Antibacterial Potential of Algae Sargassum Spp. and Chlorophyta Spp. from Ratnagiri, Maharashtra-An In Vitro Study

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ABSTRACT
Antimicrobial formulations of plant origin are of great interest as certain antibiotics have undesirable effects while many lead to the development of multidrug resistance. The aim of the present work was to screen the antimicrobial potency of two locally collected green algae belonging to Chlorophyta from a water body in Ratnagiri (Maharashtra) and two commercially procured brown algae Sargassum (sexual and vegetative forms). The algal samples were extracted using the Soxhlet extraction and methanol as a solvent. The methanolic extracts were tested against various Gram positive and Gram negative microorganisms using the well diffusion technique for antimicrobial assay. The study revealed that green algal extracts were more effective against Gram Positive organisms such as Staphylococcus, Bacillus spp. and more effective against Micrococcus spp. While the brown algal extracts were effective against Gram Negative strains, none of the extracts could however inhibit Pseudomonas spp. The algal extracts were also subjected to HPTLC fingerprinting for detecting the presence of terpenes, coumarins and flavonoids, which will help in future to establish strong correlation of these phytoconstituents and their antimicrobial potency.

Introduction:
The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to known antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (Sieradzki, Robertz, Haber, Tomasz, 1999). Efforts are on to discover new antimicrobial compounds or screen the antimicrobial potential of various kinds of sources such as plants, animals and microorganisms. Systematic screening of them may result in the discovery of novel effective compounds (Tomoko, Takashi, Hiroko, Munekazu, Totshiuyuki, Tetsuro, Hiroyu, Iriya, Tsutomu N., Kazuhito, 2002).

Algae (Latin “seaweed”, singular Alga) are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. Algae are found in diverse conditions in nature, in oceans, salt lakes, ponds etc. Algal cells produce certain phytoconstituents as defense mechanism which can act as an antagonist to certain other organisms. Hence, these can be tapped as source for production certain antibiotics. Presence of Di Peptides, Coumarins, Flavonoids and Sterols has also been reported in algae. The current work focuses on the antimicrobial activity of naturally collected green algae from water body and commercially available brown algae on microorganisms.

Many bioactive and pharmacologically active substances have been isolated from algae. For instance, extracts of marine algae were reported to exhibit antibacterial activity. Kishinhant T, Veeramuthu Duraiapandiyan, Pachiappan Permal, Savarimuthu Ignacimuthu, 2009 have also reported antibacterial activities of microalgae.

Though literature speaks diverse studies of bioactivity of marine flora, our work on testing the antibacterial efficacies and identification of various phytoconstituents using HPTLC of these algal species was comparatively a new concept and not much attempt had been made earlier on this line.

Materials & Methods:
Algae samples A and B belonging to Chlorophyta were collected from two local ponds in Ratnagiri. The samples were shade dried, cut into small pieces and powdered in a mixer grinder.

Sexual and vegetative forms of commercially available alga, Sargassum belonging to Phaeophyta were purchased from Silvia Scientific Corporation, Alibaug. These were washed thoroughly to remove any debris using distilled water; shade dried and powdered using a blender.

Preparation of extract
An extract (1%) of each alga was prepared in methanol using the Soxhlet extractor for 24 hrs at 65°C (Boiling point of Methanol). Extracts prepared were concentrated, collected in sterile vials and refrigerated for further use. The extracts were diluted with methanol so as to obtain 500 µg, 250 µg, 100 µg & 50 µg concentrations of the extracts per 50 µL of methanol, at which the activity was evaluated. Protein content of all the extracts was determined by Folin Lowry technique and the absorbance was measured at 660nm using a spectrophotometer (Lowry O.H., Rosebrough NJ, Farr AI, Randall RJ, 1951).

Test Micro-organisms
The test bacterial pathogen cultures were obtained from the stock cultures maintained in the Biotechnology Laboratory at Kishinchand Chellaram College. The test cultures used for the study were Staphylococcus aureus, Bacillus spp., Micrococcus spp., Corynebacterium diphtheriae, Escherichia coli, Pseudomonas spp., Klebsiella pneumoniae and Proteus mirabilis, whose culture density was set to 0.1 O.D. at 540 nm.

Antibacterial testing
The antimicrobial activity of various algal extracts was checked by MIC (Minimum inhibitory concentration – by tube method) and well diffusion method at various concentrations and methanol was used as a control. For well diffusion assay, medium was seeded with the test organism (1ml culture + 20 ml Medium) and a 10 mm well was made using a cork borer. The wells were filled with different algal extracts of various dilutions and screened for their antimicrobial activity against respective test organisms. The crude extract was allowed to diffuse into the wells for 10-15min at 4°C. The Petri plates were incubated at 37°C for 24hours. The diameter of zone of inhibition of each well was recorded. Methanol control was used along with algal extracts A, B, C & D. The results of antibacterial activity were tabulated as reported in Table-1.

Fingerprint analysis was performed using the method described by Hildebert Wagner & Sabine Bladt, 1929.

Samples (A, B, Sargassum sexual and Sargassum vegetative) were screened for the presence of Flavonoids, Terpenes and Coumarins using HPTLC. Selected active crude extracts were loaded on silica gel 10.0 x 10.0 cm, HPTLC plates of silica gel 60 F 254, Manufacturer E. MERCK KGAA. The profile obtained was analysed using winCATS Planar Chromatography Manager. Appropriate solvent systems and spray reagents were used for the detection. Silica plates were scanned at 254nm, 366nm and 580nm, and observations made regarding presence of cou-
Among the Gram Positive organisms, Micrococcus spp. is compared to B. fective as compared to extract A but show greater inhibition as Gram Negative organisms. Extract C and Extract D are less ef fective as compared to the other Gram Negative organisms under this study. None of the algal extracts were found to inhibit the growth of Pseudomonas spp. up to 500mg.

In our study, some of the bacterial strains did not respond to extracts, highlighting the need for further purification of the extracts to remove inhibitory substances. This could also be due to masking of antibacterial activity by the presence of some inhibi tory compounds or factors in the extract. The variation of antibiotics can be seen in the study of our extracts might be due to distribution of antimicrobial substances, which varies from species to spe cies. Nanoce M, B, Brown C (1991). Similarly, it was found that fractionation of crude extracts tested enhanced their activity against both Gram negative as well as the resistant Gram posi tive pathogens Sastry V.M.V.S. and G.R.K. Rao (1994).

**Conclusions:**

Many marine algae produce antibiotic substances capable of inhibiting bacteria, viruses, fungi, and other pathogens. It appears that the antibiotic characteristic is dependent on many factors, including the particular alga, the microorganisms, the season, and the growth conditions (Centeno POR, DL Ballantine, 1999). It may be concluded from the above findings that extracts A, B, C and D act more effectively against Gram positive organisms and some Gram negative intestinal pathogens. All the samples may contain varying amounts of coumarins, sterols, terpenoids or essential oils and flavonoids. Sample B was rich in cou marins and sample C, D have a greater amount of flavonoids. Hence compared to other algae, sample B shows less anti mi crobial activity, thus antimicrobial activity may be attributed to presence of sterols, terpene and essential oils compared to coumarins or flavonoids. The findings of the pilot study do envisage that methanol extracts of the algal samples could be utilized as a good source of antimicrobial agent in the pharma ceutical industry.

The HPTLC fingerprint analysis was carried out to throw some light on the constituents of the crude extracts, it would help study the structure – function correlation of the antimicrobial agent in the extracts.

The antibacterial activity of marine algae is generally assay ed using extracts in various organic solvents, e.g., acetone. Several extractable compounds, such as cyclic polysulphides and halogenated compounds are toxic to microorganisms and therefore responsible for the antibiotic activity of some marine algae as reported by Ohta (1979). However, an antibiotic assay of ex tracts in organic solvents probably does not reflect adequately the antibacterial activity of marine algae under natural condi tions. Identification of the active antimicrobial molecule present in these algal samples by generating an HPTLC fingerprint may aid other researchers. Antimicrobial activity shown by these ex tracts may be because of presence of terpenoids, flavonoids or coumarins, but not proteins. Extract of Sargassum could serve as a potential candidate for purification and further in vivo stud ies.

Our study showed similar results with earlier observations made by Srinavasa Rao and Parekh (1981) who have demon strated that crude extracts of Indian seaweeds were active only against Gram positive bacteria.

Overall, the present study provides enough data to show the potential of algal extracts for development of anti-pathogenic agents. Ecologically, there is likelihood that the secondary me tabolites / PUFAs produced by the different marine flora may have a role in fish growth and protection from diseases. The renewable / cultivable nature of marine flora is another advan tage for development of potential antibacterial products for use in feed or by other means administration in aquaculture. Eco nomically feasible standard operating procedures can be de veloped in preparing the extracts/fractions in large scale with reproducible antibacterial efficiency.

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<table>
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<tr>
<th>Algal Species</th>
<th>Conc. of extract (mg)</th>
<th>Zone of inhibition (mm)</th>
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<th>Gram Negative</th>
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<td></td>
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</table>

Note – (Sa) Staphylococcus aureus, (B) Bacillus spp., (M) Micrococcus spp., (Cd) Corynebacterium diphtheria, (Ec) Eschericia coli, (P) Pseudomonas spp., (Kp) Klebsiella pneumonia, (Pm) Proteus mirabilis. (-) No antimicrobial activity

A - Ratnagiri sample 1, B- Ratnagiri sample 2, C - Sargassum sexual, D - Sargassum vegetative ±indicates standard deviations in the values zones of inhibition

Table 1: The results of antibacterial activity of algal extracts A, B, C and D against Gram positive and Gram negative microorganisms.

Figure 1: Silica plate scanned at 254 nm (without chemical treatment) showing prominent quenching against green background

Key:- A – sample A, B – sample B (A and B Chlorophyta spp.) SS – Sargassum Sexual and SV – Sargassum vegetative

Figure 2: Silica plate at visible light (after derivatisation) for the detection of terpenes

Key:- A – sample A, B – sample B (A and B Chlorophyta spp.) SS – Sargassum Sexual and SV – Sargassum vegetative
Figure 3: Silica plate scanned at UV for the detection of terpenes

Key: A – sample A, B – sample B (A and B Chlorophyta spp.) SS – Sargassum Sexual and SV – Sargassum vegetative

Figure 4: Silica plate at visible light (after derivatisation) for the detection of flavonoids

Key: A – sample A, B – sample B (A and B Chlorophyta spp.) SS – Sargassum Sexual and SV – Sargassum vegetative

REFERENCE