Evaluation of antimicrobial activity of peptides isolated from *Cerastoderma* and *Didacta* bivalves habitat in the southern shores of the Caspian Sea

A Ghorbanalizadeh [1], A Moshfegh[1*], M Setorki [2]

[1] Department of Marine Biology, Lahijan Branch, Islamic Azad University, Lahijan, Iran

[2] Department of Biology, Izeh Branch, Islamic Azad University, Izeh,Iran

Received: October 2017 **Accepted:** January 2018

[Downloaded from ijaah.ir on 2026-10-14]

[DOR: 20.1001.1.2345315.2018.4.1.1.5]

[DOI: 10.29252/ijaah.4.1.1]

Abstract

The antibacterial effects of methanol, ethanol, chloroform extracts and alcalase hydrolysis of *Cerastoderma* and *Didacta* were investigated against *Salmonella typhi*, *Salmonella paratyphi* and *Staphylococcus aureus* by disk diffusion method, Minimum Inhibitory Concentration and Minimum Bactericidal Concentration. Methanolic and ethanolic extracts of *Cerastoderma* showed the highest effects against *S. typhi* (20.33 ± 0.33 and 12.33 ± 0.33) and *S. paratyphi* (22.66 ± 1.45 and 15.33 ± 0.33), however, the same effects against *S. aureus* (18.00 ± 0.00 and 17.00 ± 0.00) were observed for two bivalves. Chloroform extract of *Cerastoderma* and *Didacta* showed similar effects in controlling *S. paratyphi* (8.00 ± 0.58 vs 10.00 ± 0.57) and *S. aureus* (16.00 ± 1.15 vs 16.00 ± 1.15) in concentrations of 10 and 5 mg ml^{-1}.

Chloroform extract of *Cerastoderma* exhibited higher effect than that from *Didacta* against *S. typhi* (11.00 ± 1.73 vs 8.00 ± 0.58) and the dilution of 10 mg ml^{-1} had the most suitable performance. The enzymatic hydrolysis of the *Cerastoderma* and *Didacta* showed the same performance in controlling of *S. typhi* (13.67 ± 4.37 vs 13.33 ± 1.76) and *S. paratyphi* (17.00 ± 0.58 vs 15.33 ± 1.45). However, the enzymatic hydrolysis of *Cerastoderma* showed better effect than that of *Didacta* in controlling *S. aureus* (18.00 ± 1.15 vs 13.00 ± 2.30), and the 10 and 5 mg ml^{-1} dilutions were the most appropriate concentrations. It is concluded that *Cerastoderma* can be used as a resource with potent antibacterial compounds in the preparation of natural antimicrobial agents.

Correspondence: A Moshfegh, Department of Marine Biology, Lahijan Branch, Islamic Azad University, Lahijan, Iran (e-mail: moshfeghazam@gmail.Com).

Keywords: Antibacterial activity, Extracts, *Cerastoderma*, *Didacta*

Introduction

Bacterial and fungal diseases can cause high mortality and economic lost annually (Zasloff, 2012a). Increasing of the number of antibiotics and their uncontrolled consumption for the treatment of various infectious diseases provides the context for the development of resistant strains and thus nowadays there have been some bacterial and fungal strains without inhibition by any of the antibiotics. Therefore, the need for research and introduce of new antimicrobial compounds is of great essential. In recent years, numerous studies have been conducted to identify and introduce new antimicrobial agents from natural resources (Marshall and Arenas, 2003).

Studies about the antimicrobial compounds from soft tissues can provide valuable information for the application of new bioactive antibiotic compounds. It has been shown that the wide range of medicines obtained from the mummy's sources including peptides and sterols, terpenes, nitrogen compounds, macrolides, prostaglandins, acid-fat derivatives and other alkaloids (Bulet, Stocklin, Menin, 2004).

Among the various compounds introduced in this field, antimicrobial peptides are very suitable alternatives to the commonly used synthetic antibiotics (Zasloff, 2012b). Antimicrobial peptides are expressed by a range of animals as part of the primary defense system against pathogenic microorganisms (Yu, Sheng, Xu, 2006). Peptides have a small structure and provide a wide range of antimicrobial activity (Zasloff, 2012b; Zasloff, 2006). Increased food-related diseases such as bacterial and fungal infections and cardiovascular diseases, Diabetes, cancer and obesity have increased the consumer attitudes towards products with not only nutrition value but also beneficial health effects for the body. In addition to providing nutritional needs, other physiological roles in the body depend on the type and characteristics of peptide such as its amino acids combination. Biological products can always play an important role in medicine, and marine metabolites have become increasingly important because their structure are not only used as a model but also they have very high intrinsically drug potential. Moreover, the marine organisms involve approximately half of the biodiversity in the world. On the other hand, since life in the sea is more historic as far as in the land, aquarists had more opportunity to evolve in order to meet the conditions. This study was conducted to investigate the antibacterial activity of extracts and enzymatic hydrolysis of *Cerastoderma* and *Didacta* in the Caspian Sea.

Materials and Methods

Sample collection

The organisms (*Cerastoderma* and *Didacta* bivalves) were collected from the Caspian Sea in the city Rudsar and were sent to the Azad University of Lahijan in 2015, for the identification by observing the sub-lobe, according to the Atlas of invertebrates of the Caspian Sea book (Delinad and Nazari, 1978). They were then rinsed with seawater and transferred to the laboratory inside boxes containing intermittent layers of ice and were stored at frozen (-21 ºC) temperature until the test (Sugesh and Mayavu, 2013).

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Preparation of extracts

The hard parts of the samples were broken and their soft parts were separated. The collected soft parts were grinded and mixed individually with three different solvents including ethanol, methanol and chloroform (volumetric weight 1:1). After 24 h, the mixture was filtered and the solvent was evaporated. The obtained extract was weighted and kept at -20 ºC (Sumita, Chatterji, Das, 2009).

Enzymatic hydrolysis

The enzyme used in this study was Alcalase (Hayyan Azma Company, Iran), an alkaline enzyme (with 2.4 Unite g^{-1} Anson enzymatic activity), obtained from the bacterium Bacillus licheniformis. The freezed collected soft tissues were thawed at 4°C overnight and homogenized using a Multi grind for about 2 min. The homogenate was mixed with a buffer (pH 7) at a ratio of 2:1 (w/v) and were allowed to attain a temperature of 56°C, with stirring at minimum rpm. Alcalase was added at enzyme substrate ratio 2% (v/w) and hydrolysis allowed to proceed for 6 h. The enzyme activity was then terminated by placing the hydrolysate in a water bath at 100°C for 20 min. The hydrolysate was centrifuged at 10000 × g for 20 min. After decantation and removal of sludge, the soluble fraction was freeze-dried and stored in airtight plastic container at -20°C for further use (Awuor, Kirwa, Jackim, Betty, 2017).

Antimicrobial activity measurement

In order to evaluate the antimicrobial activity of various extracts, three bacterial species including Gram-negative pathogens: *S. typhi* and *S. paratyphi* and Gram-positive one: *S. aureus* were used. To compare the inhibitory strength of various extracts against bacterial species, the disc diffusion test was performed for the extracts prepared by ethanol, methanol and chloroform solvents, as well as an alcalase hydrolysis extract.

The antimicrobial susceptibility test (antibiogram) was used to investigate the antibacterial effects of the extracts. For this purpose, the bacterial suspensions were prepared from the overnight cultures of bacterial strains and cultured on the Mueller Hinton Agar (MHA) medium by the disk diffusion method.

For this purpose, the discs were impregnated by 10 mg ml^{-1} of extracts or enzyme hydrolysis. The concentrations of 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg ml^{-1} of the extracts were used and 20 µl of each was added to each disk. The powder obtained by the enzymatic method at a concentration of 10 mg ml^{-1} was diluted in the serum physiology, and 20 µl was added to each disk. After drying in a sterile environment and at room temperature for 3 hours, slowly dispersed with pressurized force in the agar medium and the plates were incubated at 37 °C for 24 hours.

The inhibitory zone diameter around the disk due to the antibacterial effect of the solvent was measured by the caliper. Solvents was used as the negative control.

Determine the minimum inhibitory concentration

According to the results of the disk diffusion test, *Cerastoderma* and *Didacta* extracts minimum and optimum inhibitory

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concentrations were measured at the next stage using broth dilution test. Extracts and bacteria were diluted for a series of eight tubes which included six tubes for concentrations. After culture the tubes were incubated at 37°C for 24h. the first no growth dilution was considered as MIC (Lambert, Skandamis, Coote, Nychas, 2001).

Determination of Minimum Bactericidal Concentration (MBC)

MBC is the minimum concentration of extract for the killing of bacteria. After determining the MIC, 10 µL of any tubes lacking the turbidity was cultured on the Muller Hinton Agar medium and was incubated at 37°C for 24 hours. In the absence of colony formation on the agar plate, the MBC was determined which inhibits the growth of 99% of bacterial population.

Statistical analysis

To test the normal distribution of obtained data, the Kolmogorov-Smirnov test was used. In the case of normal data, for statistical comparison between groups in the treatments (dilutions), the One-way ANOVA test was employed followed by Duncan test to compare the differences between groups. In order to compare 2 groups, the independent t-test was used at the statistical level 99%.the software Excel 2007 and SPSS 21 was used.

Results

Effects of Methanolic Extract of *Cerastoderma* and *Didacta* on *S. paratyphi*

The mean diameter of the inhibition zone in the methanolic extract of *Cerastoderma* was significantly more than gentamicin in dilutions 1 and 1/2 and it showed nearly equivalent effects to gentamicin in dilution 1/4. Furthermore, the mean diameter of the *S. paratyphi* inhibition zone for the methanolic extracts of *Didacta* was significantly less than gentamicin in all dilutions ($df = 5$, $F = 133.63$, $P = 0.00$), and only dilution 1 displayed close proximity to gentamicin. Also, comparing the diameter of the inhibition zone of two methanolic extracts of *Cerastoderma* and *Didacta* showed significant differences ($P < 0.05$). The *Cerastoderma* methanolic extract exhibited a better effect than that of *Didacta* for inhibition of *S. paratyphi* (Table 1).

Effects of Methanolic extract of *Cerastoderma* and *Didacta* on *S. typhi*

Significant difference was observed for comparing the inhibition zone among different dilutions of each of the methanolic extracts of *Cerastoderma* and *Didacta* against *S. typhi* ($df = 5$, $F = 144.76$, $P = 0.00$). The mean diameter of the inhibition zone in the methanolic extract of *Cerastoderma* in dilution 1 was significantly more than gentamicin and dilutions 1/2 and 1/4 showed a similar effect to the gentamicin. Moreover, the mean diameter of the *S. typhi* zone inhibition in the *Didacta* methanol extract in all dilutions was significantly less than that of gentamicin ($df = 3$, $F = 352.889$, $P = 0.00$). Comparing the diameter of inhibition zone of *S. typhi* in dilutions of two methanolic extracts of *Cerastoderma* and *Didacta*, significant

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difference was observed and the effects was stronger for *Cerastoderma* (Table 1).

Effects of Methanol Extract of *Cerastoderma* and *Didacta* on *S. aureus*

Comparing of the mean diameter of the inhibition zone of *S. aureus* among different dilutions of each of the extracts of *Cerastoderma* and *Didacta*, significant difference was observed (df = 4, F = 152.33, P = 0.00). In this regard, the mean diameter of the inhibition zone of methanolic extract of *Cerastoderma* and *Didacta* was significantly more than methicillin in dilutions 1 and 1/2. However, no significant difference was observed between *Cerastoderma* and *Didacta* extracts against *S. aureus* (Table 1).

Table 1. The antibacterial activity of methanol extract of *Cerastoderma* and *Didacta* against different bacteria (mm)

	S. paratyphi		***S. typhi***		***S. aureus***	
Diluation	*Cerastoderma*	*Didacta*	*Cerastoderma*	*Didacta*	*Cerastoderma*	*Didacta*
C1	22.66 ± 1.45^{Aa}	10.33 ± 0.33^{Bab}	20.33 ± 0.33^{Aa}	6.33 ± 0.33^{Ba}	18.00 ± 0.00^{Aa}	18.00 ± 0.00^{Aa}
C2	21.66 ± 0.33^{Aa}	6.00 ± 0.00^{Bb}	13.33 ± 0.67^{Ab}	2.66 ± 0.33^{Bb}	17.33 ± 0.33^{Aa}	17.00 ± 0.00^{Aa}
C3	14.00 ± 0.58^{Ab}	4.00 ± 0.67^{Bc}	12.00 ± 0.58^{Ab}	0.66 ± 0.00^{Bc}	13.33 ± 0.66^{Ab}	11.00 ± 0.57^{Ab}
C4	8.00 ± 0.00^{Ac}	3.66 ± 0.58^{Bd}	5.00 ± 0.00^{c}	-	6.67 ± 0.33^{Ac}	4.66 ± 0.33^{Ac}
C5	1.66 ± 0.33^{d}	1.00 ± 0.00^{d}	0.33 ± 0.33^{d}	-	-	2.33 ± 0.33^{d}
C6	-	-	-	-	-	0.33 ± 0.33^{e}
Gentamicin	13.00 ± 0.00^{b}	13.00 ± 0.00^{b}	13.00 ± 0.00^{b}	13.00 ± 0.00^{b}	-	-
Methicillin	-	-	-	-	14.00 ± 0.00^{b}	14.00 ± 0.00^{b}

a, b, c, … letters indicate significant difference among different dilutions in each column.

A, B, C, … letters indicate significant difference between two extracts of *Cerastoderma* and *Didacta* in each dilution against specific bacterium.

Effects of Ethanol extract of *Cerastoderma* and *Didacta* on *S. paratyphi*

Comparing the mean diameter of the inhibition zone of *S. paratyphi* among different dilutions of each ethanolic extract of *Cerastoderma* and *Didacta* significant difference was observed (df = 5, F = 59.656, P = 0.00). The mean diameter of the inhibition zone in the ethanolic extract of *Cerastoderma* was more than or equal to that of gentamicin in dilutions 1, 1/2 and 1/4. The mean diameter of *S. paratyphi* inhibition zone in the ethanolic extract of *Didacta* in all dilutions were significantly less than gentamicin (df = 4, F = 147.50, P = 0.00). For two ethanolic extracts of *Cerastoderma* and *Didacta*, significant difference was observed and *Cerastoderma* extract showed better effect (P<0.05) (Table 2).

Effects of Ethanol Extract of Cerastoderma and *Didacta* on *S. typhi*

Comparing the mean diameter of the inhibition zone of *S. typhi* in different dilutions of each of the ethanolic extracts of *Cerastoderma* and *Didacta* showed significant difference (df = 5, F = 93.35, P = 0.00). In this regard, the *Cerastoderma* and *Didacta* in dilution 1 was significantly stronger than other dilutions. Also,

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dilutions 1 of *Cerastoderma* showed no difference with gentamicin, but other dilutions of *Cerastoderma* and also the all dilutions of *Didacta* showed less effect than gentamicin. *Cerastoderma* extract showed better effect than that of *Didacta* (Table 2).

Effects of Ethanolic extract of *Cerastoderma* and *Didacta* on *S. aureus*

Comparing the mean diameter of the inhibition zone of *S. aureus* in different dilutions of each of the ethanol extracts of *Cerastoderma* and *Didacta* showed significant difference (df = 4, F = 79.25, P = 0.00). In this regard, this value for *Cerastoderma* in dilution 1 was more than methicillin (df = 6, F = 327.16 P = 0.00), and dilutions 1/2 and 1/4 of *Cerastoderma* and dilutions 1 and 1/2 of *Didacta* showed similar effect with methicillin (P>0.05). There was no significant difference between *Cerastoderma* and *Didacta* against *S. aureus* (Table 2).

Table 2. The antibacterial activity of ethanol extract of *Cerastoderma* and *Didacta* against different bacteria (mm)

	S. paratyphi		***S. typhi***		***S. aureus***	
Diluation	*Cerastoderma*	*Didacta*	*Cerastoderma*	*Didacta*	*Cerastoderma*	*Didacta*
C1	$15.33 \pm 0.33A^{a}$	$9.33 \pm 0.33Bb$	12.33 ± 0.33^{Aa}	$8.33 \pm 0.33B^{b}$	17.00 ± 0.00^{a}	13.67 ± 0.33^{a}
C2	12.00 ± 0.00^{b}	10.00 ± 0.00^{b}	9.67 ± 0.33^{b}	8.33 ± 0.33^{b}	15.00 ± 0.00^{ab}	12.00 ± 0.00^{ab}
C3	17.33 ± 0.67^{Aa}	8.33 ± 0.33^{Bb}	7.00 ± 1.00^{c}	1.00 ± 0.00^{c}	10.33 ± 0.88^{b}	5.33 ± 0.33^{b}
C4	3.66 ± 1.85^{c}	4.33 ± 0.33^{c}	4.67 ± 0.33^{d}	-	7.67 ± 0.33^{c}	4.33 ± 0.33^{b}
C5	1.00 ± 0.58^{d}	-	0.66 ± 0.33^{e}	-	-	-
C6	-	-	-	-	-	-
Gentamicin	13.00 ± 0.00^{b}	13.00 ± 0.00^{a}	13.00 ± 0.00^{a}	13.00 ± 0.00^{a}	-	-
Methicillin	-	-	-	-	14.00 ± 0.00^{ab}	14.00 ± 0.00^{a}

a, b, c, … letters indicate significant difference among different dilutions in each column.

A, B, C, … letters indicate significant difference between two extracts of *Cerastoderma* and *Didacta* in each dilution against specific bacterium.

Effects of Chloroform extract of *Cerastoderma* and *Didacta* on *S. paratyphi*

The mean diameter of the inhibition zone of *S. paratyphi* in different dilutions of each of the extract of *Cerastoderma* and *Didacta* showed significant difference (df = 3, F = 77.83 P = 0.00). In this regard, the mean diameter of the inhibition zone in the Chloroform extract of *Cerastoderma* and *Didacba* in dilutions 1 and 1/2 showed similar effect to that of gentamicin, but other dilutions showed significantly lower effects (Table 3).

Effects of Chloroform extract of *Cerastoderma* and *Didacta* on *S. typhi*

Different dilutions of each of the chloroform extracts of *Cerastoderma* and *Didacta* showed significant difference (df = 5, F = 20.676, P = 0.002). In this regard, the mean diameter of the inhibition zones in the gentamicin and the chloroform extract of *Cerastoderma* in dilution 1 was significantly higher than other dilutions, but for *Didacta* it was less than gentamicin in all dilutions (df = 3, F = 44.357, P = 0.000). The *Cerastoderma* extract at

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[DOI: 10.29252/ijaah.4.1.1]

dilution 1 significantly showed better effect than *Didacta* (Table 3).

Effects of Chloroform extract of *Cerastoderma* and *Didacta* on *S. aureus*

Comparing the mean diameter of the inhibition zone of *S. aureus* in different dilutions of each of the chloroform extracts of *Cerastoderma* and *Didacta* showed significant difference (df = 3, F = 11.429 P = 0.003). The mean diameter of the inhibition zone of chloroform extract of *Cerastoderma* and *Didacta* in dilutions 1 and 1/2 showed no difference with that of methicillin. No significant difference was observed between chloroform extracts of *Cerastoderma* and *Didacta* against *S. aureus* (Table 3).

Table 3. The antibacterial activity of chloroform extract of *Cerastoderma* and *Didacta* against different bacteria (mm)

	S. paratyphi		***S. typhi***		***S. aureus***	
Diluation	***Cerastoderma***	***Didacta***	***Cerastoderma***	***Didacta***	***Cerastoderma***	***Didacta***
C1	8.00 ± 0.58^{b}	10.00 ± 0.57^{ab}	11.00 ± 1.73^{Aa}	8.00 ± 0.58^{Bb}	16.00 ± 1.15^{a}	16.00 ± 1.15^{a}
C2	15.00 ± 1.15^{a}	13.00 ± 0.57^{a}	3.33 ± 0.88^{b}	8.00 ± 1.15^{b}	10.00 ± 1.73^{ab}	14.00 ± 2.30^{ab}
C3	$1.00 \pm 0.58B^{c}$	$8.00 \pm 0.58A^{b}$	-	4.00 ± 0.58^{c}	8.00 ± 0.58^{b}	8.00 ± 0.58^{b}
C4	-	2.00 ± 0.58^{c}	-	1.00 ± 0.58^{d}	-	3.00 ± 0.58^{c}
C5	-	-	-	-	-	3.00 ± 1.15^{c}
C6	-	-	-	-	-	-
Gentamicin	13.00 ± 0.00^{ab}	13.00 ± 0.00^{a}	13.00 ± 0.00^{a}	13.00 ± 0.00^{a}	-	-
Methicillin	-	-	-	-	14.00 ± 0.00^{ab}	14.00 ± 0.00^{a}

a, b, c, … letters indicate significant difference among different dilutions in each column.

A, B, C, … letters indicate significant difference between two extracts of *Cerastoderma* and *Didacta* in each dilution against specific bacterium.

Effects of Alcalase Hydrolysis extract of *Cerastoderma* and *Didacta* on *S. paratyphi*

Comparing the mean diameter of the inhibition zone of *S. paratyphi* in different dilutions of each hydrolysis extract showed significant difference between those from *Cerastoderma* and *Didacta* (df = 6, F = 160.352, P = 0.00) and *Cerastoderma* at dilutions 1 and 1/2 and *Didacta* in dilution 1 significantly showed higher effects than gentamicin. No significant difference was observed comparing the effect of *Cerastoderma* and *Didacta* extracts against *S. paratyphi* (Table 4).

Effects of Enzymatic Hydrolysis extract of *Cerastoderma* and *Didacta* on *S. typhi*

Comparing the mean diameter of the inhibition zone of *S. typhi* in different dilutions of each hydrolysis extract showed significant difference between those from *Cerastoderma* and *Didacta* (df = 6, F = 160.352, P = 0.00) and *Cerastoderma* at dilutions 1, 1/2 and 1/4 and *Didacta* in dilution 1 and 1/2 showed higher or equal effects than gentamicin. However, the effect of the other dilutions was significantly lower than that of gentamicin. No significant difference was observed comparing the effect of *Cerastoderma* and *Didacta* extracts against *S. typhi* (Table 4).

Effects of Enzymatic Hydrolysis extract of *Cerastoderma* and *Didacta* on *S. aureus*

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[DOI: 10.29252/ijaah.4.1.1]

Comparing the mean diameter of the zone inhibition of *S. aureus* in different dilutions of each of the enzymatic hydrolysis extracts of *Cerastoderma* and *Didacta* significant difference was observed (df = 4, F = 64.942, P = 0.000), and *Cerastoderma* at dilutions 1 and 1/2 and *Didacta* in dilution 1 significantly showed equal or higher effects than methicillin (Df = 6, F = 7.448, P = 0.001). Also, significant difference was observed comparing the effect of *Cerastoderma* and *Didacta* extracts against *S. aureus* and *Cerastoderma* showed better effect than *Didacta* (Table 5).

Table 4. The antibacterial activity of alcalase hydrolysate of *Cerastoderma* and *Didacta* against different bacteria (mm)

	S. paratyphi		***S. typhi***		***S. aureus***	
Diluation	***Cerastoderma***	***Didacta***	***Cerastoderma***	***Didacta***	***Cerastoderma***	***Didacta***
C1	17.00 ± 0.58^{a}	15.33 ± 1.45^{a}	13.67 ± 4.37^{ab}	13.33 ± 1.76^{a}	18.00 ± 1.15^{a}	13.00 ± 2.30^{ab}
C2	17.00 ± 1.57^{a}	12.00 ± 0.00^{ab}	17.67 ± 1.45^{a}	11.66 ± 2.02^{a}	$15.33 \pm 0.33A^{ab}$	8.33 ± 3.76^{Bb}
C3	11.00 ± 0.58^{b}	10.00 ± 1.15^{b}	11.00 ± 1.73^{b}	8.33 ± 0.33^{b}	7.67 ± 0.33^{b}	4.00 ± 2.30^{c}
C4	7.66 ± 0.88^{ab}	5.66 ± 0.33^{bc}	5.00 ± 1.73^{c}	5.00 ± 1.73^{c}	3.00 ± 1.15^{c}	3.00 ± 1.73^{c}
C5	1.33 ± 0.33^{c}	1.00 ± 0.57^{d}	0.67 ± 0.33^{d}	1.00 ± 0.00^{d}	-	1.33 ± 0.67^{d}
C6	0.66 ± 0.33^{c}	-	-	-	-	0.66 ± 0.33^{d}
Gentamicin	13.00 ± 0.00^{ab}	13.00 ± 0.00^{a}	13.00 ± 0.00^{ab}	13.00 ± 0.00^{a}	-	-
Methicillin	-	-	-	-	14.00 ± 0.00^{ab}	14.00 ± 0.00^{a}

a, b, c, … letters indicate significant difference among different dilutions in each column.

A, B, C, … letters indicate significant difference between two extracts of *Cerastoderma* and *Didacta* in each dilution against specific bacterium.

Table 5. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different extracts of *Cerastoderma* and *Didacta* against different bacteria (mg ml^{-1})

bivalve	**extract**	**bacteria**					
		S. aureus		***S. typhi***		***S. paratyphi***	
		MBC	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**
Cerastoderma	methanol	2.5	2.5	5	2.5	2.5	1.25
	ethanol	2.5	1.25	5	5	2.5	2.5
	chloroform	5	5	-	10	5	5
	Alcalase hydrolysis	5	2.5	2.5	2.5	2.5	1.25
Didacta	methanol	2.5	2.5	-	-	-	10
	ethanol	5	5	-	10	5	2.5
	chloroform	5	2.5	-	5	5	5
	Alcalase hydrolysis	-	-	5	2.5	2.5	2.5

Discussion

In recent years, much attention has been paid to examine the biological activities of natural products and their potential use (Kumaravel, Ravichandran, Balasubramanian, Siva Subramanian, Bilal, 2010). The development of safe and effective antimicrobial drugs over the

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[DOR: 20.1001.1.2345315.2018.4.1.1.5]
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past 71 years has evolved (Franklin and Snow, 2005). However, widespread use of antibiotics has led to the emergence of pathogens with increase resistance to antibodies (Normark and Normark, 2002). Development of drug resistance in human pathogens against commonly used antibiotics necessitates search for antimicrobial agents from other sources including natural resources, from land and sea resources (Blunt, Copp, Munro, Northcote, Prinsep, 2009). Various studies have shown significant antimicrobial activity in mollusks, seaweed and has been proposed as a source for the development of pharmaceutical substances (Defer, Bourgougnon, Fleury, 2009). Most of antibacterial activities on bivalves have been examined on *M. edulis, M. galloprovicialis, G. demissa, C. verginica* and *C. gigas* (Haug, Stensvag, Olsen, Orjan, Sandsdalen, Styrvold, 2004; Seo, Crawford, Stone, Noga, 2005). Moreover, *M. meretrix* is widely used in China and India to treat liver diseases such as Jaundice and Hepatitis A and B (Wang, Liu, Tang, Zhang, Xiang, 2006).

In the present study, the antibacterial effect of methanol, ethanol and chloroform extracts as well as alcalase hydrolysis of two Caspian Sea bivalves including *Cerastoderma* and *Didacta*, was investigated aginst *S. typhi and S. paratyphi* and *S. aureus*.

The results obtained in this study showed that *Cerastoderma* had stronger antibacterial activity than *Didacta*. Similarly, Defer 2009 showed that among three bivalves and two gastropods the highest antimicrobial activity was observed for *Cerastoderma edule* (Defer, Bourgougnon, Fleury, 2009). The lower inhibitory activity was reported from the previous studies for the extracts prepared from the separated tissues compared to the extract of the whole body. This may be due to the disruption and changes in the bioactive components during separation process. In our study, the whole soft tissues of the body were used and good antibacterial activity was observed.

Extraction method and concentrations of extract in the process of antimicrobial activity, and changes in the concentrations of the extract have influence on the antimicrobial inhibitory concentration (Shakouri, Javanmard, Mahalleh, Soheili, 2015). Sugesh and Mayavu (2013) reported good antibacterial and antifungal activity for the ethanol and methanol extract of *M. meretrix* and *M. casta* bivalves. They attributed the antimicrobial and anti-fungal activity of these extracts to the presence of amino acids and peptides in these extracts (Sugesh and Mayavu, 2013). It was found from our results that the extracts tested in this study had the highest inhibitory effect on *S. aureus*, a gram-positive bacterium possibly due to the simplicity of the cell wall of the gram-positive bacteria compared to that of gram negative ones (Donnell and Russe, 1999).

Conclusions

The results of this study indicated that *Cerastoderma* and *Didacta* extracts contain several active compounds with antimicrobials properties, however, extracts showed *Cerastoderma* higher antibacterial activity. The antimicrobial properties of the extracts can be influenced by the method of extraction,

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[DOI: 10.29252/ijaah.4.1.1]

the type of solvents used, the type of organism and also the parts used for extraction. In general, the results indicate the potential of these extracts, which is ultimately after extensive research; it can be used as a useful clinical antimicrobial agent.

Acknowledgment

The authors would like to thank the laboratory personals of Islamic Azad University of Lahijan**.**

Conflict of interests

The authors declare that there is no conflict of interest.

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[DOR: 20.1001.1.2345315.2018.4.1.1.5]

[DOI: 10.29252/ijaah.4.1.1]

بررسی فعالیت آنتی میکروبیال پپتیدهای استخراج شده از دوکفه‌ای‌های *Cerastoderma* و *Didacta* درسواحل جنوبی دریای خزر

آسیه قربانعلی زاده [۱]، اعظم مشفق [۱*]، محبوبه سترکی [۲]

[۱] گروه بیولوژی دریا، واحد لاهیجان، دانشگاه آزاد اسلامی، لاهیجان، ایران

[۲] گروه زیست شناسی، واحد ایذه، دانشگاه آزاد اسلامی، ایذه، ایران

چکیده

اثرات آنتی‌باکتریال عصاره‌های متانول، اتانول، کلروفرم و هیدرولیز آلکالاز *Cerastoderma* و *Didacta* بر باکتری‌های *Salmonella typhi*، *Salmonella paratyphi* و *Staphylococcus aureus* با روش انتشار دیسک، اندازه‌گیری هاله عدم رشد، و حداقل غلظت باکتریال مورد بررسی قرار گرفتند. عصاره متانولی و اتانولی *Cerastoderma* بالاترین اثر را در برابر *S. typhi* (۳۳/. ± ۳۳ /۲۰ و ۳۳/. ± ۱۲/۳۳) و *S. paratyphi* (۱/۴۵ ± ۲۲/۶ و ۳۳/. ± ۱۵/۳۳) نشان داد، با این حال، اثرات مشابهی در برابر *S. aureus* (۰۰/۰۰ ± ۱۸/۰۰ و ۰۰/۰۰ ± ۱۷/۰۰) برای دو گونه دو کفه‌ای مشاهده شد. عصاره کلروفرم *Cerastoderma* و *Didacta* اثرات مشابهی در کنترل *S. paratyphi* (۵۸/. ± ۸/۰۰ در مقابل ۱۰/۰۰ ± ۰/۵۷) و *S. aureus* (۱/۱۵ ± ۱۶/۰۰ در مقابل ۱/۱۵ ± ۱۶/۰۰) در غلظت ۱۰ و ۵ میلی‌گرم بر میلی‌لیتر نشان داد. عصاره کلروفرم *Cerastoderma* بالاتر از اثر *Didacta* در برابر *S. typhi* (۱/۷۳ ± ۱۱/۰۰ در مقابل ۸/۰۰ ± ۰/۵۸) اثرات بیشتری داشت و رقت ۱۰ میلی‌گرم بر میلی‌لیتر بیشترین عملکرد را داشت. هیدرولیز آنزیمی از *Cerastoderma* و *Didacta* عملکرد مشابهی را در کنترل *S. typhi* (۴/۳۷ ± ۱۳/۶۷ در مقابل ۱/۷۶ ± ۱۳/۳۳) و *S. paratyphi* (۵۸/. ± ۱۷/۰۰ در مقابل ۱/۴۵ ± ۱۵/۳۳) نشان داد. با این حال، هیدرولیز آنزیمی *Cerastoderma* اثرات بهتری نسبت به *Didacta* در کنترل(*S. aureus* ۱/۱۵ ± ۱۸/۰۰ در مقابل ۲/۳۰ ± ۱۳/۰۰) نشان داد و و رقت‌های ۱۰ و ۵ میلی‌گرم بر میلی‌لیتر مناسب‌ترین غلظت بود. نتیجه‌گیری می‌شود که سرستودرما می‌تواند به عنوان یک منبع با ترکیبات ضد باکتری قوی در تهیه مواد ضد میکروبی طبیعی استفاده شود.

کلمات کلیدی: فعالیت آنتی باکتریال، عصاره‌ها، *Cerastoderma*، *Didacta*

***نویسنده مسئول:** moshfeghazam@gmail.Com

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[DOR: 20.1001.1.2345315.2018.4.1.1.5]

[DOI: 10.29252/ijaah.4.1.1]