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Antimicrobial activity of different solvent based crude extracts from red seaweed *Tricleocarpa fragilis* (L.) Huisman & R.A. Towns from the coast of South Andaman

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Abstract
The antibacterial activity of crude extracts from red seaweed *Tricleocarpa fragilis* was obtained with different organic solvents were evaluated against five pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Bacillus cereus* and *Salmonella enterica typhimurium* by agar well diffusion method. The results revealed that crude extracts obtained by chloroform had inhibiting effect on the growth of all tested pathogens. But the maximum zone of inhibition (21 ± 0.1mm) was obtained with acetone extracts against *S. aureus*. Whereas minimum zone of inhibition (5 ± 0.3mm) was observed with the hexane extracts against *B. cerus*. But crude extracts with chloroform exhibited inhibiting effect on all the pathogens. The positive control with azithromycin showed inhibitory action against all the pathogens studied. Present results suggested that the bioactive compounds extracted from red seaweeds using chloroform as a solvent could be a major source for developing antibacterial compounds.

Keywords: Crude extract, red seaweed, *T. fragilis*, human pathogen

Introduction
In the marine environment algae are considered as one of the largest producers of biomass. The renewable resources from the sea has a wide range of application including as a major source for food, feed, manure, chemicals and importantly towards exploration of bioactive molecules with a major contribution towards livelihood for coastal community (Gopinathan and Panigrahy, 1983; Ito and Hori, 1989; Kaliapeumal and Kalimuthu, 1997; Rao and Mantri, 2006) [8, 12, 13, 29]. It is also well documented that seaweeds possess a great variety of structurally unique biomolecules and secondary metabolites with significant pharmacological and biological activities (Schwartsmann *et al*., 2001; Kolanjinathan *et al*., 2009; Manivannan *et al*., 2011; Mishra *et al*., 2016; Banu *et al*., 2018; Banu and Mishra, 2018) [30, 18, 21, 22, 3, 2]. In addition there are various antimicrobial compounds have been identified from the marine environment in comparison to the terrestrial environment (Ireland *et al*., 1988; Maheshwaran *et al*., 2013; Shimaa *et al*., 2016) [11, 20, 33]. But the quest for new bioactive compounds from marine living resources is growing day by day as several pathogens becoming fatal and resistant to the available synthetic drug. Some of the pathogenic bacteria causes skin infections, pneumonia, tetanus, typhoid, diphtheria, syphilis and meningitis with fatal effect and pose a serious threat to the human population (Kandhasamy and Arunachalam, 2008) [14]. The therapy for these infectious diseases also has certain limitations due to changing pattern of resistance in pathogens to the drugs and the consequent side effects of these synthetic drugs. Thus attempts are now being made to explore new antimicrobial drugs/compounds from natural sources, particularly from the marine environment, which is less explored in this direction but holds enormous promise in the area of new drug development. In this respect marine natural products like seaweeds are being investigated extensively as the natural compound may overcome the problems associated with drug resistance and potential side effects. The screening of marine algae towards finding such compounds with antimicrobial activity has gained momentum in recent times (Mouhssen 2013; Kolanjinathan *et al*., 2014; Mishra *et al*., 2016; Banu *et al*., 2018) [24, 17, 22, 3]. There are reports with reference to several pathogen inhibitory compounds from marine macroalgae including antibacterial (Singh and Chaudhary, 2010; Mishra *et al*., 2016) [34, 22], antifungal (De- Felicio *et al*., 2010) [6], antiviral (Perez *et al*., 2012; Bouhlal *et al*., 2010) [27, 4], antitumor (Kim and Karadeniz, 2011) [16],

Antioxidant (Kudaa *et al*., 2005; Devi *et al*., 2011) [19, 7] and anti-inflammatory activities (Vineela and Elizabeth, 2005; Inci *et al*., 2006; Patra *et al*., 2008) [35, 10, 26]. The contribution of some of the seaweed species from Andaman Sea in this respect is well documented in recent years (Mishra *et al*., 2016; Banu *et al*. 2018; Banu and Mishra, 2018) [22, 3, 2]. However, the potent antimicrobial effect of seaweed extracts resides in the efficiency of the extraction method and the solvents being used (Cox *et al*., 2010; Vinoth *et al*., 2015) [5, 36]. There are also very few studies pertaining to the antimicrobial potential of *T. fragilis* (Perez *et al*., 2012; Kehau and Anthony, 2016; Horgen *et al*., 2000) [27, 15, 9]. Thus the present study was focused to assess the *in vitro* antimicrobial activity of crude extracts with different organic solvents from the red seaweed, *Tricleocarpa fragilis* from the coast of South Andaman, India against some human pathogenic bacterial isolates.

Materials and Methods

Collection of seaweeds

Samples of red seaweed *T. fragilis* were collected by hand picking at Marina Park (Sesostris Bay; Lat. $11^0$66.927' N; Long. $92^0$74.9347' E) along South Andaman coast during low tide. The collected seaweeds were first washed with seawater and then brought to the laboratory and washed again under tap water to remove any sand particles and epiphytes followed by washing with distilled water. Following the samples were shade dried for a week at ambient temperature and dried samples were powdered using electronic blender and stored at 4 ^{0}C for further analysis.

Crude Extract Preparation

For crude extract preparation, 10gms of dried seaweed powder was put into six different conical flask (250 ml)each separately and added with 100ml of each solvent ethanol, methanol, dichloromethane, chloroform, acetone and hexane separately to each flask and kept for a week at ambient temperature. The extract was then filtered (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 stored at 4 ^{0}C in the refrigerator for further study. The concentration of prepared seaweed extract was made to a concentration of 100mg/ml by dissolving with dimethyl sulfoxide (DMSO).

Antibacterial assay

The *in vitro* antibacterial assay of the crude extracts of each solvent was carried out by agar well diffusion method against pathogenic bacteria *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 3541), *Listeria monocytogenes* (MTCC 839), *Bacillus cereus* (MTCC 430) and *Salmonella enterica typhimurium* (MTCC 1252).The bacterial strains were inoculated in nutrient broth and incubated overnight at 37 ^{0}C. The sterilized Petri plates were poured with Muller Hinton agar medium (HIMEDIA) and labelled. Following 0.1 ml of each of the test pathogens was inoculated separately and spread on the agar medium using sterile swab so as to make lawn. The agar surface was allowed to dry for five minutes. Total 8 wells were made with the help of sterilized cork borer and extract was inoculated into the respective wells as: well (1) 50µl of ethanol extract; well (2) 50µl of methanol extract; well (3) 50µl of dichloromethane extract; well (4) 50µl of chloroform extract; well (5) 50µl of acetone extract; well (6) 50µl of hexane extract; well (7) 50µl of 5% DMSO as negative control and well (8) 10µl of 1mg/ml azithromycin as positive control. The plates were then kept at an incubating temperature of 37 ^{0}C for 24 hours followed by the measurement of the zone of inhibition in each of the well and tabulated.

Result and Discussion

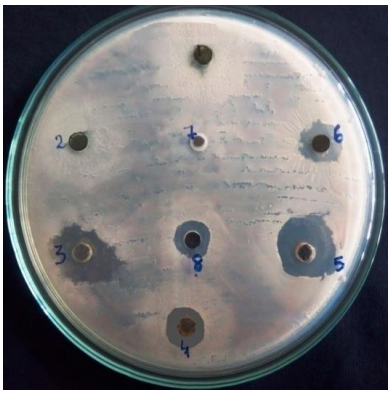
The antibacterial activity of extracted compound from *T. fragilis* with different solvents i.e. ethanol, methanol, dichloromethane, chloroform, acetone and hexane was studied by agar well diffusion method against pathogens *S. aureus*, *P. aeruginosa*, *L. monocytogenes*, *B. cereus* and *S. enterica typhimurium*. The inhibition zones (mm) exhibited by the antibacterial assay are presented in Table-1. Among the six solvent extracts tested, crude extracts with chloroform exhibited inhibition against all the pathogens assayed (Plate-1). In case of *S. aureus* it was found to be sensitive against extracts of acetone with maximum inhibition zone (21 ± 0.2mm) followed by dichloromethane (20 ± 0.1mm), hexane (15 ± 0.1mm) and chloroform (13 ± 0.3mm). The positive control in this case showed zone of inhibition as11 ± 0.1mm,while no zone of inhibition was observed for the extracts of ethanol, methanol and in negative control. In *P. aeruginosa* it was found to be susceptible only to the chloroform extract with a zone of inhibition of 12 ± 0.2mm, where positive control showed a zone of inhibition of 5 ± 0.1mm and no zone of inhibition was seen in any other extracts assayed. Similarly *L. monocytogenes* exhibited zone of inhibition against the extracts of ethanol (20 ± 0.2mm), hexane (18 ± 0.1mm), chloroform (16 ± 0.4 mm) and dichloromethane (13 ± 0.2mm). Whereas positive control had a inhibition zone of 8 ± 0.3mm, while no zone of inhibition was observed in negative control and extracts of methanol and acetone in this case. In case of chloroform extracts maximum inhibition zone of 13 ± 0.3mm against *B. cerus* was found, followed by extract of dichloromethane (10 ± 0.2mm), acetone (7 ± 0.1mm) and minimum zone of inhibition against extract of hexane (5 ± 0.3 mm). But the positive control in this case showed zone of inhibition of 16 ± 0.5mm and there was no zone of inhibition was recorded for negative control. However, *S. enterica typhimurium* was found to be sensitive only against chloroform extract with a zone of inhibition of 14± 0.2mm, whereas zone of inhibition for positive control was 12± 0.3mm and no zone of inhibition was seen against any other extracts.

The variation in antibacterial activities of the extracted compounds with different organic solvents in this case might be due to the efficacy of the eluted substances in *T. fragilis* with the respective solvent. It may also be attributed to the resistance mechanism of the pathogen by enzymatic inactivation, target sites modification in response to the active compounds in the extracts (Schwarz and Noble, 1999) [31]. As for the effectiveness of the extraction method, there are reports based on solvent effectiveness on extraction process of natural compounds from seaweeds, which supports the present extraction methodology. The present investigation is in agreement with the earlier report, where the best antibacterial activity on human pathogens was obtained from chloroform as well as isoamyl alcohol or methanol extracted compounds of red seaweed *Kappaphycus* sp., *Hypnea musciformis* and brown seaweed *Spatoglossum asperum* compared to other organic solvents (Shareef *et al*., 2012; Prasad *et al*., 2013; Pandithurai *et al*., 2015) [32, 28, 25]. Also the present study on antibacterial activity of *T. fragilis* crude extracts suggest that chloroform was the suitable organic

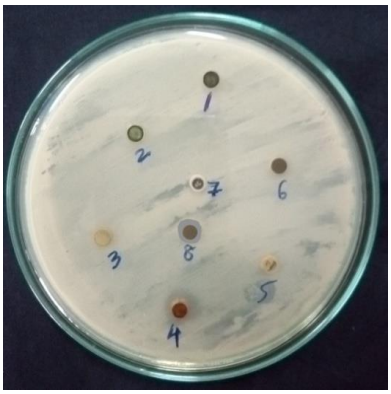
solvent for extracting the effective antibacterial compounds from *T. fragilis*, though the zone of inhibition was varied according to the solvent and test organism used in this experiment. But investigation provided evidence that *T. fragilis* can be a potential source for developing antibacterial biomolecules and develop natural therapeutic agent.

Table 1: Zone of inhibition by crude extracts of *Tricleocarpa fragilis* against human pathogens.

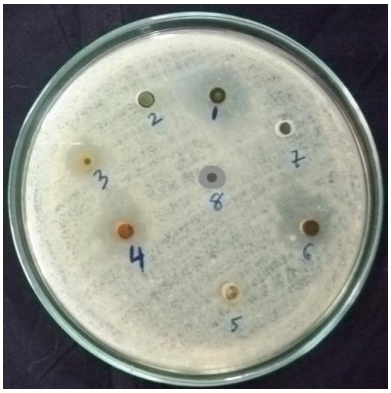
Seaweed	Solvents	Zone of inhibition (mm) in relation to bacterial pathogens				
		S. aureus	***P. aeruginosa***	***L. monocytogenes***	***B. cereus***	***S. enterica typhimurium***
Tricleocarpa fragilis	Ethanol	-	-	20 ± 0.2	-	-
	Methanol	-	-	-	-	-
	Dichloromethane	20 ± 0.1	-	13 ± 0.2	10 ± 0.2	
	Chloroform	13 ± 0.3	12 ± 0.2	16 ± 0.4	13 ± 0.3	14± 0.2
	Acetone	21 ± 0.2	-	-	7 ± 0.1	-
	Hexane	15 ± 0.1	-	18 ± 0.1	5±0.3	-
	Negative control	-	-	-	-	-
	Positive control	11 ± 0.1	5 ± 0.1	8 ± 0.3	16 ± 0.5	12± 0.3



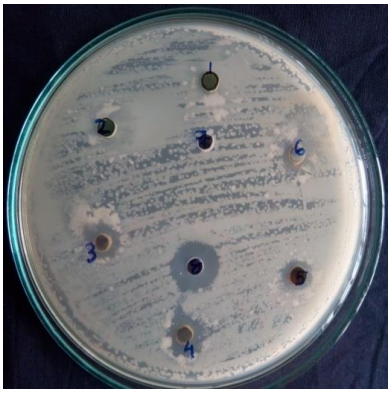
S. aureus



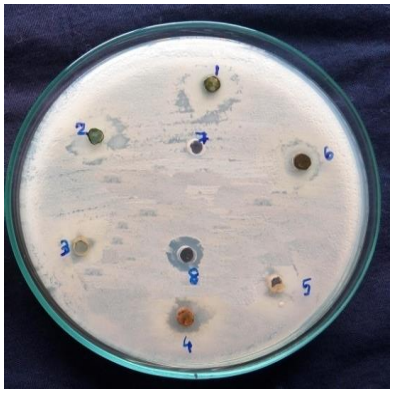
P. aeruginosa



L. monocytogens



B. cerus



S. enterica typhimurium

Well (1)-Ethanol extract; Well (2)- Methanol extract; Well (3)- Dichloromethane extract; Well (4)- Chloroform extract; Well (5)- Acetone extract; Well (6)- Hexane extract; Well (7)- Negative control and Well (8)- Positive control.

Plate 1: Inhibition zone in pathogens against extracts of *Tricleocarpa fragilis*.

Conclusion

Seaweed extracts in different solvents exhibited different antibacterial activities and it was observed from the study that chloroform solvent extraction was suitable to verify the antibacterial properties of *Tricleocarpa fragilis*. The overall antibacterial activity assessed from the above results indicates the presence of active molecules in the extractions of *T. fragilis*, which can be exploited for the production of lead molecules for use in the pharmaceutical industry.

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