International Journal of
Enteric
Pathogens

Int J Enteric Pathog. 2018 August;6(3):75-78

Original Article

http://enterpathog.abzums.ac.ir

doi 10.15171/ijep.2018.20

Antimicrobial Features of Cerastoderma and Didacna Double Basins Peptides

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Published Online May 27, 2018

Keywords: Antimicrobial peptides, Cerastoderma, Didacna

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 were 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.

Received October 15, 2017; **Revised** January 21, 2018; **Accepted** April 22, 2018

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.[2-6] 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.[5,8] 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.[9,10] 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.[11] 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.[12] 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)

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] Peterson[16] 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 Ethanolic 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.[7,18,19] 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 *Maerua edulis, Geukensia demissa, Mytilus galloprovicialis, Crassostrea gigas* and *Crassostrea verginica*.[12,20] Soft tissues of *M. meretrix* have been widely consumed in China and India for the remedy of liver diseases such as hepatitis A and B and the Jaundice disease.[9]

In the current study, the antimicrobial properties of methanolic, ethanolic, and chloroform extracts as well as alcalase hydrolysis of Cerastoderma and Didacna were assessed against *E. coli, S. paratyphi* and *S. aureus*.

The inhibition zone diameter of concentrations 1, 2, 4, 8, and 16 μg/mL of methanolic extract of Cerastoderma was significantly higher than those concentrations of Didacna extract. Similar to our results, Defer and colleagues'[21] investigation on 2 gastropods and 3 bivalve species demonstrated the highest antimicrobial properties of *Cerastoderma edule*. Methods of preparation, various concentrations of extracts used for the evaluation of antimicrobial activities and bacterial species seem to be the most important factors interfering in the activity levels.

Statistical analysis in this study elucidated that the extracts used for antimicrobial evaluation showed the highest growth-inhibitory effect against *S. aureus*, which is a gram-positive species and possibly because of the cell wall differences of the bacterium.

In general, the mean of no-growth inhibition zone for the methanolic, ethanolic, chloroform, and hydrolysis extracts of Cerastoderma and Didacna against *S. aureus* at dilutions of 16 and 8 μg/mL was significantly higher than that measured for methicillin disk.

In addition, the mean diameter of the *S. paratyphi* growth inhibition zone for dilutions of 16, 8, and 4 μg/mL of ethanolic extract from Cerastoderma was significantly higher than that of gentamicin disk, while methanolic and ethanolic extracts of Didacna had significantly lower effect than the disk. The mean diameter of the *S. paratyphi* growth inhibition zone, in the chloroform extract of Cerastoderma and Didacna, at a dilution of 8 μg/mL had a function similar to the gentamicin. Furthermore, the mean diameter of the *S. paratyphi* growth inhibition zone for the enzymatic hydrolysis extract of Cerastoderma and Didacna, at dilutions of 16 and 8 μg/mL, 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 alcalase 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.

Financial Support

This study was supported by AJA University of Medical Sciences, Tehran, Iran.

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