Antibacterial activity of green seaweeds on oral bacteria

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In present study, methanol extracts of the Chlorophycean group of seaweeds have been tested for their antibacterial activity against oral bacteria causing dental caries. Different concentrations of the extracts of the four species of seaweeds- Chaetomorpha antennina, Cladophora fascicularis, Spongomorpha indica and Ulva fasciata collected from sea coast of Visakhapatnam have been tested for their antibacterial activity against three oral pathogenic bacteria, Actinomyces viscosus (MTCC 7345), Streptococcus mitis (MTCC 2696), and Streptococcus mutans (MTCC 1943). The antibacterial sensitivity was studied by Agar disc diffusion method. Of these, U. fasciata has shown greater inhibition on all the three oral bacteria than C. antennina, C. fascicularis and S. indica. C. fascicularis has inhibited S. mitis and A. viscosus whereas C. antennina and S. indica inhibited only A. viscosus. The present findings reveal that these seaweeds have the potential antibacterial substances which can be used against oral pathogens as food additives, mouthwashes, chewing gums for preventing and treating dental caries.

Keywords: Seaweeds, Algae, Chlorophycean group, Antimicrobial, Oral bacteria, Agar disc diffusion, Dental caries.

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Introduction

The presence of nutrients, epithelial debris, and secretions makes the oral cavity a favorable habitat for a great variety of oral bacteria like Streptococci, Lactobacilli, Staphylococci, Corynebacteria, and with a great number of anaerobes, especially Bacteroides. The mouth presents a floral succession with age and corresponding ecological changes in the oral cavity. These bacteria colonizing the dental surface and gingiva have coevolved with their host to establish a highly sophisticated relationship between pathogenic and mutualistic bacteria coexisting in homeostasis. Plaque is a biofilm on the surfaces of the teeth. The accumulation of plaque results in dental caries and lead to gingivitis or periodontal diseases. Recent research has shown that oral bacteria may contribute to increased risk of heart attacks, strokes and lung disease and may be associated with premature childbirth in some women1.

The commercially available chemicals alter oral and intestinal microbiota and have adverse side effects such as vomiting, diarrhoea and tooth staining1,2. These drawbacks justify the search for new effective anticariogenic compounds that could be incorporated into dental products to complementing the mechanical removal of the biofilms and employed in caries prevention3,4. Recent studies have demonstrated the great importance of natural products, both plant extracts5,6 and isolated compounds such as essential oils7,8, parts of different plants9 and preparations of some seaweeds10 which have shown appreciable preventive effect on oral bacteria. Harder (1917) was the first to observe antimicrobial substance secreted by alga11. There are numerous reports of macro algae derived compounds that have a broad range of biological activities, such as antibacterial12-16, antifungal13,16,17, antiviral13, antineoplastic18, antifouling19, anti-inflammatory20,21, antitumoric22,23, cytotoxic24 and antimitotic activities6,9. Hence, this study was undertaken to find out the antibacterial activity of the seaweeds belonging to green algae-Chaetomorpha antennina, Cladophora fascicularis, Spongomorpha indica and Ulva fasciata of Chlorophyta (Plate 1a-d) against oral bacteria-Streptococcus mutans, Streptococcus mitis and Actinomyces viscosus, which are early invaders on tooth surfaces. These seaweeds are rich in the presence of many bioactive compounds like acrylic acid, saturated and unsaturated fatty acids20.

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Materials and Methods

Seaweed samples

The seaweeds *C. antennina*, *C. fascicularis*, *S. indica* and *U. fasciata* of Chlorophyta were collected in bulk quantity from coastal areas of Visakhapatnam-Tenneti Park, Vuda Park and Rushikonda. They were washed with sea water thoroughly to remove debris and epiphytes and brought to the laboratory, soaked in tap water for an hour and rinsed with sterile water to remove any remained animal castings, attached debris and sand particles. Excess water is drained by blotting them on the filter papers, shade dried for 5 days and they were cut into small pieces and dried in an oven at 37°C. The dried pieces were made into powder by using electrical grinder. Crude extract was prepared by methanol solvent (1:10 w/v) by using a Soxhlet extractor for 10 hrs and it was concentrated under reduced pressure by using rotary vacuum evaporator (Buchi-R420) to get their crude extracts and collected in airtight plastic vials and stored in the refrigerator for further analysis.

Microbial cultures

The oral pathogenic bacteria strains—*A. viscosus* (MTCC 7345), *S. mitis* (MTCC 2696), *S. mutans* (MTCC 1943), were obtained in lyophilized vials from the MTCC (Microbial Type Culture Collection) ans Gene Bank, Chandigarh, India. Revival of the cultures was done as per the instructions given in the MTCC catalogue. The 24 to 48 h old cultures were used in the determination of antibacterial activity of seaweeds. The concentration of the cultures was standardized by matching to the McFarland 0.5 turbidity standard.

Antibacterial activity

The antibacterial activity of the seaweeds was studied by the agar disc diffusion method. Crude extracts of seaweeds obtained were diluted with methanol to prepare 100, 50 and 25 mg/mL concentration and 5 mm diam. discs were prepared by using Whatman No.1 filter paper. They were sterilized by keeping in hot air oven at 160°C for 2 h, after cooling they were loaded with 40 µl from each

Plate 1—Seaweeds belonging to Chlorophycean group: 1a-*Chaetomorpha antennina*; 1b-*Cladophora fascicularis*; 1c-*Spongomorpha indica*; 1d-*Ulva fasciata*
stock solution i.e. 100, 50 and 25 mg/mL of the seaweed extract. They were allowed to dry for some time to evaporate the solvent from the paper disc.

**Agar disc diffusion method**

Sterile Muller-Hinton agar plates were taken and inoculated with 0.3 mL of 24 h old culture of the test microorganisms in nutrient broth with a sterile micropipette and spread with L-shaped glass rod to get the lawn of bacterial culture. Then the loaded discs were placed in the petri plates. Each plate contained three discs with three different concentrations i.e. 100, 50 and 25 mg/mL, solvent used for methanol extraction was used as a control and streptomycin (10 mcg) disc was used as standard. Plates (in triplicate) were kept in an incubator at 37°C for 24 h and later examined for clear inhibition zone around the discs and measured by using zonal scale and the results were tabulated.

**Results and Discussion**

Many research studies are available which showed the prevention of dental plaque formation and on different compounds which possess antibacterial activity against oral bacteria. The sulphated polysaccharides present in some marine algae are highly effective in preventing plaque formation by interfering with glucan deposition\(^{25,26}\). The oral preparation from *A. nodosum*, seaweed is reported to be suitable for use in prophylactic and/or therapeutic method of treating plaque and/or calculus in a mammalian individual\(^{27}\).

Seaweed extracts of methanol solvent showed different antimicrobial activity against oral pathogenic bacterial strains used in the present investigation. The obtained results of the current preliminary investigation are in agreement with those of the studies that were carried on antimicrobial activity of seaweeds by different researchers\(^ {16,25-29}\). Acrylic acid is the common antibacterial components which occur in many red, brown and green seaweeds as well as in various species of phytoplankton\(^ {30}\). Seaweeds, viz. *C. antennina, C. fascicularis, S. indica* and *U. fasciata* of Chlorophyta were tested at various concentrations by agar disc diffusion method for their antibacterial activity against oral bacteria-*A. viscosus, S. mitis* and *S. mutans*.

The antibacterial activity of *C. antennina* against oral bacteria is shown in Table 1 and Plate 2. The methanol extract of this species showed increased zone of inhibition, maximum at 100 mg/mL only on *A. viscosus*. It showed no activity on *S. mutans* and *S. mitis*. It is reported that the antimicrobial potential of this seaweed collected during the post monsoon season was high\(^ {31}\). The antibacterial activity of *C. fascicularis* against oral bacteria is shown in Table 2 and Plate 3. The methanol extract showed maximum zone of inhibition on *A. viscosus* than *S. mitis* at

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th><em>S. mutans</em></th>
<th><em>S. mitis</em></th>
<th><em>A. viscosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>2.16±0.28</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>3.83±0.28</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
<td>6.33±0.57</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Standard</td>
<td>10.83±0.28</td>
<td>10.83±0.28</td>
<td>11</td>
</tr>
</tbody>
</table>

\(-\), No activity; Mean ± S.E (standard error); diameter of halo in mm.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th><em>S. mutans</em></th>
<th><em>S. mitis</em></th>
<th><em>A. viscosus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>-</td>
<td>-</td>
<td>1.23±0.25</td>
</tr>
<tr>
<td>50</td>
<td>-</td>
<td>-</td>
<td>2.66±0.28</td>
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<tr>
<td>100</td>
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<td>2.5±0.5</td>
<td>4±0.5</td>
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<tr>
<td>Control</td>
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<tr>
<td>Standard</td>
<td>11</td>
<td>11</td>
<td>10.83±0.28</td>
</tr>
</tbody>
</table>

\(-\), No activity; Mean ± S.E (standard error); diameter of halo in mm.

Plate-2—Antibacterial activity of methanol extracts of *C. antennina*; 2a: Inhibition zones obtained against *A. viscosus*; 2b: Inhibition zones obtained against *S. mitis*; 2c: Inhibition zones obtained against *S. mutans*.
100mg/mL concentration but showed no activity on *S. mutans*. The lowest concentration of *C. fascicularis* extract to inhibit the growth of *A. viscosus* is at 25 mg/mL concentration whereas *S. mitis* was inhibited only at 100 mg/mL concentration. New compound polybrominated diphenyl ether has been isolated from the green alga *Cladophora fascicularis*. It showed antibacterial and anti-inflammatory activities. The antibacterial activity of *S. indica* against oral bacteria is shown in Table 3 and Plate 4. The methanol extract of *S. indica* showed maximum inhibition only on *A. viscosus* at 100 mg/mL concentration and minimum at 25 mg/mL concentration. It showed no activity on *S. mutans* and *S. mitis*. The methanol, ethanol and chloroform extract of *Spongomorpha indica* showed a wide varying antibacterial and antifungal activity.

The antibacterial activity of *U. fasciata* against oral bacteria is shown in Table 4 and Plate 5. The methanol extract of the species showed maximum inhibition on all the oral bacteria i.e. *S. mutans*, *S. mitis* and *A. viscosus* at 100 mg/mL concentration and lowest concentration at 50 mg/mL on *S. mutans*, *S. mitis* and *A. viscosus*. Acrylic acid isolated from the *Ulva lactuca* was responsible for the antimicrobial activity.

The present study revealed that *U. fasciata* has shown greater inhibition on all the three oral bacteria than *C. antennina*, *C. fascicularis* and *S. indica*.
C. fascicularis inhibited S. mitis and A. viscosus whereas C. antennina and S. indica inhibited only A. viscosus. The order of antibacterial activity of green algae on oral bacteria is Ulva fasciata > Cladophora fascicularis > Spongomorpha indica > Chaetomorpha antennina.

Conclusion

It can be concluded from the results that methanol extract of seaweeds belonging to Chlorophycean group from the Visakhapatnam sea coast, used in the present investigation possess significant antibacterial activity against the tested oral bacteria. However, the active components responsible for the antimicrobial activities against oral bacteria need to be evaluated further. These seaweeds have potential for the development of antibacterial agents against oral pathogens, for use in food additives, mouth-washes, chewing gums and for preventing and treating dental caries.

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