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Antibacterial activity of methanol extracts from selected seaweed of south east coast of India

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Abstract
Seaweeds offer a rich source of bioactive molecules; the present study was carried out to the antimicrobial activity of Methanol extract of three species of marine macroalgae were screened for their antimicrobial potency against seven pathogenic bacterial strains. Three seaweeds *Ulva lactuca, Sargassum wightii* and *Gracilaria edulis* were collected from four different stations (Station 1 (Chinnamuttom), Station 2 (Mandapam), Station 3 (Rameshwaram) and Station 4 (Thondi) of South East coast of India. Their antibacterial activity was evaluated. The methanolic extracts of *U. lactuca* showed high activity against *Salmonella typhii* (10±1.4) in station I. *S. wightii* inhibited *Streptococcus faecalis* (6.5± 0.7) the most in station III and *G. edulis* showed a maximum zone of inhibition against *Streptococcus faecalis* (8± 0.6) *in* station III. Among these three species *U. lactuca* significantly showed maximum antimicrobial activity than the other species ($P<0.05$). Results of the present study confirmed the potential use of seaweed extracts as a source of antibacterial compounds.

Keywords: Seaweed, antimicrobial activity, methanol, pathogenic bacteria

1. Introduction
Seaweeds are photosynthetic macroalgae that live in sea or in blackish water and classified as Rhodophyceae, Phaeophyceae and Chlorophyceae on the basis of their pigment constituents (Richard *et al.,* 1988) [19]. Seaweeds are the renewable living sources which are also used as food and fertilizer in many parts of the world. They have been screened extensively to isolate lifesaving drugs or biologically active substances all over the world. Natural products are a major resource for drug development. A large number of plants, microbes, and marine animals have been examined for bioactive secondary metabolites (Firakova *et al.,* 2007) [7]. Marine algae such as harbor endophytes and their terrestrial counterparts are a potential source of new secondary metabolites (Strobel *et al.,* 2008) [27]. Many researchers have been reported antibacterial activity of seaweeds from different localities around the world (Freile-Pelegrin and Morales, 2004; Gonzalez Del Val *et al.,* 2001; Ibtissam *et al.,* 2009; Lavanya and Veerappan, 2011; Osman *et al.,* 2010; Tuney *et al.,* 2006) [8, 9, 16, 18, 30]. Several studies have shown that seaweeds and its extracts have different biological activities, including, antitumor (Xu *et al.,* 2004) [33], antiprotozoal (Allmendinger *et al.,* 2010) [1], antiviral (Kim *et al.,* 1997) [13], antioxidant (Cox *et al.,* 2010) [5] and cytotoxic activity against the human cancer cell lines (Taskin *et al.,* 2010) [29]. Seaweed extracts were also reported to exhibit antimicrobial activity (Ballesteros *et al.,* 1992; Gonzalez del val *et al.,* 2001; Kandhasamy and Arunachalam, 2008; Karthikaidevi *et al.,* 2009; Kolanjinathan and Stella, 2009; Lavanya and Veerappan, 2011; Osman *et al.,* 2010; Sreenivasa-Rao, 1991; 1995; Seenivasan *et al.,* 2010; Tuney *et al.,* 2006; Vallinayagam *et al.,* 2009) [2, 8, 11, 12, 14, 16, 18, 26, 23, 30, 31]. seaweeds compounds such as (fatty acids, alkaloids, glycoside, flavonoids, saponins, tannins and steroids) were found to be against human bacterial pathogens (Kolanjinathan and Stella, 2009) [14], fish bacterial pathogens (Bansemir *et al.,* 2006; Kolanjinathan *et al.,* 2009) [3, 14], leaf spot disease of plant (Kumar *et al.,* 2008) [15] and marine pathogenic microorganisms (Engel *et al.,* 2006) [6]. Considering the scenario of the availability on very few records on the antibacterial activity of ethanolic extracts of macro algae, the present study was made to examine the efficacy of ethanolic extracts of selected marine macro algal species collected from southeast coast of India against bacterial pathogens.

2. Description of the Study Area

The study area Chinnamuttom lies between the coordinates of Latitude. 8°5'45"N; Longitude. 77°33'47"E. The town is located on the eastern coastal side of Kanyakumari District. Kanyakumari (formerly known as Cape Comorin), lies at the southernmost tip of East coast of India. Part of the fascination, it is the end point of the Indian peninsula where the meeting of the Bay of Bengal, the Arabian Sea and the Indian Ocean.

Mandapam (latitude 9°16'14"N; longitude 79°7'10"E), Mandapam (nearby by Rameswaram) is situated near to Bay of Bengal and close to Gulf of Mannar Biosphere. The Biosphere contains 21 islands and also rich in marine biodiversity with estuaries, mudflats, beaches, forests of the near shore environment, including marine components like algal communities, sea grasses, coral reefs, salt marshes and mangroves. The closest tourism destination of Mandapam is Rameswaram.

Rameswaram is located at the south eastern end of the Indian Peninsula at Latitude 9º 13' 9º 20' and Longitude 79o 05' 79o 15' in Ramanathapuram District. There a major Lord Siva Temple called Ramanathaswamy Temple. This is one of the major Lord Siva Temples in India.

Latitude 9˚44' 10"N to Longitude of 79˚10'45"E,Thondi coastal regions in Southeast coast of India. It is believed to be an ancient port site of pandyan kingdom (Figure.1)

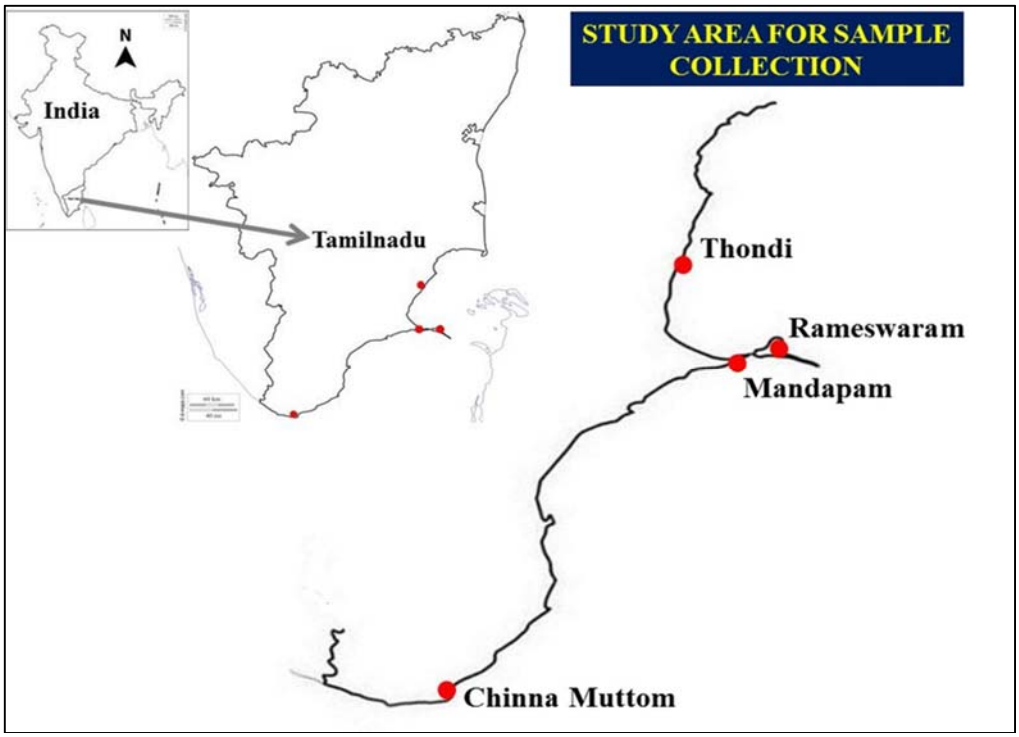

Fig 1: Sampling Locations and sampling points

3. Materials and Methods

3.1 Collection and extraction

Samples of the seaweeds *Ulva lactuca, Sargassum wightii and Gracilaria edulis* were collected during low tide from different stations of South East coast of India. The collected seaweeds were initially, washed in seawater to remove the macroscopic epiphytes, debris, again washed with fresh water to remove the surface salts, sand particles if it has any allowed to dry in the shady place for 3 to 7 days, and were identified by using standard books and manuals. The dried samples were then placed on blotting paper to remove the excess moisture before preparation of the seaweed extracts; the samples were homogenized to prior solvent extraction. Each 20 g of seaweed powder taken in 250 mL conical flask and methanol were added to get the final concentrations of the seaweeds and they were extracted by cold steep method at -10 ℃ (wright, 1998) [32].

3.2 Pathogens used for the assay

Three strains of gram positive bacteria, namely, *Bacillus cereus, Streptococcus faecalis Staphylococcus aureus* and four strains of gram negative bacteria, namely, *E-coli, Pseudomonas aeruginosa, Salmonella typhi* and *V. cholerae* were obtained from Government Medical College, Tiruchirappalli-01.The bacterial pathogens were maintained on Nutrient Agar (Hi Media, India).

3.3 Antibacterial assay

Antibacterial activity was carried out using the disc diffusion method (Murray *et al.,* 1995) [17]. The Petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA), (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. The plates were swab inoculated using sterile cotton buds with each of the previously mentioned bacterial pathogens in the concentration of 2 x 103 CFU/mL. Sterile filter paper discs 6 mm in diameters (Whatman No.1) were loaded with different extracts (100 µg/mL) and air-dried. Discs containing streptomycin were used as controls (100 µg/mL). The discs were placed on Mueller Hinton Agar (MHA). Plates were incubated for 24 hours at 37 ˚C temperature, the antibacterial assay were done in triplicates. For each algal extract zone of inhibition was recorded in millimetres and it was compared with the control and results were expressed in percentage of inhibition, all the data were statistically analysed.

3.4 Statistical analysis
The data were statistically analysed by applying a one-way ANOVA for comparison of mean values.

4. Result
The antibacterial activity of seaweeds *Ulva lactuca, Sargassum wightii,* and *Gracilaria edulis* collected from 2014-2015 different stations - namely Station 1 (Chinnamuttom), Station 2 (Mandapam), Station 3 (Rameshwaram) and Station 4 (Thondi) against clinical pathogens *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis*. *Salmonella typhii* and *Bacillus cereus* were use single letter throughout the manuscript for repeated uses of species names carried out. The results are tabulated in Tables 1-3.

Table 1: Antibacterial activity of Ulva lactuca against different bacterial pathogens in different station**.**

Pathogens	Station1	Station2	Station3	Station4
E.coli	5±0.6	3±1.0	5±0.8	3±1.0
V. cholerae	2.1±1.0	1.5±0.7	4±0.1	2±0.5
Staphylococcus aureus	3±0.5	5±1.2	6±0.6	4.1±0.1
Pseudomonas aeruginosa	4±1.4	2.1±0.5	6.2±0.7	1.5±1.1
Streptococcus faecalis	5±0.9	4±1.0	7±0.3	1.7±0.3
Salmonella typhii	10±1.4	7±1.7	5.1±1.0	2±0.5
Bacillus cereus	6±1.0	5±0.9	3±0.7	1±0.7

Table 2: Antibacterial activity of *Sargassum wightii,* against different bacterial pathogens in different station.

Pathogens	Station1	Station2	Station3	Station4
E.coli	4.8±0.8	5±0.6	4±0.5	7±1.1
V. cholerae	4.2±0.9	4±0.5	5.2±0.5	3.2±0.4
Staphylococcus aureus	5.1±0.1	6±1	6±0.1	5.8±0.9
Pseudomonas aeruginosa	4±0.5	4±0.1	3.8±0.7	4.2±0.5
Streptococcus faecalis	5±0.7	6±0.5	6.5±0.7	5.4±0.6
Salmonella typhii	4.2±0.3	4±0.5	4.5±0.5	6.2±0.6
Bacillus cereus	5±1	6±1.2	4±0.5	5±2.8

Table 3: Antibacterial activities of *Gracilaria edulis* against different bacterial pathogens in different station**.**

Pathogens	Station1	Station2	Station3	Station4
E.coli	3.7±0.7	2.5±0.8	5±0.7	5.2±0.6
V. cholerae	5±0.3	2±0.7	6.2±0.7	5.5±1.2
Staphylococcus aureus	2±0.7	7±0.1	6.4±0.3	6±0.5
Pseudomonas aeruginosa	2.3±0.1	4±0.1	6.1±0.3	3±1.0
Streptococcus faecalis	3±1.0	5±0.6	8±0.6	4±1.0
Salmonella typhii	2.1±1	2.1±1.0	7±2.8	6±0.9
Bacillus cereus	4±0.1	6±0.5	6±1.0	6±0.1

4.1 Station 1
The antibacterial activity of three seaweeds collected from the second station were compared *Ulva lactuca* showed highest activity against *S. typhii* (10±1.4mm) and lowest 2.1±1mm against *V. cholerae*. A high zone of inhibition was found in *staphylococcus aureus* (5.1±0.1mm) for *Sargassum wightii* and a low antibacterial activity was observed in *Pseudomonas aeruginosa*. (4±0.5mm). *Gracilaria edulis* had a maximum inhibition against *V. cholerae* (5±0.3mm) and a minimum against *Staphylococcus aureus* (2±0.7mm).

4.2 Station 2
In the first station the antibacterial activity of the seaweed *Ulva lactuca* was the highest against *Salmonella typhii* (7±1.7mm). It had a minimum zone of inhibition against *V. cholerae* (1.5± 0.7mm). *Sargassum wightii* had a high level of inhibition against *Bacillus cereus (*6±1.2mm) and a low of 4±0.1mm against *Pseudomonas aeruginosa*. *Gracilaria edulis* formed a high zone of inhibition against *Staphylococcus aurous* (7±0.1mm) and a low of 2±0.7mm against *V. cholerae.*

4.3 Station 3
In the third station there was a significant change in the pattern of antibacterial activity in *U. lactuca.* In the previous two stations analyzed it showed highest activity against *V. cholerae*, but in this station, the highest activity was against *Streptococcus faecalis* (7±0.3mm) and the lowest activity in *Bacillus cereus* (3±0.7mm). Similarly, *S. wightii* showed its highest inhibition against *Streptococcus faecalis* (6.5±0.7mm) and a lowest activity against *Pseudomonas aeruginosa (*3.8± 0.7mm). *G. edulis also* showed maximum activity against *Streptococcus faecalis* (8±0.6mm) and a lowest activity against *E-coli (*5± 0.7mm).

4.4 Station 4
In the fourth station *U. lactuca* inhibited *Staphylococcus aureus the* most (4.1±0.1mm) and *B. cereus* (1±0.7mm) the least. *S. wightii* had a maximum activity against *E.coli* (7±1.1mm) and a minimum activity against *V. cholerae* (3.2±0.4mm). *S.typi* was inhibited the most by *G. edulis* (6±0.9mm) and the least activity was against *Pseudomonas aeruginosa* (3±1mm).

One way ANOVA was conducted for the zone of inhibitions obtained for the three seaweeds collected from 4 stations against the pathogens tested. There was a significant difference ($P < 0.05$) in the activity of the seaweeds collected from different stations except for *Sargassum wightii*.

5. Discussion

Marine natural products or extracts with microbial activities have been isolated from a wide number of seaweeds (Chambers *et al.,* 2006). Methanolic extracts of three marine algae belongs to Rhodophyceae (*Gracilaria edulis*), Phaeophyceae (*Sargassum wightii,)* and Chlorophyceae (*Ulva lactuca*) from south east coast of India were studied for the antibacterial activity against pathogenic microbes like *Escherichia coli, Vibrio cholerae, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis. Salmonella typhii* and *Bacillus cereus.* In the present investigation Ulva *lactuca,* highly inhibited the growth of *Salmonella typhii (*10±1.4mm) to a greater extent when compared to Rhodophyta and Phaeophyta in the present investigation. Similar results were found by many other researchers (Karthigaidevi *et al*., 2009, Kolanjinathan and Stella, 2009) [12, 14] and Rizwi and Shameel (2003) [20] inferred that methanol extract of brown algae had better antibacterial activity against both gram positive and gram negative bacteria than green and red algae. Sastry and Rao (1995) [21] stated that the antibacterial activity of *S. wightii* against *S. aureus, P. vulgaris, E. coli, S. typhi, S. paratyphi* A*, S. typhiridium* and *P. aeruginosa* was mainly due to the phycoconstitutent dioctyl phthalate. In the present study *Sargassum wightii* exhibited higher activity against *E.coli* (7±1.1mm). The growth of *Streptococcus faecalis was* strongly inhibited by *Gracilaria edulis* (8±0.6mm) *among* the different stations when compared to green and brown algae. Extracts of *Gracilaria edulis* showed inhibitory activities for *Bacillus cereus* in 0.6% concentrations and was the most sensitive microorganism (Selvi and Selvaraj, 2001) [24].

Crude extracts prepared using the solvent acetone from the seaweeds of the family Chlorophyceae, Rhodophyceae and Phaeophyceae were already screened for the antibacterial activity against following pathogens: *Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Vibrio cholerae, Escherichia coli* and *Citrobacter* sp. (suresh kumar et al., 2012) [28]. Similar results regarding the activity against *Staphylococcus aureus, Salmonella typhi, Vibrio cholerae* and *Escherichia coli* were observed for the present study. The activity of the different species collected from different stations varied significantly ($P<0.05$) among the various bacterial species studied. This study clearly proves that the environment plays an important role and influences the bioactivity of the seaweeds.

6. Conclusion

In the present study, the extract of three seaweeds obtained from methanolic solvents was tested for their antibacterial activity. Among, the seaweeds tested, the *Ulva lactuca* extract exhibited the highest activity, when compared to other seaweed extracts which were studied. The present study concluded that the methanolic extract of marine green alga *Ulva lactuca* could be used for further investigation to identify actual components against human bacterial pathogens.

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