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

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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 10⁶ and 10⁹/mL, respectively¹. 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 properties²,³. Among the echinoderms, *D. setosum* is one of the most

*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.
prized delicacy in Asia, Mediterranean and Western Hemisphere countries including Barbados and Chile. 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 (Malaysia) eat D. setosum roe with boiled rice. Gonads of D. setosum also are rich in various bioactive compounds including polyunsaturated fatty acids (PUFAs) and β-carotene. 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. Moreover, β-carotene and some xanthophylls from D. setosum contains strong pro-vitamin A activity, which prevents the tumour cell development. Recently Lawrence 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 –20°C for further analysis.

**Preparation of Extract and Protein Quantification**

Ovary extract was obtained as described by Abubakar et al. 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. 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 incu-bated 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, cephalaxin 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...
Table I. Screening of antimicrobial activity of the ovary extracts from the sea urchin, *Dianema setosum*.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Methanol extract</th>
<th>Chloroform extract</th>
<th>Streptomycin</th>
<th>Ampicillin</th>
<th>Cephalexin</th>
<th>Gentamycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>9.73 ± 0.25a</td>
<td>9.00 ± 1.00b</td>
<td>35.33 ± 0.58a</td>
<td>25.00 ± 0.00a</td>
<td>24.67 ± 1.15b</td>
<td>26.00 ± 0.00b</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>10.67 ± 1.15c</td>
<td>9.33 ± 0.58b</td>
<td>20.33 ± 0.58b</td>
<td>14.33 ± 0.58a</td>
<td>9.00 ± 0.00a</td>
<td>12.00 ± 0.58b</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>16.67 ± 0.00c</td>
<td>13.33 ± 1.15b</td>
<td>13.67 ± 0.58a</td>
<td>13.17 ± 0.29a</td>
<td>21.00 ± 0.00b</td>
<td>18.90 ± 0.17d</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>17.00 ± 0.00c</td>
<td>14.67 ± 0.58a</td>
<td>15.83 ± 0.29a</td>
<td>13.00 ± 0.00b</td>
<td>36.33 ± 0.58c</td>
<td>26.67 ± 0.58e</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>16.67 ± 0.58</td>
<td>–</td>
<td>24.00 ± 0.00a</td>
<td>18.00 ± 1.00c</td>
<td>15.67 ± 0.58c</td>
<td>18.90 ± 0.17d</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10.67 ± 0.58b</td>
<td>13.33 ± 1.15b</td>
<td>22.50 ± 0.50a</td>
<td>13.00 ± 0.00b</td>
<td>21.00 ± 0.00b</td>
<td>19.67 ± 0.58d</td>
</tr>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>10.67 ± 1.00b</td>
<td>9.67 ± 1.50b</td>
<td>25.00 ± 0.00a</td>
<td>13.67 ± 0.58c</td>
<td>0.00 ± 0.00f</td>
<td>8.83 ± 0.29f</td>
</tr>
<tr>
<td><em>Acinetobacter sp.</em></td>
<td>19.33 ± 0.15</td>
<td>–</td>
<td>22.83 ± 0.29a</td>
<td>21.00 ± 1.00d</td>
<td>21.67 ± 0.58a</td>
<td>24.67 ± 0.58d</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>16.67 ± 0.58</td>
<td>9.67 ± 0.58b</td>
<td>21.00 ± 0.00c</td>
<td>11.00 ± 1.00f</td>
<td>21.67 ± 0.58a</td>
<td>25.83 ± 0.29f</td>
</tr>
</tbody>
</table>

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).
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 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.

### Acknowledgements

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### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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