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V Shajeeda Banu
Department of Ocean Studies and Marine Biology, Pondicherry University Brookshabad Campus, Port Blair, A & N Islands, India

Binita Hazarika
Department of Ocean Studies and Marine Biology, Pondicherry University Brookshabad Campus, Port Blair, A & N Islands, India

Sneha Sawhney
Department of Ocean Studies and Marine Biology, Pondicherry University Brookshabad Campus, Port Blair, A & N Islands, India

JK Mishra
Department of Ocean Studies and Marine Biology, Pondicherry University Brookshabad Campus, Port Blair, A & N Islands, India

Correspondence
JK Mishra
Department of Ocean Studies and Marine Biology, Pondicherry University Brookshabad Campus, Port Blair, A & N Islands, India

The red Seaweed, *Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott and Doty, 1960 from South Andaman, a potential source of antibacterial compounds

V Shajeeda Banu, Binita Hazarika, Sneha Sawhney and JK Mishra

Abstract
The red seaweed, *Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott and Doty, 1960 is being reported for the first time with its seasonal occurrence along the coast of Andaman & Nicobar Islands. Like other bioactive potential seaweeds, crude extract of *T. pedicellata* exhibited activity against human pathogens *Staphylococcus aureus* (MTCC 3160), *Bacillus cereus* (MTCC 430) and *Salmonella enterica typhimurium* (MTCC 1252). Ethanolic extract of *T. pedicellata* showed activity against both gram-positive and gram-negative bacteria revealing its potential as a source of bioactive compounds. Out of seven bacterial strains tested, zone of inhibition was prominent in three bacterial strains. Maximum zone of inhibition exhibited by gram-positive bacteria *S. aureus* (21mm) followed by *B. cereus* (18mm) and minimum zone of inhibition by gram-negative bacteria *S. enterica typhimurium* (14mm), when it was treated at 100µl of 100 mg/ml concentration of the crude extract.

Keywords: Macroalgae, antibacterial activity, *Trichogloeopsis pedicellata,* Andaman sea

Introduction
In the marine ecosystem, organisms develop tolerance to new environmental conditions and also have resistance to several pathogens. The marine ecosystem of Andaman Islands is isolated and unique, which remain unexplored compared to other marine ecosystems in India. The unexplored sea life of this region may have the potential for rich source of active biomolecules, particularly from the sources like seaweeds. These marine macroalgae also have served as a major source material for structurally unique natural products with pharmacological and biological activities (Schwartsmann *et al*., 2001; Manilal *et al*., 2010; Chiheb *et al*., 2009) [1, 22, 5]. They have been used since ancient times as food, fodder, fertilizer and as natural source of medicine. At present these marine algal resources has become raw materials for many industrial products including agar, algin and carrageenan, in addition as widely consumed food stuff as it contains carotenoid, vitamins, minerals and dietary fibres (Ito and Hari, 1989) [15]. Simultaneously, the bioactive potential of marine algae are well known as it is found that the chemical compounds and the metabolites from them inhibit or limit the development and growth of other competitive microorganisms and exhibit antibacterial (Shimizu, 1996; Kolanjinathan *et al*., 2009; Lustigman and Brown, 1991; Freile-Peligrin *et al*., 2004; Chander *et al*., 2014; Karthikeyan *et al*., 2015) [33, 19, 21, 11, 4, 16], antiviral (Wang *et al*., 2008) [36], antiprotozoal (Vonthron-Senecheau *et al*., 2011) [35], antifungal (Mhadhebi *et al*., 2012; Genovese *et al*.,2013) [23, 12], anti-coagulant (Dayong *et al*., 2008) [7], anti-cancer (Kim *et al*., 2011) [17], anti-fouling (Bhadury and Wright, 2004) [3] and antioxidant (Yangthong et. al., 2009; Devi *et al*., 2011) [37, 9] activities. In addition, bromophenols from red seaweeds show significant activities against various pathogenic bacteria too (Oh *et al*., 2008) [25]. Also the study on the role of crude extracts of many marine algal species suggest that not only they have inhibition activity against pathogenic bacteria but also have bacteriostatic and bactericidal properties (Hornsey and Hide, 1985; Padmakumar and Ayyakkannu, 1997; Gorban *et al*., 2003; Kim *et al*., 2007; Adaikalaraj *et al*., 2012; Chander *et al*., 2014; El Shafay *et al*., 2016) [14, 26, 13, 18, 2, 4, 10].
The studies involving effect of marine macroalgae in antimicrobial activity is regarded as an indicator to detect their potent pharmaceutical capacity. There are numerous studies on antimicrobial activity of seaweed extracts, but very limited reports are available from the Andaman Sea (Chander *et al*., 2014; Mishra *et al*., 2016; Deepa *et al*., 2017; Shajeeda and Mishra, 2018a, b) [4, 24, 8, 31-32].

As reported there are about 1,153 seaweed species in Indian waters (Rao and Mantri, 2006) [28], of which about 300 estimated species are available in Andaman Sea, India (Palanisamy, 2012) [27]. So, exploring seaweeds for antimicrobial activity from Andaman Sea might lead to the discovery of better alternatives to conventional drugs, which pose a serious threat to the humankind in terms of emergencea of hazardous traits of antibiotic resistance bacterial pathogens. Present study was thus aimed at evaluating the antimicrobial activity of red algae, *Trichogloeopsis pedicellata* (M. Howe) from the Andaman Sea, which is also being reported for the first time from Indian waters.

T. pedicellata is being reported for the first time from the South Andaman coast, which was found at Marina Park (Sesostris Bay); Carbyn's Cove area (opposite to Hornbill Nest resort), Kodiyaghat. The species was mostly available in the lower littoral zone, which remains attached by its holdfast to rocky substrates and grow luxuriantly in solitary or with other macroalgae species at times. The occurrence of the species in the study area was observed for a very short period during December to April following post monsoon. Thallus of the species was highly slimy and slippery in its natural condition. This species has its homotypic synonym as *Liagora pedicellata* (M. Howe, 1920) and reported along the coast of Florida (North America); Caribbean Islands; Bermuda, Canary Islands of Atlantic coast; Belize, Costa Rica (Central America); Venezuela etc. (Abbott and Doty, 1960) [1]. However the bioactive potential of this species is not well documented yet. Thus in the present investigation, attempt was made to evaluate the antimicrobial activity of *T. pedicellata* from the coast of South Andaman.

Materials and Methods

Collection of Seaweeds

The seaweed, *T. pedicellata* (Plate - 1) was collected from Kodiyaghat (Lat. 11.53'N; Long. 92.72'E) along the coast of south Andaman during low tide period in sterile sample covers and brought to the laboratory. The sample was washed thoroughly under running tap water to remove any attached debris, epiphytes and sand particles and then the final washing was done using sterile distilled water.

Plate 1: *Trichogloeopsis pedicellata* (M. Howe) I.A. Abbott and Doty, 1960 at Kodiyaghat, South Andaman.

Crude Extract Preparation

For crude extraction 500 gm of seaweed was grinded with 1000 ml absolute ethanol using mortar and pestle. The aliquot was transferred into a 2 litre conical flask and kept for one week at ambient temperature by sealing the flask. The extract was then filtered with Whatman No. 1 filter paper and solvent was evaporated under reduced pressure at 45 ^{0}C using the rotary evaporator (Buchi RII Rotavapour). The resulting crude extract was made up to a concentration of 100mg/ml with Dimethyl sulfoxide (DMSO) and stored at 4 ^{0}C for further study.

Antibacterial assay

The *in-vitro* antibacterial activity of the crude extract was carried out by agar well diffusion method against seven pathogenic bacteria i.e. *Escherichia coli* (MTCC 443), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 3541), *Bacillus cereus* (MTCC 430), *Salmonella enterica typhimurium* (MTCC 1252), *Listeria monocytogenes* (MTCC 839) and *Bacillus subtilis* (MTCC 121).The bacterial strains were inoculated in nutrient broth and incubated overnight at 37 ˚C. The sterilized Petri plates were poured with Muller Hinton agar medium (HIMEDIA) and labelled. Following 0.1 ml of test pathogen was inoculated and spread on the agar medium using sterile swab so as to make lawn. The agar surface was allowed to dry for five minutes. The wells were made with the help of sterile cork borer. The prepared algal extract with the concentration of 100mg/ml was assayed for its bioactivity. From this aliquot 25µl, 50µl and 100µl of crude extract was loaded to each well separately. The negative control was carried out by loading 100µl 5% DMSO into the well. Whereas, the positive control was 10µl of 10mg/ml azithromycin. The plates were kept for 24 hours at an incubating temperature of 37 ^{0}C and after 24 hours the zone of inhibition was measured and tabulated.

Results and Discussion

The results of the antibacterial activity of the crude extracts against indicator bacterial strains are given in Table - 1. As depicted the ethanol based crude extracts was effective only on two gram-positive species *B. cereus* and *S. aureus*, when it was assayed with 25µl 50µl and 100µl of the extract. But the crude extract was effective on gram-negative species, *S. enterica typhimurium*, when it was treated with 100µl of the extract. However, extracts did not have any effect on other gram-positive and gram-negative species assayed in the investigation.

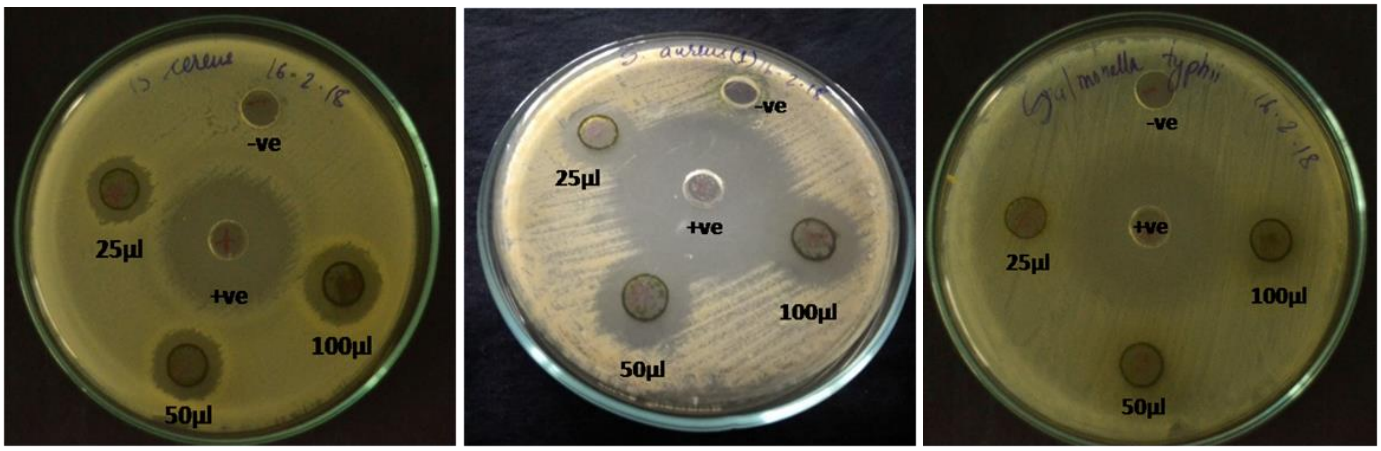
The maximum zone of inhibition of 21 mm was exhibited against gram-positive bacteria, *S. aureus* followed by 18 mm inhibition zone by *B. cereus*, whereas minimum zone of inhibition of 14mm was exhibited by gram-negative bacteria, *S. enterica typhimurium* at 100µl of crude extract with the concentration of 100mg/ml of sample (Table – 1).

Table 1: Antibacterial activity of *Trichogloeopsis pedicellata* against human pathogens.

Bacteria	Pathogen	Zone of inhibition (mm)/ Concentration of crude extract		
		25µl	50µl	100µl
Gram-positive bacteria	*Bacillus cereus*	15	16	18
	Bacillus subtilis	-	-	-
	Listeria monocytogenes	-	-	-
	Staphylococcus aureus	17	19	21
Gram-negative bacteria	*Escherichia coli*	-	-	-
	Pseudomonas aeruginosa	-	-	-
	Salmonella enterica typhimurium	-	-	14

The *in-vitro* assay suggest that the crude extracts did not have any activity against two species of gram-positive bacteria, *Listeria monocytogenes* and *Bacillus subtilis* and gram-negative bacteria, *E. coli* and *P. aeruginosa* and (Plate – 2). Among all the pathogens, *S. aureus* found to be the most sensitive with the widest inhibition zone in response to the

crude extract. There was no zone of inhibition seen in the negative control. Whereas in the positive control with Azithromycin, there was distinct inhibition zone. The present findings revealed that the extracted molecules from *T. pedicellata* have prominent activity against two species of gram-positive bacteria and only one species of gram-negative bacteria.



B. cereus ***S. aureus*** ***S. enterica typhimurium***

Plate 2: Zone of inhibition by crude extracts by *in-vitro* assay.

Though the study by Lavanya and Veerappan (2011) [20] suggested that the crude extracts of brown seaweed have higher activity than the red seaweed. In the present investigation, comparative study of activity by brown, red and green algae extracts was not carried out. But the present findings showed that the prominent zone of inhibition (21mm) against *S. aureus* by the ethanolic extract of red algae *T. pedicellata* was well exhibited. Similar activity was recorded against *B. cereus* and *S. enterica typhimurium*. Cox *et al*. (2010) [6] also reported antimicrobial activity of red and green seaweed extracted with ethanol and acetone significantly ($p< 0.05$) increased.

Veeragurunathan *et al*. (2008) [34] reported highest antibacterial activity (12mm at 75µl) of ethanolic extract of *H. musciformis* against *E. coli*, which is a gram negative bacteria. On contrary in the present study highest antibacterial activity (21mm at 100µl) of ethanolic extract of *T. pedicellata* against *S. aureus* and minimum zone of inhibition (14mm) against *S. enterica typhimurium*. Selvi and Selvaraj (2000) [30] reported that ethanol extract of *Hypnea* sp. had no antibacterial activity against *P. aeruginosa* even at the higher concentration of 200µg/disc of crude algal extracts, which is in concurrence with the present study as there was no antibacterial activity against the gram-negative species. Among all the seven bacteria tested in the present study, *S. aureus* showed highest zone of inhibition suggesting that the pathogen is more sensible to ethanol extracts of red algae, which supports previous results.

Out of seven bacterial strains investigated, zone of inhibition was detected only in three bacterial strains. As suggested, gram positive bacteria are more sensitive to algal extracts than gram negative bacteria, which were also observed in the present study that *S. aureus* and *B. cereus* showed maximum zone of inhibition while *S. enterica typhimurium* showed comparatively less. Other strains like gram-negative bacteria *Escherichia coli* and *P. aeruginosa* and gram-positive bacteria *L. monocytogenes* and *B. subtilis* did not show any activity. The study suggests the possibility that different quantities of active secondary products may have been synthesized under different conditions pertaining to the pathogenic strains. The more susceptibility of a particular group of bacteria such as gram-positive bacteria may be due to the difference in their cell wall structure and their composition and the active compounds in the algal extracts may go and bind to the cell wall of the microbes leading to inhibition of its growth. Also in the outer membrane of gram-negative bacteria due to the presence of lipopolysaccharides, it might acts as a barrier to many environmental substances including antibiotics or any bioactive compounds. So it is less sensitive to the algal extracts. Seaweeds are the potential sources of bioactive compounds and should be investigated for natural antibiotics. However it is can be suggested that the red algae, *Trichogloeopsis pedicellata* can be a potential source of antimicrobial compounds for treating microbes causing infections and pose a threat to human kind.

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