FROM BIOWASTE TO BIODRUG

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ABSTRACT

Several activities other than fishing were identified as impacting the marine and coastal environment like waste dumping. These activities result in several forms of habitat degradation including pollution and degradation of water quality. The promotion of healthy aquatic ecosystems is fundamental for limiting pollution.

Echinoderms, which got caught unintentionally while fishing, were either thrown in the fish landing centre as biowaste or used as animal feed. But it can be best used as a pharmacological source by identifying bioactive compounds from them to be used as biodrugs.

Marine organisms are known for their source of structurally novel and biologically active metabolites. The present study is aimed at identifying the anti-bacterial potential of Seaurchin, Temnopleurus alexandri, thrown out as biowaste in the fish landing centre. The anti-bacterial activity of the ethyl acetate extract (20, 200, 2000, 5000ppm concentrations) of T.alexandri was tested with 7 bacterial strains i.e., Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa. Except for B.subtilis and K.pneumoniae the extract was effective in controlling the E.faecalis, S.aureus, E.coli, P.vulgaris and P.aeruginosa. Minimum inhibitory concentrations were also found. The chemical composition of ethyl acetate extract was also identified by GCMS.

Key Words: Biowaste, Pharmacology, Echinoderms, Antibacterial effect, Bioactive compound

INTRODUCTION

Marine organisms represent excellent source for bioactive compounds. Bioactive chemical compounds can be classified as primary metabolites and secondary metabolites, depending on its biosynthetic origin, biochemical role and general occurrence. The secondary metabolites have various functions, it is likely that some of them may be pharmacologically active on humans and useful as medicines. A majority of pharmacologically active secondary metabolites have been isolated from echinoderms. Echinoderms seem to have secondary metabolites which are antimicrobial in nature.

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thrown in the fish landing centre as biowaste or used as animal feed. But it can be best used as a pharmacological source by identifying bioactive compounds from them to be used as biodrugs. The present study is thus aimed at assessing the antibacterial activity of the ethyl acetate extract from thus collected sea urchin, *Temnopleurus alexandri*.

**MATERIAL AND METHODS**

**Collection of animals**

Sea urchin, *T. alexandri* were collected from fish landing centre, Chennai coast, which was thrown as waste. Authentication of the echinoid was done with Zoological Survey of India (ZSI), Chennai (India).

**Extraction**

Shade dried specimens were immersed in ethyl acetate (1:3 w/v). Extract was obtained by cold percolation and concentrated under reduced pressure using rotary evaporator at 40°C. Finally crude extract was obtained. The crude extract was stored at 4°C until further use.

**Microorganisms**


**Antimicrobial assay**

Antimicrobial activity was carried out using disc diffusion method. Petri plates were prepared with 20 ml of sterile Muller Hinton Agar (MHA) (Hi-media) for bacteria. The extract was dissolved in 2% DMSO. Antibacterial sterile (empty) (Sigma) discs were used to load the extract of the required concentration. The test cultures (bacteria 10^8 CFU/ml) were swabbed on top of the solidified media and allowed to dry for 10 min. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using DMSO. Streptomycin and ampicillin (10µg/dish) were used as positive controls. The plates were incubated for 24 hr at 37°C for bacteria. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

**Minimum Inhibitory Concentration (MIC)**

MIC was performed according to the standard reference method. MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. Three replications were maintained.

**Gas Chromatography-Mass spectrometry (GC-MS) Analysis**

The crude extract was quantified using gas chromatograph (GCMS-Shimadzu) equipped with a DB-5 ms column (mm inner diameter 0.25 mm, length 30.0m, film thickness 0.25µm) mass spectrometer (ion source 200°C, R170eV) programmed at 40-650°C with a rate of 4°C/min. Injector temperature was 280°C; carrier gas was He(20 psi), column flow rate was 1.4ml/min, injection mode—split.

**RESULTS AND DISCUSSION**

Ethyl acetate extract of *T. alexandri* had very good antibacterial activity for many bacteria tested almost on par with ampicillin, except with *K.pneumoniae* and *B.subtilis* (Table 1). The Zone of inhibition (in mm) were found to be 18mm for *S.aureus*, 14mm for *P.aeruginosa* and 12mm for both *E.faecalis*
and *P. vulgaris*, and 11 mm for *E. coli*, all at the concentration of 5000 ppm of extract. Of all the concentrations tested, 5000 ppm was found to have greater antibacterial activity than the other concentrations (5, 20, 200, 2000 ppm) used. The zone of inhibition was found to increase with increased concentration of the extract. MIC (Table 2) was found to be as low as 5 ppm for *P. aeruginosa*, 50 ppm for *S. aureus*, 250 ppm for *E. coli*; 500 ppm for *E. faecalis* and 1250 ppm for *P. vulgaris*. GC-MS analysis revealed presence of ethylphenyl phenyl ether, mono(2-ethylhexyl)phthalate, delta 3,5-cholestadiene, 2,4,6-tris(1-phenylethyl) phenol etc.

**Table 1: Antibacterial activity of ethylacetate extract of T. alexandri**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Strep</th>
<th>Amp</th>
<th>DMSO</th>
<th>20ppm</th>
<th>200ppm</th>
<th>2000ppm</th>
<th>5000ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>32</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>20</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>E. coli</td>
<td>30</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>30</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>31</td>
<td>18</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>S. aureus</td>
<td>29</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>11</td>
<td>18</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>31</td>
<td>16</td>
<td>-</td>
<td>10</td>
<td>10</td>
<td>12</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table 2: Minimum inhibitory concentration (MIC)**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Minimum concentration observed in ZOI (in ppm)</th>
<th>Range of concentration in MIC plates (in ppm)</th>
<th>Minimum inhibitory concentration (in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>200ppm</td>
<td>200-3.125</td>
<td>50</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2000ppm</td>
<td>2000-31.25</td>
<td>250</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>20ppm</td>
<td>20-0.3125</td>
<td>5</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>2000ppm</td>
<td>2000-31.25</td>
<td>500</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>5000ppm</td>
<td>5000-78.25</td>
<td>1250</td>
</tr>
</tbody>
</table>

The results obtained from the present study revealed antibacterial activity by the ethyl acetate extract of *T. alexandri*. Highest activity was observed with the maximum dose of extract and the zone of inhibition was increasing with respect to increasing dose. Echinoderms have already been reported to contain pharmacologically active compounds with respect to antihistaminic, cytotoxicity, antiangiogenicity, angiogenicity and antibacterial activity. The ophuroid *Ophoplocas januarii* from Argentina.
contained one new antiviral steroidal sulfate. Similarly; Neothyoside is an antifungal triterpene diglycoside from the Gulf of California holothurians Neothyone gibbosa. The individualistic or the synergistic action of the compounds present in the extract, as evidenced by GCMS, could have been responsible for the antibacterial potential. Since antibacterial agents that possess antibacterial activity are of interest in the field of pharmacology, further fractionation, purification, and identification of the exact bioactive compound present in the present ethyl acetate extract is of much importance.

CONCLUSION
The present study clearly have demonstrated that the ethyl acetate extract of T. alexandri had antibacterial activity against many bacteria and the major components identified by GC-MS analysis could have been responsible for the antibacterial activity of the ethyl acetate extract of T. alexandri.

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REFERENCES