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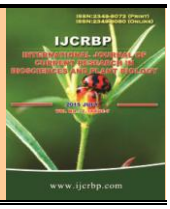

Original Research Article

Isolation, Characterization and Application of Fucoidan from Weeds

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Abstract

Aquatic weeds, *Egeria densa, Analipus japonicas, Chaetomorpha antennina, Enteromorpha* sp., and a common aquatic weed, *Eichhornia crassipes* were collected from Munabam beach Coast of Arabian Sea, air dried and ground to powder form. The powdered sample was soaked in Methaonol:Chloroform mixture and extracted by 75% Ethanol. The partially purified fucoidan content was separated by DEAE cellulose ion exchange chromatography which was confirmed by Thin Layer Chromatography (TLC) and Fourier Transform/Infra Red Spectroscopy (FTIR) technique. The weeds were tested for their Antibacterial activity against seven pathogenic bacteria such as *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Enterococcus sp., Proteus vulgaris, Staphylococcus aureus* and *Bacillus sp.* in Muller Hinton plates. Industrially and economically important enzymes produced by the weeds were also determined. These weeds were also screened for their phytochemical activity. On the whole, the weeds are found to have antibacterial and anticoagulant activity.

Keywords

Anti-bacterial activity

Anticoagulant

Aquatic weeds

Fucoidon

Introduction

Aquatic weeds are considered as source of bioactive compounds and produce a great variety of secondary metabolites. They are characterized by a broad spectrum of biological activities. Although, a little has been done to define an ecological role for these compounds they may, therefore, possess chemical defenses to prevent the colonization of their surface. The uses of marine natural products are able to inhibit bacteria development offer a rich pharmacological potential. Numerous reports show that macro algae present a broad range of biological activities such as antibacterial, antifungal, antiviral and anti-inflammatory effect. Compounds with cytostatic, antiviral, antihelminthic, and antifungal activities have been detected in green, brown and red algae and other aquatic weeds. Marine bacteria often produce anticancer and antibacterial substances as a means of maintaining relationships between epiphytic micro environments, inhibiting competing organisms and microbial pathogens (Avendano-Herrera et al., 2005). Many of these secondary metabolites are halogenated. The present study was carried out to find out the presence of fucoidon in selected weedy plant species and its antibacterial and anticoagulant activity.

Materials and methods

Sample collection

The weeds were collected by hand picking during the period of November–December 2014 from Munabam

beach in Kerala. The samples were cleaned of epiphytes and necrotic parts. Then the cleaned samples were rinsed with sterile distilled water to remove any associated debris. The cleaned fresh materials were air-dried as described by Gonzalez Del Val et al. (2001). The aquatic weedy plants were identified as *Egeria densa* Planch. (Seaweed, W1), *Analipus japonicus* (Harvey) M.J.Wynne, 1971 (Aquatic weed, W2), *Chaetomorpha antennina* (Bory de Saint-Vincent) Kützing, 1847 (Seaweed, W3), *Eichhornia crassipes* (Mart.) Solms (Aquatic weed, W4) and *Enteromorpha* sp. (Seaweed, W5).

Extraction of the weeds

The samples were shade dried for 15 days and then pulverized into fine powder using mortar and pestle. Different solvents were used successively with gradient polarity (chloroform, methanol and ethanol) for extraction. The extracts were evaporated to complete dryness by vacuum distillation and stored in refrigerator for further use (Akinyemi et al., 2000; Mohanta et al., 2007; Patra et al., 2008). Extracts using chloroform, methanol and ethanol solvents were prepared from each weed powder and used for the analysis.

Phytochemical analysis

The phytochemical analyses of the weeds (W1, W2, W3, W4 and W5) were performed for the presence different phytochemicals like carbohydrates, tannins saponins, flavanoids, alkaloids, quinines, glycosides, terpenoids, phenols and coumarins.

Antimicrobial assay

The powder of weeds was ground with methanol using mortar and pestle. The ground material was then filtered using Whatman filter paper. The crude extract of each weed sample was aseptically added to the wells using micropipette to sterile Muller Hinton Agar plates. Then the plates were kept for 48 h incubation. The zone of inhibition was observed and measured. Antibacterial assay was carried against the bacterial species, *Escherichia coli, Enterobacter aerogenes, Pseudomonas aeruginosa, Enterococcus sp., Proteus vulgaris, Staphylococcus aureus* and *Bacillus sp.*

Extraction and purification of fucoidan

Moist samples were air dried at 37°C for 2 weeks and then grinded to powder form. The powder was soaked in Methanol-chloroform mixture (1:1 v/v) for 24 h and then filtered to get defatted sample. The extraction procedure was carried out with 85% ethanol (w/v) at 70°C to get partially purified fucoidan.

Ion-exchange chromatography

The sample of fucoidan from the weeds W1, W2, W3, W4 and W5 (30 mg) were dissolved in 0.05 M sodium acetate buffer and then fractionated by ion-exchange chromatography on a DEAE-cellulose column (16.5 × 1.3 cm), previously equilibrated and washed with the same buffer, followed by separation of SP fractions, also using the same buffer containing NaCl at different concentrations (0.50, 0.75 and 1.00 M). Fucoidan fractions (5 mL) from each sample were collected.

Thin layer chromatography (TLC)

TLC was performed on a silica gel aluminum plate (silica gel 60 F254) to fractionate active compound of the extracts. The chloroform: methanol (10:1 v/v) was mobile face. The TLC plate was placed in a tightly packed jar containing iodine crystals to observe the spots. The separated spots were marked and the R*f* value was calculated.

Anti-thromboplastic activity

Six sterile glass slides (C, W1, W2, W3, W4 and W5) were taken. Then a drop of blood was added to all six slides. C was labeled as control. Each plant extract of 5µl was added to the slides W1, W2, W3, W4 and W5 respectively. The clotting time was noted carefully and tabulated.

Characterization of fucoidan by FTIR spectroscopy

The qualitative investigation of the purified fucoidan was done by FTIR spectroscopy (Shimadzu, Japan) described by Kemp (1991). The spectra were recorded between 4000 and 400 cm^{-1} wave number and the trembling was recorded as a graphic representation.

Enzyme production from weeds

The plant samples were checked for the presence of amylase, lipase, cellulase and urease using standard procedures.

Results and discussion

Phytochemical analysis of weeds

Qualitative analysis of extract of the weeds, *Egeria densa, Analipus japonica, Chaetomorpha antennina, Eichhornia crassipes* and *Enteromorpha* sp. are shown in Table 1. Methanol extraction of each sample showed more phytochemical activity than other extractions. These phytochemical analysis in the weeds *Egeria densa, Analipus japonica, Chaetomorpha antennina, Eichhornia crassipes* and *Enteromorpha* sp. showed the presence of flavanoids, carbohydrate, alkaloids, phenols, quinines and saponins were obtained from methanol extraction. The same kind of results has been observed in ethanol, aqueous and methanol extraction of *Chaetomorpha antennina* by Subathra et al. (2013).

Table 1. Phytochemical analysis of the extracts of weeds.

TEST	Extract tested														
	W 1			W2			W3			W4			W5		
	Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform	Ethanol	Methanol	Chloroform
Carbohydrate	-	-	-	+	-	-	-	+	+	-	+	-	+	+	-
Tannins	-	-	+	-	+	+	+	-	-	+	-	-	-	-	-
Saponins	-	-	+	-	+	+	+	-	+	-	-	-	-	+	-
Flavanoids	+	+	-	+	-	-	-	+	-	+	+	+	+	+	-
Alkaloids	-	+	-	+	+	-	-	+	-	-	+	-	-	-	+
Quinines	+	+	+	-	-	-	+	-	-	-	-	-	+	-	-
Glycosides	-	+	+	+	-	-	-	+	+	-	+	-	+	+	+
Cardiac glycosides	+	-	-	-	+	+	+	+	-	-	-	+	-	+	-
Terpanoids	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-
Phenols	-	+	+	+	-	+	-	+	-	-	+	-	-	-	-

W1: *Egeria densa*; W2: *Analipus japonicus*; W3: *Chaetomorpha antennina*; W4: *Eichhornia crassipes*; W5: *Entomorpha* sp.

Table 2. Antimicrobial activity of the methanolic extract of weeds.

Test bacteria	Zone of inhibition (mm)				
	W1	W2	W3	W4	W5
Escherichia coli	25	16	10	14	9
Enterobacter aerogenes	Nil	14	12	21	6
Pseudomonas aeruginosa	14	20	22	34	15
Enterococcus sp.	24	27	18	22	26
Proteus vulgaris	Nil	9	nil	nil	11
Staphylococcus aureus	12	Nil	15	Nil	9
Bacillus sp.	13	12	16	24	Nil

W1: *Egeria densa*; W2: *Analipus japonicus*; W3: *Chaetomorpha antennina*; W4: *Eichhornia crassipes*; W5: *Entomorpha* sp.

Table 3. R_f values of weed extracts in TLC.

Sl.no	Extract	R_F value
1	*Egeria densa*	0.64
2	*Analipus japonicus*	0.50
3	*Chaetomorpha antennina*	0.54
4	*Eichhornia crassipes*	0.56
5	*Entomorpha* sp.	0.52

Anti bacterial activity of weeds against pathogenic bacteria

The antibacterial activity of methanol extract of five weeds against seven strains of bacteria is presented in Table 2. The zone of inhibition ranged from 10-35mm. The maximum activity of 34mm was recorded in the ethanolic extract of *Eichhornia crassipes* against *Pseudomonas aeruginosa*. The results of the present

study are in line with the study conducted by Vallinayagam et al. (2009). The phytochemical analysis *Eichhornia crassipes* showed high levels of phenol.

Ion exchange chromatography

Methanol: chloroform extraction of weeds was subjected to ion exchange chromatography on the DEAE cellulose column procedure. The DEAE cellulose was the anion exchanger which separate positively charged molecules. Different fractions were obtained and were proved by TLC.

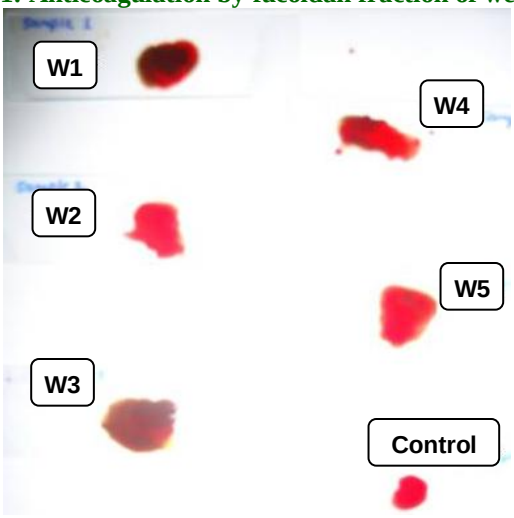
Thin layer chromatography

The bands obtained showed the R_f value similar to the standard R_f value (0.51) of fucoidan. Fucoidan is a sulphated polysaccharide has anticoagulant activity which when combines with iodine vapour to produce blue color bands. The R_f value of the obtained bands were similar to the standard fucoidan (Nuri Muahiddah, 2010).

Antithrombotic activity

Extracted fucoidan fraction showed maximum anticoagulant activity. Control was coagulated within 10 minutes. The fraction which showed maximum anticoagulant activity was *Egeria densa* because of the high amount of fucoidan (Shanthi et al., 2014).

Fig. 1: Anticoagulation by fucoidan fraction of weeds.



Characterization of fucoidan by FTIR spectroscopy

Functional groups of fractions can be analyzed using FTIR spectroscopy. The FTIR spectroscopy showed that the fucoidan bands were formed in the region of wave number between 1500 and 1000. The purified galactose rich sulphated fucoidan fraction exhibiting anti-coagulation activity was characterized by FTIR spectra. The maximum peak of fucoidan content in *Egeria densa* was seen in the region 1646.507 – 1552.797, in *Analipus japonicas* 1629.832 – 1245.847, in *Chaetomorpha* 1631.092 – 1552,694, in *Eichhornia crassipes* 1263.145 – 1626.013 and *Enteromorpha* 1238.382- 1635.502, as compared with the study on characterization of galactose rich fucoidan with anticoagulation potential isolated from *Turbinaria decurrens* Bory de Saint-Vincent occurring along the coast of Gulf of Mannar (Pamban), India (Shanthi et al., 2014).

Table 4. Antithrombolytic activity of fucoidan fraction from weed extracts.

Sl. No.	Blood (µg)	Extract	Clotting time (in minutes)
1	5	*Egeria densa*	27
2	5	*Analipus japonicas*	20
3	5	*Chaetomorpha antennina*	25
4	5	*Eichhornia crassipes*	20
5	5	*Enteromorpha* sp.	25

Fig. 2: FTIR result obtained for *Egeria densa.*

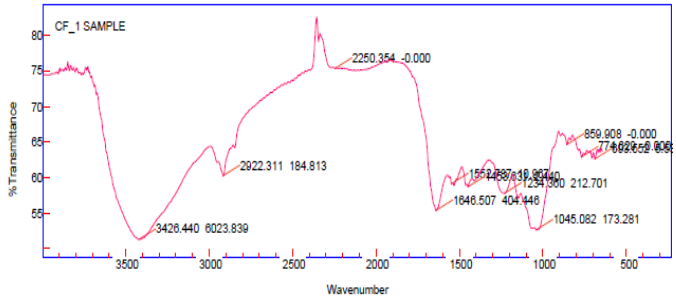
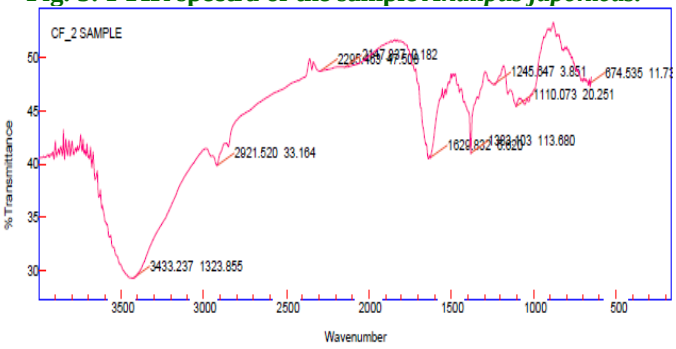


Fig. 3: FTIR spectra of the sample *Analipus japonicas.*



Enzyme production

Amylase is an industrially important enzyme was produced after 2 days of incubation, iodine solution was

added in the samples and all the plates showed negative result except *Egeria densa*. It shows that amylase is least metabolic important enzyme in the tested plant materials. After 2 h of incubation due to hydrolysis of tween 80 free acids is released and pH decreases and the color change was observed. *Egeria densa*, *Analipus japonicas*, *Chaetomorpha antennina* and *Enteromorpha* sp. showed positive results for lipase. Cellulose is a compound which is mainly present in plants. Cellulase enzyme degrades cellulose. Cellulase is an industrially important enzyme produced by different plants. All the samples showed positive result for cellulase. After 2 days of incubation, all the test samples were urease positive.

Fig. 4: FTIR spectra of sample *Chaetomorpha antennina.*

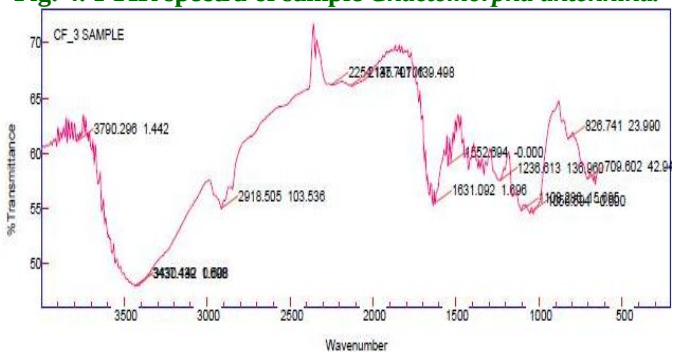


Fig. 5: FTIR spectra of sample *Eichhornia crassipes.*

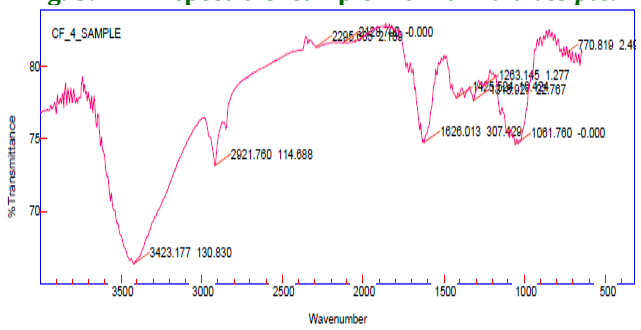
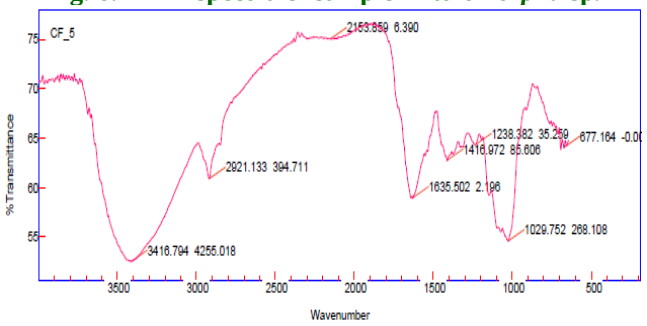


Fig. 6: FTIR spectra of sample *Enteromorpha* sp.



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