Larvicidal and anti-bacterial efficacy of *Kappaphycus alverazii* methanol extract from Mandapam coast

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Abstract

The marine seaweeds are one of the vital living resources. The present study is focused on antibacterial and larvicidal activity of marine red algae *Kappaphycus alverazii*. The antibacterial activity of methanol extract, was analysed for in vitro antibacterial activity against selected test pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Bacillus cereus*.

The methanol extract of red algae *Kappaphycus alverazii* showed maximum zone of inhibition (32 ±3.1) against test pathogen of *Escherichia coli*. The in vitro mosquito larvicidal activity was performed with different concentrations using *Anopheles stephensi*. Methanol extract exhibited high mortality rate effective against *Anopheles stephensi* after 24 hours of exposure.

Keywords: *Kappaphycus alverazii*, Antibacterial activity, Larvicidal activity.

Introduction

In traditional remedies, seaweeds are in use as in food diets. Seaweeds help as an essential source of bioactive natural materials such as in Japan and Korea and China. Numerous metabolites isolated from marine algae own bioactive effects. Aquatic organisms are a rich basis of fundamentally innovative and biologically energetic metabolites1. The group of plants are marine microalgae that live in brackish water environment or water.

The support of land living plants like seaweed contains photosynthetic pigments from sun light and nutrients. In the coastal areas, fresh and dry seaweeds are widely consumed by societies. Marine ecologies seaweeds are straight exposed and are susceptible to ambient microbes such as bacteria, fungi and viruses2. The origin of seaweeds from coastal region among high flow to low flow and subtidal wave area up to a penetration of 0.01% photosynthetic sunlit is available. Based on the colours, seaweeds can be classified into three different algae. They were divided based on the colour of the algae Red [Rhodophyta], green [Chlorophyta] and brown [Phaeophyta]. Red algae in the genus *Kappaphycus alverazii* have been intentionally introduced throughout the tropics for mariculture3. A red alga of *Kappaphycus alverazii* farming was introduced in the Philippines in 1960s through native diversities of its rough populations and it prolonged further to other portions of the biosphere with dissimilar refinement technologies5,6.

*Kappaphycus alverazii* is one of the more prominent tropical red algae in the world and also consumes one of the wildest increasing rates, bright to double in size each fifteen to thirty days. The algae are quite sturdy and firm, growing up to 2 meters in height. Branches are bulky and highly irregular, forming dense tangles of seaweed. Besides, *Kappaphycus alverazii* is identified for its capability to produce carrageenan7. Red algae (Rhodophyta) are a different eukaryotic lineage. Environmentally, red algae are essential as primary producers, providers of structural environment for other nautical organisms and they play a vital part in the primary formation and preservation of coral reefs. Specific red algae are economically significant as providers of nutrition and gels8. The hydrophilic colloid of carrageenan forms by the aqueous extraction of *Kappaphycus alverazii* 9. Carrageenan has many applications, such as thickening and stabilizing agents. Food crops include chocolate milk, cottage cheese, whipped cream, instant products, yogurt, frozen desserts, sauces. Apart from that, a pharmaceutical application includes cosmetics10.

Material and Methods

Sample Collection: The red seaweed sample of *Kappaphycus alverazii* was collected from mandapam coast, Rameshwaram, Tamilnadu. Collected samples were washed with salt water. Then the collected samples were shade dried for ten days and powdered using mixer grinder.

Algal extract preparation: Numerous extracts were prepared according to the procedure of Indian Pharmacopoeia14. The powdered algal samples were subjected to Soxhlet extraction of methanol. Then the extracts were concentrated by rotary evaporator to obtain pure extract. The algal extract was weighed and stored in the refrigerator for future use.

Test pathogens: Pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Bacillus cereus* were used for antibacterial activity.

Antibacterial activity: Methanol extract of red seaweed *Kappaphycus alverazii* was used for antibacterial study. Extracts were dissolved with DMSO (Dimethyl sulphoxide)15. Different concentrations (50, 100 and 150
mg/ml) of extracts were tested for these bacterial pathogens *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Bacillus cereus*. Well diffusion method was used for antibacterial activity. The inoculated culture plate was tested by different pathogen bacterial strain using streak plate method. Algal extracts were poured into the well using micropipette. Later on, the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. After, those plates were incubated for 24 hours at 37°C for bacteria. The outcomes (results) were noted by determining the diameter of inhibition zone after 24 hours of incubation.

**Larvicidal Activity:** The larvicidal bioactivity was done. Five batches of twenty in 240 millilitre (ml) of H2O and 1.0 ml of algal extract concentration were taken for larvicidal activity. Acetone and polysorbate 80 were used for control. The quantities of dead larvae were calculated after twenty-four hours of exposure, and the percentage of mortality was reported. In dose-dependent bioassay, 100% mortality of larvae increase was chosen in experimental media. Toxicity test for red algae was achieved by insertion of 20 mosquito larvae in 200 millilitres (ml) of Milli-Q water and 250 ml of methanol in a glass beaker. According to the desired concentrations (62.5, 125, 250, 500 and 1000 ppm), algal extracts were dissolved with Milli-Q water as a solvent. A set of control group included in each test (methanol and double distilled water) with individual concentration. Mortality of 4th stage of instar larvae was assessed after 24 hours to determine the acute toxicities on of *Anopheles stephensi*.

**Results and Discussion**

The purpose of this study was to assess the antibacterial activity of methanol extract of *Kappaphycus alverazii* on selected bacterial species viz. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia* and *Bacillus cereus*. The red algae of *Kappaphycus alverazii* showed the maximum and most considerable antibacterial activity against *Escherichia coli*. Most of the screened workers of *kappaphycus alverazii* showed maximum activity of antibacterial activity. The inhibition zones are measured in terms of millimetres (mm). For control, a standard antibiotic was used, and the results were compared with control.

In the present study, the observed mortalities effects of mosquito larvae are observed in 21, 40, 61, 83 and 100% at the concentration of 62.5, 125, 250, 500 and 1,000 ppm respectively against *Anopheles stephensi*. After 24 hours of exposure, the maximum parasite mortality was found in *Anopheles stephensi* (LC50=168.75 ppm, r²=0.992). In the concurrent assay, control (Milli-Q water) showed zero mortality. The χ² values were important at p<0.05 level. Overall mortality was detected for methanol extract of *Kappaphycus alverazii* for the mosquito larvae *Anopheles stephensi* at 1,000 ppm.

<table>
<thead>
<tr>
<th>Seaweed</th>
<th>Concentration Milligram (mg)</th>
<th>Zone of inhibition in test pathogens in millimetre(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Kappaphycus alverazii</em></td>
<td>50</td>
<td>14 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>25 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>34 ± 3.1</td>
</tr>
</tbody>
</table>

**Table 1**

**Inhibition zones of *Kappaphycus alverazii* (Methanol extract)**

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentrations (ppm)</th>
<th>Per cent mortality* (ppm)±SE</th>
<th>LC50 (UCL–LCL) (ppm)</th>
<th>Slope</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>1000</td>
<td>100±0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>81±2.39</td>
<td>168.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>63±4.13</td>
<td>(141.67-201.01)</td>
<td>63</td>
<td>0.992</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>40±0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>21±2.67</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

(MilliQ water) Control- Zero mortality.
Lethal concentrations (LC50) that destroys 50 percentage (%) of the exposed larvae. Upper confidence limit (UCL).
Lower confidence limit (LCL), r² regression coefficient, P<0.05, Significant level- *Mean value of five replicates.
Conclusion
The marine micro red algae of *Kappaphycus alvarezii* possess secondary metabolites with extensive range of activities. Antimicrobial activity of red algae collected from mandapam coast showed excellent result in *Escherichia coli* followed by *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus* and *Klebsiella pneumoniae* in bacterial pathogen. *Escherichia coli* showed good results in zone of inhibition in antibacterial activity in methanol extract of *Kappaphycus alvarezii*. Mosquito larvicidal activity has shown good mortality rate at the concentration of 1000 (ppm) and r² 0.992.

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References

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