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Invitro antimicrobial potentials of marine *Oscillatoria* species

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ABSTRACT

The in vitro antimicrobial efficacy of the marine cyanobacteria was examined against gram-positive and gram-negative bacteria. The pyridine and n- butanol extract of Oscillatoria subrevis and O. amphibia exhibited broad spectrum of antibacterial activity. The study revealed that O. subbrevis (18, 15, 14 and 10 mm) showed the better results against Vibrio pathogens tested. The organic solvents of cyanobacterial extracts of pyridine, n-butanol showed the activity against selected five Vibrio pathogens. The different activity in different extracts suggests different compounds with different polarities.

Key words: antimicrobial efficacy, cyanobacteria , organic solvents, *Vibrio* pathogens.

INTRODUCTION

Cyanobacterial blooms have a wide range of social environmental and economic impacts. They are structurally diverse and widely distributed throughout the world and are later known as blue green algae. Because of the growing bacterial resistant against commercial standard and reverse antibiotics the search for the new active substances with antimicrobial activity against hospital – based *Staphylococcus aur*eus (MRSA – stains) is increasing importance. The extracts were prepared from cyanobacterial biomass with solvents of different polarity for their biological activity in various in-vitro test systems. [11], were studied for centuries, marine algae have been used as a food source and as a treatment for a variety of condition in most part of the marine algae are still used in folk medicine for the treatment of a variety of diseases. The world distribution and used of marine algae as a food source must have contributed to its popularity [17]. Furthermore, cyanobacterial biogenic particularly cytotoxic compounds could provide leads for further development of new therapeutic agents for a variety of diseases and for the development of new antibiotics.

Today infections disease is the main cause of death in developing countries worldwide and they hold the second position after heart diseases. Because of the growing bacterial resistance against commercial standard and reverse antibiotics the search for the new active substances with

antimicrobial activity against hospital based MRSA strains, gram positive and gram negative bacterial pathogens is of increasing importance [14]. Recently we investigated and screened the extracts from cyanobacterial mass with organic solvents for their biological activity in various in-vitro test systems.

MATERIALS AND METHODS

Culturing and growth condition

Three cyanobacterial strains namely *Oscillatoria* sp, *O. amphibia*, *O. subbrevis* and *O. chlorina* were collected from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and using ASN III medium exposed to 3000 lux light intensity for 16 h: 8 h. Light: Dark cycle. The isolates were identified morphologically using the manuals of [4] and [6].

Clinical strain tested

Table 1. Strains used in the study

Pathogenic strains	Source
Bacterial strains (Gram positive and Gram negative bacteria): *Bacillus cereus*	MTCC 6909
Enterobacter sp.	Personal isolate
Enterococcus faecalis	ATCC – BAA 2128
Staphylococcus aureus	ATCC – BAA 1720
Salmonella typhi	Personal isolate
Fungal strains: *Aspergillus flavus*	MTCC 6323
A. koeningii	Personal isolate
A. quercinus	Personal isolate
A. oryzae	Personal isolate
A. wentii	Personal isolate
Candida albicans	MTCC 6954
Helminthosporium sp.	Personal isolate
Humicola sp.	Personal isolate
Verticillium sp.	Personal isolate
Curvularia senegalensis	Personal isolate
Vibrio cultures: *Vibrio alginolyticus*	Personal isolate
V. anguillarium	Personal isolate
V. cholerae	Personal isolate
V. fluvialis	Personal isolate
V. mimicus	Personal isolate

Extract preparation

The mid log phase cyanobacterial cells were separated and rinsed with sterile water to remove any associated debris. According to the methods of [10] and [21] pyridine, n – butanol, ethyl

acetate, and aqueous were used to extract cyanobacterial metabolites and were preserved at ±4^{o} C.

Antimicrobial Assay

Screening of antibacterial activity

Antibacterial activity was assessed with the radial diffusion assay as described by [7]. Briefly, an overnight cultures (18 h) of bacteria was grown at 37^{o}C in tripticase soy broth with 1% NaCl and then washed three times by centrifugation at 590 X g for 10 min at 4^{o}C, followed by suspension with cold phosphate – buffered saline (pH 7.0). The bacterial suspension was adjusted to an optical density (OD $_{570}$) of 0.1 (10^{8} CFU/mL). One mL of bacterial suspension was mixed with autoclaved one hundred ml of LB broth containing 1.5% low EEO agarose, 0.5% NaCl, 200 mM phosphate buffer (pH 6.7), and 100mg/mL streptomycin sulphate at 48^{o} C, and poured into sterile petri dishes. For the radial diffusion assays, 50 µl (5 mg dry weight/mL) of algal crude extracts were transferred into 5- mm diameter wells. Streptomycin sulphate was used as a positive control and solvents were used as a negative control. The plates were incubated at 37^{o}C for 18 h. After incubation, the clearing zone diameters were observed.

Screening of antifungal activity

Petri dishes with 10 ml of potato dextrose agar were prepared, and mixed with 0.1 ml of 48 h broth cultures of test fungi cultures. The wells were made and filled with 100 µl of cyanobacterial extracts. The inoculated plates were incubated for 48 h at 37^{o}C. After incubation, the diameter of the inhibition zone was measured [1]. For the agar well diffusion assays, 50 µl (5 mg dry weight/ mL) of algal crude extracts were transferred into 5-mm diameter wells. Kanamycin was used as a positive control and organic solvents were used as a negative control. The plate was incubated at 37^{o}C for 48 h. After incubation the results were observed.

Screening of antivibrio activity

The sterile petri dishes with 10 ml nutrient agar (pH – 8.5) were prepared, and mixed with 0.1 ml of 18 h broth cultures of *Vibrio*. For the agar well diffusion assays, 50 µl (5 mg dry weight/ mL) of algal crude extracts were transferred into 5- mm diameter wells. The inoculated plates were incubated for 48 h at 37^{o}C. After incubation, the diameter of the inhibition zone was measured [1]. Ciprofloaxin was used as a positive control and organic solvents were used as a negative control.

RESULTS

Antibacterial activity of cyanobacterial species

The antibacterial activity of the extract from the marine cyanobacterial cultures of *Oscillatoria sp, O. subbrevis, O. amphibia* and *O. chlorina* were assayed against a panel of gram positive, gram negative bacteria and methicilline resistance *Staphylococcus aureus* (MRSA) strains. Four solvent systems (Ethyl acetate, pyridine, n-Butanol and water) were used to extract the selected cyanobacterial culture extracts. The maximum zone of inhibition was observed in n-butanol and pyridine extracts *O. subbrevis, O. amphibia* and *O. chlorina* against *Salmonella typhi* and MRSA *Staphylococcus aureus* with inhibition zones of 11, 10, 8, 7, 6 and 5 mm in diameter respectively (Table 2). The growth of *Salmonella typhi* and *Staphylococcus aureus*

were strongly inhibited by the n-Butanol and pyridine extract. However, the water and ethyl acetate extracts of cyanobacterial cultures showed weak antibacterial activity in tested.

Table 2. Antibacterial activity of selected cyanobacterial species

Name of the Cyanobacteria	Zone of inhibition (dia in mm)				
	Bacillus cereus	***Enterobacter* sp.**	***Enterococcus faecalis***	***Staphylococcus aureus***	***Salmonella typhi***
Oscillatoria sp. (SGBRA 05)					
Ethyl acetate	-	-	-	-	-
Pyridine	-	7	-	5	6
n-Butanol	-	3	3	6	7
Water	-	-	-	-	-
O. subbrevis (SGBRA 04)					
Ethyl acetate	-	-	-	-	-
Pyridine	6	6	8	10	-
n-Butanol	8	8	7	7	2
Water	-	-	-	-	-
O. amphibia (SGBRA 06)					
Ethyl acetate	-	-	-	-	-
Pyridine	4	6	-	4	11
n-Butanol	3	4	5	2	7
Water	-	-	-	-	-
Oscillatoria chlorina (SGBRA 07)					
Ethyl acetate	2	2	-	-	-
Pyridine	5	6	4	8	5
n-Butanol	-	8	6	5	8
Water	-	-	-	-	-

Antifungal activity of cyanobacterial species

Antifungal activity of ethyl acetate, pyridine, n-Butanol and aqueous extracts of cyanobacterial isolated were tested by using agar well diffusion method against fungal pathogens. Among the four cyanobacteria, Oscillatoria species were the most potential species because of this ability to grow fast and even the agar plates high productivity of antifungal compounds in the culture media. The maximum zone inhibitions was observed in n- butanol and pyridine extract of *Oscillatoria subbrevis*, *O. amphibia* and *O. chlorina* with inhibition zone of 10, 9, 8, and 7.5 diameter in mm (Table 3). The growth of *Candida albicans*, *Aspergillus wentii* and A. flavus were strongly inhibited by the pyridine and n-butanol. However, the ethyl acetate and water cyanobacterial culture showed weak antifungal activity in tested pathogens.

Antivibrio activity of selected cyanobacterial cultures

The predominant cyanobacteria studies were tested against five *Vibrio* pathogens i.e., *V. alginolyticus, V. fluvialis, V. mimicus, V. anguillarium and V .cholerae*. The organic solvents of cyanobacterial extracts of pyridine, n-butanol showed the activity against selected five *Vibrio* pathogens. Among these cyanobacterial cultures the maximum inhibition zones (18, 15, 14 and 10 dia in mm) observed with *Oscillatoria subbrevis* (Table 4).

Table 3. Antifungal activity of selected cyanobacterial species

Name of the Cyanobacteria	Zone of inhibition (dia in mm)				
	Aspergillus oryzae	***Aspergillus flavus***	***Aspergillus koeningii***	***Candida albicans***	***Aspergillus wentii***
Oscillatoria sp. (SGBRA 05)					
Ethyl acetate	-	-	-	-	-
Pyridine	7.5	7.5	-	7	-
n-Butanol	7.5	7.5	7	6	9
Water	-	-	-	-	-
Oscillatoria subbrevis (SGBRA 04)					
Ethyl acetate	-	-	6	-	-
Pyridine	7.5	10	8	8	10
n-Butanol	8	9	9	10	9
Water	-	-	-	-	-
Oscillatoria amphibia (SGBRA 06)					
Ethyl acetate	-	-	-	-	-
Pyridine	9	9	7.5	7	6
n-Butanol	7.5	7.5	9	8	7.5
Water	-	-	-	-	-
Oscillatoria chlorina (SGBRA 07)					
Ethyl acetate	-	-	-	-	-
Pyridine	7.5	-	8	6	-
n-Butanol	9	9	7.5	7.5	9
Water	-	-	-	-	-

Table 4. Antivibrio activity of selected cyanobacterial species

Name of the Cyanobacteria	Zone of inhibition (dia in mm)				
	Vibrio alginolyticus	***V. fluvialis***	***V. mimicus***	***V. anguillarium***	***V. cholerae***
Oscillatoria sp. (SGBRA 05)					
Ethyl acetate	8	6	8	7.5	10
Pyridine	14	15	18	15	10
n-Butanol	10	10	10	10	10
Water	-	-	-	-	-
Oscillatoria subbrevis (SGBRA 04)					
Ethyl acetate	-	-	-	-	-
Pyridine	10	9	10	12	11
n-Butanol	10	12	10	10	9
Water	-	-	-	-	-
Oscillatoria amphibia (SGBRA 06)					
Ethyl acetate	10	8	10	-	-
Pyridine	15	13	15	10	-
n-Butanol	15	10	15	10	-
Water	-	-	-	-	-
Oscillatoria chlorina (SGBRA 07)					
Ethyl acetate	7.5	7.5	7.5	10	10
Pyridine	15	20	15	15	15
n-Butanol	15	20	15	15	15
Water	-	-	-	-	-

The growth of *V. fluvialis, V. mimicus* was also strongly inhibited by the n-butanol and pyridine extract of *O. amphibia* and *O. chlorina* species. However, the ethyl acetate extract showed no significant antivibrio activities. Positive control (Ciprofloaxin mg/ml) was assayed for reference purposes and also we observed the negative control as selective solvent as pyridine and n-butanol.

DISCUSSION

In recent years, an increasing number of natural marine products have been reported to display biological activities [12]; [19]. [7] summarized that the antibacterial activity of the extract of from the marine green algae *U. lactuca* were assayed against a panel of gram positive, gram negative bacteria, one fungal species, and three MRSA strains. The most susceptible organisms were MRSA CCARM 3561, MRSA CCARM3115, and MRSA CCARM3089, with inhibition zones of 27, 26 and 24 mm, respectively. The growth of *E. coli* D31 (17mm), *E. aerogenes* (20 mm), *S. typhimuriumm* (18 mm), *V. paraheamolyticus* (18 mm), and *E. tarda* (18mm) were also strongly inhibited by the ethyl – ether extract of cyanobacteria. The revealed that the growth of *S.typhi* (11mm) and *St. aureus* (10 mm) were strongly inhibited by n- butanol, pyridine extract.

Methanol, n – Proponol, petroleum ether, ethanol, chloroform, and water was employed for the extraction antimicrobial compounds from cyanobacteria. Antifungal activity against *C. albicans* was determined using the standard serial broth micro – dilution assay of [22]. Maximum antifungal activities in case of pyridine and n-Butanol extraction as observed in the present study are in accordance with earlier reports [16]; [20]. The present study deals with, the maximum zone of inhibition was observed in pyridine and n- butanol extracts of *O.subbrevis* against *Aspergillus wentii* and *Candida albicans* (10 dia in mm).

Vibrio fluvialis was isolated from aquatic environments in India [18] and in other countries [3]; [15]. The vibrio species are highly susceptible to fluoro quinolones [2]; [8]; [13]; [9] analysed MIC values of 13 antibiotics and reported that chloramphenical, nalidixic acid, oxytetracycline, trimethoprim and ciprofloaxacin appear to be effective against susceptible micro – organisms by inhibiting protein synthesis. Enteropathogenic gram negative *Bacilli* such as *Salmonella, Vibrio* sp. and *Aeromona*s species are all susceptible to the quinolones [5]. The study revealed that all the test organisms were susceptible to ciprofloaxcin, and the *O. subbrevis* (18, 15, 14 and 10 mm) showed the better result against *Vibrio* pathogens tested. The organic solvents of cyanobacterial extracts of pyridine, n-butanol showed the activity against selected five *Vibrio* pathogens.

The present study focused on the commercial application of selected cyanobacterial isolates such *Oscillatoria* sp, *O. subbrevis, O. smphibia* and *O. chlorina.* Very promising were obtained on the crude cyanobacterial extracts against pathogenic bacteria, fungus and *Vibrio* isolates. Among these various solvents used for the extract, pyridine and n- butanol showed better results.

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