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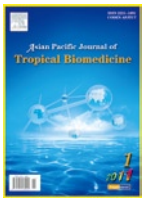

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Antimicrobial and hemolytic activity of seaweed extracts *Ulva fasciata* (Delile 1813) from Mandapam, Southeast coast of India

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ABSTRACT

Objective: To evaluate the *In vitro* antimicrobial and hemolytic activity of marine macroalgae *Ulva fasciata* (*U. fasciata*) collected from Mandapam coastal waters, and to identify certain seaweed extracts that can act as an alternative of commonly used antibiotics. **Methods:** Seaweeds *U. fasciata* was collected from Gulf of Mannar, Southeast Coastal Region, Mandapam, Tamil Nadu and was screened for antimicrobial and hemolytic activity. Methanol, butanol and aqueous extracts were tested against selected fish pathogens, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Proteus* sp. *Vibrio alginolyticus* (*V. alginolyticus*) and *Enterobacter* sp. and fungal pathogens *Rhizopus* sp., *Aspergillus flavus*, *Aspergillus* sp., *Aspergillus niger* and *Candida* sp. The extract was subjected to TLC to determine the presences of peptides and amide groups. And the hemolytic activity was assayed. **Results:** Maximum of 16 mm inhibition zone was observed against *V. alginolyticus* and the minimum 12 mm against *Enterobacter* sp., respectively. *U. fasciata* showed poor activity against the fungal pathogens. The present results showed the use of seaweeds as an antimicrobial agents for pharmacology or as a health-promoting food for aquaculture. **Conclusions:** The screening result confirms that these seaweeds can be further studied and used as possible source of antimicrobial compounds.

1. Introduction

Antibiotic treatment of bacterial diseases in fish culture has been applied for many years. The occurrence of antibiotic resistant bacteria associated with fish diseases is a worldwide problem in aquaculture, which has received considerable attention in the last years and continues to increase due to the absence of more effective and safer use of antibiotics. The prevention and treatment of these infectious diseases by applying products from marine organisms appears to be a possible alternative resource. Hence, the interests in marine organisms as a potential and promising source of pharmaceutical agents has increased during the last years[1-3]. There are numerous reports concerning the inhibiting activities of macroalgae against human pathogens, fungi and yeasts, but only few show data about effects against fish pathogens[4-7]. Therefore, the aim of the present study was to investigate the antimicrobial activity of extracts of marine algae *Ulva fasciata* (*U. fasciata*) against five fish pathogenic bacteria that are often the cause of bacterial diseases in aquaculture. The possible use of active seaweeds for prevention or treatment of the bacterial fish diseases should be discussed.

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2. Materials and methods

2.1. Collection of the algal material

The seaweeds *U. fasciata* was collected in bulk quantity from Mandapam coastal area, east coast of India. Seaweeds exposed on sand and rocks were collected in plastic bags and brought to the laboratory. Seaweed was washed thoroughly with running water to remove epiphytes, animal castings, attached debris and sand particles. The final washings were done using distilled water and then they were dried under shade.

2.2. Chemical extraction

The chemical extraction was done by following the method of Sreenivasa-Rao and Parekh (1981). 5 g of dried plant powder was extracted in soxhlet apparatus using methanol, butanol and aqueous (200 mL) as solvents for 8 h at 65 ℃. The

resulting extracts were concentrated to dryness in a rotary evaporator under reduced pressure and were stored at 4 ℃ until use.

2.3. Antibacterial assay

Antibacterial activity was determined against five fish pathogens *Aeromonas hydrophila* (*A. hydrophila*), *Pseudomonas fluorescens* (*P. fluorescens*), *Proteus* sp., *Vibrio alginolyticus* (*V. alginolyticus*), *Enterobacter* sp., using the paper disc assay method of El-Masry *et al*[8].

2.4. Antifungal assay

Antifungal activity was determined against five fish pathogens *Aspergillus flavus*, *Candida* sp., *Aspergillus niger*, *Rhizopus* and *Aspergillus* sp., using the paper disc assay method described by El-Masry *et al*[8].

2.5. Thin layer chromatography (TLC)

The soluble fractions showed ninhydrin positive spots. They were subjected to purification by gel permeation chromatography Sephadex LH 20 using methanol as eluent and monitored by TLC. Diagnostic TLC was performed on methanol and chloroform extracts. They were also spotted and plates developed in varying proportions. Specific color reagent ninhydrin was used for detecting the compounds.

2.6. Determination of hemolytic assay and protein

The crude extracts of 11 fractions were assayed for their hemolytic activities on chicken and goat blood. Chicken and goat bloods were obtained from a nearby slaughterhouse, using 2.7% ethylenediaminetetraacetic acid (EDTA) solution as an anticoagulant at 5% of the blood volume, and was brought to the laboratory. The blood was centrifuged thrice at 5000 rpm for five minutes; 1% erythrocyte suspension was prepared by adding 99 mL normal saline to 1 mL of packed erythrocytes. The micro hemolytic assay was performed in 96-well 'V' bottom microtitre plates. Serial two-fold dilutions of the crude mucus were made in 100 μL of normal saline. Then 100 μL of 1% erythrocyte was added to all wells. For positive control, 100 μL of distilled water and for negative control 100 μL of normal saline were added respectively to the 1% red blood cell (RBC) suspension. The plate was gently shaken and allowed to stand for two hours at room temperature. The presence of uniform red color suspension in the wells was considered to be positive hemolysis and a button formation in the bottom of the wells showed a lack of hemolysis. The reciprocal of the highest dilution of the crude toxin showing hemolytic pattern (hemolytic unit) was divided according to the protein content to obtain the specific hemolytic unit and the protein was estimated by the method of Lowry *et al*[9,10].

3. Results

3.1. Antimicrobial assay

The antibacterial activity of extracts of the *U. fasciata* was presented in the Table 1. The extracts of the *U. fasciata* shows a strong inhibition in the growth of tested bacteria. The maximum zone of inhibition was observed against *V. alginolyticus* and minimum in *Enterobacter* sp. The extracts were ineffective against the other bacteria and the fungal strains. The comparative antibacterial effect of the extracts of *U. fasciata* by using standard drug tetracycline is also shown in Table 1.

Table 1
Antimicrobial activity of *U. fasciata* against fish pathogens.

Name of the fish pathogen	Activity of control	Zone of inhibition (mm)		
		Methanol	Butanol	Aqueous
V. alginolyticus	+Ve	16	11	–
Proteus sp.	+Ve	–	–	–
A. hydrophila	+Ve	–	9	–
P. fluorescens	+Ve	–	–	–
Enterobacter sp.	-Ve	12	–	–
Rhizopus	+Ve	–	–	–
Aspergillus sp.	+Ve	–	–	–
A. flavus	+Ve	–	–	–
A. niger	-Ve	–	–	–
Candida sp.	+Ve	–	–	–

3.2. Thin layer chromatography

Thin layer chromatography profiling was done for the samples of nerves tissue and mucus extract in solvent system of butanol, acetic acid and water (BAW) in proportions of 5:1:4. The plates when developed in both the solvent systems showed light pink spots when the TLC plate is sprayed with ninhydrin. The plate with fractions developed in BAW as the solvent system and was sprayed with ninhydrin, showing pink spots indicating the presence of amino acids and peptides and the result is shown in Figure 1.

Figure 1. Thin layer chromatography.
M – Methanol extract; B – Butanol extract; Aq – Aqueous extract.

3.3. Protein estimation

The amount of protein present in the extract of *U. fasciata* was 11.6%.

3.4. Haemolytic assay

The crude extract as well as the fractions produced pronounced hemolytic activity on chicken and goat blood. Hemolytic factors were present in the crude sample as well

as in all the fractions, but differed considerably depending on the type of blood used. Chicken blood, was the most vulnerable to lysis provoked by the *U. fasciata*. The crude extract of chicken blood showed maximum of 64 HU/mg for *U. fasciata* and the goat blood showed maximum of 32 HU/mg (Figure 2).

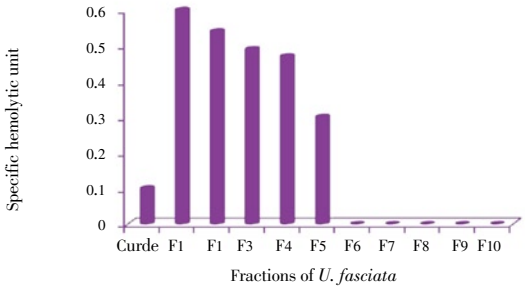

Figure 2. Hemolytic activity of *U. fasciata* extract.

4. Discussion

Seaweeds are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterised by a broad spectrum of biological activities. Compounds with cytostatic, antiviral, anthelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae[1,3]. Shanmughapriya *et al*[11] has reported fourteen seaweeds collected from the intertidal zone of Southwest coast of India with antibacteria and anitfungus activities against ten human pathogen bacteria and one human pathogen fungus using the well diffusion test in the casitone agar medium. Several solvents were used for the extraction of freeze-dried seaweed powder. The present data revealed that considerable inhibition zones were observed from the dichloromethane extracts. Kolanjinathan *et al*[12] has reported the antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. Furthermore, Selvin and Lipton[13] reported that the green alga *U. fasciata* exhibited broad spectrum antibacterial activity whereas the red alga *Hypnea musicformis* showed narrow spectrum antibacterial activity. In contrast, Lima-Filho *et al*[14] reported that *U. fasciata* has not any antimicrobial activity against tested organisms. However, the results obtained by the aforementioned authors suggest the production of antimicrobial substances by the same species varies remarkable differences may be due to several factors.

The crude extract as well as the fractions produced pronounced hemolytic activity on chicken and goat erythrocytes. Hemolytic factors were present in the crude sample as well as in all the fractions, but differed considerably depending on the type of blood used. Gerasimenko *et al*[15] has studied the antimicrobial and hemolytic activity of low molecular metabolites of brown seaweed *Laminaria cichorioides*. The results clearly show that seaweeds are an interesting source for biologically active compounds that may be applied for prophylaxis and therapy of bacterial fish diseases additionally or instead of commercial antibiotics. It might be an alternative approach to use the extracts, fractions or purified compounds from algae as drugs or fish feed components. However, further investigations regarding toxicity, stability and metabolism of seaweeds and seaweed components must be carried out.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Lindequist U, Schweder T. Marine biotechnology. In: Rehm HJ, Reed G, editors. *Biotechnology*. Weinheim: Wiley-VCH; 2001, p. 441-484.
[2] Mayer AMS, Hamann MT. Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanism of action. *Comp Biochem Physiol C Toxicol Pharmacol* 2002; **132**: 315-339.
[3] Newman DJ, Cragg GM, Snader KM. Natural products as source of new drugs over the period 1981-2002. *J Nat Prod* 2003; **66**: 1022-1037.
[4] Sridhar KR, Vidyavathi N. Antimicrobial activity of seaweeds. *Acta Hydrochim Hydrobiol* 1991; **5**: 455-496.
[5] Mahasneh I, Jamal M, Kashasneh M, Zibdeh M. Antibiotic activity of marine algae against multi-antibiotic resistant bacteria. *Microbios* 1995; **83**: 23-26.
[6] de Val AG, Platas G, Basílio A, Cabello A, Gorrochategui J, Suay I, et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001; **4**: 35-40.
[7] Liao WR, Lin JY, Shieh WY, Jeng WL. Antibiotic activity of lectins from marine algae against marine vibrios. *J Ind Microbiol Biotech* 2003; **30**: 433-439.
[8] El-Masry HA, Fahmy HH, Abdelwahed ASH. Synthesis and antimicrobial activity of some new benzimidazole derivatives. *Molecules* 2000; **5**: 1429-1438.
[9] Kanagarajan U, Bragadeeswaran S, Venkateshvaran K. On some toxinological aspects of the starfish *Stellaster equestris* (Retzius, 1805). *J Venom Anim Toxins Incl Trop Dis* 2008; **14**: 435-449.
[10] Lowry OH, Rosebrogh NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275.
[11] Shanmughapriya S, Manilal A, Sujith S, Selvin J, Segal-Kiran G, Natarajaseenivasan K. Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Ann Microbiol* 2008; **58**(3): 535-541.
[12] Kolanjinathan K, Ganesh P, Govindarajan M. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. *Eur Rev Med Pharmacol Sci* 2009; **13**: 173-177.
[13] Selvin J, Lipton AP. Biopotentials of *Ulva fasciata* and *Hypnea musciformis* collected from the peninsular coast of Indian. *J Mar Sci Technol* 2004; **12**(1): 1-6.
[14] Lima-Filho JVM, Carvalho AFFU, Freitas SM. Antibacterial activity of extracts of six macroalgae from the northeastern Brazilian Coast. *Braz J Microbiol* 2002; **33**: 311-313.
[15] Gerasimenko NI, Chaykina EL, Busarova NG, Anisimov MM. Antimicrobic and hemolytic activity of low molecular metabolites of brown seaweed *Laminaria cichorioides* (Miyabe). *Appl Biochem Microbiol* 2010; **46**(4): 426-430.