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ANTIBACTERIAL ACTIVITY OF *SARGASSUM LONGIFOLIUM* AND *GRACILARIA CORTICATA* FROM GULF OF MANNAR AGAINST SELECTED COMMON SHRIMP PATHOGENS

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ABSTRACT

The antibacterial activity of crude extracts of *Sargassum longifolium* and *Gracilaria corticata* collected from the Gulf of Mannar Coast, Tamilnadu, India against common shrimp pathogens were tested by disc diffusion method. Three different solvents namely aqueous, ethanol and methanol were used for extraction. The qualitative phytochemical analysis revealed the presence of carbohydrates, proteins, alkaloids, glycosides, terpenoids, flavonoids, tannin, saponins and steroids in the crude extracts. Different concentration of the crude extracts of *Sargassum longifolium* and *Gracilaria corticata* showed antibacterial activity against common shrimp pathogens such as *Aeromonas hydrophilia, Pseudomonas aeruginosa, Vibrio cholerae, V. harveyi*, and *V. parahaemolyticus*. The methanol extract showed better results than the other extracts. Methanol extract of *Gracilaria corticata* showed maximum activity against *P. aeruginosa* (20.75 ± 0.5) and *V. parahaemolyticus* (18.75 ± 0.5 mm) at 800 µg/disc. *V. cholerae* has highly sensitive (21.25 ± 0.5 mm) against methanol extract of *S. longifolium* (800 µg/disc). Aqueous extracts of both seaweeds showed low activity against all the tested bacteria. These results show the potentials of seaweeds *G. corticata* and *S. longifolium* for screening of antibacterial compounds.

KEYWORDS: Antibacterial activity, Seaweeds, *Gracilaria corticata, Sargassum longifolium,* and Phytochemical compounds.

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INTRODUCTION

Aquaculture has become a very fast growing sector in food productions. Bacterial disease is a serious commercial loss in aquaculture. Bacteria such as *Vibrio* spp*., Pseudomonas* spp*. Flavobacterium* spp. and *Aeromonas* spp*.* are the most common bacterial pathogens of shrimps[1]. Wide ranges of chemicals particularly antimicrobial agents are used in shrimp farming to prevent diseases. The problem of antibiotic resistance and its epidemiological consequences, led to the exploration of several alternate approaches for disease management in aquaculture systems. One such method is screening of antibacterial compounds from seaweeds. The marine environment is the habitat of diverse groups of microorganisms. Seaweeds are constantly in contact with potentially dangerous microbes and they have apparently defended against the microbial thread[2]. Seaweeds are considered as diverse source of secondary metabolites having a broad spectrum of biological activities with cystostatic, antiviral, antihelminthic, antifungal, antitumour and antibacterial activities[3,4]. Seaweeds are the source of amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes and cyclic polysulphides[5]. Several works have been carried out on the extracts from seaweeds and their extracts were reported to exhibit antibacterial activity[6,7]. There are many reports on the antibacterial activity of seaweeds against human pathogens, but little information was available for aquatic bacteria. Hence the present study was designed to study the antibacterial activity of *Gracilaria corticata* and *Sargassum longifolium* against some shrimp pathogens.

MATERIALS AND METHODS

(i) Seaweeds

The red alga *Gracilaria corticata* and the brown algae *Sargassum longifolium* were obtained from Central Marine Fisheries Research Institute (CMFRI), Mandapam, Tamil Nadu, India. The material was cleaned of epiphytes, washed with distilled water, shadow dried and stored at 4°C until use.

(ii) Bacteria

Shrimp pathogens such as *Aeromonas hydrophilia, Pseudomonas aeruginosa, Vibrio cholerae, V. harveyi* and *V. parahaemolyticus* (isolated from diseased shrimp) were used. They were obtained from the Department of Microbiology Laboratory, Srimad Andavan Arts and Science College, Tiruchirappalli, Tamil Nadu, India, and the bacterial strains were maintained in nutrient agar medium (Hi Media, India) at 4°C.

(iii) Preparation of seaweed extracts[8]

Three hundred grams of shade dried sample of seaweed were chopped and pulverized. The pulverized sample of 100 g of red alga *Gracilaria corticata,* and brown alga *Sargassum longifolium* was separately extracted in 250 mL of solvents such as methanol, ethanol and water in a one liter capacity round bottom flask (Soxhlet apparatus) in a water bath at 65ºC until the solvent become colourless. The extracts were filtered through Whatman No.1 filter paper and concentrated. Finally, it was collected in air–tight plastic bags and stored in the refrigerator (4ºC) for further studies.

(iv) Antibacterial assay[9]

The antibacterial assay was carried out using a standard disc diffusion technique. Known quantity of the extract was dissolved in 1 mL DMSO (Dimethyl sulfoxide) and make up to the final concentration of 10 mg/mL. Sterile paper discs were loaded with 10, 20, 40 and 80 µL (100, 200, 400 and 800 µg of the mass) of different crude extracts using micropipettes and were allowed to dry under aseptic conditions. The discs were placed onto the petri plates containing solidified Muller Hinton agar medium (Hi Media) smeared with 0.1 mL of bacterial culture in exponential phase (0.6 OD at A_{590} nm). After incubation at 37^0C for 24 hours, the diameters of the clear zones around the discs were measured as antibacterial activity. Four

replicates were maintained for each experiment and the mean values expressed with ± standard deviation. The standard antibiotic disc (streptomycin sulphate, 10 µg/Disc; HiMedia, India) and solvents used for reconstituting the crude extracts were loaded on paper discs were used as control.

Phytochemical analysis

The extracts that obtained were subjected to qualitative analysis by the methods described[10, 11, 12]. Phytochemical analysis was conducted to determine the presence of flavonoids, alkaloids, steroids, glycosides, terpenoids, saponins, tannins, carbohydrates and proteins.

RESULTS

The antibacterial activity of the extracts of *Gracilaria corticata* was presented in the Table-1. All three extracts showed antibacterial activity against the tested bacteria. Methanol extract showed higher activity against *P. aeruginosa* (20.75 mm diameter inhibition zone) at the concentration of 800 µg/disc. At the lowest concentration (100 µg/disc) the tested bacteria has resist against the aqueous and ethanol extracts. Aqueous extract showed highest activity against *V. harveyi* (10.5 ± 0.58 mm zone; 800 µg/disc), but, aqueous extract has less activity against *V. cholerae* and *V. parahaemolyticus*. Ethanol extract of *G. corticata* displayed maximum activity against *V. cholerae* (19 ± 0.82 mm zone; 800 µg/disc), but it has less activity against *P. aeruginosa* (16.5 ± 1.73 mm zone; 800 µg/disc). Antibacterial activity of seaweed *Sargassum longifolium* extract was tested against shrimp pathogens and the results are summarized in Table-2. Among the solvents used for the extraction of antibacterial activity, maximum antibacterial activity was found in the crude methanol extracts. The methanol extract showed maximum activity against *V. cholerae* (21.25 mm zone; 800 µg/disc), but it was low against *P. aeruginosa* (17 mm; 800 µg/disc). The ethanol extracts showed high antibacterial activity against the bacteria *P. aeruginosa* (16.75± 1.5 mm zone; 800 µg/disc), and it was low against *Aeromonas hydrophilia* (15 ± 1.15 mm zone; 800 µg/disc). The aqueous extract showed maximum activity against *A. hydrophilia* (13.5 ± 1.29 mm zone; 800 µg/disc). Overall, the antibacterial activity in the crude aqueous extracts of seaweeds has low activity against all the tested bacteria. Table-3 shows the qualitative analysis of phyto compounds present in the seaweed extracts. All three extracts showed carbohydrates, proteins, tannin and steroids. The ethanol extract of *S. longifolium* has glycosides and saponins. Methanol extract showed the maximum number of active components such as proteins, tannin, flavonoids and terpenoids. Methanol and ethanol extracts of *G. corticata* has carbohydrates, proteins, glycoside, terpenoids, tannin, saponins and steroids. Aqueous extract contains carbohydrates, proteins, tannin and steroids as bioactive compounds.

Table 1
Antibacterial activity of Gracilaria corticata against common shrimp pathogens

Organism	Aqueous				Ethanol				Methanol			
	100	200	400	800	100	200	400	800	100	200	400	800
Aeromonas hydrophilia	ND	ND	7.75 ± 0.96	9.75 ± 0.96	ND	ND	11.75 ± 1.26	14.75 ± 0.95	ND	12 ± 1.15	15.25 ± 0.57	17.5 ± 0.58
Pseudomonas aeruginosa	ND	ND	6.75 ± 0.5	10 ± 1.15	ND	7.5 ± 0.58	15.25 ± 1.26	16.5 ± 1.73	8 ± 0.82	10.25 ± 1.5	17.5 ± 1	20.75 ± 0.5
Vibrio cholerae	ND	ND	6.25 ± 0.5	8.5 ± 1	ND	8 ± 0	17 ± 0	19 ± 0.82	ND	9.75 ± 2.06	13.25 ± 0.95	16.5 ± 0.58
Vibrio harveyi	ND	6.25 ± 0.5	7.75 ± 0.5	10.5 ± 0.58	ND	8.25 ± 0.5	16.25 ± 0.96	18.5 ± 0.58	7.75 ± 0.5	14.751. ±0. 73	16.25 ± 0.95	18.25 ± 0.5
Vibrio parahaemolyticus	ND	ND	6.5 ± 0.58	8.75 ± 0.5	ND	8 ± 0.82	16 ± 0.82	17.75 ± 0.96	8.25 ± 0.5	14.25 ± 0.95	16.75 ± 0.5	18.75 ± 0.5

The zone of inhibition was reported as mean value (mm) with standard deviation.
The zone diameter was measured along with the diameter of the antibiotic disc.

Table 2
Antibacterial activity of Sargassum longifolium against common shrimp pathogens

Organism	Aqueous				Ethanol				Methanol			
	100	200	400	800	100	200	400	800	100	200	400	800
Aeromonas hydrophilia	6.75 ± 0.5	8.25 ± 0.96	10.25 ± 0.96	13.5 ± 1.29	ND	9.75 ± 1.26	12.5 ± 0.58	15 ± 1.15	8.25 ± 0.96	10.5 ± 1	14 ± 0.82	18.75 ± 1.26
Pseudomonas aeruginosa	ND	6.25 ± 0.5	10.5 ± 1	11.75 ± 1.26	ND	11.25 ± 1.26	14.5 ± 1.29	16.75 ± 1.5	ND	10 ± 1.41	14 ± 1.15	17 ± 0
Vibrio cholerae	ND	ND	8.25 ± 0.5	11 ± 0.82	8.25 ± 0.5	10 ± 1.4	13.75 ± 0.5	15.75 ± 0.95	8.25 ± 0.5	12.75 ± 2.06	16.5 ± 1	21.25 ± 0.5
Vibrio harveyi	ND	ND	7 ± 1.15	10.75 ± 0.96	8 ± 0.82	10.25 ± 1.5	12.75 ± 0.95	15.25 ± 0.95	ND	9.75 ± 1.5	13.5 ± 1	18.5 ± 0.58
Vibrio parahaemolyticus	ND	ND	7.25 ± 1.5	12.25 ± 1.5	ND	9 ± 1.4	12 ± 0.82	15.5 ± 1	ND	9.5 ± 1.91	13.75 ± 1.5	17.75 ± 0.96

The zone of inhibition was reported as mean value (mm) with standard deviation.
The zone diameter was measured along with the diameter of the antibiotic disc.

Table 3
Preliminary phytochemical analysis of seaweed extracts

S.No	Metabolites	*Sargassaum longifolium*			*Gracilaria corticata*		
		Aqueous	Ethanol	Methanol	Aqueous	Ethanol	Methanol
1.	Carbohydrates	+	+	+	+	+	+
2.	Protein	+	+	+	+	+	+
3.	Alkaloids	-	-	-	-	-	-
4.	Glycoside	-	+	+	-	+	+
5.	Terpenoids	-	-	+	-	+	+
6.	Flavonoids	-	-	+	-	-	-
7.	Tannin	+	+	+	+	+	+
8.	Saponins	-	+	+	-	+	+
9.	Steroids	+	+	+	+	+	+

+ - Positive
- - Negative

DISCUSSION

In the present study we observed the antibacterial activity of the seaweeds such as *S. longifolium* and *G. corticata* against some common shrimp pathogens. Several researchers reported that marine algae have antibacterial activity[5,13,14,15] against pathogenic bacteria. Organic solvents are commonly used for extraction of active compounds from algae. In this study three different solvents (methanol, ethanol and aqueous) used for extraction of

active compounds from the seaweeds. Among the solvent used methanol extract showed highest antibacterial activity against tested bacterial pathogens. Aqueous extracts have a low potent effect on the shrimp pathogens. Methanol extract of *S. longifolium* showed maximum activity against *Vibrio cholerae* at 800µg/disc. It is revealed that the antibacterial activity of the methanolic extracts of *S. longifolium* is more efficient than that of ethanolic and aqueous extracts against the shrimp pathogens tested. Similarly, earlier researcher[15] observed methanolic extraction gives higher antimicrobial activity than other extracts. Earlier report showed chloroform extracts of *S. latifolium* has highest antibacterial activity against *V. alginolyticus, V. parahaemolyticus* and *V. harveyi*[16]. In this study, both ethanol and methanol extracts of *Gracilaria corticata* showed high inhibition activity against all the tested bacteria, highest antibacterial activity was against *P. aeruginosa* followed by *V. parahaemolyticus* and *V. harveyi*. Aqueous extract of *G. corticata* has less activity against all the shrimp pathogens tested. Reports are available that both the aqueous and ethanol extracts of *G. corticata* could not inhibit bacteria[17], but other researchers were reported aqueous extract of *G. corticata* has maximum zone of inhibition against *Proteus mirabilis* (17 mm). The ethanolic extract of seaweed *G. edulis* inhibits fish pathogens such as *Escherichia coli, P. aeruginosa, Staphylococcus aureus* and *Streptococcus faecalis*[18]. Earlier reports are available that methanol extracts showed strong antibacterial activity[19]. The effectiveness of extraction methods highlighted that methanol extraction yield higher antibacterial activity[20]. The results of the study would suggested that a particular solvent is required to extract some antimicrobial substances within the algal plant and therefore the inhibitory activity is higher.

Antimicrobial activity of the seaweed is depends on the solvents used for their extraction. Many researchers reported influence of different extraction solvents on the content of compounds in extracts[21]. Solvents solubility efficiency is strongly dependent on material used for extraction[22,23]. Qualitative phyto-compounds of the extracts also reflect the soluble property of the bioactive compounds present in the seaweeds. In this study, methanol extracts of both seaweeds showed the maximum number of phyto-compounds such as carbohydrates, proteins, glycoside, terpenoids, tannin, saponins and steroids, but, the aqueous extract have only few compounds which indicates the seaweed contains phyto-compounds are water insoluble and they are soluble in organic solvents. Methanol has effectively soluble the compounds from the seaweeds than ethanol. Seaweeds are known to produce many secondary metabolites, including bioactive compounds with various activities[24]. It is clear that using organic solvents provides a higher efficiency in extracting compound than water based methods[25]. Previously, phyto- compounds such as carbohydrates, steroids, tannin, saponins, fats, gums and proteins were extracted from *G. corticata* and *S. wightii* in varying concentration[26]. Earlier researchers observed alkaloids, terponoids, steroids, coumurins, tannins, saponins, flavonoids, quinons, proteins, carbohydrates, glycosides and catachin in methanol extracts of *G. corticata*[27]. Acetone and ethanol was suitable solvent for extracting the antibiotic principle from *S. boveanum*[28]. The activity of crude algal extracts is often attributed to the complex mixture of active compounds. The present study results were direct evidence of these algae containing antibacterial activities which is due to its bioactive secondary metabolites. The methanol extract showed higher percentage of bioactive metabolites; hence, it showed superior activity than the other extracts. Similar report was observed by many researchers[26,27]. The present study results were positively indicates the antibacterial potential of methanolic extracts of *G. corticata* and *S. longifolium,* and it was concluded that the seaweeds are the potential source of new marine pharmacological products to the aquaculture industries which may be a good substitute for antibiotics in shrimp aquaculture. Further, it is also necessary for successful separation, purification and characterization of biologically active compounds from the seaweeds.

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