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Antimicrobial Activity of Cyanobacteria Isolated from Freshwater Lake

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Abstract: The present study was aimed to collect and identify the cyanobacteria from Samuthiram Lake, Thanjavur, Tamilnadu, India. Totally 46 species of freshwater cyanobacteria were collected and cultured in BG_{11} medium. Based on their growth characteristics, five species namely *Oscillatoria latevirns, Phormidium corium, Lyngbya martensiana, Chrooccocus minor* and *Microcystis aeroginosa* were selected for the production of antimicrobial agents against organisms like *Bacillus subtilis, Staphylococcus aureus, Streptococcus mutants, Escherichia coli, Micrococcus mutans, Klebsiella pnuemoniae, Saccharomyces cerevisiae* and *Candida albicans*. Antimicrobial activity of ethanol and acetone on Gram-negative bacteria, methanol extracts on Gram-positive bacteria, ethanol extracts on both Gram-positive and Gram-negative organisms was observed. In addition, *Oscillatoria latevirens, Chrococcus minor and Microcystitis aeroginosa* were found to have antifungal activity on *Candida albicans*.

Key words: Antimicrobial activity · Freshwater cyanobacteria · Antifungal activity

INTRODUCTION

Cyanobacteria, the blue green algae are an assemblage of gram negative eubacteria widely distributed throughout the world. Cyanobacteria are rich sources of structurally novel and biologically active metabolites. Recent studies indicate the presence of some bioactive compounds in the freshwater blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities [1, 2, 3].

In general, isolation of bioactive compounds from cyanobacteria is done with two objectives. One is to discover new compounds for pharmaceutical, agricultural or biological application. The other is for the better understanding of the interactions of individual organisms within their natural communities. For each of these purposes, there is a need to screen new organisms [4]. Biologically active substances were proved to be extracted from microalgae. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity. Temperature of incubation, pH of the culture medium, incubation period, medium constituents and light intensity are the important factors influencing the production of antimicrobial agents [5].

The aim of the present work was to study the antimicrobial activity of cell extracts of various cyanobacteria *in vitro* against some selected Gram-positive, Gram-negative bacteria and pathogenic fungi.

MATERIALS AND METHODS

Cyanobacterial Samples: Water samples containing cyanobacteria were collected from various sites of Samuthiram lake, Thanjavur, Tamil Nadu, India, during Jan. 2011. Samples were isolated and identified by standard microbiological methods [6, 7, 8]. To study the antimicrobial activity of cyanobacterial species such as *Oscillatoria latevirens, Phormidium corium, Lyngbya martensiana, Chrococcus minor* and *Microcystis,* microorganisms like *Bacillus subtilis*, *Staphylooccus aureus, Streptococcus mutans, E. coli, Micrococcus luteus, Klebsiella pneumoniae, Saccharomyces cerevisiae* and *Candida albicans* were tested and they were obtained from Govt. Hospital, Trichy. Bacterial strains were inoculated on to in nutrient broth and incubated at 37°C for 24 hrs. The yeast and fungal strains were also inoculated on to glucose peptone broth and incubated at 30°C for 5 days.

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Preparation of the Algal Extracts: Ten days old algal cultures were centrifuged and the pellets were collected, weighted and used for extraction of antimicrobial agents. 0.5 g of each of the five algal pellets was extracted in 10 ml of either acetone, ethanol, methanol or diethyl ether. All the extracts were preserved at +4C [9].

Determination of the Inhibitory Effect of the Algal Extracts: Antibacterial and antifungal activities of cyanobacteria extracts were tested by agar well diffusion method. Nutrient agar, plates were inoculated with 100 µl of a 24 h broth culture of the test bacteria or 100 µl of a 5 day glucose peptone broth culture of the test fungi and yeast. Two wells (6 mm) were made and filled with 100 µl extract. The inoculated plates were incubated for 24 h at 37°C for bacteria and inoculated for 3 days at 30°C. for fungi. The diameter of the inhibition zone was measured with calipers and the results were recorded [10]. Comparing the antimicrobial activity of cyanobacteria with standard antibiotics (erythromycin, tetracycline and amoxicillin) and fungicides (itraconazole and polyoxylin) was also done.

RESULTS AND DISCUSSION

Totally 43 species of cyanobacteria were isolated and based on their growth characteristic 5 species:- *Oscillatoria latevirens, Phormidium corium, Lyngbya martensiana, Chroococcus minor* and *Microcystis aeroginosa* were selected for antibacterial and antifungal activity testing (Table 1). It is clear from the study that the diameter of the inhibition zone depends mainly on the type of the algal species, type of the solvent used and the tested bacterial and fungal organisms.

Concerning the antibacterial effects, the results clearly indicated that acetone extracts of *Oscillatoria latevirens* and ethanol extracts of *Phormidium corium* gave the highest antimicrobial activity against *Staphylococcus aureus* & *Streptococcus mutans and Micrococcus mutans & S. aureus* respectively. The results also indicated that these two extracts had a moderate activities towards *Klebsiella pneumoniae*. The antibacterial effect of *Microcystis aeroginosa* and *Chrococcus minor* extracts against *Staphylococcus aureus* and *E. coli* than that of *Bacillus subtilis* and *Micrococcus mutants*. At the same time extract of *Lyngbya martensiana* had an antibacterial effect towards *Bacillus subtilis Staphylococcus aureus* and *E. coli* but negative effect towards *Streptococcus mutans, Micrococcus mutans, Klebsiella pneumoniae* (Table 1). These results go in harmony with those obtained [11], who found that some microalgae had high biological activity against *Bacillus subtilis, B. thungiensis, Bacillus megaterium, E. coli, Pseudomonas aeruginosa, Candida tropicalis* and *Sauatromyces cerevisiae*.

Table 1: Antibacterial and antifungal activities of different cyanobacteria extracts

		Diameter of inhibition zone (cm)							
		Bacterial species				Fungal species			
Algal species	Organic solvent	*B. subtilis*	*S. aureus*	*S. mutans*	*E.0coli*	*M. mutans*	*K. pneumonia*	*S. cerevisae*	*C. albicans*
Oscillatoria latevirens	Ethanol	2.2	2.4	3.0	2.0	1.8	2.0	1.8	1.2
	Acetone	R	3.8	2.0	R	1.6	1.2	1.8	R
	Diethyl ether	2.0	R	1.8	1.0	1.2	R	1.6	2.0
	Methanol	1.0	1.5	3.3	4.0	2.0	2.0	1.5	0.9
Phormidium corium	Ethanol	1.0	2.2	2.0	1.2	2.5	1.8	1.0	0.6
	Acetone	1.2	4.6	4.6	2.0	1.2	2.5	2.5	1.2
	Diethyl ether	R	1.0	R	1.8	1.0	1.5	1.8	1.5
	Methanol	1.0	R	0.6	2.0	1.6	2.0	1.2	0.8
Lyngbya martensiana	Ethanol	2.5	1.8	R	1.6	1.0	R	1.4	1.0
	Acetone	1.5	2.0	2.2	1.8	2.2	2.2	0.8	1.2
	Diethyl ether	2.2	1.6	R	1.8	1.4	R	1.6	1.5
	Methanol	1.8	2.0	1.2	1.6	R	1.8	1.2	2.5
Chrococcus minor	Ethanol	R	1.0	2.0	R	1.8	R	1.6	2.0
	Acetone	1.8	3.4	3.0	2.2	1.0	1.0	1.8	2.4
	Diethyl ether	1.0	2.0	R	1.8	2.0	2.2	1.6	0.6
	Methanol	2.0	1.8	1.5	R	2.0	1.6	1.0	0.8
Microcystis aeruginosa	Ethanol	R	1.8	R	2.0	1.8	1.5	1.0	1.5
	Acetone	1.0	3.3	1.5	3.0	1.6	R	1.6	1.2
	Diethyl ether	R	1.8	1.5	2.0	1.8	1.0	2.6	2.0
	Methanol	2.0	2.5	1.6	R	2.6	2.0	1.8	2.4

R – Resistant; ND – Not Detected

Table 2: Diameters of inhibition zone (cm) exhibited by test microorganisms against standard antibiotics and fungicides

		Diameter of inhibition zone (cm)				
		Standard antibiotics			Standard fungicides	
	Test organism	Erythromycin	Tetracycline	Amoxicillin	Itraconazole	Polynaxylin
Bacterial species	*Bacillus subtilis*	3.2	2.8	5.2	ND	ND
	S. aureus	R	R	R	ND	ND
	S. mutans	2.8	2.2	3.0	ND	ND
	E. coli	4.8	4.3	3.8	ND	ND
	M. mutans	R	2.0	2.2	ND	ND
	Klebsiella pneumoniae	3.0	R	2.0	ND	ND
Fungal species	*Saccharomyces cerevisiae*	ND	ND	ND	R	R
	Candida albicans	ND	ND	ND	2,6	1,8

R – Resistant; ND – Not Detected

The results also proved that ethanol was the best solvent for the extracting the antibacterial and antifungal agents from *Oscillatoria latevirens,* while acetone was the best organic solvents for extracting antibacterial and antifungal agents from *Phormidium corium* and *Lyngbya martensiana* (Table 1). The same results were also obtained [12, 13]. It was reported that extracts of *Oscillatoria, Phormidium* and *Lyngbya* obtained by different solvents exhibited antimicrobial activity on both Gram positive and Gram negative organisms [14, 15].

Many investigators mentioned that the acetone extract of cyanobacteria revealed antibacterial activity on *E. coli, Bacillus subtilis and Pseudomonas aeruginosa* [16, 17]. The results also proved that acetone and ethanol were the best solvents for extracting the antibacterial and antifungal agents from *Lyngbya martensiana* and *Oscillatoria latevirens*. However, the methanolic extract of cyanobacteria has been investigated [18] for *in vitro* antimicrobial activity against *Proteus vulgaris, Bacillus cereus, E. coli, Pseudomonas aeruginosa, Aspergillus niger* and *A. flavus* using agar cup plate method. The present study also supported that methanol extract showed moderate activities.

Table 1 also clearly shows that the antifungal activities of nearly all the extracts of cyanobacteria towards tested fungi gave positive results but with varying degrees. Acetone extract of *Phormidium corium,* methanol extract of *Lyngbya martensiana* and diethyl ether extract of *Microcystis aeruginosa* gave the largest inhibition zones on the plates of the tested fungi. The fungicidal activity of *Anabaena* against a set of phytopathogenic fungi was examined [19].

The antimicrobial activity of the test microorganisms against standard antibiotics and fungicides are shown in Table 2. It was found that the effect of standard antibiotics was more than that of algal extracts on *Bacillus subtilis* and *E. coli*. While, the antibacterial effect of algal extracts on *S. aureus, S. mutans* and *M. mutans* were higher than those of standard antibiotics. On the other hand, the effect of the standard antibiotics on *Klebsiella* was variable and was more or less similar to that of the investigated algal extracts.

By comparing, the antifungal effect of all tested cyanobacteria, it was more or less positive against *Sacchromyces cerevisiae*. While itraconazole and polynoxylin showed no effect against the tested fungi. In this study the antimicrobial activity of microalgae could be explained by presence of cyclic peptides, alkaloids and lipopolysacchairdes. The antimicrobial activities of *Bacillus subtilis, S. aureus, E. coli, Pseudomonas aeruginosa, Saccharomyces cerevisiae* and *Candida albicans* against standard anbitiocis and fungicides were studied [20].

It can be concluded that the extracts obtained by various solvents used in this study had antibacterial and antifungal activities and that these extracts could be much more effective when compared with contemporary antibiotics and fungicides.

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