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Preliminary comparative antimicrobial activity of two macroalgae extracts from South Eastern Coast of Tamil Nadu

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ABSTRACT

Secondary metabolites in marine algae are becoming the focus of biological researchers due to its varied therapeutic values. Preliminary comparative screening studies have been performed between Gracilaria. crassa and Asparagopsis taxiformis, which belongs to Rhodophyta group of marine algae, for the presence of bioactive components by extracting them with suitable solvents and studying their antimicrobial activities using disc diffusion method. Gracilaria crassa was found to have better antimicrobial potency compared to Asparagopsis taxiformis. An attempt has also been made to separate the unknown antimicrobial compounds using Thin Layer Chromatography (TLC) and purify the extracts using column chromatography since not much work has been carried out in these species.

Keywords: *Gracilaria. crassa*, *Asparagopsis taxiformis*, antimicrobial activities, TLC, Column chromatography

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INTRODUCTION

Today, the general public is aware of the widespread economic significance of marine vegetation sources. But this recognition doesn't go back very far. Before the two world wars, the study of marine organisms was a matter of little more than academic interest. The crisis caused by the cut – off of German potash supplies during World War I and a similar crisis with respect to Japanese agar during World War II awoke the United States to the industrial utilization of sea weeds and therefore gave useful purpose to marine algology in the public eye. This has brought the seashore within reach of the most inland dweller. Seaweeds or the marine algae are the dominant autotrophic producers found in the marine environment. They have a unique ability to produce numerous secondary metabolites with diverse structures, capable of acting against various pathogenic bacteria, virus, fungi etc.

Our study is limited to Rhodophyta group of marine algae which are one of the largest producers of biomass in the marine environment. The main products of red seaweeds are agar agar and carrageen used mostly in baking, confectionary and as an emulsifying agent in pharmaceutical industry [1]. The bioactive compounds produced by certain sp. of Gracilaria from the Rameshwaram coastal regions were studied and they were found to exhibit antimicrobial activity against several pathogens [2]. Since not much work has been carried out in these species, this present investigation aims at screening both *Gracilaria. crassa* and *Asparagopsis taxiformis* for the presence of bioactive components, extracting them with suitable solvents and studying their antimicrobial activities. An attempt has also been made to separate and purify the extracts.

MATERIALS AND METHODS

About 1 kg of the two species of seaweeds namely *Gracilaria crassa* and *Asparagopsis taxiformis* were collected fresh from the south east coast of Tamil Nadu (Rameshwaram) during the month of December, 2007. Cleaning and drying of the samples were performed as mentioned by Pereiara *et al* [3]. 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 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.

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 [4].

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*. [5] 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 Thin layer chromatography.

RESULT AND DISCUSSION

Table 1 Antimicrobial activity from various extracts of *Asparagopsis taxiformis*

S.No	Test Organisms	Zone of Inhibition (mm)				
		Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol
1	*Escherichia coli*	-	-	-	-	-
2	*Staphylococcus aureus*	-	-	-	-	-
3	*Citrobacter sp.,*	-	-	-	-	-
4	*Klebsiella sp.,*	-	-	-	-	-
5	*Pseudomonas sp.,*	-	-	-	-	-
6	*Proteus sp.,*	-	-	-	-	-
7	*Salmonella sp.,*	-	-	-	-	*6.6*
8	*Candida albicans*	-	-	-	-	-

Legend: `- ´ sign indicates the absence of zone of inhibition

Table 2 Antimicrobial activity from various extracts of *Gracilaria crassa*

S.No	Test Organisms	Zone of Inhibition (mm)				
		Petroleum ether	Benzene	Chloroform	Ethyl acetate	Methanol
1	*Escherichia coli*	*8*	-	*9*	-	*9*
2	*Staphylococcus aureus*	-	-	-	-	*9*
3	*Citrobacter sp.*	*9*	-	*8*	-	*9*
4	*Klebsiella sp.*	*9*	-	*8*	-	*8*
5	*Pseudomonas sp.*	-	-	-	-	-
6	*Proteus sp.*	-	-	-	-	-
7	*Salmonella sp.*	-	-	-	-	-
8	*Candida albicans*	-	-	*8*	-	*10*

Table 3 Antimicrobial activity of purified methanol extract through silica gel column against *Gracilaria crassa*

S.No	Test organisms	Zone of inhibition(mm)							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	*Escherichia coli*	10	11	12	8	9	12	-	-
2	*Staphylococcus aureus*	10	9	9	9	9	12	10	9
3	*Citrobacter sp.*	8	8	-	8	9	9	12	8
4	*Klebsiella sp.*	9	8	7	-	10	10	9	8
5	*Pseudomonas sp.*	-	-	-	-	-	-	-	-
6	*Proteus sp.*	-	-	-	-	-	-	-	-
7	*Salmonella sp.*	-	-	-	-	-	-	-	-
8	*Candida albicans*	8	8	8	-	8	9	10	-

In the present study an attempt has been made to extract the bioactive substances with antimicrobial (antibacterial and antifungal) properties from the marine algae *Gracillaria crassa* and *Asparogopsis taxiformis.* The main purpose of selecting these marine algae is not only the availability of these in pure culture as bulk but also very few work has been carried out with these organisms that are present in the South East Coast of Tamil Nadu. Out of the various solvents used for extraction only the methanolic extract of *Asparagopsis taxiformis* contained antibacterial substance (Table 1). From the results of table 1, it could be inferred that the antimicrobial compounds could be one or two in comparatively low concentration. In contrast another species of *Asparagopsis* namely *A. armata* was reported to have a broad spectrum of activity against many Gram negative bacteria [6]. The probable reason for *A. taxiformis* not showing any similar activity could be due to the species variation. The green alga *Gracilaria crassa* was studied for its antimicrobial potency by extracting the metabolites using various organic solvents and water. The petroleum ether, chloroform and methanol extract showed antimicrobial activity towards all the test microorganisms. However the zone size was comparatively bigger in size (Table 2). Since the methanolic extract of *G.crassa* showed activity against several test cultures of bacteria suspecting the presence of many compounds of antimicrobial property, different ratio of chloroform: methanol was tried as mobile phase to obtain a possible better resolution of the components of methanolic extract. And chloroform: methanol ratio of 80: 20 was found to be better; the same ratio of the solvents was used for eluting the compounds from silica gel column also. The fractions obtained from silica gel column separation also showed smaller but definite zones of inhibition (Table 3). These results necessitate concentration of the crude as well as eluted fractions. But various fractions obtained from the column had antimicrobial property, there could be probably few to many compounds with antimicrobial property.

CONCLUSION

In this paper, we have reported the antimicrobial properties of bioactive components extracted from the marine algae *Gracillaria crassa* and *Asparogopsis taxiformis*. Further investigation is required to study the nature of compounds with antimicrobial properties with reference to *Gracilaria crassa;* whereas with *Asparagopsis taxiformis*, the study can be extracted to collect the samples during different seasons of a year and confirm its antimicrobial potency. Intensive studies must be carried out to unravel its unexhausted reserve of bio-metabolites.

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