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Studies on the Antimicrobial Potency of the Marine Algae - *Gracilaria corticata var cylindrica* and *Hydroclathrus clathratus*

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Abstract:

Microbial diseases cause severe economic loss worldwide. Due to increase in microbial resistance against antibiotics, there arises a need for new antimicrobials with high efficacy and no side effects. Nowadays, the focus has been targeted to seaweeds which proves to be a promising source of antimicrobials. Hence, the present aim of the study was to identify marine algae with antimicrobial potency. *Gracilaria corticata var cylindrica* and *Hydroclathrus clathratus*, which belongs to marine algae group were screened for the presence of bioactive components by extracting them with suitable solvents and studying their antimicrobial activities using disc diffusion method. An attempt has also been made to separate the unknown compounds using TLC and purify the extracts using column chromatography since not much work has been carried out in these species. Both the algal extracts showed inhibition against human pathogens confirming its potential as a natural source of antibiotic.

Keywords:

*Gracilaria corticata var cylindrica*l; *Hydroclathrus clathratus*; Antimicrobial Activities; TLC; Column Chromatography

1. INTRODUCTION

For many years, marine phycology has been the Cinderella of this area in science; indeed almost all of its practioners even on a world scale could be counted on the fingers of ones hand. Natural products of macro algal metabolites play a valuable role in drug discovery process [1]. More than 150000 seaweed species are found in the oceans of the globe but only a few of them was identified [2]. About 2400 natural products have been isolated from macroalgae belonging to the classes Rhodophyceae, Phaeophyceae and Chlorophyceae [3]. Harder [4] was the pioneer to observe the antimicrobial potentials of seaweeds. Selvi and Selvaraj [5] reported the antibacterial activity of some Indian seaweeds. Special attention has been reported for antibacterial and antifungal activities related to marine algae against several pathogens [6–9]. Considering the scenario of meager availability on the antimicrobial activity of aqueous extracts of macroalgae, the present study was made to examine the efficacy of extracts of selected marine macro algal species *Gracilaria corticata var cylindrica* and *Hydroclathrus clathratus*, collected from Rameshwaram against human pathogens. These activities are dependent on many factors, such as the species of seaweed, the region of the thallus, the microorganisms, the season and the growth conditions [10–12].

2. MATERIALS AND METHODS

About 1 kg of the two species of seaweeds namely *Gracilaria corticata var cylindrica* and *Hydroclathrus clathratus* were collected fresh from the south east coast of Tamil Nadu (Rameshwaram) during the month of December, 2007. The sea weeds were identified as per the identification manual [13, 14]. **Figure 1** Cleaning and drying of the samples were performed as mentioned by Pereira *et al.* [15]. The washed samples were shade dried, powdered and stored in sterile containers under refrigeration until use.

Extraction of hundred grams of powdered biomass with solvents of increasing polarity ranging from 200ml petroleum ether to water through benzene, chloroform, ethyl acetate and methanol for 72 hours at room temperature with intermittent stirring for every twenty four hours was made in succession. This procedure was carried out until the extract became colorless in order to select a suitable solvent/and method to extract the antibacterial substances. Then the extracts were combined keeping each extraction type separate and concentrated using Rotavapor under reduced pressure at 45C and stored in the refrigerator for further analysis.

Strains used for testing antimicrobial activity were Staphylococcus aureus (MTCC, 740), Salmonella typhimurium (MTCC, 98), Escherichia coli (GM242) and Candida albicans (MTCC, 227). The remaining isolates such as Klebsiella sp, Proteus sp, Citrobacter sp and Pseudomonas sp were collected from PSG Institute of Medical Science & Research, Coimbatore.

Around 100 μl of all the extracts thus were injected into empty sterilized filter paper disc having a diameter of 5 mm. The discs impregnated with the mother solvents of each extracts served as the control and were placed on the same plate. Antimicrobial activities of algal extracts were tested separately using disc diffusion method [16].

Antimicrobial activities were conducted in triplicates and the results are expressed as mean $\pm$ SD using SPSS-14.

Thin layer chromatography of each sample was performed on Merck TLC F254 plates, with Chloroform: Methanol in the ratio of varying concentrations such as 10: 90, 80:20, 20:80 and 90:10.as mobile phase. The separated components were visualized under ultraviolet light of 254 nm.

Purification of the crude extracts was carried out as per the procedure of Vairappan *et al.* [17] with few modifications. The seaweed extracts with antibacterial activity were fractionated by silica gel column chromatography (chloroform and methanol).The fractions were eluted with chloroform: methanol and were further subjected to antimicrobial assay and TLC.

3. RESULT AND DISCUSSION

Marine algae have been the group of organisms that has received the most attention from marine natural product scientist over the last 30 years. However, since many of algal metabolites were described before the current pharmacological bioassays became available, the potential of most of these metabolites remain unexplored. There are hardly few reports on screening these seaweeds from south east coast of Tamil Nadu for their antimicrobial potency; hence the present study is focused on the evaluation of the crude extract of the seaweeds to detect antimicrobial activity.

The powdered algal samples were extracted with a series of solvents of increasing polarity and the crude extracts were screened for their antimicrobial properties. From **Table 1**, it is observed that out of all the solvents, only in methanolic extract of *Hydroclathrus clathratus*, the antibacterial compound have been extracted and no activity against fungi was observed It could either be due to the presence of a compound with a broad spectrum of antibacterial activity or could be due to the presence of more than one compound each having its own target of action. When the extraction of the powdered sample of *Gracilaria corticata var cylindrica* using variety of solvents was performed,

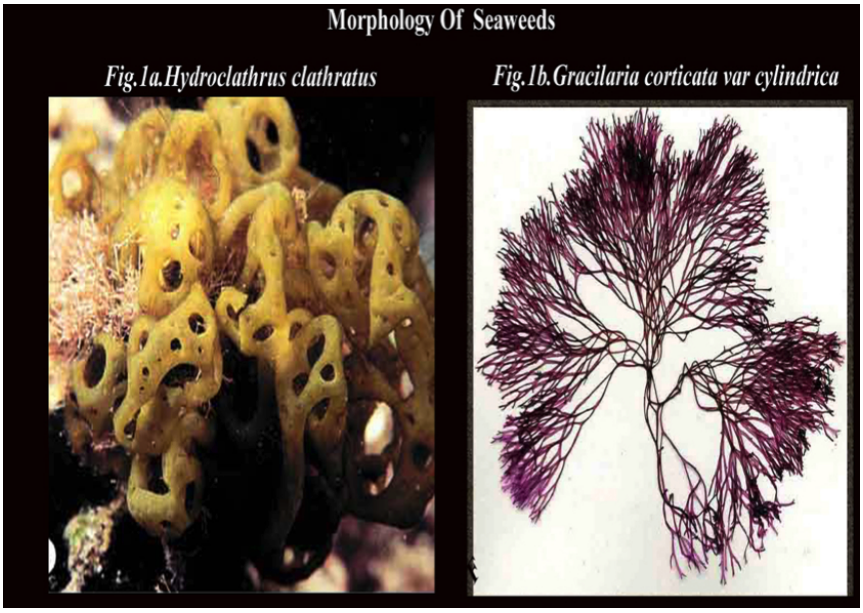

Figure 1. Morphology of sea weeds

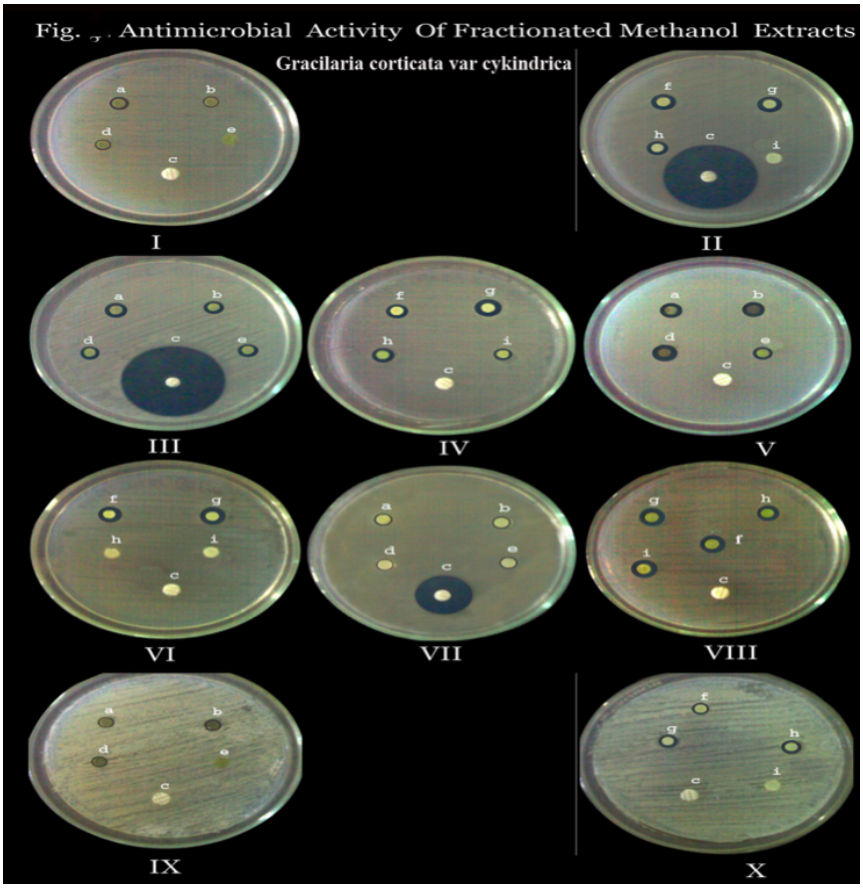

Figure 2. Antimicrobial activity of fractionated methanolic extracts *Gracilaria corticata var cylindrica*

Table 1. Antibacterial And Antifungal Activity Of Various Extracts From *Hydroclathrus clathratus*

	Zone of Inhibition (mm)				
Test Organisms	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol
Escherichia coli	-	-	-	-	-
Staphylococcus aureus	-	-	-	-	10±0
Klebsiella sp	-	-	-	-	-
Citrobacter sp	-	-	-	-	-
Salmonella typhi	-	-	-	-	10.6±0.50
Pseudomonas sp	-	-	-	-	-
Proteus sp	-	-	-	-	-
Candida albicans	-	-	-	-	-

Table 2. Antibacterial And Antifungal Activity Of Various Extracts From *Gracilaria corticata var cylindrica*

	Zone of Inhibition (mm)				
Test Organisms	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol
Escherichia coli	9.0±0.40	-	9.0±0.40	-	10.0±2.0
Staphylococcus aureus	-	-	-	-	9.1±0.9
Klebsiella sp	9±0	-	8±0	-	10.0±1.0
Citrobacter sp	-	-	8.8±1.76	-	10±1.0
Salmonella typhi	-	-	-	-	-
Pseudomonas sp	-	-	-	-	-
Proteus sp	-	-	-	-	-
Candida albicans	-	-	9±0.40	-	9±1.0

petroleum ether, chloroform and methanol extract contained antimicrobial compound as shown in **Table 2**.

Thin layer chromatography (TLC) was performed with different combinations of chloroform: methanol to get better resolution. A ratio of 80:20 could yield a better resolution of the compounds and the same ratio of the solvents was used in silica gel column chromatography also. The fractions collected from the column at regular intervals were screened for antimicrobial properties and almost all fractions showed zone of inhibition against the test culture used (**Table 3**, **Figure 2**). This result indicates a definitive presence of a mixture of antimicrobial compounds in the crude extracts of algae.

Table 3. Results Of Antimicrobial Activity Of Purified Methanol Extract Through Silica Gel Column Against Gracilaria Corticata Var Cylindrica

Test Organisms	Zone of inhibition (mm)									Figure
	a	b	c	d	e	f	g	h	i	
Staph. aureus	8	-	-	-	-	10	10	9	-	I, II
Escherichia coli	10	9	-	9	9	10	11	10	8	III, IV
Citrobacter sp.	10	10	-	10	9	9	10	-	-	V, VI
Klebsiella sp.	-	-	-	-	-	11	11	10	11	VII, VIII
Pseudomonas sp.	-	-	-	-	-	-	-	-	-	
Proteus sp.	-	-	-	-	-	-	-	-	-	
Salmonella sp.	-	-	-	-	-	-	-	-	-	
Candida albicans	8	8	-	9	-	8	9	10	-	IX, X

4. CONCLUSION

In the present study an initial evaluation has been made with the crude extracts. An attempt has also been made to partially purify the active principle(s). So in future, these purified fractions can be used for GCMS and NMR studies. In order to find the compounds responsible for the activity and elucidate their structures so that they can be further synthesized chemically to form potent antibiotic.

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