In Vitro Antibacterial Activity of Dictyota dichotoma (Hudson) Lamouroux

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Abstract

Seaweeds are regarded as a prospective source of bioactive compound. In the present study, the algae *Dictyota dichotoma* have been extracted using hexane, chloroform and ethanol and were tested for antibacterial activity against the standard culture of gram positive and gram negative bacterial strains. The results of the present study showed a proficient activity of the algae against all the bacterial strains under hexane extract. Thus the test report implied *Dictyota dichotoma* to possess good antimicrobial activity.

Key Words: Seaweeds, *Dictyota dichotoma*, Antibacterial activity, Inhibition Zone.

Introduction

In many Asian countries, marine algae have been used as food and medicine. Marine algae possess abundant biological activities which indeed lead in the discovery of new pharmaceutical agents and novel bioactive compounds. This brought a high demand in the national products of marine algae (Bluden, 2001; Iwamoto *et al.*, 2001). The marine seaweed *Dictyota* belongs to Kingdom : Chromista, Subkingdom : Harosa, Infrakingdom : Heterokonda, Phylum : Ochrophyta, Subphylum : Phaeista, Infraphylum : Limnista, Superclass : Fucistia, Class : Phaeophyceae, Order : Dictyotales, Family : Dictyotaceae (Guiry and Guiry, 2011).

A wide defensive action against the herbivores in the marine environment was expressed by the dictyotale species due to the production of large quantity of bioactive secondary metabolites (De Paula *et al.*, 2001). High cholesterol level, some kind of inflammatory disorders, breast cancer was thought to be ameliorated by the consumption of marine algae (Fitton and Helen, 2003). As greater variety of secondary metabolites were produced by the marine macroalgae, it has become a very promising alternative source of bioactive compounds and which inturn characterize the important factors of biological behaviours such as antifungal, antibacterial and antiviral properties (Marinho-Sorianto *et al.*, 2006).

Materials and Methods

Collection of Seaweed Samples

A large and well sufficient quantity of marine macroalgae was collected from Mandapam, Gulf of Mannar. Using distilled water, the samples were then washed thoroughly to remove excess salts and epiphytes. It is then packed in sterilized polyethylene bags and was transferred to the laboratory in an icebox for the experimental work. Again with sterile distilled water, the samples in the laboratory were washed, air dried and

oven dried at 45°C to 50°C which were then cut in to small pieces and then grounded well to a fine powder using tissue grinder. The identification of the sample was done according to Aleem (1978) and Guiry (2011).

Preparation of Solvent Extracts

In a soxhlet apparatus, 5g measure of the shade dried algae sample was placed and extract was obtained using hexane, chloroform and ethanolic solvents. The concentration of the entire part of the algal extracts was varied to examine bioassay studies.

Antibacterial Assay

The well diffusion method is used to determine the antibacterial activity (Perez *et al.,* 1990). The various solvent extracts were dissolved in the DMSO to a final concentration of 100 mg/ml. In the nutrient broth with the incubation period of 8h at 37°C, suspension of each bacterial strain was done. These cultured bacterial strains were then seeded in the Nutrient Agar (NA) plates for 8mm diameter, wells were cut in each of these plates using sterile cork borer. The different concentrations (500, 1000, 1500 and 2000 µg/ml) of the plant extracts were added carefully in to the wells using sterilized dropping pipettes and being allowed to diffuse at room temperature for 2h. The plates were then incubated at 37°C for 18-24h.

The test was carried out by Triplicate method. The positive and negative control used was Gentamicin (10µg/disc) and the solvent DMSO respectively. Through the measurement of the diameter of the inhibition zone, antimicrobial activity was evaluated.

Microorganism Used

Antibacterial activity was examined against the standard culture of Gram positive and Gram negative bacterial strains. . Gram positive strains such as methicillin resistant Staphylococcus aureus (MTCC 3381), Bacillus cereus (MTCC 430), Micrococcus luteus (MTCC 2470). Gram negative strains such as Escherichia coli (MTCC 739), Aeromonas hydrophila (MTCC 1739). These bacteria were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Results and Discussion

The algae taken for the present study were examined against some Gram positive and Gram negative bacterial strains under three different extracts. The observation of the result exhibited a high zone of inhibition against *Staphylococcus aureus* under hexane extract. Fresh and dried Sample of *U . rigida* were extracted by Tuney *et al,.* (2007). It was reported the fresh material extraction had shown efficient inhibitory activity against the bacteria *S. Aureus* whereas the dried sample shown nil activity against the same strain. The bacterial strain, *Bacillus cereus* was observed to be inhibited under all extracts. The algal extract of hexane and chloroform had shown almost similar level of inhibition against the bacteria *Micrococcus luteus*. Antibacterial activity with pure sterol compounds were examined against adhesive Gram-positive bacteria (*B. subtilis*) and Gram-negative bacteria (*V. alginolyticus, V. parahaemolyticus, V. mimicus,* and *P. aeruginosa*). Still a very close and deep zone of inhibition could be reached with hexane extract. As like in the case of the measure of inhibition level against the bacteria *Escherichia coli* but a greater state of inhibition was observed with ethanol extract at 2000 µg concentration. Also a good zone of inhibition were reported against *E. faecalis* and *E. Coli* (Tuney *et al,.* 2007). Against *Aeromonas hydrophila*, almost all the three extracts showed a similar range of inhibition. The gram negative bacterial strains have been selected because of its varied characteristics

prevailing in the marine environment (Iyyapparaj *et al.*, 2012). Das *et al.*, 2006 reported as the cell wall of the negative bacterial strain is being highly adapted for the greater salinity environment. In which, hexane and chloroform extracts showed a dominant role at its peak concentration. The overall view of zone of inhibition stood good against all the bacterial strains under hexane extract. Hence the power of algae *Dictyota dichotoma* could be well expressed under hexane extract.

Table 1: Antibacterial activity of *Dictyota dichotoma* crude extract against the tested pathogens

S.No	Test samples	Con (µg)	Zone of inhibition diameter(mm)				
			S.aureus	***B.cereus***	***M.luteus***	***E.coli***	***A.hydrophila***
1	Hexane	500	–	8.5±0.1	12.0±0.0	8.5±0.1	–
		1000	10.0±0.0	9.0±0.2	12.0±0.0	–	10.0±0.0
		1500	10.5±0.7	10.0±0.0	13.0±0.0	10.5±0.7	10.0±0.0
		2000	11.0±0.0	10.5±0.7	13.0±0.0	10.0±1.4	10.5±0.7
2	Chloroform	500	–	10.0±0.0	13.0±0.0	10.0±0.0	–
		1000	–	10.0±0.0	11.5±0.7	10.5±0.7	10.0±0.0
		1500	–	10.0±0.0	10.0±0.0	10.5±0.7	10.0±0.0
		2000	–	10.0±0.0	10.0±0.0	10.5±0.7	11.0±0.0
3	Ethanol	500	–	10.0±0.0	10.5±0.7	–	–
		1000	–	10.0±0.0	10.5±0.7	–	–
		1500	–	10.0±0.0	10.0±0.0	10.0±0.0	10.0±0.0
		2000	–	10.0±0.0	10.5±0.7	11.0±0.0	10.0±0.0
4	Gentamicin (positive control)	10	28.0±0.9	27.0±0.6	29.7±0.7	28.8±1.4	28.3±1.8

Mean±SD (n=3)
S.aureus – Staphylococcus aureus
B.cereus – Bacillus cereus
M.luteus – Micrococcus luteus
E.coli – Escherichia coli
A.hydrphila – Aeromonas hydrophila

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