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ANTIBACTERIAL ACTIVITY OF SEAWEEDS FROM VERAVAL COAST

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ABSTRACT
Antibacterial activity of seaweed species *Caulerpa racemosa, Ulva lactuca*, *Sargassum whitti*, *Dictyota bartayresiana*, *Hypnia musciformis* and *Gracilaria edulis* available at Veraval region of Gujarat state was tested against three fish pathogenic *bacterial* species *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Vibrio alginolyticus* with disk diffusion method using Muller Hilton agar. Higher antibacterial activity against *A.hydrophila* was noted in *G.edulis* (2.73 ± 0.66) followed by *H. musciformis* (2.03 ± 1.07) and *S. whitti* (1.72 ± 0.84). *S. whitti* (1.03 ± 0.61) extract showed high antibacterial activity against *P. aeruginosa* followed by *G. edulis* (1.03 ± 0.75). While *H. musciformis* (3.37 ± 1.02) extract showed high antibacterial activity against *V. alginolyticus* followed by *G. edulis* (2.23 ± 0.64). Results show that red and brown *seaweeds* have highest antibacterial activity against all three fish pathogens than green seaweeds.

INTRODUCTION

Pathogenic bacteria in water bodies cause heavy mortality in wild as well as cultured aquatic animals. These problems are usually tackled by following preventive methods or by treating animals with drugs or chemicals. Due to increased number of diseases, the use of antimicrobial agents has increased significantly in aquaculture practices (Burridge *et al*., 2010).

Due to indiscriminate use of antibiotics to evade from diseases in aquaculture, the microorganisms have developed new strategies to evade the action of antibiotics, leading to multiple drug-resistant bacterial strains (Perez *et al*., 2016). Hence developing cheaper and effective natural antimicrobial agents with better potential, less side effects than antibiotics, good bioavailability and minimal toxicity is necessary (Thanigaivel *et al*., 2015). Seaweeds are one of the abundant natural resources in marine ecosystems classified as red algae (Rhodophyta), brown algae (Phaeophyta) or green algae (Chlorophyta) depending on their nutrient and chemical composition. Fresh and dry seaweeds are extensively consumed by people especially living in the coastal areas (Shelar *et al*., 2012) and are already in use as traditional medicine for a long time (Taskin *et al*., 2007). Hence they have recently received significant attention for their capacity to provide a rich source of primary and secondary metabolites (Tuney *et al*., 2006), which have been characterized as a broad spectrum of antibacterial agents (Cox *et al*., 2010; Lavanya and Veerappan, 2011), antiviral (Gomez *et al*., 2010), anticancer compounds (Boopathy and Kathiresan, 2010), antioxidant compounds (Vinayak *et al*., 2011), antifouling compounds (Manilal *et al*., 2010) and pharmaceutical preparations (Yuvaraj *et al*., 2011). Seaweeds are also used in agriculture seed treatment for organic farming practice (Ambica and Sujatha, 2016). Seaweeds have been recognized as potential sources of the antibiotic substances (Shimaa *et al*., 2016). Among them fatty acids, terpenes, carbonyls and bromo-phenol compounds in seaweeds are responsible for antibiotic activity (Aubert *et al*., 1979).

Diverse literature available on antibacterial activity of seaweeds but antibacterial efficacy study of seaweeds on fish pathogens are comparatively a new concept (Choudhury *et al*., 2005). Seaweeds from the west coast of India are well known for diversity and its antibacterial activities (Manilal *et al*., 2009). In this context, the objective of the study was to evaluate the antimicrobial activity of different seaweeds collected from Veraval coast (Gujarat) against important pathogenic bacteria of fish.

MATERIALS AND METHODS

Sample collection and preparation

Six different seaweed species (*Caulerpa racemosa, Ulva lactuca*, *Sargassum whitti*, *Dictyota bartayresiana*, *Hypnia musciformis* and *Gracilaria edulis*) were collected from the intertidal region of the Veraval Coast. The seaweeds were washed thoroughly with sea water to remove extraneous materials and brought to the laboratory in plastic bags. Then samples were sun dried for 7 days, ground in an electric mixer and stored in refrigerator.

Extract preparation

The crude extracts from the seaweeds were prepared by solvent extraction method. Ethanol was used as solvent. The crude extracts were kept for evaporation to remove the solvent by intermittent shaking. The dried extracts were used for assay (Raman, 2006).

Bacterial strains used

In the present study, antibacterial activity of seaweeds was examined against *Aeromonas hydrophila, Pseudomonas aeruginosa* and *Vibrio alginolyticus.*

Preparation of algal discs

Whatman No. 1 sterile filter paper discs of 6 mm diameter were impregnated with seaweed extract. Control discs were prepared by just loading the sterile discs with the solvent (ethanol) alone. The positive control discs were prepared by the impregnation of the sterile discs with 0.1% concentration of the ampicillin antibiotic. Triplicates were maintained for all the tests, including controls.

Antibacterial assay

The agar disc diffusion method was followed for antibacterial susceptibility test (Bauer et *al*., 1966). The sterilized petri plates were poured with Muller Hinton (MH) agar medium and labelled. The discs were inoculated with 0.1 ml of test organisms (viz., *A. hydrophila, P. aeruginosa* and *V. alginolyticus)* and spread on the agar medium using sterilized "L" rod so as to make lawn. The agar surface was allowed to dry for five minutes. To prepare the algal extract impregnated discs 500 μg of each extract was dissolved in 50 μl ethanol (Choudhury et *al*., 2005). After allowing to evaporate, the discs were placed on MH agar test plates. The zone of inhibition was measured around the disc (mm diameter) after 24 hours of incubation at 37ºC.

Statistical analysis

All the data were expressed as means ± standard error (SE). The experiment was carried out in triplicates. The zone of inhibition of bacteria around the disc was measured and scored positive (+) if it is < 2 mm, double positive (+ +) if the zone is ≥2 mm, triple positive (+ + +) if the zone of inhibition is ≥7 mm, and negative (-ve) if there is no inhibition of microbial growth (Thompson *et al.*, 1985).

RESULTS AND DISCUSSION

Seaweed extracts can become an alternative eco-friendly approach for disease control in aquaculture (Romero *et al.*, 2012). Several scientists evaluated antibacterial activities of seaweed against several humans as well as fish pathogen and obtained promising result. In the present study antimicrobial activity of six different seaweed species against three fish pathogenic bacteria was carried out by disk diffusion method using MH agar. The zone of inhibition (mm) of ethanol extracted seaweeds were measured against three different fish pathogens which are presented in Table 1. The zone of inhibition in this study ranged from 0.00 - 3.37mm. Higher antibacterial activity against *A. hydrophila* was noted in *G. edulis* (2.73±0.66) followed by *H. musciformis* (2.03±1.07) and *S. whitti* (1.72±0.84). *S. whitti* (1.03 ± 0.61) extract showed high antibacterial activity against *P. aeruginosa* followed by *G. edulis* (1.03 ± 0.75). While *H. musciformis* (3.37 ± 1.02) extract showed high antibacterial activity against *V. alginolyticus* followed by *G. edulis* (2.23±0.64).

Results of present study showed that among six different seaweeds, red and brown seaweeds have higher antibacterial activity than green seaweeds. Similar results also reported by Rao (1991). In this study, red seaweed show higher antibacterial activity than brown seaweed, while, Caccamese *et al*. (1985) reported that brown seaweed have higher antibacterial activity than red seaweed. Natrah *et al*. (2015) also observed that brown seaweeds contain more antibacterial activity than red and green seaweeds against different aquaculture pathogens. Nanthinidevi *et al*. (2014) observed that purified extract of *S. whitti* showed antibacterial activity against *A. hydrophila*, while in the present study, *G. edulis* showed higher antibacterial activity against *A. hydrophila* (2.73±0.66), followed by *H. musciformis* (2.03±1.07) and *S. whitti* (1.72±0.84). The higher antibacterial activity against *P. aeruginosa* was observed in the ethanolic extract of *G. edulis* followed by *S. whitti*, whereas similar result was observed by Kolanjinathan *et al*. (2009), who found antibacterial activity of ethanolic extract of *G. edulis* aginst *P. aeruginosa*, *E. coli*, *S. aureus* and *S. faecalis*. Cox *et al*. (2010) also observed significantly stronger activity of brown seaweeds against *P. aeruginosa*. Elsie *et al*. (2010) observed ethanol extract of *S. whitti* showed higher antibacterial activity compared to *G. edulis* against *P. aeruginosa*.

Least antibacterial activity was observed in green seaweed against all the three fish pathogenic bacteria in present study.

Table 1: Zone of Inhibition (Mean±SE) of different seaweeds extracts against fish pathogenic bacteria

Seaweeds	Fish pathogenic Bacteria		
	Aeromonas hydrophila	*Pseudomonas aeruginosa*	*Vibrio alginolyticus*
Green algae			
Caulerpa racemosa	(-) 0.00±0.00	(-) 0.00±0.00	(+) 1.57±1.38
Ulva lactuca	(-) 0.00±0.00	(-) 0.00±0.00	(+) 1.91±1.74
Brown algae			
Sargassum *whitti*	(+) 1.72±0.84	(+) 1.03±0.61	(+) 1.33±0.69
Dictyota bartayresiana	(+) 0.90±0.67	(+) 0.83±0.38	(+) 0.87±0.33
Red algae			
Hypnia musciformis	(+ +) 2.03±1.07	(+) 0.77±0.50	(+ +) 3.37±1.02
Gracilaria edulis	(+ +) 2.73±0.66	(+) 1.03±0.75	(+ +) 2.23±0.64
Positive control (Ampicillin)	(+ +) 5.93±1.34	(+ +) 2.59±0.41	(+ +) 2.09±0.92
Negative control (Ethanol)	(-) 0.00±0.00	(-) 0.00±0.00	(-) 0.00±0.00

Positive (+) = the zone of inhibition is < 2 mm; Double positive (+ +) = the zone is ≥2 mm; Triple positive (+ + +) = the zone of inhibition is ≥7 mm and ; Negative (-ve) = no inhibition zone

Similar results were observed by Cox *et al*. (2010) and Gupta *et al*. (2010). While Singh and Chaudhry (2010) found higher inhibition zone of methanol extract of green algae *Pithophora oedogonia* against *Bacillus subtilis* and *Staphyllococcus aureus*. Seenivasan *et al*. (2010) observed antibacterial activity of extracts of *Ulva fasciata* against *E. coli* whereas in the present study *U. lactuca* and *C. racemosa* showed antibacterial activity against *V. alginolyticus* and no antibacterial activity was observed against *A. hydrophila* and *P. aeruginosa*.

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