

# Antibacterial and hemolytic effects of aqueous and organic extracts from different tissues of sea urchin *Echinometra mathaei* on pathogenic streptococci

Soodabeh Kazemi · Behrooz Heidari · Mehdi Rassa

Received: 3 February 2016 / Accepted: 2 September 2016 / Published online: 10 September 2016  
© The Author(s) 2016. This article is published with open access at Springerlink.com

**Abstract** In this study, the antibacterial and hemolytic effects of the gonadal tissue, test, spines and Aristotle's lantern of the sea urchin (*Echinometra mathaei*) were investigated. The aerobic Gram-positive pathogenic bacteria *Streptococcus mutans* and *Streptococcus sobrinus* were selected because of their importance in oral diseases. The samples of sea urchin were collected from an intertidal zone in the Persian Gulf and then dissected. Finally, the crude extracts of the gonadal tissue, test, spines and Aristotle's lantern of the samples were separately prepared by the solvents, including phosphate-buffered saline (PBS), 96 % ethanol (Et) and 80 % acetonitrile (ACN). The antibacterial activities of the extracts were evaluated using the well diffusion method in two concentrations of 1500 and 600  $\mu\text{g well}^{-1}$ . The antibacterial effects against streptococcal species were observed in the organic extracts (Et and ACN) of the gonads and test while the extracts of spines and Aristotle's lantern showed no antibacterial activity. The hemolytic activity of the PBS, 96 % Et, and 80 % ACN extracts from the sea urchin were evaluated using horse red blood cells. Hemolytic activity was observed only in the 80 % Et and ACN extracts of the gonad and test. In conclusion, the organic extracts of gonadal tissue showed the most inhibitory activity on the growth of *S. mutans* and *S. sobrinus*.

**Keywords** Sea urchin · Antibacterial effects · Gonad

## Introduction

Microbial populations in seawater and sediments may be as high as  $10^6$  and  $10^9$  per milliliter, respectively (Austin 1988). Marine invertebrates are therefore constantly exposed to high concentrations of bacteria, fungi and viruses, many of which may be pathogenic. The survival of these organisms depends on efficient antimicrobial mechanisms to protect themselves against microbial infections. During the last decade, there has been an increment in research on marine crustaceans, sponges, jellyfish, sea anemones, corals, bryozoans, molluscs, tunicates and echinoderms with particular interest on their secondary metabolites containing desirable antimicrobial properties (Bhakuni and Rawat 2005; Casas et al. 2011; Haug et al. 2002).

Echinoderms are benthic organisms, which are constantly exposed to relatively high concentrations of the pathogens which can be harmful to the organism (Haug et al. 2002). Sea urchins as an echinoderm belong to

---

S. Kazemi · B. Heidari (✉) · M. Rassa  
Department of Biology, Faculty of Science, University of Guilan, Namjoo St, 4193833697 Rasht, Iran  
e-mail: Bheidari@guilan.ac.ir

B. Heidari  
Department of Marine Sciences, Caspian Sea Basin Research Center, University of Guilan, Rasht, Iran

the class Echinoidea of the phylum Echinodermata, which also include starfish, sea cucumbers, sea lilies, and brittle stars. All sea urchins have a hard calcareous shell called “test”, which is covered with a thin epithelium and is usually armed with spines. The test contains the various polyhydroxylated naphthoquinone pigments such as spinochromes and echinochrome A that their bactericidal effect has been reported too (Shankarlal et al. 2011). The mouth, located on the underside, consists of five calcareous plates called “Aristotle’s lantern” in honor of the Greek naturalist and philosopher. The Aristotle’s lantern leads to the digestive tract, which empties through the anus located on the top of the test (Kato and Scheroeter 1985).

The human oral cavity contains a number of different habitats, including the teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils, which are colonized by bacteria (Dewhirst et al. 2001). Oral diseases caused by aerobic and anaerobic bacteria are of the most important problems in the entire world. Dental caries is one of the most common chronic infectious diseases in the world (Aas et al. 2008; Beighton 2005; Chhour et al. 2005; Anusavice 2002; Becker et al. 2002). The streptococcal species are the major causative agents of the dental caries. They are the most common pathogens isolated from human dental plaque and their prevalence has been reported in epidemiological studies which suggest they have an important role in the development and progression of dental caries (Nishikawara et al. 2006; Okada et al. 2005).

The two species responsible for the initiation of dental caries are *Streptococcus mutans* and *Streptococcus sobrinus* (Okada et al. 2005; Ghasempour et al. 2013; Nascimento et al. 2004). They are a group of bacterial species characterized by their ability to produce extracellular glucans from sucrose and by their acid production in animal and human studies (Nishikawara et al. 2006; Okada et al. 2005; Ghasempour et al. 2013).

Antimicrobial activity in several species of echinoderms collected from the Gulf of California, Mexico, Caribbean and Coast of Norway has been reported (Haug et al. 2002; Rinehart et al. 1981; Bryan et al. 1994). Also, a variety of antimicrobial factors, including steroidal glycosides (Andersson et al. 1989), polyhydroxylated sterols (Iorizzi et al. 1995), lysozymes (Canicatti and Roch 1989; Stabili and Pagliara 1994), complement-like substances (Leonard et al. 1990) and antimicrobial peptides (Beauregard et al. 2001) have also been isolated from echinoderms. Unfortunately, in most of these studies, the whole bodies or body walls have been tested for the antimicrobial activity in the echinoderms (Abubakar et al. 2012).

The recent findings suggest that marine echinoderms are a potential source of new types of antibiotics for pharmaceutical development. The pathogenic bacteria resistance to the conventional antibiotics calls for a more focus on new antimicrobials metabolites. On the other hand, there is a high potential of marine biological reserves in the Persian Gulf. Hence, this study focused on the screening and comparing the antibacterial effects of various extracts from different tissues of sea urchin from the Persian Gulf.

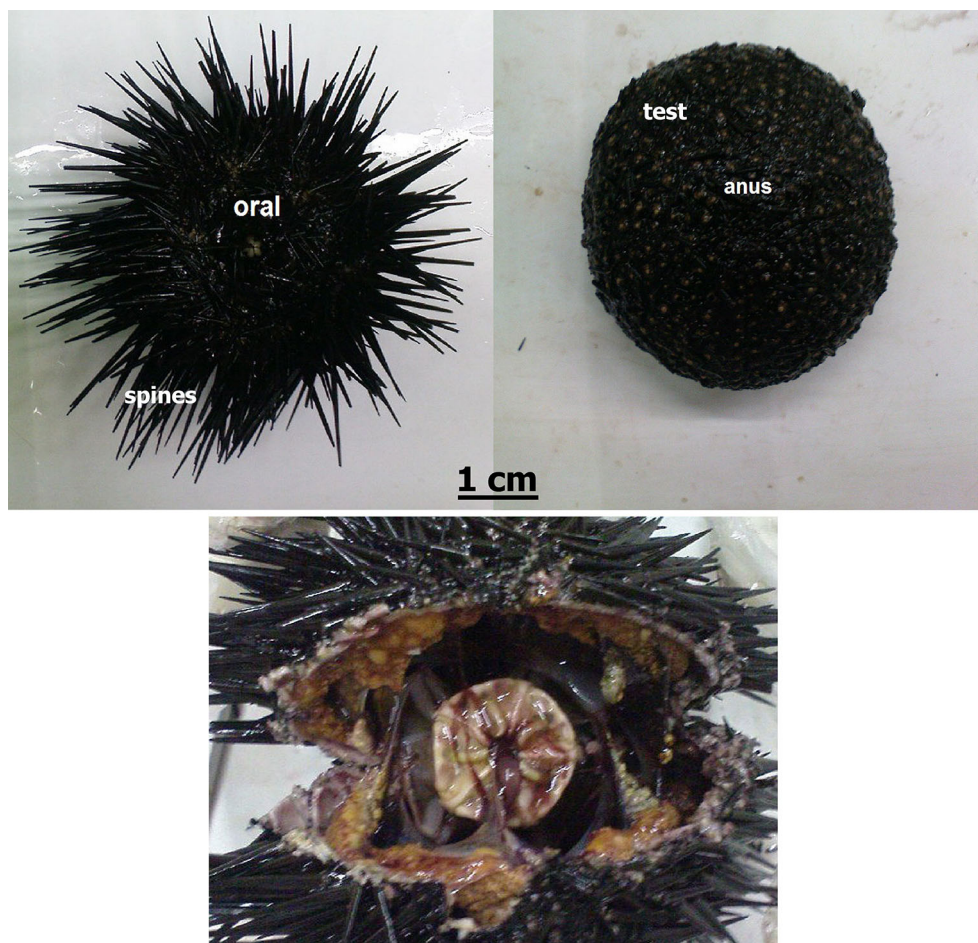
## Materials and methods

### Sampling and preparation of specimens

Live specimens of the sea urchins ( $N = 10$  specimens) were collected from an intertidal zone of Qeshm Island in the Persian Gulf. The samples were transferred on ice to the Marine Biology Laboratory of the University of Guilan. After dissection, all tissues of animals, including spines, test, gonads and the Aristotle’s lantern (Fig. 1) were separately frozen in liquid nitrogen for 5 min and kept at  $-70$  °C until extraction.

### Specimen and sex determination

The specimens were identified using the test and spines of the sea urchin. After taking digital photographs of test and spines through a stereo microscope, identification of species was done using the diagnostic keys (Price 1981). The sex determination of samples was carried out by the histological method with routine hematoxylin and eosin staining.



**Fig. 1** The different tissues of sea urchin that were used for extract processing

### Preparation of extracts

#### *Phosphate-buffered saline (PBS) extract*

All tissues were separately homogenized (Misonix Sonicator 3000, USA) with PBS ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.1 M; NaOH, 0.2 M) (1:5 w/v) for 5 min. The samples were shaken (Infros, RFI-150) on ice for 2 h (120 rpm) and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was collected, stored at 4 °C, and the residue was again centrifuged in two volumes of the PBS. Then, the combined supernatants were centrifuged and lyophilized by freeze-dryer (Christ Alpha 1-2/LD, Osterodeam Harz, Germany) for 9 h (−55 °C and 0.02 mbar), and finally were kept at −70 °C until antibacterial test.

#### *Ethanol (Et) and Acetonitrile (ACN) extracts*

The organic extracts were prepared with 96 % Et and 80 % ACN (Merck-Schuchardt, Germany). As above, all tissues were separately homogenized in Et and ACN as solvents (1:5 w/v) for 5 min. Subsequently, the solutions were shaken at 40 °C for 24 h (120 rpm) and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatants were collected and the residues were again centrifuged in two volumes of the solvents. After centrifuging the combined supernatants, they were lyophilized by freeze-dryer for 9 h (−55 °C and 0.02 mbar) and kept at −70 °C until antibacterial test.



## Bacterial strains

The Gram-positive bacteria *S. mutans* (ATCC 35668) and *S. sobrinus* (ATCC 27607) purchased from the Persian Type Culture Collection (PTCC, Tehran, Iran). The strains were grown on blood agar (Biolife, Italy) containing 7 % fresh horse blood and maintained at 37 °C for 48 h.

## Antibacterial test

Antibacterial activities of the aqueous (PBS) and organic extractions were assessed by the well diffusion method. *S. mutans* and *S. sobrinus* were cultured on blood agar for 24 h. Based on Kiani et al. (2014), the crude aqueous and organic extracts with two concentrations, 600 and 1500 µg, were dissolved in 40 µl of d.d. H<sub>2</sub>O and dimethyl sulfoxide (DMSO) (Merck, Germany) as solvents, respectively. Afterwards, the dissolved extracts were transferred into the wells with diameter of 5 mm punched out on plates and incubated at 37 °C for 24 h. The antibacterial activities were determined by measuring the diameter of the inhibitory zone (mm). Antibiotics, such as vancomycin (30 µg disc<sup>-1</sup>), tetracycline (30 µg disc<sup>-1</sup>) and bacitracin (15 µg disc<sup>-1</sup>) were used as positive controls. DMSO and d.d.H<sub>2</sub>O were also tested as negative control to ensure that they do not interfere with the tests.

## Hemolytic assay

To test whether the sea urchin contains factors that display a toxic effect on eukaryotic cells, the hemolytic activity in the aqueous and organic extracts of the test, gonad, Aristotle's lantern and spines were determined. The assessment was performed using the method described by Haug et al. (2002). Briefly, heparinized horse red blood cells (RBCs) washed three times with phosphate-buffered saline (PBS; pH = 7) and suspended in PBS to a hematocrit value of 10 %. Samples were diluted to a protein concentration of 500 µg.ml<sup>-1</sup>. The test mixture consisted of 40 µl PBS, 50 µl extract, and 10 µl of the RBC suspension. After incubation at 37 °C for 1 h, the mixture was centrifuged at 3000 rpm for 10 min. Finally, absorbance of the supernatants (60 µl) was measured at 550 nm. Baseline hemolysis and 100 % hemolysis were defined as the amount of hemoglobin released in the presence of PBS and 0.1 % Triton X-100 (Sigma), respectively.

## Statistical analysis

The raw data were initially tested against the assumptions of normality and homogeneity of variance. The differences between various extracts were assessed using one-way ANOVA followed by Duncan test with a confidence level of 95 % using IBM SPSS Statistics 19 in Windows 7. Statistical independent *T* test was used to determine the significant differences between the two concentrations of 600 and 1500 µg well<sup>-1</sup>. All experiments (treatments and controls) were carried out triplicate and all data were expressed as the mean ± SD.

## Results

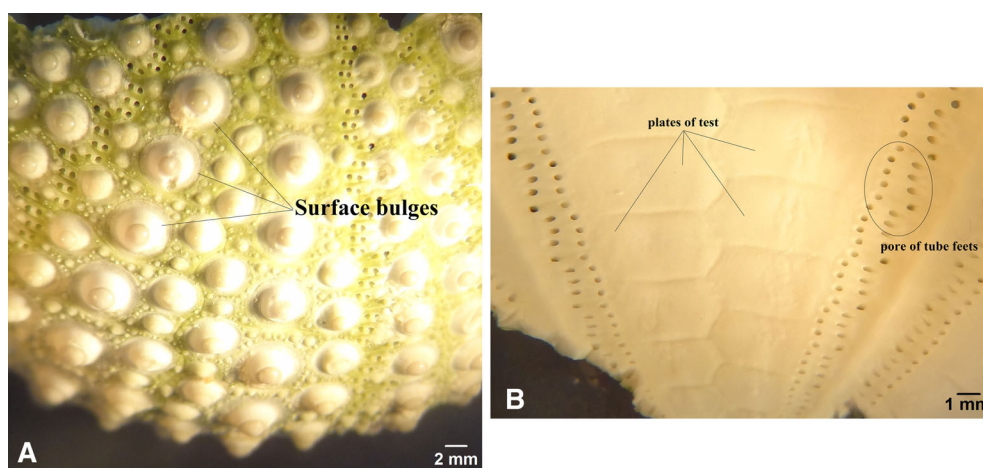
### Specimen and sex determination

The biometric data of samples were brought in Table 1. Based on the diagnostic keys of Price (1983), the photographs of test and spines belong to *Echinometra mathaei* (de Blainville 1825) (Figs. 2, 3). Also, histological sections of the gonads showed that the studied samples were female gender (Fig. 4).

**Table 1** Biometric data of the sea urchin *Echinometra mathaei*

Weights (g)						
Aristotle's lantern	Spines	Gonads	Test	Total weight	Width (cm)	Height (cm)
3.86 ± 0.53	21.98 ± 3.86	3.51 ± 0.43	21.69 ± 5.70	82.21 ± 10.94	5.50 ± 0.75	2.97 ± 0.31





**Fig. 2** The inner (a) and outer (b) surfaces of sea urchin test that were used to determine the species



**Fig. 3** The spines of sea urchin *Echinometra mathaei*. a Long and short conical spines in aboral side. b Long and short paddle-shaped spines in oral side

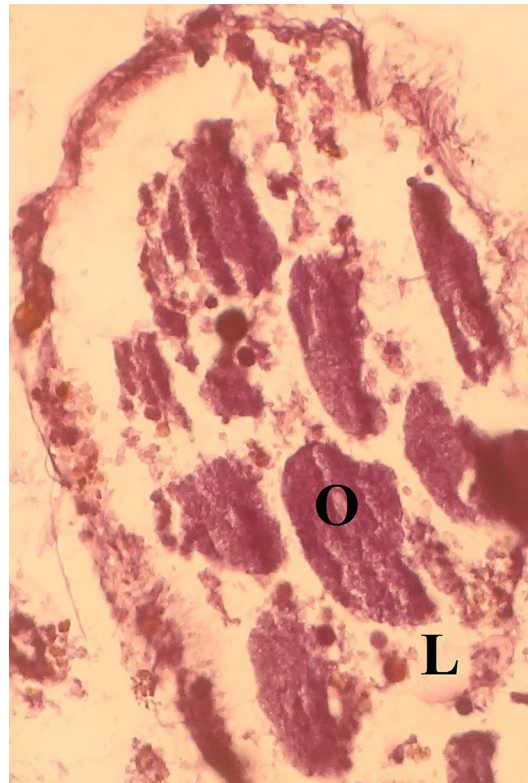
#### Antibacterial activity of the extracts

The results showed that antibacterial activities were found in some tissues of the echinoderm species *Echinometra mathaei* tested against streptococci. Tables 2 and 3 represent the detailed antibacterial activities of the different extracts, positive and negative controls on *S. mutans* and *S. sobrinus*.

#### *Streptococcus sobrinus*

Both in a concentration of 600 and 1500  $\mu\text{g well}^{-1}$ , the extracts of Aristotle's lantern and spines had no antibacterial effects (Table 2) while the ACN and Et extracts of the gonads and the test showed inhibitory effects against *S. sobrinus* (Fig. 5). The highest antibacterial activity belongs to an ACN extract of the gonad and an Et extract of the test with diameter of inhibitory zone  $11 \pm 1.73$  and  $10.33 \pm 2.52$  mm, respectively. The comparison of two concentrations of ACN extracts showed that the extracts with concentration of 1500  $\mu\text{g well}^{-1}$  had more significant effect than of 600  $\mu\text{g well}^{-1}$  ( $p = 0.016$ ,  $F = 16.00$ ).





**Fig. 4** Histological section of the sea urchin ovary. *O* mature eggs, *L* lumen ( $\times 400$ )

**Table 2** Antibacterial activity of aqueous and organic extracts of various tissues of *E. mathaei* against *S. mutans* and *S. sobrinus*

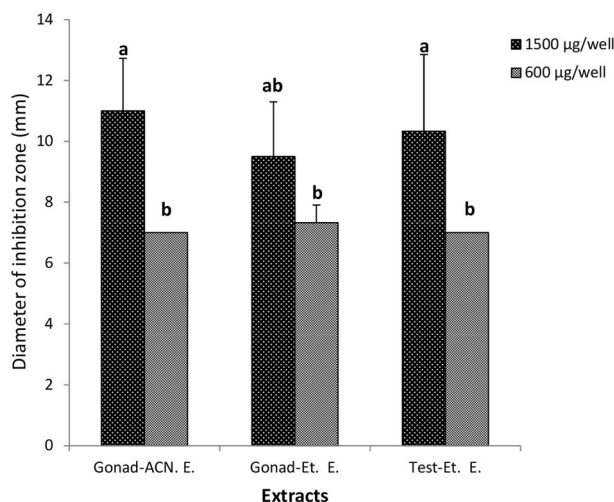
Tissues	Concentration $\mu\text{g}/\text{well}$	Diameter of Inhibitory Zone (mm)					
		Acetonitrile (80 %)		Ethanol (96 %)		PBS	
		<i>S. mutans</i>	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>S. sobrinus</i>	<i>S. mutans</i>	<i>S. sobrinus</i>
Gonads	1500	11.33 $\pm$ 1.53	11.00 $\pm$ 1.73	12.33 $\pm$ 2.52	9.50 $\pm$ 1.80	–	–
	600	8.67 $\pm$ 0.58	7.00 $\pm$ 0.00	7.67 $\pm$ 0.58	7.33 $\pm$ 0.58	–	–
Test	1500	–	–	–	10.33 $\pm$ 2.52	–	–
	600	–	–	–	7.00 $\pm$ 0.00	–	–
Spines	1500	–	–	–	–	–	–
	600	–	–	–	–	–	–
Aristotle's lantern	1500	–	–	–	–	–	–
	600	–	–	–	–	–	–

– No activity

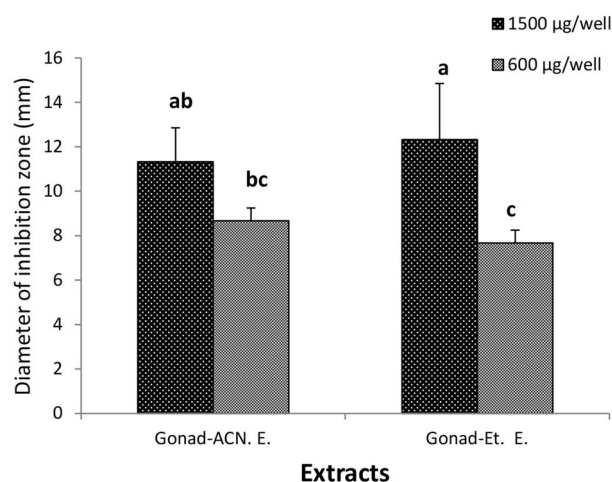
**Table 3** Antibioqram tests on *S. mutans* and *S. sobrinus*

Diameter of inhibitory zone (mm)			
Control	$\mu\text{g}/\text{disc}$	<i>S. mutans</i>	<i>S. sobrinus</i>
Vancomycin	30	23.67 $\pm$ 0.58	28.33 $\pm$ 1.15
Tetracyclin	30	27.67 $\pm$ 0.58	34.00 $\pm$ 1.00
Bacitracin	15	–	–
d.d.H <sub>2</sub> O ( $\mu\text{L}/\text{well}$ )	40	–	–
DMSO ( $\mu\text{L}/\text{well}$ )	40	–	–

– No activity



**Fig. 5** The mean diameter of inhibitory zone for *S. sobrinus* under the effect of different extracts of *Echinometra mathaei*. Mean values bearing different superscripts are significantly different ( $p < 0.05$ )



**Fig. 6** The mean diameter of inhibitory zone for *S. mutans* under the effect of different extracts of *Echinometra mathaei*. Mean values bearing different superscripts are significantly different ( $p < 0.05$ )

### *Streptococcus mutans*

Both in a concentration of 600 and 1500  $\mu\text{g well}^{-1}$ , the extracts of Aristotle's lantern, spines and test had no antibacterial effects (Table 2). The ACN and Et extracts of the gonad with concentration of 600 and 1500  $\mu\text{g well}^{-1}$  had significantly antibacterial different effect in which ACN extract showed the maximum inhibitory zone in both concentrations ( $p = 0.018$ ,  $F = 6.19$ ,  $df = 11$ ) (Fig. 6). The T-test analysis showed that the extracts with concentration of 1500  $\mu\text{g well}^{-1}$  had more significant effect than of 600  $\mu\text{g well}^{-1}$  ( $p = 0.047$ ,  $F = 2.571$ ).

### Hemolytic activity

The hemolytic activity of the PBS, 96 % ethanol and 80 % ACN extracts from the Aristotle's lantern, spines, test and gonad of sea urchin were evaluated using horse red blood cells. The ACN and Et extracts of the gonads and an Et extract of the test showed hemolytic activity while the other extracts had no hemolytic effects (Table 4).

**Table 4** Hemolytic activity in extracts from sea urchin, *E. mathaei*, against red blood cells

Tissues	Extracts	Hemolytic activity (%)*
Gonads	PBS	–
	ACN (80 %)	30
	Ethanol (96 %)	20
Test	PBS	–
	ACN (80 %)	–
	Ethanol (96 %)	10
Spines	PBS	–
	ACN (80 %)	–
	Ethanol (96 %)	–
Aristotle's lantern	PBS	–
	ACN (80 %)	–
	Ethanol (96 %)	–

\* The data are presented as % hemolysis compared to control (Triton X-100)

### Controls

The negative controls (DMSO and d.d.H<sub>2</sub>O) did not show any activity against streptococci while the antibiotics showed the different antibacterial effects (Table 3).

### Discussion

In this study, it was trying to evaluate the antibacterial effects of various extracts in the different tissues of sea urchin *E. mathaei* on oral streptococci.

It has been discovered that echinoderms have stronger antibacterial effects than Porifera, Mollusca, Bryozoa or Annelida (Ridzwan et al. 1995). Antibacterial activity has previously been described in some species of echinoderms (Haug et al. 2002; Kiani et al. 2014; Ridzwan et al. 1995). Haug et al. (2002) studied the antibacterial activity in different parts of the green sea urchin *Strongylocentrotus droebachiensis*, the common starfish *Asterias rubens*, and the sea cucumber *Cucumaria frondosa* against Gram-positive and negative bacteria. They showed antibacterial activities in the extracts of several tissues from *A. rubens* and *C. frondosa*. The coelomocytes of the sea urchin *Paracentrotus lividus* showed antibacterial activity against *Vibrio alginolyticus* (Stabili et al. 1996). Rinehart et al. (1981) examined 83 unidentified species of echinoderms from the west coast of Baja California and the Gulf of California and found 43 % of them had antimicrobial activity. In the same study, 58 % out of 36 unidentified Caribbean species showed antimicrobial activity. Out of 22 species of echinoderms collected from the northern Gulf of Mexico, 80 % had antimicrobial activity (Bryan et al. 1994). This study demonstrated the presence of antibacterial factors in several tissues such as gonads and test of *E. mathaei*. Whether the same antibacterial factors are responsible for the activity in all organs, is unknown. However, it seems that the antibacterial factors have an important function as a first line of defense against pathogenic microorganisms.

A variety of antimicrobial compounds, including steroidal glycosides (Prokof'eva et al. 2003; Iorizzi et al. 2001), polyhydroxylated sterols (Iorizzi et al. 1995), naphthoquinone pigments Service and Wardlaw (1984), lysozymes (Canicatti and Roch 1989; Stabili and Pagliara 1994), complement-like substances (Leonard et al. 1990), and antimicrobial peptides (Li et al. 2010) have been isolated from echinoderms. In this study, ethanol and acetonitrile (organic) as well as PBS (aqueous) extracts from different tissues of the sea urchin were examined for antibacterial activity. These extracts with different solvents allowed us to determine the presence of antibacterial substances potentially present in the lipid or the water-soluble fraction. Since the antibacterial effects with organic solvents has been found, so protein factors could be responsible for such effects. Antimicrobial peptides (AMPs) are evolutionarily conserved small molecular weight proteins of the innate





immune response, with a broad spectrum of antimicrobial activities against bacteria, viruses, and fungi (Mookherjee and Hancock 2007). In the green sea urchin, *S. droebachiensis*, the purification and characterization of two antibacterial peptides from coelomocyte extracts has been done (Li et al. 2008). Also, Ridzwan et al. (2012) studied the body wall's proteins of three species of sea cucumber and at least 11 types of proteins (20–125 kDa) were identified.

The organic and PBS extracts of Aristotle's lantern and spines in *E. mathaei* had no absolutely antibacterial effects on streptococci. It seems that these tissues have just a physical role against environmental stresses including prey and predator and have no chemical function. The ethanol extract from the test showed antibacterial activity on *S. sobrinus* which might be due to the innate immunity parameters and also related to the presence of bacterial symbionts (Strahl et al. 2002) living on the organisms.

In the sea urchin, *E. mathaei*, the highest antibacterial activity against two streptococcus strains was detected in the organic extract of the gonads. Due to the exposure of the sea urchin eggs against the environmental pathogens, maternal transfer of immune parameters, especially antimicrobial peptides is not out of mind. Hence, the high level of antibacterial effects in gonadal tissue of *E. mathaei* is inevitable.

Just as antibacterial effects aqueous extract of the test and gonads, the studied species showed the hemolytic activity of course with lower activity. Several hemolytic factors have been isolated from echinoderms, including proteins (Canicatti and D'Ancona 1990), lectins (Hatakeyama et al. 1994), complement-like factors (Bertheussen 1983), and a variety of saponins (Iorizzi et al. 2001). From a pharmaceutical point of view, it is an advantage when antibacterial drugs have no side effects such as hemolytic activity (Haug et al. 2002). In this study, although some antibiotics showed more antibacterial effects than the tested extracts, but cannot be confirmed the superiority of antibiotics compared to the extracts because of purifying bioactive compounds of the extracts was not carried out.

## Conclusions

This study indicated that the sea urchin, *E. mathaei*, as an echinoderm, can be a source of novel antibiotics. The growth inhibition of *S. sobrinus*, and *S. mutans*, generally was controlled by the organic extracts of gonads, whereas the ethanol extract of test showed antibacterial effect against *S. sobrinus*. The extracts of the sea urchin should be further analyzed to isolate and purify as well as determine the chemical structure of the antibacterial compounds for their application in the medicine and dentistry sciences.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

## References

- Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, Leys EJ, Paster BJ (2008) Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol* 46:1407–1417
- Abubakar L, Mwangi C, Uku J, Ndirangu S (2012) Antimicrobial activity of various extracts of the sea urchin *Tripneustes gratilla* (Echinoidea). *Afr J Pharmacol Ther* 1:19–23
- Andersson L, Bohlin L, Iorizzi M, Riccio R, Minale L, Moreno-Lopez W (1989) Biological activity of some saponins and saponin-like compounds from starfish and brittle-stars. *Toxicon* 27:179–188
- Anusavice K (2002) Dental caries: risk assessment and treatment solutions for an elderly population. *Compend Contin Educ Dent Suppl* 23:12–20
- Austin B (1988) *Marine microbiology*. Cambridge University Press, London 222
- Beauregard KA, Truong NT, Zhang HY, Lin WY, Beck G (2001) The detection and isolation of a novel antimicrobial peptide from the echinoderm *Cucumaria frondosa*. *Adv Exp Med Biol* 484:55–62
- Becker MR, Paster BJ, Leys EJ, Moeschberger ML, Kenyon SG, Galvin JL, Boches SK, Dewhirst FE, Griffen AL (2002) Molecular analysis of bacterial species associated with childhood caries. *J Clin Microbiol* 40:1001–1009
- Beighton D (2005) The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Community Dent Oral Epidemiol* 33:248–255
- Bertheussen K (1983) Complement-like activity in sea urchin coelomic fluid. *Dev Comp Immunol* 7:21–31
- Bhakuni DS, Rawat DDS (2005) *Bioactive marine natural products*. Springer, New York 382



- Bryan PJ, McClintock JB, Watts SA, Marion KR, Hopkins TS (1994) Antimicrobial activity of ethanol extracts of echinoderms from the northern Gulf of Mexico. In: *Echinoderms* edited by David B, Guille A, Fefar J-P, Roux M, Rotterdam. Through Time Balkema, pp 17–23
- Canicatti C, D'Ancona G (1990) Biological protective substances in *Marthasterias glacialis* (Asteroidea) epidermal secretion. *J Zool* 222:445–454
- Canicatti C, Roch P (1989) Studies on *Holothuria polii* (Echinodermata) antibacterial proteins. I. Evidence for and activity of a coelomocyte lysozyme. *Experientia* 45:756–759
- Casas SM, Comesana P, Cao A, Villalba A (2011) Comparison of antibacterial activity in the hemolymph of marine bivalves from Galicia (NW Spain). *J Invertebr Pathol* 106:343–345
- Chhour K-L, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N (2005) Molecular analysis of microbial diversity in advanced caries. *J Clin Microbiol* 43:843–849
- Dewhirst FE, Paster BJ, Tzellas N, Coleman B, Downes J, Spratt DA, Wade WG (2001) Characterization of novel human oral isolates and cloned 16S rDNA sequences that fall in the family *Coriobacteriaceae*: description of *Olsenella* gen. nov., reclassification of *Lactobacillus uli* as *Olsenella uli* comb. nov. and description of *Olsenella profusa* sp. nov. *Int J Syst Evol Microbiol* 51:1797–1804
- de Blainville HMD (1825) Oursin, Echinus (Actinozoaires). In: Levrault FG (ed) *Dictionnaire des Sciences Naturelles*. Strasbourg & Paris, pp 59–98
- Ghasempour M, Rajabnia R, Irannejad A, Hamzeh M, Ferdosi E, Bagheri M (2013) Frequency, biofilm formation and acid susceptibility of *Streptococcus mutans* and *Streptococcus sobrinus* in saliva of preschool children with different levels of caries activity. *Den Res J* 10:440–445
- Hatakeyama T, Kohzaki H, Nagatomo H, Yamasaki N (1994) Purification and characterization of four Ca<sup>2+</sup>-dependent lectins from the marine invertebrate *Cucumaria echinata*. *J Biochem-Tokyo* 116:209–214
- Haug T, Kjuul AK, Styrvold OB, Sandsdalen E, Olsen MO, Stensvag K (2002) Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea) and *Asterias rubens* (Asteroidea). *J Invertebr Pathol* 81:94–102
- Iorizzi M, Bryan P, McClintock J, Minale L, Palagiano E, Maurelli S, Riccio R, Zollo F (1995) Chemical and biological investigation of the polar constituents of the starfish *Luidia clathrata*, collected in the Gulf of Mexico. *J Nat Prod* 58:653–671
- Iorizzi M, DeMarino S, Zollo F (2001) Steroidal oligoglycosides from the asteroidea. *Curr Org Chem* 5:951–973
- Kato S, Scheroeter SC (1985) Biology of the Red Sea Urchin, *Strongylocentrotus franciscanus*, and its fishery in California. *Mar Fish Rev* 47:1–20
- Kiani N, Heidari B, Rassa M, Kadkhodazadeh M, Heidari B (2014) Antibacterial activity of the body wall extracts of sea cucumber (Invertebrata; *Echinodermata*) on infectious oral streptococci. *J Basic Clin Physiol Pharmacol* 25:367–373
- Leonard LA, Strandberg JD, Winkelstein JA (1990) Complement-like activity in the sea star *Asteriasforbesi*. *Dev Comp Immunol* 14:19–30
- Li C, Haug T, Styrvold OB, Jørgensen TØ, Stensvag K (2008) Strongylocins, novel antimicrobial peptides from the green sea urchin, *Strongylocentrotus droebachiensis*. *Dev Comp Immunol*. 32(12):1430–1440
- Li C, Haug T, Moe MK, Styrvold OB, Stensvag K (2010) Centrocins: isolation and characterization of novel dimeric antimicrobial peptides from the green sea urchin, *Strongylocentrotus droebachiensis*. *Dev Comp Immunol* 34:959–968
- Mookherjee N, Hancock REW (2007) Cationic host defence peptides: innate immune regulatory peptides as a novel approach for treating infections. *Cell Mol Life Sci* 64(7–8):922–933
- Nascimento MM, Lemos JA, Abranches J, Gonçalves RB, Burne RA (2004) Adaptive acid tolerance response of *Streptococcus sobrinus*. *J Bacteriol* 186:6383–6390
- Nishikawara F, Katsumura S, Ando A, Tamaki Y, Nakamura Y, Sato K, Nomura Y, Hanada N (2006) Correlation of cariogenic bacteria and dental caries in adults. *J Oral Sci* 48:245–251
- Okada M, Soda Y, Hayashi F, Doi T, Suzuki J, Miura K, Kozai K (2005) Longitudinal study of dental caries incidence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in pre-school children. *J Med Microbiol* 54:661–665
- Price ARG (1981) Studies on the echinoderm fauna of the western Gulf. *J Nat Hist* 15:1–15
- Price ARG (1983) Echinoderms of Saudi Arabia: Echinoderms of the Arabian Gulf coast of Saudi Arabia. *Fauna Saudi Arabia* 5:28–108
- Prokof'eva NG, Chikina EL, Kicha AA, Ivanchina NV (2003) Biological activities of steroid glycosides from starfish. *Comp Biochem Physiol B* 134:695–701
- Ridzwan BH, Kaswandi MA, Azman Y, Fuad M (1995) Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. *Gen Pharmacol* 26:1539–1543
- Ridzwan BH, Hanis ZF, Daud JM, Althunibat OY (2012) Protein profiles of three species of Malaysian sea cucumber; *holothuria edulis* Lesson, *H. scabra* Jaeger and *stichopus horrens* selenka. *Eur J Sci Res* 75(2):255–261
- Rinehart KL Jr, Shaw PD, Shield LS, Gloer JB, Harbour GC, Koker MES (1981) Marine natural products as sources of antiviral, antimicrobial, and antineoplastic agents. *Pure Appl Chem* 53:795–817
- Service M, Wardlaw AC (1984) Echinochrome-a as a bactericidal substance in the celomic fluid of *Echinus esculentus* (L.). *Comp Biochem Physiol B Biochem Mol Biol* 79:161–165
- Shankarlal S, Prabu K, Natarajan E (2011) Antimicrobial and antioxidant activity of purple sea urchin shell (*Salmacis virgulata* L. Agassiz and Desor 1846). *Am-Euras J Sci Res* 6:178–181
- Stabili L, Pagliara P (1994) Antibacterial protection in *Marthasterias glacialis* eggs, characterization of lysozyme-like activity. *Comp Biochem Physiol* 109:709–713
- Stabili L, Pagliara P, Roch P (1996) Antibacterial activity in the coelomocytes of the sea urchin *Paracentrotus lividus*. *Comp Biochem Physiol* 113(3):639–644
- Strahl ED, Dobson WE, JrLL Lundie (2002) Isolation and screening of brittle star-associated bacteria for antibacterial activity. *Curr Microbiol* 44:450–459

