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In Vitro Screening Of Antibacterial Activity Of Green Seaweed (*Chetomorpha Linum*) Against Fish Bacterial Pathogens

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Acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous extract of green seaweeds Chetomorpha linum was screened for antibacterial activity by well diffusion methods against five fish bacterial pathogens Pseudomonas aeruginosa, Vibrio alginolyticus, Aeromonas hydrophila, Vibrio parahaemolyticus, Enterobacter aerogenes. In our observations the highest inhibition zone was recorded in methanol extract (28 mm) and ethyl acetate extract (20 mm) of C. linum against Pseudomonas aeruginosa. No activity was recorded in chloroform, distilled water and negative control against all tested pathogens. Results from this survey demonstrate that antimicrobial activities are prevalent among extracts from marine algae, suggesting that antimicrobial chemical defenses are widespread among marine plants. The present study provides enough data to show the potential of algae extract for development o anti-pathogenic agents for use in aquaculture.

Key words: Solvents, *Chetomorpha linum*, Bacterisl Pathogens.

Introduction

Seaweeds are living source of the marine environment. Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. Extracts from marine organisms have led to the discovery of a variety of secondary metabolites with antimicrobial activities against fish pathogen. However, these activities provides little indication of the extent to which these compounds serve the host organisms as defenses against harmful macro microorganisms (Engel *et al*., 2002 and 2006).

Most of the bioactive substances isolated from marine algae are chemically classified as brominated aromatics. Nitrogen- heterocyclic, nitrosulphuric- heterocyclic, sterols, dibutanoids, protein, peptides and sulphated polysaccharides. The secondary metobolites directly act as antibiotics in aquaculture. In aquaculture the bacterial pathogens create with fish diseases is a worldwide problem. Hence, the interest in macro algae as a potential and promising source of pharmaceutical agents has increased during the last years (Lindequist and Schweder, 2001; Mayer and Hamann, 2002; Newman *et al*., 2003; Bansemir *et al*., 2006; Engel *et al*., 2006; Kim *et al*., 2007; Choudhury *et al*., 2005; Osman *et al*., 2010; Tukmechi *et al*., 2010; Cox *et al*., 2010).

Some substances extracted from marine green algae have been shown to have many pharmacological activities (Awad, 1998 and 2000; Sukatar *et al*., 2006). *Chetomorpha linum* is especially green macroalgae found throughout the world in the upper intertidal zone of seashores. However, the distribution of such antimicrobial activity within algal thalli has not been studied. The present study investigated the antimicrobial activity against fish pathogens extracted from *Chetomorpha linum* from Vellar Estuary, Port Novo, South east of India.

Materials and methods

Sample collection

Seaweed *Chetomorpha linum* (Chlorophycea) (Fig.1.) was collected from vellar estuary (Lat 11° 29° N; Long 79° 49 °E). The fresh samples were washed with sea water and fresh water to remove salt, epiphytes, microorganisms and other suspended materials. The samples were shade dried until constant weighed

obtained and ground in an electric mixer. The powdered samples were then stored in refrigerator for future use.

Chemicals and Medias

Chemicals: Acetone, Methanol, Chloroform, Diethyl ether, Ethyl acetate, hexane.

Media compositions:

Composition of ZoBell marine broth (g/l)

Peptone……5.00
Yeast extract……1.00
Ferric citrate…….0.10
Sodium chloride……..19.45
Magnesium chloride……8.80
Sodium sulfate…….3.24
Calcium chloride……..1.80
Potassium chloride……0.55
Sodium bicarbonate……..0.16
Potassium bromide……0.08
Strontium chloride……0.034
Boric acid……..0.022
Sodium silicate…….0.004
Sodium fluoride……0.0024
Ammonium nitrate……..0.0016
Disodium phosphate……0.008
Final pH (at 25°C) 7.6±0.2

Composition of ZoBell Marine Agar (g/l)

Sodium Chloride……....19.40
Potassium Bromide……0.08
Magnesium Chloride…….8.80
Strontium Chloride……...0.034
Bacteriological Peptone…….5.00
Boric Acid……..0.022
Sodium Sulfate……..3.24
Disodium Phosphate…….0.008
Calcium Chloride……..1.80
Sodium Silicate…….0.004
Yeast Extract…….1.00
Sodium Fluoride……0.0024
Potassium Chloride……...0.55
Ammonium Nitrate……..0.0016
Sodium Bicarbonate…….0.16
Ferric Citrate…….0.10
Bacteriological Agar…….15.00
Final pH 7.6 ± 0.2 at 25ºC

Fig.1. *Chetomorpha linum*

Fish baterial pathogens	Solvents																				
	A			M			C			H			EA			DEE			Aq		
	50	P	N	50	P	N	50	P	N	50	P	N	50	P	N	50	P	N	50	P	N
Pseudomonas aeroginosa	12	30	-	28	30	-	-	30	-	10	30	-	20	30	-	12	30	-	-	30	-
Vibrio alginolyticus	10	30	-	24	30	-	-	30	-	8	30	-	14	30	-	12	30	-	-	30	-
Aeromonas hydrophila	-	12	-	-	12	-	-	12	-	-	12	-	14	12	-	-	12	-	-	12	-

Preparation of extract

The extraction was carried out with different solvents in the increasing order of polarity. Namely Acetone, Methanol, Chloroform, Diethyl ether, Ethyl acetate, hexane and aqueous (1: 4 w/v), and kept for two weeks at room temperature and the extracts were collected and concentrated. The concentrates were reconstituted with their respective extracts (5 mg mL^{-1}).

Antibacterial assay

The antibacterial activity was valuated using well- cut diffusion technique (el-masry *et al*., 2000). In vitro antibacterial studies were carried out against five fish pathogens *Pseudomonas aeruginosa, Vibrio alginolyticus, Aeromonas hydrophila, Vibrio parahaemolyticus, Enterobacter aerogenes*. Wells were punched out using a sterile (7mm) cork borer in zoobal marine agar media plates inoculated with the test microorganisms. Seaweed extract transferred into each well on (50 µl). For each microorganism controls were maintained where pure solvents for negative control and chlorofenical for positive control. The plates were later incubated at 37°C for 24 h. The inhibition zones were measured excepting the (-mm) well. Every zone of inhibition was recorded in millimeters. Experiment was carried out five times. Inhibition zones > 15mm moderate and from 1 to 5 mm as weak activities.

Results

The antimicrobial activity of seaweed (*Chetomorpha linum*) using seven different solvents (Acetone, Methanol, Chloroform, Diethyl ether, Ethyl acetate, hexane and water) were tested against 5 fish pathogens viz., *Pseudomonas aeruginosa, Vibrio alginolyticus, Aeromonas hydrophila, Vibrio parahaemolyticus, Enterobacter aerogenes* were presented in Table.1.

Table.1.Antibacterial activities of green seaweed C*hetomorpha linum* using well method in µl (Results in diameter)

Vibrio parahaemolyticus	-	10	-	8	10	-	-	10	-	-	10	-	10	10	-	-	10	-	-	10	-
Enterobacter aerogenes	-	30	-	12	30	-	-	30	-	-	30	-	20	30	-	14	30	-	-	30	-

A-Acetone; **M**-Methanol; **C**-Chloroform; **H**-Hexane; **EA**-Ethyl acetate; **DEE**-Diethyl ether; **Aq**- Aqueous
P- Positive control (Chlorophenical-20 µl); **N**- Negative control (Solvents- 20 µl)

In our observations the highest inhibition zone was recorded in methanol extract (28 mm) and ethyl acetate extract (20 mm) of *C. linum a*gainst *Pseudomonas aeruginosa*. No activity was recorded in chloroform, distilled water and negative control against all tested pathogens and acetone extract *Aeromonas hydrophila, Vibrio parahaemolyticus, Enterobacter aerogenes;* methanol extract *Aeromonas hydrophila;* hexane extract *Aeromonas hydrophila, Vibrio parahaemolyticus, Enterobacter aerogenes* and diethyl ether *Aeromonas hydrophila, Vibrio parahaemolyticus* Maximum inhibition zone (<10 mm) was recorded in Acetone extract against *Pseudomonas aeruginosa*; Methanol extract against *Pseudomonas aeruginosa, Vibrio alginolyticus,* and *Enterobacter aerogenes*; Ethyle acetate extract of all tested pathogens except *Vibrio parahaemolyticus*; Diethyl ether extract against *Pseudomonas aeruginosa, Vibrio alginolyticus* and *Enterobacter aerogenes.* Minimum activity (>5mm) was recorded in Acetone extract against *Vibrio alginolyticus*; Methanol extract against *Vibrio parahaemolyticus* and *Aeromonas hydrophila*; Hexane extract against *Pseudomonas aeruginosa* and *Vibrio alginolyticus*; Ethyl acetate extract against *Vibrio parahaemolyticus.*

Discussion

Bansemir *et al*., 2006 was reported dichloromethane extract of chlorophyceae members *Codium taylorii, Ulva rigida* and *Valonia utricularis* no activity was recorded against *Aeromonas hydrophila*. Contrast our work ethyle acetate extract of chlorophyceae member of *C. linum* shows high activity (14mm) against *Aeromonas hydrophila*. Choudhury *et al*., 2005 reported that methanol extract of *Enteromorpha compressa* and *Ulva fasciata* shows no activity against *Enterobacter aerogenes., Vibrio alginolytics, Aeromonas hydrophila*. Contrast of our work ethyle acetate extract *of C. linum* shows high activity (14mm) against *Aeromonas hydrophila* and methanol extract of *C. linum* show high activity against, *Vibrio alginolytics* (24 mm) and *Pseudomonas aroginosa* (28 mm). Kolanginathan *et al*., 2009 reported that ethanol extract of *Caulerpha peltada* no activity was recorded against *Enterobacter aerogenes* and moderate activity was recorded against *Enterobacter aerogenes*. Contrast of our work ethyle acetate extract of *C. linum* shows high activity (20mm) against *Enterobacter aerogenes* and methanol extract of *C. linum* against *Pseudomonas aroginosa* (28 mm). Kim *et al*., 2007 find out ethyl ether extract of *Ulva lactuca* shows high activity against *Vibrio parahaemolyticus*. Similarly our work ethyl acetate extract of *C. linum* shows high activity against *Vibrio parahaemolyticus* (10 mm).

Conclusion

Overall, the present study provides enough data to show the potential of algae extract for development o anti-pathogenic agents for use in aquaculture. In nature environment establish growth of *Chetomorpha linum* provide food for fish and also act as antibiotic natural drugs for wiled fishes.

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