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Antibacterial activity of ovary extract from sea urchin *Diadema setosum*

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Abstract. – OBJECTIVE: Sea urchin gonad is considered as a highly prized delicacy in several countries. It is also rich in valuable bioactive compounds including polyunsaturated fatty acids (PUFAs) and β-carotene. This study was undertaken to examine the antimicrobial properties of the ovary extract from sea urchin *Diadema setosum* against selected Gram-negative and Gram-positive bacteria.

MATERIALS AND METHODS: The ovary extract was obtained using two different solvents such as methanol and chloroform. The obtained extract was used to examine its potential antimicrobial properties against the following 11 bacterial species using the disc diffusion method: Gram-negative bacteria (*Salmonella typhi, Salmonella typhimurium, Shigella flexneri, Pseudomonas aeruginosa, Aeromonas hydrophila, Acinetobacter sp, Citrobacter freundii and Klebsiella pneumonia*) and Gram-positive bacteria (*Bacillus subtilis, Staphylococcus epidermidis* and *Staphylococcus aureus*). The activity was measured in terms of zone of inhibition (mm).

RESULTS: The methanol extract exhibited a higher zone of inhibition against all the bacteria taken for examination. Whereas, the ovary extract obtained by chloroform did not show any antimicrobial activity against *S. typhi*, *S. epidermidis*, *C. freundii* and *K. pneumonia*. The results indicated that the ovary extract obtained by methanol extracts are capable of inhibiting the growth of pathogenic microbes taken for analysis. Moreover, the result indicates the presence of antimicrobial agents in sea urchin ovary.

CONCLUSIONS: The study suggests that the ovary extract of *D. setosum* may be a potential source of antimicrobial agent for pathogenic microorganisms.

Key Words:
Antimicrobial activity, Sea urchin, *Diadema setosum*, Ovary.

Introduction

In the modern world, the techniques on the development of drugs and therapeutic agents are greatly improved by various new discoveries and formulations of medicines from various natural resources. However, throughout the world, infectious diseases are still a limiting factor for public health. To combat this, many researches have been undertaken to find out an effective method to prevent or cure diseases. Pharmacological industries have developed a huge number of drugs and antibiotics. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Hence several attempts have been made to explore new antimicrobial drugs from natural resources of land and water. But, till date, these drug discoveries are not well explored from marine resources due to lack of knowledge on the medicinal qualities of the marine resources including plants and animals.

Microbial populations in seawater and sediments may be as high as 10^6 and 10^9/mL, respectively[1]. Marine invertebrates are, therefore, constantly exposed to high concentrations of bacteria, fungi and viruses, many of which are pathogenic. The survival of these organisms depends on the efficient antimicrobial mechanisms to protect themselves against various microbial infections. During the last decade, there has been an increase in research on marine crustaceans, molluscs and echinoderms, with particular interest on their secondary metabolites which have desirable antimicrobial properties[2,3]. Among the echinoderms, *D. setosum* is one of the most

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widely distributed sea urchin. In the Indo-West Pacific Ocean, where it occurs from the Red Sea (Gulf of Suez, Gulf of Aqaba, northern and southern Red Sea) and the east coast of Africa to Japan and Australia[4]. This species can be available in both tropical waters and temperate zones including Malaysia[5].

Sea urchin gonad is considered as a highly prized delicacy in Asia, Mediterranean and Western Hemisphere countries including Barbados and Chile[6]. The brown gonads are colored due to the presence of carotenoids and polyhydroxylated naphthoquinones such as echinochrome A, which has a potent antioxidant activity. *Diadema setosum* has not yet been used as a common edible species in Malaysia. However, it is reported that an indigenous tribal people "Bajau Laut" from Sabah region (Malayisa) eat *D. setosum* roe with boiled rice[7]. Gonads of *D. setosum* also are rich in various bioactive compounds including polyunsaturated fatty acids (PUFAs) and β-carotene[7]. PUFAs consisted of eicosapentaenoic acid [(EPA, C20:5) (n-3)] and docosahexaenoic acid [(DHA C22:6) (n-3)], have a significant preventive effects on arrhythmia, cardiovascular diseases and cancer[8]. Moreover, β-carotene and some xanthophylls from *D. setosum* contains strong pro-vitamin A activity, which prevents the tumour cell development[9]. Recently Lawrence[10] observed that *D. Setosum* contains high level of arachidonic acid (AA) and EPA. These existing findings encouraged researchers to explore the medicinal values of sea urchin species. Hence, in this study, we attempted to examine the antimicrobial properties of sea urchin through its ovary extract due to the presence of valuable bioactive compounds in the ovary.

Materials and Methods

Ovary Collection and Maintenance

Matured adults of *D. setosum* were collected from Tanjung Dawai, Sungai Petani, Kedah Darul Aman, Malaysia and transported to the laboratory. The ovaries were dissected out and stored at –20ºC for further analysis.

Preparation of Extract and Protein Quantification

Ovary extract was obtained as described by Abubakar et al[11]. Four grams of ovary was homogenized and extracted with 40 mL of 70% methanol or chloroform in a shaker (90 rpm/min at 10°C for 24 h). Then, the crude extract was centrifuged (12.000 g for 5 minutes at 4°C) and the supernatant was collected, followed by passing through a 0.2 μm millipore filter and collected in a Beckman tube, and stored at –20°C. This sterile filtrate was used for the antimicrobial assay through agar disc diffusion method.

Bacteria

Gram-negative bacteria (*Salmonella typhi, Salmonella typhimurium, Shigella flexneri, Pseudomonas aeruginosa, Aeromonas hydrophila, Acinetobacter* sp, *Citrobacter freundii* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Bacillus subtilis, Staphylococcus epidermidis* and *Staphylococcus aureus*) were used for the antimicrobial assays. These bacteria were grown at room temperature in their respective nutrient broth.

Antimicrobial Assay

Antibacterial activity of the extract was tested against various bacterial species using disc diffusion method[10]. In brief, 20 mL sterile nutrient agar was poured into Petri dishes, and then allowed them to solidify at 37°C. Then, 100 μL of a 24 h broth cultured bacteria was inoculated. Discs of Whatman (No. 1) filter paper were cut using an office punching machine and autoclaved at 121°C for 15 min. Each sterile disc was then dipped in 100 μL of the ovary extract and placed on the agar plate using flame sterilized forceps. After 30 min, the plates were inverted and incubated at 37°C for 16-18 h. The diameter of the zone of inhibition was measured in millimetre (mm). Clear zone of inhibition around the discs indicated the antimicrobial activity. The assay was performed in three replicates and the data were presented as average of three replicates ± standard deviation. One hundred μg of streptomycin, ampicillin, cephalexin and gentamicin were used as positive control, whereas the same volume of nuclease-free de-ionized water used as a negative control.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The methanolic extract of sea urchin ovary showed better antimicrobial activity. Thus, it was further subjected to examine the minimum inhibitory concentration (MIC) to obtain the lowest concentration of ovary extract, which can inhibit

the growth of bacteria. One hundred μL of the sea urchin ovary extract was serially two-fold diluted with 70% methanol. The well plate was incubated for 18-24 h. The growth of inhibition was observed visually based on the turbidity of the mixture. Minimum bactericidal concentration (MBC) was determined to identify the lowest concentration at which the ovary extract kills the bacteria. Once the MIC was determined, the inoculum of higher concentration was sub cultured on the nutrient agar and incubated for 18-24 h. The agar plate that does not have any bacterial colony was considered as the point of MBC.

Statistical Analysis

One way analysis of variance and Duncan's multiple-range tests were employed to analyze the data collected for zone of inhibition. Differences between means were considered significant when $p < 0.05$.

Results

In this study, two solvents were used to extract and screen sea urchin ovary for antimicrobial activities. Methanol and chloroform extracts were prepared from the ovary of *D. setosum*. The results of the antibacterial activity of ovary extracts of *D. setosum* are presented in Table I. These extracts were then screened against 11 bacterial species namely, *S. typhi, S. typhimurium, S. flexneri, P. aeruginosa, A. hydrophila, Acinetobacter* sp, *C. freundii, K. pneumoniae, B. subtilis, S. epidermidis* and *S. aureus* by using disc diffusion method.

The methanol extract exhibited the highest antimicrobial activity against all the tested microorganisms compared to the chloroform extract. Maximum zone of inhibition (19.33 mm) for the methanol extract was observed against *K. pneumoniae* followed by *A. hydrophila* with an inhibition zone of 18.33 mm. Chloroform extract of sea urchin ovary showed antimicrobial activity against *B. subtilis, S. flexneri, S. typhimurium, P. aeruginosa, A. hydrophila, Acinetobacter* sp and *S. aureus* only. But it did not inhibit growth of *S. typhi, S. epidermidis, C. freundii* and *K. pneumoniae*. For comparison among the activities, four antibiotics (streptomycin, ampicillin, cephalexin and gentamicin) were used as positive controls.

The MIC and MBC of methanol extract of sea urchin are shown in Table II. The results revealed that a concentration of 50 mg/mL inhibit-

Table I. Screening of antimicrobial activity of the ovary extracts from the sea urchin, *Diadema setosum*.

Bacteria	Zone of inhibition (mm)					
	Methanol extract	Chloroform extract	Streptomycin	Ampicillin	Cephalexin	Gentamycin
Bacillus subtilis	9.73 ± 0.25^{d}	$9.00 \pm 1.00^{a,b}$	35.33 ± 0.58^{a}	25.00 ± 0.00^{a}	24.67 ± 1.15^{b}	26.00 ± 0.00^{c}
Salmonella typhi	$16.67 \pm 1.15^{b,c}$	–	31.00 ± 0.00^{b}	24.83 ± 0.29^{a}	25.00 ± 1.00^{b}	37.30 ± 0.58^{a}
Shigella flexneri	$17.00 \pm 0.00^{b,c}$	$9.33 \pm 0.58^{a,b}$	20.33 ± 0.58^{f}	14.33 ± 0.58^{e}	19.00 ± 0.00^{d}	25.67 ± 0.58^{c}
Salmonella typhimurium	$17.33 \pm 1.15^{b,c}$	$13.33 \pm 1.15^{a,b}$	13.67 ± 0.58^{h}	13.17 ± 0.29^{f}	18.90 ± 0.17^{d}	26.33 ± 1.15^{c}
Staphylococcus epidermidis	15.67 ± 0.58^{c}	–	14.67 ± 0.58^{g}	15.83 ± 0.29^{d}	26.67 ± 0.58^{a}	37.67 ± 0.58^{a}
Pseudomonas aeruginosa	$16.67 \pm 2.08^{b,c}$	$11.33 \pm 0.58^{a,b}$	24.00 ± 0.00^{d}	18.00 ± 1.00^{c}	15.67 ± 0.58^{e}	28.17 ± 0.76^{b}
Aeromonas hydrophila	$18.33 \pm 0.58^{a,b}$	$10.00 \pm 1.00^{a,b}$	22.50 ± 0.50^{e}	13.00 ± 0.00^{f}	15.67 ± 0.58^{e}	17.33 ± 0.58^{e}
Acinetobacter sp.	10.67 ± 0.58^{d}	$9.67 \pm 1.50^{a,b}$	25.00 ± 0.00^{c}	13.67 ± 0.58^{f}	0.00 ± 0.00^{f}	8.83 ± 0.29^{f}
Citrobacter freundii	$18.00 \pm 1.00^{a,b}$	–	10.33 ± 0.58^{i}	0.00 ± 0.00^{h}	0.00 ± 0.00^{f}	0.00 ± 0.00^{g}
Klebsiella pneumoniae	19.33 ± 1.15^{a}	–	22.83 ± 0.29^{e}	21.00 ± 1.00^{b}	19.67 ± 0.58^{d}	24.67 ± 0.58^{d}
Staphylococcus aureus	$16.67 \pm 0.58^{b,c}$	$9.67 \pm 0.58^{a,b}$	21.00 ± 0.00^{f}	11.00 ± 1.00^{g}	22.67 ± 0.58^{c}	25.83 ± 0.29^{e}

Note: "–" indicates no zone of inhibition. Each value is the mean and standard deviation of three replicates. Values bearing different superscripts in the each column are significantly different ($p < 0.05$).

Table II. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanolic extract of sea urchin, *Diadema setosum* test microorganisms.

Bacteria	MIC (mg/ml)	MBC (mg/ml)
Bacillus subtilis	50.00	100.00
Salmonella typhi	12.50	25.00
Shigella flexneri	25.00	50.00
Salmonella typhimurium	6.25	12.50
Staphylococcus epidermidis	3.13	6.25
Pseudomonas aeruginosa	50.00	100.00
Aeromonas hydrophila	6.25	12.50
Acinetobacter sp.	25.00	50.00
Citrobacter freundii	50.00	100.00
Klebsiella pneumoniae	25.00	50.00
Staphylococcus aureus	12.50	25.00

ed the growth of *B. subtilis* and *C. freundii*. The lowest MIC value of 3.13 mg/mL was observed in *S. epidermidis* followed by 6.25 mg/mL in *S. typhimurium* and *A. hydrophila*. The lowest MBC of 6.25 mg/mL was observed in *S. epidermidis* followed by 12.5 mg/mL in *S. typhimurium* and *A. hydrophila*. The highest MBC (100 mg/mL) was recorded in *B. subtilis, P. aeruginosa* and *C. freundii*. The result indicated that *S. epidermidis* has the lowest MIC (3.13 mg/mL) and MBC (6.25 mg/mL) than the other tested bacteria.

Discussion

This study elucidates the antimicrobial activity of ovary extract of *D. setosum*. Antibacterial activity has previously been described in a wide range of echinoderm species[12-15]. In most of the species studied, the whole body or body walls were tested for activity. Antimicrobial activity has also been reported in egg extracts of echinoid *Paracentrotus lividus*[16] and the asteroid *Marthasterias glacialis*[17]. In the latter study, the antibacterial compound was shown to be a lysozyme. The egg extracts of other marine invertebrates have also been shown to exhibit antimicrobial activity[17.]

In this study, ovary of sea urchin was extracted in two solvents to obtain different components. Antimicrobial activity was observed in both the methanol and the chloroform extracts of the ovary, however the higher inhibition was exhibited by the methanol extracts. This suggested that the antimicrobial components might be present in the sea urchin ovary.

The antimicrobial susceptibility showed that, sea urchin ovary extract has the higher zone of inhibition against a few bacteria compared to the conventional antibiotics such as streptomycin, ampicillin, cephalexin and gentamicin. For example, ampicillin showed a very high antibacterial activity against *B. subtilis* and *S. typhi*. However, the methanol extract of sea urchin showed better zone of inhibition against *S. flexneri, S. typhimurium, A. hydrophila, K. pneumoniae, C. freundii* and *S. aureus*. *Citrobacter freundii* was not inhibited by ampicillin, cephalexin and gentamicin. The methanol extract of sea urchin ovary showed inhibition against these bacteria.

The MIC test results showed that a minimum concentration of 3.13 mg/mL was found to inhibit the growth of *S. epidermidis*. The Gram-negative bacteria have higher MIC and MBC values compared to the Gram-positive bacteria. This is because Gram-negative bacteria have thick cell wall, made up of lipids and polysaccharides thus increasing its resistance to antimicrobial agents.

Conclusions

The results obtained clearly reflect that the ovary extract of *D. setosum* has excellent antimicrobial properties against a vast variety of pathogenic and non-pathogenic bacteria. Further studies on this sea urchin extract should channel into producing a novel drug in the near future.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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