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Screening and Characterization of Marine Seaweeds and its Antimicrobial Potential against Fish Pathogens

M. L. Maheswaran
Centre of advanced study in Marine
Biology, Faculty of Marine Sciences,
Annamalai University, Parangipettai,
Tamilnadu, India.

S. Padmavathy
Department of Zoology and
Microbiology, Thiagarajar College,
Madurai, Tamilnadu, India.

B. Gunalan
Centre of advanced study in Marine
Biology, Faculty of Marine Sciences,
Annamalai University, Parangipettai,
Tamilnadu, India.

M. L. Maheswaran, S. Padmavathy, B. Gunalan

ABSTRACT

Bacterial diseases are responsible for heavy mortality in wild and cultured fish. The problems in the farms are usually tackled by preventing disease outbreaks or by treating the actual disease with drugs or chemicals. The use of antimicrobial agents has increased significantly in aquaculture practices. In the present study, the seaweed samples were tested against wide range of fresh water fish pathogens. It is well documented that all the five seaweed samples were effective against fish pathogens. It is documented that, the present study is initiated to explore the bioactive potential of major seaweeds infested along Gulf of Mannar as a potential source of marine bioprospecting in future. This study aims to pave way for the identification of new bioactive compounds for treating the dreadful diseases and thereby help to reduce the multidrug resistant organisms.

Keywords: Seaweed, fish pathogen, Gulf of Mannar, Antimicrobial activity.

1. Introduction

Fish is among the most important sources of protein to human consumption, thus the study of the signs and lesions, induced by fish diseases, helps the protection in our national economy. Infectious diseases of cultured fish are among the most notable constraints on the expansion of aquaculture and the realization of its full potential [1, 2, 3]. Many pathogens such as parasites, fungi, viruses and bacteria can cause skin disease in fish, but bacteria are of particular interest because infections are common and often deadly if untreated. Some of the most obvious signs of bacterial dermatitis include development of reddened lesions, sores, or ulcers on the body, reddening of the base of the fins and dulling or darkening of skin color. The distribution of the skin lesions is quite variable and may include the head, face, operculum, mouth, back, trunk or body wall including the lateral line and caudal peduncle [4].

The signs of disease vary somewhat depending on the species of bacteria and the species of fish. A common sign in all the cases is exophthalmia and hemorrhages in the eye, hemorrhages around the opercula and at the base of the fins. It was non – hemolytic type which apparently caused a number of massive fish kills of estuarine fish along the Gulf coast of Florida and Alabama. It was suggested that there was probably some underlying stress although this was not identified. In these fish the above signs plus swollen abdomens filled with a bloody fluid, hemorrhagic enteritis and pale livers were noted. Most bacteria that infect fish are Gram-negative, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Flavobacterium columnare*, *Vibrio* and *Pseudomonas* species. The major groups of Gram-positive that cause disease in fish are *Streptococcus* [5]. Marine bacteria with antagonistic activity could be employed to combat epizootics in aquaculture systems [6, 7, 8] as probiotics or biocontrol agents at prophylactic or curative doses.

Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. But, the pathogenic bacteria becoming resistant to drugs are common due to indiscriminate use of antibiotics. It becomes a greater problem in the treatment against resistant pathogenic bacteria. Marine organisms are a rich source of structurally novel and biologically active metabolites. Marine plants have long been recognized as producers of biologically active substances.

Correspondence:
B. Gunalan
Centre of advanced study in Marine
Biology, Faculty of Marine Sciences,
Annamalai University,
Parangipettai, Tamilnadu, India.
Tel: +04144-243223

Potential activity of some marine plants like mangroves, seaweeds, seagrasses and lichen have been reported from both India and elsewhere [9, 10, 11, 12]. Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. Nearly 50 lakhs (50,000,00) species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation [13, 14, 15] apart from their potential ecological/industrial significances such as controlling reproduction, settlement/bio fouling and feeding deterrents [16]. In this background, the present study intended to evaluate activity range and potency of seaweeds collected from the peninsular coast of India.

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antibacterial, antifungal, antitumorals and anti-inflammatory activities [17, 18, 19]. The bioactive properties of seaweeds are generally assayed using extracts in various organic solvents, e.g. acetone, methanol-toluene, ether and chloroform-methanol. Invariably, seaweeds have been proven to be potent source of antimicrobial compounds. This potential have also been proved in "in captivity" control of shrimp bacterial pathogens [16]. Ethanolic extract of 8 species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad spectrum antibacterial and antifungal activities [21]. The algal extracts such as *Enteromorpha compressa*, *Cladophorosis zoolingeri*, *Padina gymnospora*, *Sargassum wightii* and *Gracilaria corticata* were active against gram positive and gram negative bacteria (Rao *et al.*, 1991). Based on the earlier findings, it could be inferred that the bioassay guided fractionation and purification of natural products from seaweeds may come up with potent biological compounds. In this background, the present study was initiated to explore the bioactive potential of major seaweeds infested along southwest coast of India as a potential source to control fish pathogens.

2. Materials and Methods

2.1 Sample Collection

Diseased fresh water fishes were collected from fish market at Anna Nagar, Madurai. They were used as a source for the study of the disease causing organisms. The different fresh water fishes used for the present study were given as follows.

- | | |
|-----------------------|--------------------------------|
| 1. Paarai Fish | - <i>Alectis indicus</i> |
| 2. Jilebi Kendai Fish | - <i>Oreochromis niloticus</i> |
| 3. Kuravai Fish | - <i>Channa punctatus</i> |
| 4. Ooli Fish | - <i>Sphyraena barracuda</i> |

2.2 Isolation and Characterization of Fish Pathogens

The fish infecting microorganisms were isolated and identified from various infected fresh water fishes. The infected area like gill region and infected external body surface was carefully scraped from the dorsal body using sterilized cotton buds. The ventral skin and mucus was not collected to avoid intestinal and sperm contamination. The samples were crushed in sterile distilled water and spread plated on nutrient agar plates. The plates were incubated at 37 °C for 24 hrs. Different types of colonies from the respective fishes were selected on nutrient agar plates were identified based on the colonial morphology. They were purified by repeated streaking on fresh nutrient agar plate and stored at 4 °C for further characterization.

2.3 Identification of Bacterial Fish Pathogens using Selective Media

For identification the stored pure cultures of fish pathogens were streaked on selective/differential media which includes Blood Agar, Mac Conkey Agar, Salmonella Shigella Agar, Xylose Lysine Deoxycholate Agar, Cysteine Lactose Electrolyte Deficient agar, Thiosulphate Citrate Bile salt Agar etc. Based on the results obtained the organisms were identified using selective biochemical tests as per standard methods.

2.4 Biochemical Characterization of the Fish Pathogens

The fish pathogens were confirmed by the following biochemical reactions such as motility, indole production, catalase, oxidase, glucose fermentation, citrate utilization, Triple sugar iron agar test, methyl red, Voges Proskauer and Urease test.

2.5 Collection and Identification of Seaweeds

Five different seaweed samples were collected during the low tidal conditions at depths of 1 to 3 m from different locations at Gulf of Mannar, southeast coastal region, Mandapam (9°16'47"N 79°7'12"E) with the help of divers. To avoid air contamination, seaweed samples were collected aseptically below surface level, using sterilized wide mouthed glass bottle containers. The containers and its accessories were steam sterilized for 15 minutes at 15 lbs. Marine algae were collected by hand picking from the submerged marine rocks and transferred to the laboratory and were identified at Department of Marine and Coastal Studies, Madurai Kamaraj University, Mandapam, Tamil Nadu, India.

2.6 Sample Preparation

Algal samples were cleaned of epiphytes and extraneous matter, and necrotic parts were removed. Plants were washed with sea water and then in fresh water. The seaweeds were transported to the laboratory in sterile polythene bags at 0 °C temperature. In the laboratory, samples were rinsed with sterile distilled water and were shade dried, cut into small pieces and powdered in a mixer grinder.

2.7 Aqueous Extraction of Seaweeds

Aqueous extracts were prepared by transfer of 1 g of the powder to sterile wide mouthed screw-capped bottles of 50 ml volume. 10 ml of sterile deionised distilled water was added to the powdered samples which were allowed to soak for 24 hrs at room temperature, after heating the extracts for an hour at 100 °C. The mixture were then centrifuged at 2000 rpm for 10 min at 4 °C. The supernatants were filtered through a sterile funnel containing sterile Whatman No. 1 filter paper.

2.8 Solvent Extraction of Seaweeds

One gram of each seaweed sample was extracted with 4ml of the solvents. The dried sample were soaked in the solvents for 48 h in sterile wide-mouthed screw-capped bottles of 50 ml volume and then covered with aluminium foil. The mixture was then centrifuged at 2000 rpm for 10 min at 4 °C. The supernatants were filtered through a sterile funnel and sterile what man filter paper no. During this procedure filtration should be carried out very quickly in order to avoid the evaporation of the solvent sample. Extract obtained was used for screening of their antimicrobial potential. The experimental procedure was carried out with different solvent system such as methanol, dichloromethane, diethyl ether, ethyl acetate, hexane, benzene, ethanol, n-butanol, chloroform.

2.9 Antibioqram Pattern of Fish Pathogens

The agar well diffusion assay was used for testing the antimicrobial activity of crude seaweed extracts. In this technique, the identified fresh water fish pathogens were used. This assay method was described by Schillinger and [22] which was used for testing the antagonistic activities of solvent extracted seaweed. Required wells each of 7mm in diameter were made in the agar using well cutter and 100µl of the crude extract of different seaweeds were transferred into each well using solvent as control. The plates were incubated for 24 hrs at 30 °C and examined for clear inhibition zone around the well. The assay was carried out in duplicate for all the identified fish pathogens [22].

2.9.1 Standard Antibiotic disc Assay

To compare the effect of antagonistic activity of seaweed extracts, the isolated fresh water fish pathogens were subjected to antimicrobial assay to find their resistant and sensitive pattern to a group of selected antibiotics discs by Kirby – Bauer method [24]. The following commercial antibiotics are used for this assay Erythromycin, Tetracycline, Penicillin, Streptomycin.

2.9.2 Determination of dry weight

The dry weight of the crude seaweed extract was determined by

taking 1 ml of the sample in the previously weighed watch glass. Then the sample was allowed to evaporate to dryness. Final weight was calculated by which dry weight of the seaweed sample was calculated.

2.9.3 Chemical Characterization

The crude extract of different seaweeds was analyzed by Fourier Transform Infra-Red Spectroscopy (FTIR) 22.24.

2.9.4 FTIR Analysis

FT-IR - 8400 S, SHIMADZU model Fourier Transmission Infra-Red Spectroscopy was used for the analysis of the solvent extracted potential seaweed sample. The spectrum was taken in the mid IR region of 400 – 4000 cm⁻¹. The spectrum was recorded using ATR (Attenuated Total Reflectance) technique. The sample was directly placed in the Potassium bromide crystal and the spectrum was recorded in the transmittance mode.

3. Results

Fresh water diseased fishes were collected from fish market in Anna Nagar, Madurai. The different fishes used for the present study were Paarai Fish (*Alectis indicus*), Jilebi Kendai Fish (*Oreochromis niloticus*), Kuravai Fish (*Channa punctatus*) and Ooli Fish (*Sphyraena barracuda*).

Table: 1 Enumeration of Microorganisms from Fresh Water Fishes

S. No	Fresh Water Fishes	Sample analysed	Bacterial colonies (CFU /ml)			
			10 ⁻²	10 ⁻⁴	10 ⁻⁶	10 ⁻⁸
1	<i>Alectis indicus</i> (Paarai Fish)	Gill Region	TNTC	TNTC	TNTC	13
		Body surface	TNTC	TNTC	TNTC	25
2	<i>Oreochromis niloticus</i> (Jilebi Kendai)	Gill Region	TNTC	TNTC	190	12
		Body surface	TNTC	TNTC	TNTC	27
3	<i>Channa punctatus</i> (Kuravai Fish)	Gill Region	TNTC	TNTC	TNTC	68
		Body surface	TNTC	TNTC	TNTC	96
4	<i>Sphyraena barracuda</i> (Ooli)	Gill Region	TNTC	TNTC	TNTC	87
		Body surface	TNTC	TNTC	TNTC	105



Plate 1: Isolation of Organisms from Different Edible Fish

The infected area like gill region and external body surface were used for isolation and enumeration of pathogens using nutrient agar medium by spread plate technique and enumerated for finding out the colony forming units. The organisms were subcultured based on the differences in colony morphology in fresh nutrient slants and stored at 4 °C for further characterization (Plate – 1; Table.1). The isolated fresh water fish pathogens were then identified by

using selective biochemical tests and selective media as per the standards methods. The bacterial fish pathogens were tentatively identified from different ornamental fishes were *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Salmonella typhi*, *Shigella sp.*, *Pseudomonas aeruginosa* and *Escherichia coli* (Table. 2, 3, 3.1, 3.2 & 3.3 & 4; Plate- 2).

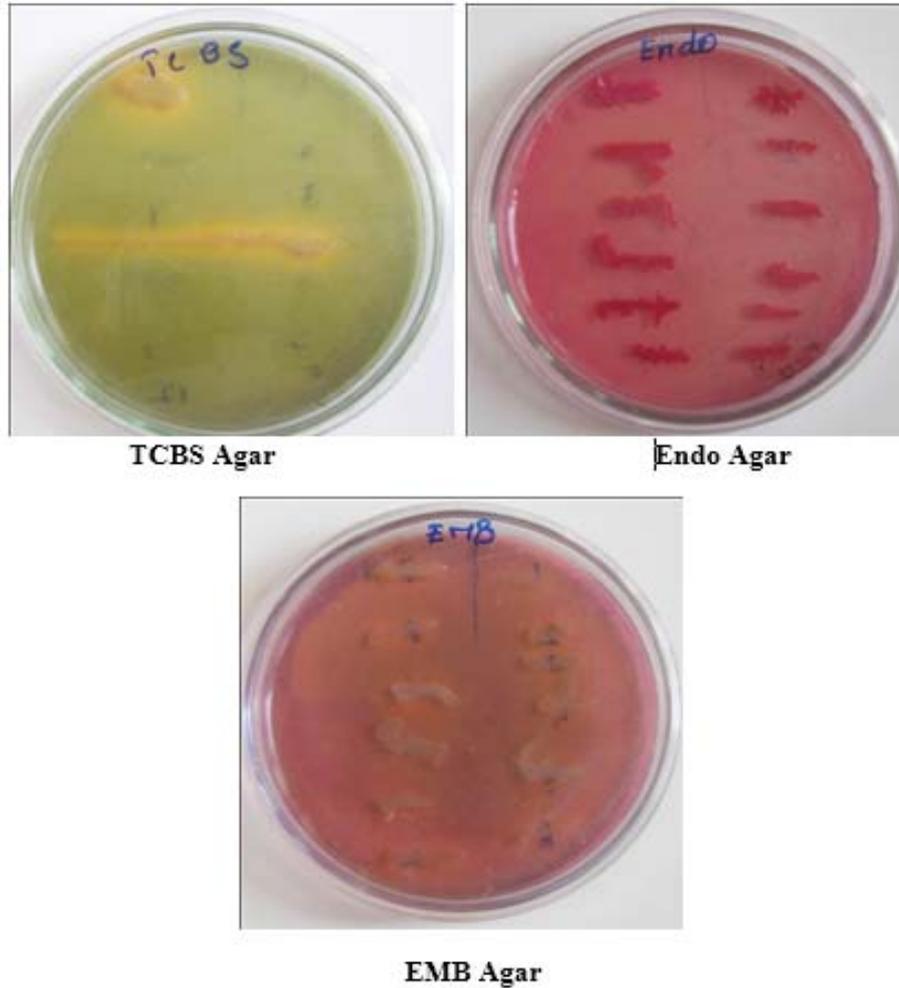


Plate 2: Identification of Fish Pathogens using Selective/Differential Media

Table 2: Cultural Characteristics of Fish Pathogens on Selective/Differential Media

S. No.	Organisms	Selective / Differential media	Color of the colonies
1.	<i>Vibrio cholera</i>	TCBS Agar	Yellow color
2.	<i>Vibrio parahaemolyticus</i>	TCBS Agar	Green color
3.	<i>Aeromonas hydrophila</i>	TCBS Agar	Yellow color
4.	<i>Aeromonas hydrophila</i>	Blood agar	β - Haemolysis
5.	<i>Shigella sp.</i>	SS agar	Pink color
6.	<i>Salmonella sp.</i>	SS agar	Colorless with black centered colony
7.	<i>Pseudomonas aeruginosa</i>	Flo agar & Tech agar	Bluish & Yellowish green (Fluorescent colony)
8.	<i>Escherichia coli</i>	EMB agar	Metallic Sheen
9.	<i>Escherichia coli</i>	Mac Conkey Agar	Pink colored colonies

Table 3: Biochemical tests for *Pseudomonas* sp.

S. No	Organisms	Catalase test	Denitrification Test	Growth at 4 °C	Growth at 41 °C	Triple Sugar Iron Agar Test			
						Slant	Butt	H ₂ S	Gas
1.	<i>P. aeruginosa</i>	Negative	Gas formation	Absence of growth	Presence of growth	Alkaline	Alkaline	-	-

#Note: Alkaline - Red color, Acid - Yellow color

Table 3.1: Biochemical tests for *Vibrio* sp. and *Aeromonas hydrophila*

S. No	Organisms	Oxidase test	Motility test	Growth on NaCl (80 g/l)	Triple Sugar Iron Agar Test			
					Slant	Butt	H ₂ S	Gas
1.	<i>V. cholerae</i>	Positive	Absence	Absence	Alkaline	Acid	-	-
2.	<i>V. parahaemolyticus</i>	Positive	Absence	Presence	Alkaline	Acid	-	-
3.	<i>A. hydrophila</i>	Positive	Presence	Absence	Alkaline	Acid	-	-

#Note: Alkaline- Red color, Acid -Yellow color

Table 3.2: Biochemical tests for *Salmonella* sp. and *Shigella* sp.

S. No	Organisms	Triple Sugar Iron Agar Test			
		Slant	Butt	H ₂ S	Gas
1.	<i>Shigella</i> sp.	Alkaline	Acid	-	-
2.	<i>Salmonella</i> sp.	Alkaline	Acid	+	-

#Note: Alkaline - Red color, Acid - Yellow color

Table 3.3: Biochemical tests for *E. coli*

S. No	Organisms	Indole	Voges Proskauer	Oxidase Test	Triple Sugar Iron Agar			
					Slant	Butt	H ₂ S	Gas
1.	<i>E. coli</i>	+	-	-	Acid	Acid	-	+

#Note: Alkaline - Red color, Acid - Yellow color

Table 4: Identification of fish pathogens from fresh water fishes

S. No	Name of the Fish	Identified Organisms
1.	<i>Alectis indicus</i> (Paarai Fish)	<i>Aeromonas hydrophila</i>
2.	<i>Oreochromis niloticus</i> (Jilebi Kendai)	<i>Pseudomonas aeruginosa</i>
3.	<i>Channa punctatus</i> (Kuravai Fish)	<i>Vibrio cholera</i>
		<i>Vibrio parahaemolyticus</i>
4.	<i>Sphyraena barracuda</i> (Ooli)	<i>Shigella</i> sp.
		<i>Salmonella</i> sp.
		<i>Escherichia coil</i>

Five different seaweed samples were collected during the low tidal conditions at depths of 1 to 3 m from different locations at Gulf of Mannar, southeast coastal region, Mandapam, India. They were identified at Department of Marine and Coastal Studies, Madurai Kamaraj University, Mandapam, Tamil Nadu, India. The collected

marine seaweed samples were identified as *Sargassum wightii*, *Caulerpa racemosa*, *Caulerpa sertularioides*, *Padina gymnospora* and *Chaetomorpha antennina* (Plate: 3).

Sample preparation was done by cleaning seaweed samples free of epiphytes and extraneous matter, and necrotic parts. Plants were

washed with sea water and then in fresh water. Finally, samples were rinsed with sterile distilled water and were shade dried, cut

into small pieces and powdered in a mixer grinder.



Caulerpa racemosa



Sargassum wightii

Plate 3: Collection of Marine Seaweed Samples

Aqueous extracts were prepared by transfer the powder to sterile wide mouthed screw-capped bottles containing sterile deionised distilled water which were allowed to soak for 24 hrs. at room temperature, after heating the extracts for an hour at 100 °C. The mixture were then centrifuged at 2000 rpm for 10 min at 4 °C. The supernatants were filtered through a sterile funnel containing sterile Whatman No. 1 filter paper (Plate: 4). Solvent extraction of seaweeds was carried out using one gram of each seaweed sample was extracted with 4ml of the solvents. The dried sample were

soaked in the solvents for 48 hrs. and then covered with aluminium foil. The mixture was then centrifuged at 2000 rpm for 10 min at 4 °C. The supernatants were filtered through a sterile funnel and sterile Whatman filter paper No. 1. Extract obtained was used for screening of their antimicrobial potential. The experimental procedure was carried out with different solvent system such as methanol, dichloromethane, diethyl ether, ethyl acetate, hexane, benzene, ethanol, n-butanol, chloroform (Plate:4).



Plate 4: Extraction of Crude Seaweed Extracts using Different Solvents

Extract obtained by above mentioned method was used for screening of their antimicrobial potential. The different solvent samples were taken as control sample (Plate: 5). Well diffusion

assay was used for testing the antimicrobial activity of crude seaweed extracts. In this technique, the identified fresh water fish pathogens were used (Table: 5 – 9; Fig: 1 – 5; Plate: 6 & 7).

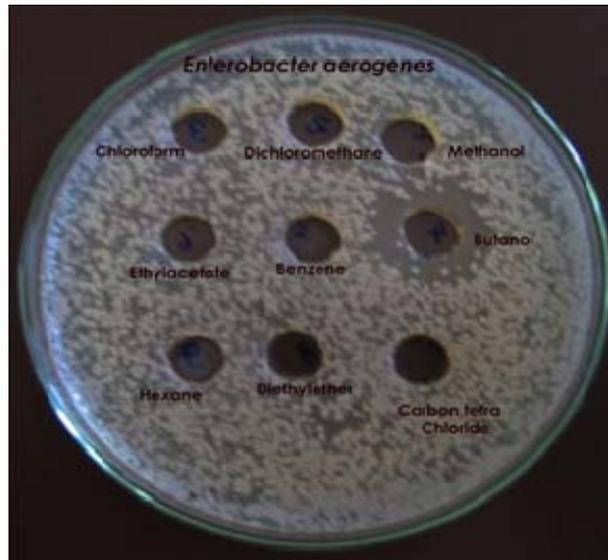
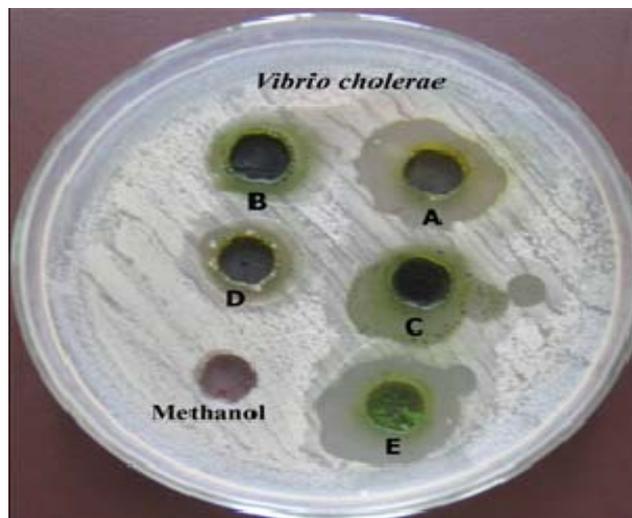


Plate 5: Antagonistic Activity of Different Solvents against Fish Pathogens



A - *Sargassum wightii*, B - *Caulerpa racemosa*, C – *Caulerpa sertularioides*, D- *Padina gymnospora*, E - *Chaetomorpha antennina*

Plate 6: Antagonistic Activity of Crude Seaweed Extracts against Fish Pathogens

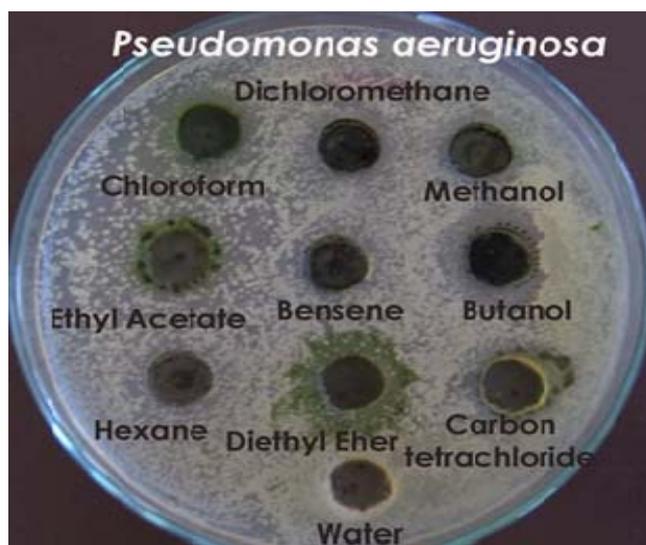


Plate 7: Antagonistic Activity of Crude *Sargassum wightii* extracts against Fish Pathogens

Table 5: Antagonistic activity of *Sargassum wightii* against fish pathogens

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
<i>Escherichia coli</i>	24	23	23	27	19	20	12	18	13	-
<i>Pseudomonas aeruginosa</i>	11	23	11	19	17	18	11	20	21	-
<i>Salmonella typhi</i>	20	19	18	19	20	24	22	22	23	-
<i>Shigella sp.</i>	19	20	18	20	24	25	21	19	18	-
<i>Vibrio cholerae</i>	21	20	19	18	20	17	18	20	25	-
<i>Vibrio parahaemolyticus</i>	23	12	23	23	21	19	19	20	12	-
<i>Aeromonas hydrophila</i>	18	20	20	24	24	20	26	19	20	-

Table 6: Antagonistic activity of *Caulerpa racemosa* against fish pathogens

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
<i>Escherichia coli</i>	20	20	13	20	17	15	13	15	18	-
<i>Pseudomonas aeruginosa</i>	19	25	21	10	19	19	19	11	19	-
<i>Salmonella typhi</i>	21	19	10	20	19	20	13	19	14	-
<i>Shigella sp.</i>	20	19	21	17	12	20	12	10	20	-
<i>Vibrio cholerae</i>	20	23	15	11	11	24	20	17	18	-
<i>Vibrio parahaemolyticus</i>	24	20	20	18	12	26	27	13	15	-
<i>Aeromonas hydrophila</i>	23	19	19	23	20	25	25	16	10	-

Table 7: Antagonistic activity of *Caulerpa Sertularioides* against fish pathogen

Test Organisms	Solvents used									
	Methanol	Dichloro Methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
<i>Escherichia coli</i>	20	19	19	19	19	19	19	20	12	-
<i>Pseudomonas aeruginosa</i>	23	24	23	23	23	23	17	18	11	-
<i>Salmonella typhi</i>	21	20	24	24	24	24	24	19	19	-
<i>Shigella sp.</i>	21	21	23	23	23	23	19	21	21	-
<i>Vibrio cholera</i>	20	19	18	20	25	19	20	23	23	-
<i>Vibrio parahaemolyticus</i>	20	21	19	18	21	21	20	20	20	-
<i>Aeromonas hydrophila</i>	26	19	24	24	19	26	24	26	19	-

Table 8: Antagonistic activity of *Padina gymnospora* against fish pathogens

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloro form	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl ether	Hexane	Water
<i>Escherichia coli</i>	23	26	17	18	20	25	21	26	19	-
<i>Pseudomonas aeruginosa</i>	24	27	19	20	25	24	19	27	17	-
<i>Salmonella typhi</i>	18	24	18	23	27	19	20	20	25	-
<i>Shigella sp.</i>	23	11	23	24	25	26	19	24	27	-
<i>Vibrio cholerae</i>	18	23	24	26	23	19	20	20	22	-
<i>Vibrio parahaemolyticus</i>	18	20	25	22	22	23	30	18	20	-
<i>Aeromonas hydrophila</i>	11	17	12	12	20	18	23	26	18	-

Table 9: Antagonistic activity of *Chaetomorpha antennina* against fish pathogens

Test Organisms	Solvents used									
	Methanol	Dichloro methane	Chloroform	Butanol	Benzene	Ethyl acetate	Carbon tetra chloride	Diethyl Ether	Hexane	Water
<i>Escherichia coli</i>	19	24	18	11	20	20	18	17	15	-
<i>Pseudomonas aeruginosa</i>	20	19	18	19	20	24	22	22	23	-
<i>Salmonella typhi</i>	21	20	19	18	20	17	18	20	25	-
<i>Shigella spp.</i>	21	21	20	18	17	19	20	20	22	-
<i>Vibrio cholera</i>	20	19	18	20	25	19	20	25	24	-
<i>Vibrio parahaemolyticus</i>	23	24	25	26	19	19	19	22	12	-
<i>Aeromonas hydrophila</i>	24	26	23	19	20	24	17	12	14	-

The isolated fresh water fish pathogens were subjected to antimicrobial assay to find their resistant and sensitive pattern to a group of selected antibiotics discs by Kirby – Bauer method. The

following commercial antibiotics are used for this assay Erythromycin, Tetracycline, Penicillin, Streptomycin (Table: 10).

Table 10: Anti-Microbial Activity Using Standard Antibiotic Disks Against Fish Pathogens

S. No	Fish Pathogens	Standard Antibiotic Disk (Zone of Inhibition in mm)			
		Streptomycin	Tetracycline	Penicillin	Erythromycin
1	<i>Escherichia coli</i>	15	-	-	10
2	<i>Pseudomonas aeruginosa</i>	15	-	-	-
3	<i>Salmonella typhi</i>	10	-	-	-
4	<i>Shigella spp.</i>	12	-	-	-
5	<i>Vibrio cholera</i>	14	-	-	-
6	<i>Vibrio parahaemolyticus</i>	15	-	-	-
7	<i>Aeromonas hydrophila</i>	17	-	-	-

The dry weight of the crude seaweed extract was determined by taking the sample in the previously weighed watch glass. Then the sample was allowed to evaporate to dryness. Final weight was calculated by which dry weight of the seaweed sample was

calculated (Table: 10).

Fourier Transmission Infra-Red Spectroscopy was used for the analysis of the solvent extracted potential seaweed sample (*Sargassum wightii* and *Caulerpa sertularioides*).

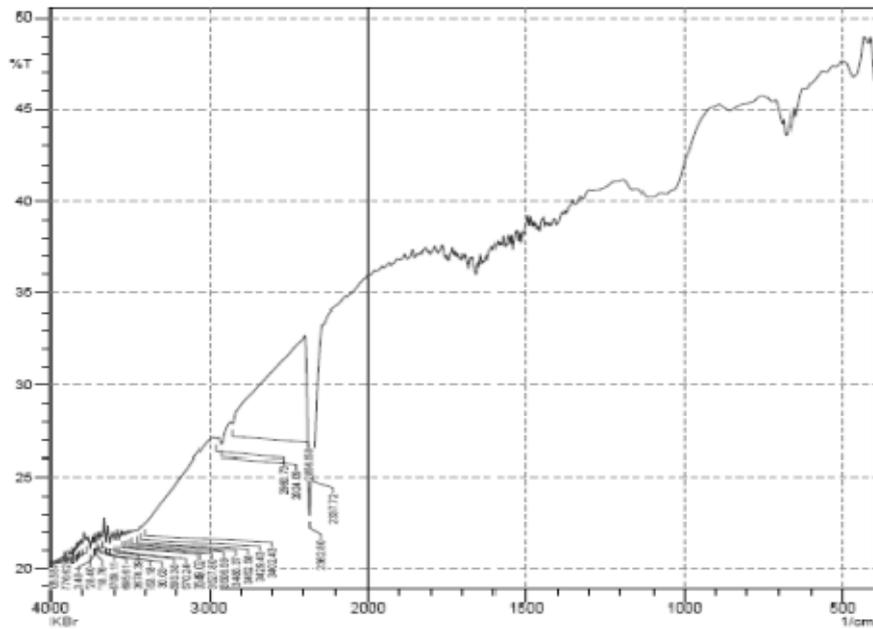


Fig 6: Infra-Red Spectrum Analysis of *Sargassum wightii* Extracts

The spectrum was taken in the mid IR region of 400 – 4000 cm^{-1} (Fig: 6 & 7). The noise peaks were not considered in the spectral

data and the peaks showed at high intensity were considered for the determination of functional groups.

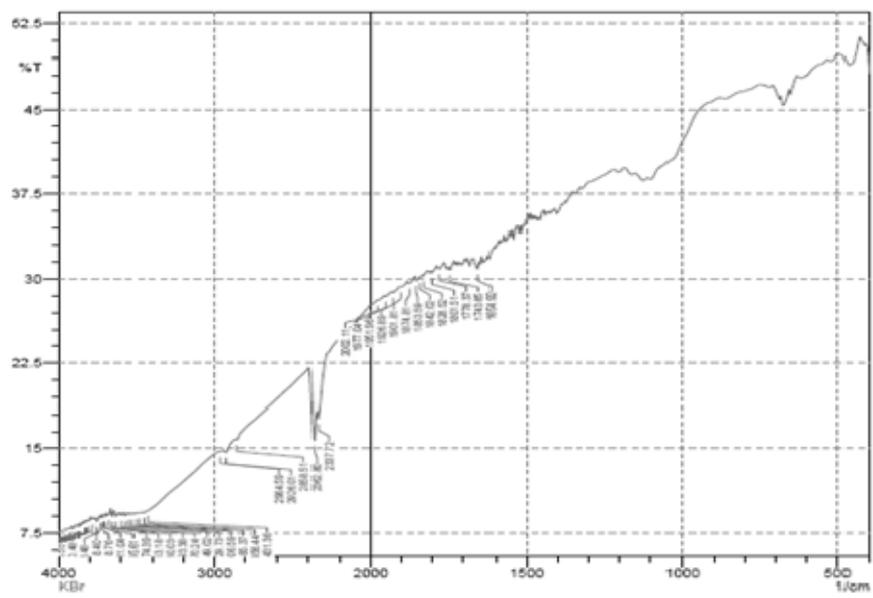


Fig 7: Infra-Red Spectrum Analysis of *Caulerpa Sertularioides* Extracts

4. Discussion

Fish losses from infectious diseases are a significant problem in aquaculture worldwide. The ability of antibiotics to protect fish against infection caused by fish pathogens is a recent investigation. The results of early investigations [25, 26] revealed a potential for antimicrobial peptides to protect against fish infections. Similarly the results of the preliminary characterization of the antibacterial component revealed that it is a heat stable compound also. These antimicrobial agents from marine habitats are excellent sources in producing several antibiotics.

Many species of vibrios have been isolated from diseased fish; however, the most important include *Vibrio cholera*, *V. parahaemolyticus* and *Aeromonas hydrophila* which coincides with present report on the pathogens from diseased fresh water fishes. In

addition, we reported the presence of gram negative enteric organism from the edible fishes which can transmit diseases to humans through improperly cooked fish products [27] reported on transmit of diseases by enteric organisms from edible fishes by food consumption. In the present investigation the bacterial fresh water fish pathogens were tentatively identified as *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Salmonella typhi*, *Shigella* spp., *Pseudomonas aeruginosa* and *Escherichia coli*. [28]. *Aeromonas hydrophila* are common inhabitants of soil and water and commonly are found on the surface of fishes, particularly on the gills. The stress of crowding, handling, spawning, or holding fish at above-normal temperatures, as well as the stress of external injury, facilitates the transmission of fish diseases. The enumeration of fish pathogens of the current study

revealed that the body surface are possessed with more amount of microbial load in comparison with the gill region of the edible fishes [8]. The metabolic and physiological capabilities of marine organisms that allow them to survive in complex habitat types provide a great potential for production of secondary metabolites, which are not found in terrestrial environments. Thus, marine algae are among the richest sources of known and novel bioactive compounds [29, 30].

Five different seaweed samples were collected during the low tidal conditions at depths of 1 to 3 m from different locations at Gulf of Mannar, southeast coastal region, Mandapam, India. They were identified as *Sargassum wightii*, *Caulerpa racemosa*, *Caulerpa sertularioides*, *Padina gymnospora* and *Chaetomorpha antennina*. Aqueous extracts and solvent extracts were prepared for testing the antimicrobial potential of the collected seaweed samples. The experimental procedure was carried out with different solvent system such as methanol, dichloromethane, diethyl ether, ethyl acetate, hexane, benzene, ethanol, n-butanol, chloroform. Extract obtained by above mentioned method was used for screening of their antimicrobial potential by well diffusion assay. Extract obtained by above mentioned method was used for screening of their antimicrobial potential. The different solvent samples were taken as control sample. Well diffusion assay was used for testing the antimicrobial activity of crude seaweed extracts fish pathogens. Based on the results obtained for antagonistic activity, it is documented that though several solvents were utilized. The following order of the solvents showed high degree of activity against test organisms i.e. Methanol, dichloromethane, benzene, carbon tetrachloride and hexane respectively. This order of activity in case of solvents was found to be revealed for the entire seaweed samples. While inferring the broad spectrum activity of *Sargassum wightii*, *Caulerpa racemosa*, *Caulerpa sertularioides*, *Padina gymnospora* and *Chaetomorpha antennina* against fresh water fish pathogens, among the five different seaweeds two samples namely *Sargassum wightii* and *Caulerpa sertularioides* showed wide spectrum activity and high degree of zone of inhibition against the fish pathogens. In aqueous extracts antagonistic activity for all test organisms was not obtained. This is in accordance with earlier reports of [31].

Antibacterial activities of two species of seaweed (*Caulerpa sertularioides*, *Grateloupia lithophila*) tested against bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *S. epidermidis*, *Bacillus sp.* which showed that the extracts of algal species processed moderate antibacterial activity. We have evaluated the antimicrobial potential of from both aqueous and solvent extract. Different solvents: Methanol, Ethanol, Butanol, Chloroform, Acetone, Dichloromethane, water were used. Present work revealed the presence of moderate antimicrobial activity in almost both the seaweed extract and was compared to the standard drugs used [31]. The activity of seaweed extracts were assayed against bacterial fish pathogens belonging to the species *Vibrio parahaemolyticus*, *Salmonella sp.*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Vibrio vulnificus*, *Aeromonas hydrophila* and *Shigella spp.* with the aim of evaluating the possible use of these crude seaweed extracts for controlling diseases in aquaculture. Inhibition tests on solid medium showed that, in general, the majority of fish bacterial pathogens were strongly sensitive to all the used seaweed samples. Comparing with all extracts, among the five different seaweeds two samples namely *Sargassum wightii* and *Caulerpa sertularioides* showed wide spectrum activity and high degree of zone of inhibition against the

fish pathogens.

The same observations recorded in the experiments conducted using cell-free supernatant fluids of marine bacteria demonstrated the involvement of antibiotic substances in the inhibition of fish pathogens [32]. Seaweed extracts are considered to be a rich source of phenolic compounds [33, 31]. The large majority of these (about 60%) are terpenes, but fatty acids are also common (comprising about 20% of the metabolites), with nitrogenous compounds and compounds of mixed biosynthesis each making up only about 10% [34]. Fatty acids are isolated from micro algae that exhibited antibacterial activity [35]. Many workers revealed that the crude extracts of Indian seaweeds are active against Gram-positive bacteria [36]. Methanolic extracts of fifty-six seaweeds collected from South african coast, belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae showed antibacterial activity [37] showed that crude extracts of Indian seaweeds were active only against Gram positive bacteria. Ethanol extracts from 56 Southern African seaweeds from the divisions Chlorophyta (green), Phaeophyta (brown) and Rhodophyta (red) scored highest antibacterial activity for Phaeophyta [38]. Similar results were reported by [38] and [39] for screening studies on seaweeds of Mediterranean and Eastern Sicily coast respectively. Bacterial infection causes high rate of mortality in human population and aquaculture organisms. For an example, *Bacillus subtilis* is responsible for causing food borne gastroenteritis. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* cause diseases like mastitis, abortion and upper respiratory complications, while *Salmonella sp.* causes diarrhoea and typhoid fever [40, 41]. *P. aeruginosa* is an important and prevalent pathogen among burned patients capable of causing life-threatening illness [42].

In the present investigation, the seaweed samples were tested against wide range of fresh water fish pathogens. It is well documented that all the five seaweed samples were effective against fish pathogens. Similar studies have been documented by [43]. Bacterial diseases are responsible for heavy mortality in wild and cultured fish. The problems in the farms are usually tackled by preventing disease outbreaks or by treating the actual disease with drugs or chemicals. The use of antimicrobial agents has increased significantly in aquaculture practices [44]. Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture. Decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives [45].

Preventing disease outbreaks or treating the disease with drugs or chemicals tackles these problems. Nowadays, the use of antibiotics increased significantly due to heavy infections and the pathogenic bacteria becoming resistant to drugs is common due to indiscriminate use of antibiotics. It becomes a greater problem of giving treatment against resistant pathogenic bacteria [46]. Moreover the cost of the drugs is high and also they cause adverse effect on the host, which include hypersensitivity and depletion of beneficial microbes in the gut [47]. Decreased efficiency and resistance of pathogen to antibiotics has necessitated the development of new alternatives [46]. Many bioactive and pharmacologically important compounds such as alginate, carrageen and agar as phycocolloids have been obtained from sea-weeds and used in medicine and pharmacy [48].

Given the economic potential of this seaweed and the importance of 1, 3-glucanase and chitinase as potential determinants in the resistance of plants to fungal diseases [49], the presence of these proteins in the lectin-rich fraction obtained from the red seaweed *H. musciformis* was analyzed and the activity against the human pathogen yeasts *Candida albicans* and *C. guilliermondii* was assessed. The Kirby Bauer assay using commercial antibiotic discs against investigated fresh water pathogens such as *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Aeromonas hydrophila*, *Salmonella typhi*, *Shigella* spp., *Pseudomonas aeruginosa*, and *Escherichia coli* showed resistance pattern to penicillin, erythromycin and tetracycline and sensitivity to streptomycin alone. Resistance to different antibiotics and transferable plasmid encoding multiple drug resistance has been observed in *Aeromonas salmonicida* [49].

The dry weight of the crude seaweed extract was determined by evaporating the dryness. Final weight was calculated by which dry weight of the seaweed sample was calculated. Fourier Transmission Infra-Red Spectroscopy was used for the analysis of the solvent extracted potential seaweed sample (*Sargassum wightii* and *Caulerpa sertularioides*). The spectrum was taken in the mid IR region of 400 – 4000 cm⁻¹. FTIR spectral data for major peaks of *Sargassum wightii* showed the presence of C =N stretch, C- H stretch and hydrogen bonded O –H stretch which showed the presence of nitriles, aldehydes and phenols or alcoholic groups in the crude extract. FTIR spectral data for predominant peaks of *Caulerpa sertularioides* showed the presence of C =N stretch, C- H stretch, hydrogen bonded O –H stretch, C=O stretch and N=O bend which indicated the presence of functional groups such as nitriles, aldehydes and phenols or alcoholic groups, esters and nitro groups. Marine macro algae collected from the Mandapam coast of India have been shown to possess a number of biological activities. In our studies five different seaweed samples were collected and identified as *Sargassum wightii*, *Caulerpa racemosa*, *Caulerpa sertularioides*, *Padina gymnospora* and *Chaetomorpha antennina* and checked for their antimicrobial activity. These seaweeds are currently undergoing preliminary investigations with the objective of screening of biologically active species. Furthermore, the encouraging biological activities seen in this study show that the Indian coastline is a potential source of variety of marine organisms worthy of further investigation.

The use of seaweeds in the development of pharmaceuticals, nutraceuticals, and botanicals as a source of pigments, bioactive compounds and antiviral agents is in full expansion. Ecologically and commercially seaweed are important as the potential source as food supplement of 21st century because it contains proteins, lipids, polysaccharides, minerals, vitamins and enzymes. They are being used for food, medicine industry and other uses such as integrated aquaculture with fishes or mollusc culture and also have been tried to remove heavy metals in cleaning waste water. Further studies are needed to be extended on the purification and chemical characterization of the bioactive products. This will enhance the implementation of natural products in aquaculture to act as potential agent for treating and controlling the fish borne diseases.

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