Antimicrobial Features of Cerastoderma and Didacna Double Basins Peptides

Abdolmajid Ghasemian1,2, Farshad Nojoomi2, Zahra Najafi-olya3, Hossein Rajabi-Vardanjani4*

1Department of Microbiology, Fasa University of Medical Sciences, Fasa, Iran
2Microbiology Department, Faculty of Medicine, AJA University of Medical Sciences, Tehran, Iran
3Department of Bacteriology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
4Researcher of Shahrekord University of Medical Sciences, Shahrekord, Iran

Abstract
Background: In recent years, high attention has been given to the biological activities of natural compounds and their potential antimicrobial properties.

Objective: In this study, the antibacterial properties of the extracts from tissue and peptides of Cerastoderma and Didacna were studied.

Materials and Methods: samples of Cerastoderma and Didacna were collected and washed. Then, the soft tissues were cut and powdered, and concentrations of 16, 8, 4, 2, 1 and 0.5 of chloroform, ethanol and methanol, and in addition extract of enzymatic hydrolysis were prepared, and their antibacterial activities against Staphylococcus aureus, Escherichia coli and Salmonella paratyphi were investigated. The disc diffusion method was used for the evaluation of strains susceptibility. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were investigated for bacterial growth inhibition.

Results: Methanolic and ethanolic extracts from Cerastoderma demonstrated higher growth inhibitory effects compared to those from Didacna on E. coli and S. paratyphi and exhibited similar activities against S. aureus at concentrations 16 and 8 ug/mL. In addition, chloroform extracts of Cerastoderma and Didacna displayed similar inhibitory effects on S. paratyphi and S. aureus at concentrations 16 and 8 ug/mL which was a suitable effect, and the extract from Cerastoderma was more effective. MIC and MBC of methanolic extracts at the lowest level, especially against S. aureus.

Conclusion: It was revealed that Cerastoderma and Didacna extracts were effective as antibacterial compounds on S. aureus, E. coli and S. paratyphi species as natural agents.

Background
Antibacterial and antifungal compounds isolated from marine organisms have been used for many years, however scientific investigations have been scarce in this regard.1 Antimicrobial investigations on soft tissues can elucidate the substantial information on the efficiency of new bioactive compounds. Difficulties in eradication of drug-resistant infections have been a critical issue in recent years.2,3 Great therapeutic properties of aquatic sources including sterols, peptides, terpenes and nitrogen compounds, macrolides, acid-fat derivatives, prostaglandins, and other alkaloids have been revealed.7 Bacterial infections cause many deaths annually.3,4 By the increasing the rate of antibiotics uncontrolled consumption and resistance to them, the application of antimicrobial peptides seem proper strategy as alternative broad-spectrum antibiotics being produced in human body as primary antimicrobial compounds.5,6 Furthermore, marine organisms encompass approximately half of the biodiversity world and because of far sea history and vast volume, they can be deeply investigated as a gold opportunity.10 Creatures in the sea have compounds which possibly play a leading role as natural and novel therapeutic compounds. This study was performed to evaluate the antibacterial properties of extracts from Cerastoderma and Didacna, the sea creatures from the Persian Gulf.

Materials and Methods
Preparation of Extracts
After sample collection, oysters were crushed and their soft body tissues were separated and cut. The extracts were prepared as described by Sharma et al.11 Methanol, ethanol and chloroform (volumetric weight 1:1) solvents were used for the extraction of effective compounds.

Enzymatic Hydrolysis
An Alcalase 2.4 L enzyme (Hayyan Azma Co., Iran)

*Corresponding Author:
Hassan Rajabi-Vardanjani,
Tel: +989394514860;
Fax:+9882884555;
Email: bacteriology94@gmail.com

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was used, an alkaline enzyme which has 2.4 Unite/g Anson enzymatic activity, and is obtained from Bacillus licheniformis. The enzyme was kept at 4°C until use in the experiment. After the enzymatic hydrolysis of tissues in phosphate buffer at pH 8.5, at 95°C, the samples were centrifuged at 8000 rpm for 20 minutes and the supernatant was collected.13

Antimicrobial Activity Measurement
The disc diffusion method was used for antimicrobial evaluation of methanolic, ethanolic, and chloroform extracts, in addition to enzymatic hydrolysis of extract on the Mueller-Hinton agar (MHA) medium. Then, 10 mg of extract or enzymatic hydrolysis powder was inoculated on 1 mL of each solvent using 20 µL of each extract at concentrations 16, 8, 4, 2, 1 and 0.5 µg/mL.

After drying each sterile medium at room temperature for 3 hours, the plates were incubated at 37°C for 24 hours. The inhibitory zone diameters were measured by a caliper.

The Minimum Inhibitory and Bactericidal Concentrations
The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of methanolic and ethanolic extracts were evaluated using broth dilution test using 8 dilutions. After bacterial suspension preparation, an equal amount of MHA was added and 128 µg/mL of extract was added to the first tube and diluted. Afterward, they were incubated at 37°C for 24 hours. The first dilution without any growth was considered as MIC.14

MBC was measured by culture of 10 µL of first tube with no growth, and incubation at 37°C for 24 hours. If no growth was observed on MHA, it was concluded as the MBC indicating inhibition of 99% of bacterial population.

The Chemical Mixture
The evaluation of protein, carbohydrate and lipid contents was confirmed following previous studies including Bergmann and Feeney,15 Peterson16 and DuBois et al,17 respectively.

Data Analysis
Kolmogorov-Smirnov method was applied for normal distribution and evaluation of the treatments and one-way ANOVA test and Duncan test were used, after testing the homogeneity of variances, to compare the group means.

Independent t test was used at the statistical level 99% as the statistical test between 2 groups. Excel 2007 and SPSS version 21.0 were used for data analyses.

Results
Effects of Methanolic Extract of Cerastoderma and Didacna
The Cerastoderma and Didacna compounds exhibited significant differences in their effects on S. aureus, and Cerastoderma extract inhibited S. paratyphi more significantly compared to Didacna extract. In addition, the methanolic extract of Cerastoderma significantly inhibited the bacterial growth compared to gentamicin in dilutions 1 and 2 µg/mL, but such inhibition was not observed against S. paratyphi.

Effects of Ethanoi Extracts of Cerastoderma and Didacna
Salmonella paratyphi inhibition by Cerastoderma and Didacna extracts was significantly different (df = 5, F = 59.656, P = 0.00). Growth inhibition by Cerastoderma extract was significantly higher than that by gentamicin in dilutions 1 and 4, but it was lower regarding ethanolic extract of Didacna.

In addition, effects of Cerastoderma and Didacna extracts on S. aureus was statistically significant (df = 4, F = 79.25, P = 0.00). The Cerastoderma extract in dilution 1 exhibited significantly higher effect than methicillin.

Effects of Chloroform extracts of Cerastoderma and Didacna
Effects of chloroform extracts of Cerastoderma and Didacna on S. paratyphi were statistically significant; as in dilution 1, Cerastoderma and Didacna extracts demonstrated significantly higher and lower effects than gentamicin, respectively.

The effects of chloroform extracts of Cerastoderma and Didacna were significantly different on S. aureus (P = 0.003) and interestingly, in dilution 1 both compounds inhibited S. aureus significantly higher than methicillin.

Effects of Enzymatic Hydrolysis Extracts of Cerastoderma and Didacna
Salmonella paratyphi and E. coli growth inhibition was significantly different in used dilutions of alcalase hydrolysis of Cerastoderma and Didacna (df = 6, F = 160.352, P = 0.00); as Cerastoderma extract in dilution 1 inhibited S. paratyphi significantly higher than gentamicin.

Furthermore, their different effects on S. aureus was statistically significant (P = 0.000), as Cerastoderma and Didacna exhibited higher effects than methicillin.

MIC and MBC Levels
The MIC and MBC of methanolic extracts of Cerastoderma and Didacna against E. coli were 64 µg/mL and 128 µg/mL, and against S. paratyphi were 128 µg/mL and >12 µg/mL, respectively; but their MIC and MBC against S. aureus were 32 µg/mL and 64 µg/mL, respectively.

In addition, the MIC and MBC of ethanolic extracts of Cerastoderma and Didacna against E. coli and S. paratyphi were both 128 µg/mL and >128 µg/mL, respectively, however their MIC and MBC against S. aureus were 64
μg/mL and 128 μg/mL, respectively.

Discussion
In recent years, high attention has been given to the biological activities of natural products, especially antimicrobial potential of them. This is because of increasing and widespread resistance against antibiotics commonly prescribed by pathogens. Various researches have demonstrated a high level of antimicrobial activity conferred by mollusks and seaweed and have thus proposed these resources to be consumed to develop their pharmaceutical properties. Several previous studies have evaluated antibacterial activities conferred by bivalves mostly against *Escherichia coli*, *Salmonella paratyphi* and *Staphylococcus aureus*. Further, the methanolic extract of *Cerastoderma* and *Didacna* were significantly higher than gentamicin.

Conclusion
In this study, the mean diameter of growth inhibition zone for *E. coli*, regarding the methanolic extract of *Cerastoderma*, was observed in dilution of 16 mg/mL, and alc alase hydrolysis of *Cerastoderma* at dilutions 16 and 8 μg/mL significantly demonstrated higher effect than gentamicin. The results of the present study emphasized that soft tissue extracts of *Cerastoderma* and *Didacna* contain several effective compounds exhibiting antimicrobial properties. The antimicrobial characteristics of these extracts could be influenced by the method of extraction and nature of solvents applied. The results demonstrated the potential of these extracts, and their possible potential to be useful for clinical purposes as antibacterial agents can be verified by further surveys.

Authors' Contributions
Study design and laboratory work: FN and AG; Data analysis: HRV and ZN.

Ethical Approval
This study was approved by Shahrekord University of Medical Sciences.

Conflict of Interest Disclosures
The authors declare that they have no conflict of interests.

References


