PRELIMINARY PHYTOCHEMICAL AND ANTI BACTERIAL SCREENING OF FRACTIONS OF GRACILARIA CORTICATA AGAINST THE SELECTED BACTERIAL STRAINS

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ABSTRACT

Gracilaria corticata is a red alga which is abundantly present in many seacoasts around the world. Phytochemical analysis of fraction 3 of crude methanolic extract of Gracilaria corticata has shown presence of carbohydrates, alkaloids, tannins, flavinoids, terpenoids and proteins. The anti bacterial activity of fraction 3 of crude methanolic extract of Gracilaria corticata was tested against certain human pathogens such as Bacillus subtilis MTCC 441, Bacillus cereus MTCC 492, Staphylococcus aureus MTCC 121, Streptococcus pyogenes MTCC 442, Klebsiella pneumonia MTCC 530, Salmonella typhi MTCC 531 and Escherichia coli MTCC 443. The predominant activity was observed against all the organisms tested except Klebsiella pneumonia. Studies suggested that potential anti bacterial compounds can be isolated from Gracilaria corticata.

KEYWORDS: Seaweeds; Gracilaria corticata; Phytochemical screening; antibacterial action.

INTRODUCTION

The concept of finding newer drugs is now a days focused to marine resources especially for anti microbial and anti cancer drugs.[¹- ²] Seaweeds have been used as medicine for many
ailments since ancient times. During the management of infectious diseases, there is repeated administration of antibiotics that is leading to multiple drug resistance which is very common problem especially in nosocomial infection. The Pharmaceutical importance of seaweeds is wide spread all over the world. Many studies were reported earlier on the antimicrobial study of marine algae. The screening of the phyto constituents reveals the chemical nature of seaweeds. The present study was undertaken to perform phytochemical screening and to investigate the antibacterial activities of fractions of methanolic extracts of *Gracilaria corticata* screened against selected organisms.

**MATERIALS AND METHODS**

*Collection, identification and processing of alga*

The seaweed *Gracilaria corticata* was collected from Rameswaram coast (Palk Bay) and identified at CMFRI, Mandapam camp, Tamilnadu, India. The collected sample of seaweed was washed with fresh water to remove the impurities present on the alga. Then the alga was dried in shade until it dried completely. The dried alga was finely powdered. The grinded powder was pooled and stored in room temperature.

*Extraction of alga*

The powdered alga was packed in Soxhlet apparatus and bioactive principles were identified by hot continuous percolation using methanol. The crude extract was fractionized by using column chromatography over silca gel G 120 eluted by mobile phase methanol – dichloromethane and the fractions were collected. Totally 6 fractions were collected and the chemical constituents was confirmed by various chemical tests.

*Strains used*

The following strains were collected from Microbial type culture and collection (MTCC), Chandigarh, India. *Bacillus subtilis* MTCC 441, *Bacillus cereus* MTCC 492, *Staphylococcus aureus* MTCC 121, *Streptococcus pyogenes* MTCC 442, *Klebsiella pneumonia* MTCC 530, *Salmonella typhi* MTCC 531 and *Escherichia coli* MTCC 443.

*Antibacterial screening*

The lyophilized culture was sub cultured and the concentration of working stock culture was found as $10^{-6}$ CFU/ml. Specified quantity of Mueller Hinton agar was prepared and the plates were plated in aseptic condition. To detect the antibiotic susceptibility for fractions of drugs agar well diffusion technique was used and for susceptibility test for standard ciprofloxac
disc, agar disc diffusion technique was used. DMSO was used as solvent to dissolve the extracts to achieve concentration of 500 µg/ml. After incubating for 24 h at 37°C, the zone of inhibition was measured and the activity was compared with that of the standard ciprofloxacin disc (5mcg/disc).

**Statistical analysis**

All the experiment were performed six times (n= 6) and the data was subjected to one way analysis of variance (ANOVA), the level of significance is P < 0.05 using Graph pad Instat software system. The test values were compared with standard drug values by using Dunnet’s test (post test).

**RESULTS AND DISCUSSION**

The phytochemical analysis of methanolic extract of *Gracilaria corticata* showed the presence of the various phyto constituents such as carbohydrates, alkaloids, proteins, tannins, flavinoids and terpenoids. Totally 6 fractions of the extract were obtained through column chromatography. Among the 6 fractions of crude extract fraction 3 was found to contain more active constituents (Table 1). Therefore, fraction 3 was considered for antimicrobial studies. The activity of crude extracts was compared with standard ciprofloxacin activity. From the observation it was evident that the methanolic extract of *Gracilaria corticata* showed spectrum of activity against all tested organisms except *Klebsiella pneumonia* (Table 2). Earlier report by Kolanjinathan and Stella in 2010[9] demonstrated that 5 mg/ml of methanolic extract exhibited better activity against *Staphylococcus aureus* followed by *Streptococcus pyogenes, Streptococcus epidermis, Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Pseudomonas aeruginosa*. Kolanjinathan et al., 2009[1] reported that ethanol extracts of *Gracilaria edulis* inhibited most of the tested bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus faecalis*. However, earlier reports showed that the seasonal variations also influence the active principles.[10] Therefore, further investigation is necessary to know about the seasonal variations on the antibacterial activity.
Table.1: Various phytochemical constituents in fractions 3 of methanolic extract of *Gracilaria corticata*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Phytoconstituents</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Proteins</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Falvonoids</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>Present</td>
</tr>
</tbody>
</table>

Table.2: Spectrum of anti bacterial effect of fraction 3 of methanolic extract of *Gracilaria corticata*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction 3 - Crude extract</td>
</tr>
<tr>
<td><em>Escherichia coli</em> MTCC 443</td>
<td>14 ± 2.2</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em> MTCC 441</td>
<td>12 ± 1.4</td>
</tr>
<tr>
<td><em>Bacillus cereus</em> MTCC 492</td>
<td>12 ± 2.1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> MTCC 121</td>
<td>10 ± 1.2</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> MTCC 442</td>
<td>9 ± 2.1</td>
</tr>
<tr>
<td><em>Klebsiella pneumonia</em> MTCC 530</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> MTCC 531</td>
<td>8 ± 1.6</td>
</tr>
</tbody>
</table>

*Each value is the mean of 6 batches with standard deviation.

Figure.1: Antibacterial effect of fraction 3 of methanolic extract of *Gracilaria corticata* compared with standard ciprofloxacin disc.
CONCLUSION
In this preliminary research work, we had demonstrated the antibacterial effect of *Gracilaria corticata* against certain bacterial strains but further detailed study is required for isolating and establishing the antibacterial constituents from *Gracilaria corticata*.

CONFLICTS OF INTEREST
The author declares that he has no financial or non-financial conflicts of interest.

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REFERENCES