.
Tentacle	Bacteria	5	0.035	–	5	0.114	–
	Concentration (mg mL^{-1})	2	0.028	*	2	0.008	**
	Total	7		7			
Disc	Bacteria	5	0.025	–	5	0.051	–
	Concentration (mg mL^{-1})	2	0.091	–	2	0.101	–
	Total	7		7			
Total	Bacteria	5	0.055	–	5	0.035	–
	Concentration (mg mL^{-1})	2	0.010	*	2	0.090	**
	Total	7		7			

The variable parameters in this analysis were type of bacteria and the concentrations of each extracts. This statistical analysis was done in three levels of significance

Ms mean square, *Df* degrees of freedom, *Sig*. significant

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, – no significant level

Their results confirmed that these compounds from sea anemone had sufficient antibacterial activity and the best inhibition belong to *A. hydrophila* (Gunasundari et al. 2013).

The novelty of this research is that this research is the first report on the antimicrobial activity of sea anemone at the Persian Gulf; until now, we have not seen any article that studies the antimicrobial activity of this marine animal. Also, the antibiofilm activity of marine animals has not been described until now and this consider as another novelthy of current research.

However, a few researches were also carried out on the antimicrobial activity of toxins produced by the marine sea anemone. Ghosh et al. (2011) studied the antimicrobial activity of the toxin produced by *H. magnifica* and *S. meritensis* and identified that some extracts showed highest inhibition against *S. aureus* (69.23%) and *S.* Typhi (63.16%) (Ghosh et al. 2011).

Table 3 Chemical composition of *Stichodactyla haddoni* (Haddon's sea anemone) extracts obtained by GC–MS

No.	Compounds	Formula	Ret. time	Area	%, of total
1	Propane, 2,2-dimethoxy-	$C_5H_{12}O_2$	2.995	2.048e+4	0.063
2	Methyl isobutyl ketone	$C_6H_{12}O$	3.598	3.099e+4	0.095
3	3-Benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylonitrile	$C_{11}H_7F_4NS$	3.900	2.038e+4	0.062
4	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$	4.722	9.636e+4	0.295
5	*p*-Xylene	C_8H_{10}	5.477	2.071e+4	0.063
6	Cyclohexane, 1,3,5-trimethyl-2-octadecyl	$C_{27}H_{54}$	16.327	1.811e+4	0.055
7	1-Heptadecyne	$C_{17}H_{32}$	18.352	1.308e+5	0.400
8	1-Hexadecanol	$C_{16}H_{34}O$	18.545	2.525e+5	0.772
9	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	18.998	3.180e+5	0.972
10	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	20.334	4.633e+4	0.142
11	Methyl stearidonate	$C_{19}H_{30}O_2$	20.577	3.171e+5	0.969
12	Methyl 16-methyl-heptadecanoate	$C_{19}H_{38}O_2$	20.919	1.319e+5	0.403
13	Methyl 5,8,11,14,17-eicosapentaenoate	$C_{21}H_{32}O_2$	22.253	3.277e+5	1.002
14	*cis*-7,10,13,16-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	23.845	1.874e+5	0.573
15	Methyl 7,10,13,16,19-docosapentaenoate	$C_{23}H_{36}O_2$	23.941	2.261e+5	0.691
16	17-(1,5-Dimethylhexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[	$C_{27}H_{46}O$	31.056	2.922e+6	8.933
17	Campesterol	$C_{28}H_{48}O$	32.958	1.792e+6	5.480

John et al. (2015) evaluated the inhibitory effect of *S. haddoni* and *Anthopleura elegantissima* extracts (diethyl ether) against Gram-positive and Gram-negative bacteria.

In the current research, we evaluated the antibacterial and antibiofilm activity of one species of sea anemone against six antibiotic-resistant bacteria. This research is the first report on antimicrobial activity of sea anemone (Persian Gulf environment). The Persian Gulf is a diver's marine ecosystem in the world, but a few researches were performed on the extraction of bioactive compounds from this marine ecosystem. Our results on the antibacterial activity of three parts of *S. haddoni* extracts against planktonic forms of six bacteria confirmed that these extracts have sufficient inhibition. However, the rate of inhibition against different bacteria varied. Also, the effect obtained with agar well plate method for antimicrobial assay was stronger than that obtained with diffusion method. This result is expected because the diffusion of sea anemone extracts into agar well is better than paper disc; thus, the zone of inhibition will be bigger than disc.

The values recorded in this study for the antibacterial activity of extracts are according to the values reported by the mentioned researcher (Bragadeeswaran et al. 2011; Bhosale et al. 2002). Although the method for extraction of bioactive compounds in this research was different than another researcher, a few differences in the results were observed in this study compared to the published research. The MIC and MBC recorded in this study were low, and these results confirmed that these extractions can be active in lower concentration.

The antibiofilm activity of bioactive compounds from marine animals was low investigated. Bragadeeswaran et al. (2011) studied the antifouling activity of two sea anemone extracts (which include *Heteractis aurora* and *H. magnifica*) against seven bacterial biofilms. They concluded that the crude extract of *H. magnifica* showed a maximum inhibition zone (18 mm) against *Pseudomonas sp*. and *E. coli*. The minimum inhibition zone (3 mm) was observed against *P. aeruginosa*, *Micrococcus sp*., and *B. cereus* for methanol, acetone, and dichloromethane extracts, respectively (Bragadeeswaran et al. 2011). Also, Bhosale et al. (2002) have reported antimicrobial property of marine organisms against bacteria that were isolated from test panels biofilm (Bhosale et al. 2002).

In this research, the antibiofilm activity of the sea anemone was investigated. Three parts of *S. haddoni* were selected for this analysis that include tentacle, disc, and a mixture of disc and tentacles. The results confirmed that the inhibition of biofilm structures of six pathogenic bacteria by these extracts was higher than the destruction of biofilms of these bacteria. In another hand, when the bacterial biofilms are forming, the extracts have better inhibitory effects than formed biofilms. These results show that the antimicrobial

compounds from *S. haddoni* were effective on bacterial attachment. However, when the bacteria were established on the surface, these inhibitory effects decrease dramatically.

The data obtained from antibiofilm activity and inhibition of the metabolic activity of bacteria by *S. haddoni* extracts confirmed that Gram-positive bacteria (*B. cereus*, *S. aureus*) were more resistant than Gram-negative bacteria (*E. coli*, *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*). Since, the Gram-positive bacteria have thick cell wall compare to Gram-negative bacteria then the antimicrobial agents have low killing effect on Gram-positive bacteria. This study is the first report on antibiofilm activities of S. haddoni and until now, there are not any publications in this field.

The antimicrobial constitutes of *S. haddoni* were determined by GC–MS. The results confirmed that the major compounds of *S. haddoni* extract were aliphatic compounds. These compounds were described also by another researcher as antimicrobial agents, some examples were explained as follows: Tamokou et al. (2011) analyze the chemical structures of *Brillantaisia lamium.* They found that this plant has four compounds including: Aurantiamide acetate, Lupeol, Lespedin, Sitosterol 3-O-β-D-glucopyranoside and a mixture of sterols: Campesterol, Stigmasterol, and β-sitosterol. These compounds exhibited both antibacterial and anti-fungal activities that varied with a microorganism (MIC = 6.25–1000 μg/ml) (Tamokou et al. 2011).

In this study, we detect Campesterol in sea anemone; this material was reported by Takoma et al as antibacterial agents.

Kumar et al. (2011) studied the antibacterial activity of alga *Spirulina*, also they determined the chemical composition of this alga. Their results revealed that the major antibacterial compounds in this alga were 1-Octadecene and 1-Heptadecane. In our study, we identified these materials as antimicrobial agents in sea anemone (Kumar et al. 2011).

Conclusion

Data obtained (according to studies of other research groups) revealed that the marine organisms and especially sea anemone are an important source of bioactive compounds that can be used to prevent bacterial biofilm. The results of this preliminary study confirmed that *S. haddoni*'s extract had sufficient inhibitory effect against principal pathogenic bacteria (e.g., *Pseudomonas*). The possible application of these bioactive materials originates from this particular sea anemone can be proposed for future studies (of microbial ecology and biomedical application).

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