

Potential Antimicrobial Activity of Cyanobacteria *Oscillatoria Boryana* and *Oscillatoria Pseudogeminata* Isolated from Odisha Coast, India

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Cyanobacteria as proven source of bioactive secondary metabolites have recently found immense application in pharmaceutical sector. The goal of this study was to find out the antimicrobial property in two species of cyanobacteria, *Oscillatoria boryana* and *Oscillatoria pseudogeminata* against different pathogenic microorganisms. Aqueous and different solvent extracts (acetone, methanol, benzene) were prepared and assayed for antimicrobial activity against four bacterial (*Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholerae* and *Escherichia coli*) and four fungal (*Aspergillus niger*, *Candida albicans*, *Penicillium verrucosum* and *Fusarium oxysporum*) pathogens. The study results recorded highest antimicrobial activity in methanol extracts of *O. boryana* showing significant zone of inhibition against *V. cholerae* (20 ± 0.74 mm) and *C. albicans* (17 ± 0.80 mm). Least antimicrobial activity was detected in aqueous extract among all the test species. The purified active fractions were analysed using GC-MS, where dominant compounds that are responsible for the inhibitory activity such as n-hexadecanoic (15.42%) 5-Methyl-2-phenyl indolizine (13.3%), Acetic acid (12.47), 5-Nitro-3-cyano-2(1H)-pyridone (10.37 %), 9-Oxo-9,10-dihydro acridine-4-yl (6.8%) were detected. Thus the obtained results suggests that studied species of cyanobacteria can be prospective in biotechnology as an alternative sources of antimicrobial substances.

Keywords: Antimicrobial; Bioactive; Cyanobacteria; Extract; MIC.

Cyanobacteria are photosynthetic organism found in a variety of ecosystems, including fresh, marine, brackish water and terrestrial environments. Cyanobacteria were found to produce different types of secondary metabolites which inhibit the growth of other microorganisms in their surrounding.^{1,2} Cyanobacteria possess natural bioactive substances that are difficult to produce by chemical synthesis³. These compounds has a wide range of biological activities such as antimicrobial,

antioxidant, anticancer etc^{4,5-7}. The search for antibiotics and other pharmacologically active substances in cyanobacteria has received more attention at present time⁸. A number of literatures are available on antimicrobial substances produced from microalgae and cyanobacteria⁹. However, the compounds responsible for antimicrobial properties in cyanobacteria are yet to be identified for its maximum efficacy^{10,11}. In the marine environment cyanobacteria were found to synthesize a large

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array of chemical compounds due to biotic and abiotic stress¹². Antimicrobial substances produced by cyanobacteria are of interest for research not only protective for pathogens, but also as pharmacological important bioactive secondary metabolites¹³. Moreover, the differential antimicrobial activity of cyanobacteria is correlated to the selected species and the solvents used in experiments¹⁴⁻¹⁷. Despite the fact that several work has been carried out on antimicrobial property of cyanobacteria of different habitats but a limited reports were available on the species found across Odisha coast. Thus the current study intends to find out the antimicrobial properties of two selected predominant species (*Oscillatoria boryana* and *Oscillatoria pseudogeminata*), obtained from Odisha coast.

MATERIALS AND METHODS

Collection, culture condition and isolation of cyanobacteria

The test organism (Cyanobacteria) were collected from different sites across Odisha coast (Fig 1A & B) by using a plankton net (2i) and the samples attached to different substratum using forceps. The samples were isolated to unialgal culture by repeated subculture and serial dilution, observed under trinocular research microscope (Make: Dewinter Model: "SELECT" DGI-1000) and identified by standard procedure¹⁸⁻²⁰. The isolated cyanobacterial species were grown in 1 litre flasks with aeration to obtain large biomass using ASN III medium with illumination of 30 imol photon m⁻² s⁻¹ along with light/dark cycle of 16 h/8h at 25±1°C temperature.

Test Cyanobacteria

Morpho-taxonomic identification

Oscillatoria boryana: Class- Cyanophyceae; Order- Nostocales; Family- Oscillatoriaceae; Genus- *Oscillatoria*; Species- *boryana*

Trichome coiled like a screw at the apices or totally, sometimes straight compressed near cross walls, 6-8µ broad, sometimes slight granulation observed adjacent to cross walls, cells at the apices 4-6µ elongated or rounded or more or less pointed, not capitates, calyptra absent, the breadth of the trichome varies very much between 3.3-5.0µ, 4-6.6µ, 5-7µ and 6-8µ (Fig.1B-i).

Oscillatoria pseudogeminata: Class- Cyanophyceae; Order- Nostocales; Family- Oscillatoriaceae; Genus- *Oscillatoria*; Species- *pseudogeminata*

Thallus blue-green in colour, trichomes coiled, ends are not attenuated, cells are 1.3-2.2µ broad, about 2.6µ long, the cross walls are not squeezed but thickened, non granulated and cells are spherical, calyptras absent (Fig.1B-ii).

Cyanobacterial extract preparation

The cyanobacterial cultures (10-14days old) were harvested by centrifugation (3500 rpm for 15min) and dried at 45 °C in a hot air oven. The dried samples were grounded using a sterile mortar and pestle. Then, 0.5 g of grounded sample was mixed with 10 ml of solvents (acetone, methanol, benzene and sterile water) used and kept overnight at room temperature in a rotary shaker for complete extraction. At 4000rpm for 15minutes the extracts were centrifuged and concentrated at 50°C under reduced pressure. The dried residue was dissolved in DMSO to obtain final concentrations of 1mg/ml, and used for antimicrobial activity²¹.

Pathogenic strains

Four pathogenic bacteria i.e two Gram +ve (*Staphylococcus aureus* MTCC-96, *Bacillus subtilis* MTCC-441) and two Gram -ve (*Vibrio Cholerae* MTCC-3906, *Escherichia coli* MTCC-443) and four pathogenic fungi (*Candida albicans* MTCC 183, *Aspergillus niger* MTCC-1344, *Penicillium verrucosum* MTCC-1758, *Fusarium oxysporum* MTCC-284) were used for the experimental purpose. These microorganisms were procured from Institute of Microbial Technology, Chandigarh and kept in the Department of Biotechnology, Maharaja Sriram Chandra Bhanja Deo University, Takatpur, Mayurbhanj, Odisha.

Antibacterial assay

The agar cup method²² is used for the antibacterial study, using Mueller-Hinton agar (MHA) media for bacteria. Petriplates with 20ml of sterilised solid media was inoculated with 20µl of test pathogens (10⁶ CFU/ml) were uniformly swabbed. Six mm diameter wells were formed and 50 µl of cyanobacterial extracts (DMSO, 1 mg/ml) were put into the wells and placed for incubation at 37 ± 1 °C for 24 hours. The inhibition zone formed around the wells was measured after incubation period. The average value of diameter of three

plates was recorded. Ampicillin at a concentration of 100µg/ml are used as positive control.

Antifungal assay

The antifungal activity study was carried out on potato dextrose agar medium (PDA).²² Sterilized potato dextrose agar (PDA) was poured into sterile petriplates (20ml), solidified and inoculated with 20µl of fungal test pathogens (10⁶ CFU/ml) and swabbed uniformly. Six mm diameter wells were formed and 50 µl of cyanobacterial extracts (DMSO, 1 mg/ml) were put into the wells and placed for incubation at 25 ± 2 °C for 72 hours. The inhibition zone formed around the wells was measured after incubation period. The average value of diameter of three plates was recorded. Clotrimazole at a concentration of 100µg/ml are used as positive control.

Study of Minimum inhibitory concentration (MIC)

MIC experiment was done using 96 well micro titer plates containing broth medium. The first series of well was given 50 µl of Mueller-Hinton broth (MHB) and 50 µl bacteria culture as negative control. Starting from second series of wells until eighth row were embedded with various concentration of extract (15.62, 31.25, 62.5, 125, 250, 500 and 1000µg/ml) by two fold dilution in MHB media with pathogenic microorganisms as control and kept at 37° C for 24 h and subsequently were analysed with microplate reader (make-TECAN, model-M200PRO) and interpreted.²³⁻²⁵

Gas Chromatography/Mass Spectrometry (GC-MS) analysis

The GC-MS evaluation was determined at Analytical Chemistry, Triyat Scientific Co., Nagpur, Maharashtra, India. The GC-MS analysis was done

using a Agilent 7890A Gas Chromatography system equipped with a HP-5 column (30ml x 250µm ID, 0.25µm thick film) and Agilent 5975C inert Mass Spectrometer Detector with Helium as carrier gas. The sample extract was injected into the injector port and the Helium gas expel the sample down the column where different chemical constituents get separated. The injection port was maintained at 80 °C for 4 minutes. The run time was continued for 40 minutes. The flow rate of 1.50ml/min; injector and column oven temperature of 280°C and 80°C; injector split ratio of 20:1 was set. GC-MS mass spectrum interpretation was conducted and compared with the reference data base from NIST.

Statistical analysis

Statistical analysis of results is performed. The values are expressed as mean with standard deviations (±SD) and were subjected to one way ANOVA, and the results were the average of three replicates (p < 0.05).

RESULTS

Antibacterial Activity

The results obtained in the evaluation of the antibacterial activity of different solvent extracts of two marine cyanobacteria *Oscillatoria boryana* and *Oscillatoria pseudogeminata* against four pathogenic bacteria (*S. aureus*, *B. Subtilis*, *V. cholerae* and *E. coli*) strains using the agar well diffusion method was shown in Table 1(A&B). Differential antimicrobial activity was observed in separate solvent extracts against particular pathogen. The maximum zone of inhibition of 20mm was detected against *V. cholerae* using



Fig. 1A. Sample collection sites across Odisha Coast

methanol extracts of *O. boryana*, followed by 18mm and 17mm with *B. Subtilis* and *S. aureus* respectively (Table 1A). A Smaller zone of inhibition of 9mm was found in aqueous extract of *O. Pseudogeminata* against *B. Subtilis* and *E.*

coli (Table 1B). Further, larger inhibition zone was observed in methanol extract than by acetone and benzene whereas minimum zone of inhibition was found in aqueous extract in both the test species of marine cyanobacteria. The study result reveals

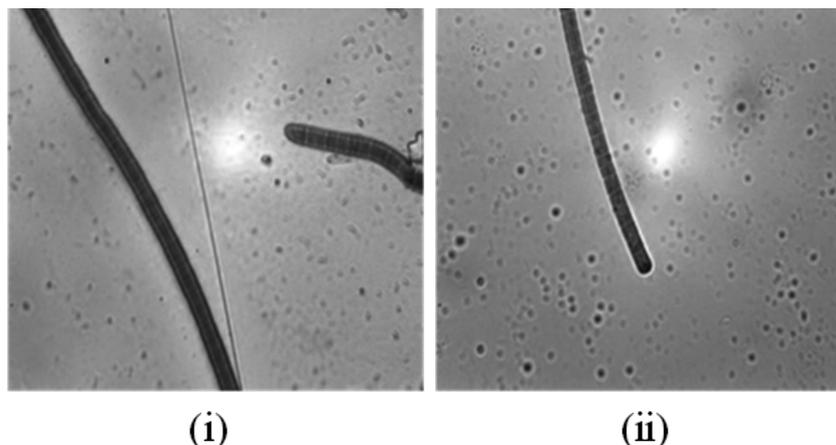


Fig.1B. Microscopic Photograph of two test cyanobacteria (i) *Oscillatoria boryana*
(ii) *Oscillatoria pseudogeminata*

Table 1A. Antibacterial and antifungal activity of different solvent extract of *Oscillatoria boryana* with bacterial and fungal pathogens

Bacterial and Fungal Pathogens	Acetone	Inhibition zone diameter (mm)		
		Methanol	Benzene	Aqueous
<i>S. aureus</i>	16±0.82	17 ±0.73	13±0.75	11±0.76
<i>B. subtilis</i>	15±0.77	18±0.76	14±0.79	10±0.83
<i>V. cholerae</i>	16 ±0.79	20±0.74	14±0.78	10±0.80
<i>E. coli</i>	14±0.81	15 ±0.78	13 ±0.76	10±0.75
<i>A. niger</i>	14±0.76	16±0.81	10±0.75	09±0.77
<i>C. albicans</i>	12±0.79	17±0.80	13±0.77	10±0.81
<i>P. verrucosun</i>	13 ±0.84	15±0.76	12±0.80	10±0.77
<i>F. oxysporum</i>	12±0.81	15 ±0.76	11 ±0.73	10±0.82

Table 1B. Antibacterial and antifungal activity of different solvent extracts of *Oscillatoria pseudogeminata* with bacterial fungal pathogens

Bacterial and Fungal Pathogens	Acetone	Inhibition zone diameter (mm)		
		Methanol	Benzene	Aqueous
<i>S. aureus</i>	12±0.73	13±0.81	11±0.74	10±0.79
<i>B. subtilis</i>	10±0.75	12±0.76	10±0.77	09±0.77
<i>V. cholerae</i>	11±0.82	11±0.79	10±0.79	11±0.75
<i>E. coli</i>	10±0.77	11±0.84	11±0.81	09±0.77
<i>A. niger</i>	12±0.82	14±0.80	12±0.79	11±0.75
<i>C. albicans</i>	11±0.83	13±0.84	10±0.76	11±0.77
<i>P. verrucosun</i>	10±0.75	11±0.82	10±0.78	09±0.76
<i>F. oxysporum</i>	12±0.77	12±0.79	09±0.82	08±0.72

that the methanolic extract of *O. borynana* found to have significant antibacterial activity in *V. cholerae* and in contrary negligible activity was marked in aqueous solvent in agar well diffusion assay.

Antifungal Activity

The results exhibiting the in vitro antifungal activity of different solvent extracts of two marine cyanobacteria *O. borynana* and *O. pseudogeminata* against four fungal pathogens (*A. niger*, *C. albicans*, *P. verrucosum* and *F. oxysporum*) strains using the agar cup method was shown in Table 1A&B. Maximum zone of inhibition of 17mm against *C. albicans* and 14mm against *A. niger* in *O. borynana* and *O. pseudogeminata*

respectively in methanol extract and the minimum zone of inhibition of 9mm was found in aqueous extract of *O. borynana* against *A. niger* and 8mm in *O. pseudogeminata* against *F. oxysporum* (Table 1A& B).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was estimated at a concentrations range of 1000µg/ml to 15.62µg/ml and optimum dilution showing inhibition was observed as MIC value (Table 2). The MICs of the extracts were measured using serial dilutions up to 1000µg/ml of extracts in DMSO. The MIC detected for *O. borynana* was 31.2 µg/ml and was 125µg/ml for *O. pseudogeminata*.

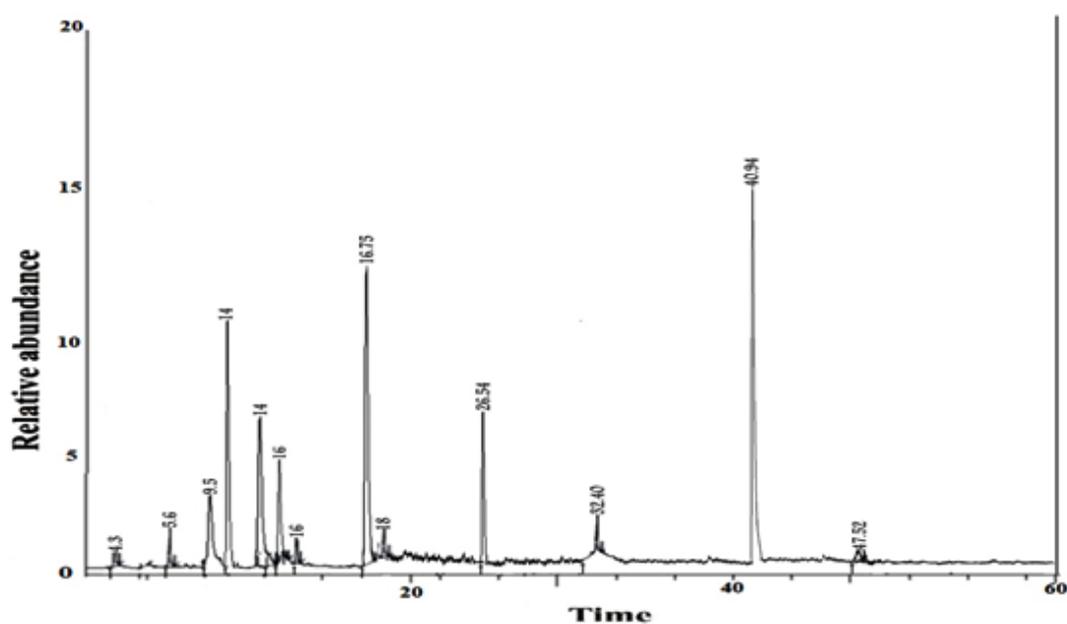


Fig. 2. GC/MS analysis of methanolic extract of *O. borynana*

Table 2. Minimum inhibitory concentration (MIC) of *O. borynana* and *O. pseudogeminata* against bacterial and fungal pathogens

Pathogens	Minimum inhibitory concentration (µg/ml)							
	<i>Oscillatoria borynana</i>				<i>Oscillatoria pseudogeminata</i>			
	Acetone	Methanol	Benzene	Aqueous	Acetone	Methanol	Benzene	Aqueous
<i>S. aureus</i>	125	125	500	500	250	250	500	500
<i>B. subtilis</i>	250	62.5	250	250	500	125	500	500
<i>V. cholerae</i>	125	31.2	250	500	500	500	250	250
<i>E. coli</i>	250	125	500	500	500	500	500	1000
<i>A. niger</i>	125	125	500	500	250	125	500	250
<i>C. albicans</i>	250	62.5	250	250	250	125	500	500
<i>P. verrucosum</i>	125	125	250	500	500	500	500	500
<i>F. oxysporum</i>	500	125	500	500	250	250	1000	1000

Table 3. Compounds identified in methanol extract of *O. boryana* as revealed by GC-MS analysis.

No	Compounds name	Mol. Formula	Mol. weight (g/mol)	Ret. Time RT (mins)	Peak area (%)
1	Propane,1,1,3-triethoxy-	C ₉ H ₂₀ O ₃	176.25	4.3	0.92
2	Benzoic acid	C ₇ H ₆ O ₂	126.12	5.6	0.78
3	Undecanoic acid	C ₁₁ H ₂₂ O ₂	186.29	9.5	1.73
4	5-Nitro-3-cyano-2(1H)-pyridone	C ₅ H ₄ NH(O)	165.11	14	10.37
5	Acetic acid	C ₂ H ₄ O ₂	60.05	14	12.47
6	9-Oxo-9,10-dihydro acridine-4-yl	C ₁₃ H ₉ N	179.13	16	6.8
7	Hexadecanoic acid	C ₁₈ H ₃₆ O ₂	284.48	16	5.02
8	5-Methyl-2-phenyl indolizine	C ₁₅ H ₁₃ N	207.27	16.75	13.3
9	Phytol	C ₂₀ H ₄₀ O	128.17	18	1.58
10	Silicic acid	Si(OH) ₄	96.11	26.54	5.02
11	Benzenemethanol	C ₇ H ₈ O	108.14	32.40	1.9
12	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.4	40.94	15.42
13	Erucic acid	C ₂₂ H ₄₂ O ₂	338.57	47.52	0.87

The MIC result thus reveals that *O. boryana* has significant antimicrobial property as compared to *O. Pseudogeminata*.

GC-MS Analysis of methanolic extract of *O. boryana*

The chemical constituents of methanol extract of *O. boryana* detected by GC-MS was presented in (Fig. 2, Table 3). Major chemical components present in the methanol extract of *O. boryana* indicated the occurrence of many novel bioactive compounds. Thirteen compounds are detected in methanolic extract of *O. boryana* with the highest value of n-hexadecanoic acid (15.42 %) followed by 5-Methyl-2-phenyl indolizine (13.3%). The other prevailing compounds were Acetic acid (12.47%), 5-Nitro-3-cyano-2(1H)-pyridone(10.37%), 9-Oxo-9,10-dihydro acridine-4-yl (6.8%), Hexadecanoic acid and Silicic acid 5.02% each. The analysis also revealed the presence of few biologically active compounds in low concentration such as Benzenemethanol (1.9%), Undecanoic acid (1.73 %), Phytol(1.58%), Propane,1,1,3-triethoxy (0.92 %), Erucic acid (0.87%) and Benzoic acid (0.78 %). The study results infers that possibly these chemical compounds are important in terms of their antimicrobial activity^{27,28}.

DISCUSSION

Unregulated use of antibiotics gives rise antibiotic resistance in bacteria, fungi and thus raises an issue of health hazards. The alarming

increase in microbial contamination has boosted the search for new, novel and secure candidate to fight against infection. Thus more specific research on identification of bioactive compounds derived from various microalgae and cyanobacteria is of interest at present. Cyanobacteria are potential organism for producing a variety of bioactive secondary metabolites. These compounds derived from cyanobacteria were found to possess antimicrobial activity. Based on this fact; two species of marine cyanobacteria *Oscillatoria boryana* and *Oscillatoria pseudogeminata* were studied for antimicrobial activity. The findings of present work explained that among the two test cyanobacteria, methanol extract of *O. boryana* exhibited significant antimicrobial activity than *O. pseudogeminata*. In the similar pattern,²⁹also reported that *Oscillatoria brevis* has antibacterial activity against *S. aureus*(ATCC 13565);*B. cereus*(EMCC 1080); *S. typhi* (ATCC25566); *P. aeruginosa* (NRRL B-272) and *K. Pneumonia* (LMD 7726). Earlier study by Sethubathi and Prabu³⁰ which is in agreement to our result revealed that the crude extracts of *Oscillatoria* sp.; *Phormidium* sp. and *Lyngbya majuscula* were found to possess antimicrobial activity against pathogens such as *S. mutants*; *S. aureus*; *P. aeruginosa*; *B. Subtilis* and *K. pneumonia*. The MIC value as recorded in the present piece of work was promising and the cyanobacterial extracts showed significant antimicrobial potency against *V. cholerae*; *B. subtilis* and *C. albicans* which match

to the work carried out by Wijesekara and Manage³¹ with *Oscillatoria boryana* extract showing maximum antimicrobial activity against *S. aureus* as compared to *S. typhi*. Moreover, the findings of the present investigation on GC-MS analysis showed the presence of n-hexadecanoic acid, Acetic acid, 5-Nitro-3-cyano-2(1H)-pyridoneectas predominant compounds which might be responsible for antimicrobial activity in *Oscillatoria boryana*, which matches to the work carried out by Mundt *et al.*³² and Shanab^{33,34}. Similarly, El-Syad *et al.*³⁵ detected presence of hexadecanoic acid and bis(2-ethylhexyl) phthalate in the phenolic extract of *Spirulina* and in addition, phytol was previously found in a marine cyanobacteria, *N. muscorum* and in the *S. platensis*³⁶ showing antimicrobial property. Recently, Hobby *et al.*³⁷ stated that modification of phospholipid composition by fatty acids suppress the antimicrobial compounds. However, there is no any specific reason established between chemical classes and their role in bioactive properties such as antimicrobial activity³⁸. The saturated fatty acids might be binding the cell membrane and alter the cellular construct thereby enhancing in antimicrobial activity. Thus, deeper study is required to focus on the bioactivity of metabolites synthesise in cyanobacteria for the production of novel antimicrobial compounds that maybe useful for commercial purpose.

CONCLUSION

This study demonstrates that both the cyanobacteria have the potential to produce potent antimicrobial compounds that act against human pathogens. Among different solvents used, the methanolic extract of both the species of *Oscillatoria* showed better activity, inhibiting the growth of test pathogens. The present research has shown that the antimicrobial activity of different species of cyanobacteria is dependent on the solvents used to make the extracts and the effect of those solvents. It is thought that the findings obtained from this investigation can be used for future research and for the production of antibacterial drugs of cyanobacterial origin. Moreover, this study has paved the way towards demonstrating the presence of potential high-value pharmaceutical antimicrobials having ample scope

for further investigation towards drug development in pharmaceutical and health care industry.

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Conflict Of Interest

The authors declares no conflict of Interest.

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