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Antimicrobial Activities of Some Selected Cyanobacteria from Fresh Water Bodies of Sri Lanka

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Abstract: The aim of the study was to investigate the anti-pathogenic activities of selected cyanobacteria isolated from freshwater bodies of Sri Lanka. Ethanolic extract of six uni algal cultures *i.e.,Oscillatoria* sp., *Synechococcus* sp., *Dermocarpa* sp., *Chroococcus*sp., *Nostoc* sp. and *Microcystis*sp. were tested against five plant pathogens and seven human pathogens using agar disk diffusion method. Human pathogens include *Escherichia coli* ATCC 35218, *E. coli* ATCC 25922, *Staphylococcus aureus*ATCC 25923, *Pseudomonas aeruginosa*ATCC 27853, *EntarococcusfaecalisATCC* 29212, *Klebsiellapneumoniae*and *Candida albicans*. Plant pathogens include *Colletotricummusae, Colletotricumcoccodes, Pomopsis*sp., *Tricoderma*sp., and *Cladosporiumcladoplorioides*. The cyanobacterial extract at the rate of 0.01 g/ml and 0.025 g/ml did not show any zone of inhibition on pathogenic fungi and bacteria. In the present study, at the rate of 0.05 g/ml extract concentration, *Synechococcus* sp. showed the zone of inhibition against highest 7 pathogens such as *E. coli ATCC 35218, E. coli ATCC 25922, P. aeruginosa*ATCC 27853, *K. pneumonae, E. faecalis ATCC 29212, C. musae,* and *C. coccodes.* Cyanobacteria *Oscillatoria*sp. showed the zone of inhibition against six pathogens *i.e*., *E. coli* ATCC 35218, *E coli* ATCC 25922, *S. aureus*ATCC25923, *K. pneumonia, C. musae*and *C. coccodes.* However,*Nostoc* sp. showed zone of inhibition against 5 pathogens *i.e*., *E. coli* ATCC 35218, *E. coli* ATCC 25922, *S. aureus*ATCC 25923, *K. pneumonae*and *E. faecalis*ATCC 29212. At the same time cyanobacteria *Chrococcus*sp. and *Microcystis* sp. showed zone of inhibition against three pathogens each *i.e.,E. coli* ATCC 35218, *E. coli ATCC 25922* and *K. pneumonae.* After increasing the cyanobacteria extract concentration to 0.1 g/ml, the zone of inhibition also increased. However, *Dermocarpa*sp. did not show any inhibition at any concentration. The present study revealed that cyanobacterial extract can be an effective source in pharmaceutical use against human and plant pathogens.
Keywords: Cyanobacteria, Anti-pathogenic, Minimum Inhibition Zone (MIZ), Sri Lanka.

INTRODUCTION

Since ancient time, nature has provided diverse amount of pharmacologically active compounds. There is a wide spread belief that green medicines are healthier and harmless or safer than synthetic ones because of their limited side effects [1]. Cyanobacteria have a significant attraction as natural source of bioactive molecules with a broad range of biological activities [2]such as antibiotics, antiviral, antitumourals, antioxidant and anti-inflammatory compounds [3]. Cyanobacteria have been regarded as a good candidate for drug discovery with applications in agriculture [4], industry [5] and especially in pharmaceuticals [6]. Researchers have been claimed that consumption of cyanobacteria are beneficial to health due to its chemical composition including compounds like essential amino acids, vitamins, natural pigments and essential fatty acids, particularly γ-linolenic acid, a precursor of the body's prostaglandins. Cyanobacteria believed to be rich in antioxidants and phycobiliproteins [7,8] (PBPs) which are the unique photosynthetic pigments. These pigments have been widely used as natural colorants in foods, cosmetics, and pharmaceuticals particularly as substitutes for synthetic dyes [9]. In addition, PBPs are also used in the field of immunology due to their fluorescent properties. In our previous study it has already been reported that some cyanobacteria are rich in antioxidants [8].

Screening of cyanobacteria for antibiotics and other pharmacologically active compounds, has received ever-increasing interest as a potential source for new drugs [10,11]. Cyanobacteria isolated from local habitats has more adaptive and tolerance ability [12] and seem to be a source of potential new active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms [13].

However, the use of antimicrobial agents has increased significantly almost in all sectors. Massive use of antibiotics created problems including solubility, palatability, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few [14]. Simultaneously, decreased efficacy and resistance of pathogens to antibiotics has necessitated development of new alternatives [15].

The present study was carried out to test antipathogenic (antibacterial and antifungal) activities of cyanobacteria isolated from different freshwater bodies of Sri Lanka.

METHODOLOGY

Cyanobacteria strains

Six unialgal cultures representing three climatic zones were isolated [12] from different freshwater bodies of Sri Lanka. The isolates were identified as *Oscillatoria* sp. (dry zone), *Synechococcus* sp. (dry zone), *Dermocarpa* sp. (wet zone), *Chroococcus*sp. (intermediate zone), *Nostoc* sp. (wet zone) and *Microcystis*sp. (intermediate zone).

Culturing and Semi mass culturing

Cyanobacterial culturing and sub culturingwas carried out by the method of Hossain*et al.*[8].

Harvesting biomass

Cyanobacteria biomass was harvested from 39 days old culture by centrifugation (2000 rpm). Cyanobacterial pellets were oven dried overnight at 60 ^{o}C. Dry biomass was made fine powder using mortar and pestle. Samples were kept in the refrigerator until it was used for analysis.

Preparation of extract impregnated discs

From the six cyanobacterial strains, 0.01 g/ml, 0.025 g/ml, 0.05 g/ml ethanol extracts were prepared and placed on actively growing pathogenic cultures on Petri plates using 5 mm size filter paper disk. In brief, biomass of cyanobacteria (0.1g, 0.05g and 0.02g) with 2 ml ethanol was placed for sonication (35 KHz, 20 min). The filter paper was punched with the punching machine to prepare the discs. The discs were autoclaved and were impregnated with different concentration of ethanolic extract and allowed 5 min for absorption.

Plant and human pathogens

Five plant pathogen fungi (*Colletotricummusae, Colletotricumcoccodes, Pomopsis*sp.*, Tricoderma*sp. and *Cladosporiumcladoplorioides*) were obtained from Department of Botany, University of Peradeniya Sri Lanka. Also the test human pathogens such as *Staphylococcus aureus*ATCC 25923, *Escherichia coli* 25922, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Klebsiellapnuemoniae* and *Candida albicans*were obtained from Department of Microbiology, Faculty of Medicine, University of Ruhuna, Sri Lanka.

In vitro antimicrobial activity

The *in vitro* antimicrobial activity of the extract was measured by employing agar disc diffusion method. The discs (impregnated with extract and control) were placed aseptically over the actively growing pathogen culture on potato dextrose agar (PDA) or nutrient agar (NA) plates and incubated. After incubation, the zone of inhibition around the disc was measured by millimeter scale. The experiment was carried out in triplicates.

STATISTICAL ANALYSIS

Statistical analyses were done using MINITAB-16 and SPSS-16 statistical software packages.

RESULTS AND DISCUSSION

Zone of inhibition against human and plant pathogens

Human and plant pathogens were cultured at three different concentration of cyanobacteria extracts (0.01 g/ml, 0.025 g/ml, 0.05 g/ml). Out of these three concentrations only 0.05 g/ml showed the zone of inhibition. However, the concentration at 0.01 g/ml and 0.025 g/ml did not show any zone of inhibitions. Out of all six cyanobacteria extracts selected in the present study five strains showed the zone of inhibitions. But *Dermocarpa* sp. didn't show any zone of inhibition.

In the present study cyanobacteria strain *Synechococcus* sp. showed the zone of inhibition against highest number of pathogens (7 pathogens) *i.e., Escherichia coli ATCC 35218, Escherichia coli ATCC 25922, Pseudomonas aeruginosa*ATCC 27853,*Klebsiellapneumoniae, Enterococcus faecalis ATCC 29212, Colletotricummusae,* and *Colletotricumcoccodes*(Table 1). At the same time, *Oscillatoria*sp. showed the zone of inhibition against six pathogens *i.e.,Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Staphylococcus aureus*ATCC25923, *Klebsiellapneumoniae, Colletotricummusae,* and*Colletotricumcoccodes* (Table 1). *Nostoc* sp. showed zone of inhibition against five pathogens *i.e.,Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *Staphylococcus aureus*ATCC 25923, *Klebsiellapneumoniae*and

*Enterococcus faecalis*ATCC 29212 (Table 1).Both*Chroococcus*sp. and *Microcystis* sp. showed zone of inhibition against the same three pathogens *i.e.*, *Escherichia coli ATCC 35218, Escherichia coli ATCC 25922* and*Klebsiellapneumoniae*(Table 1). However, *Dermocarpa* sp. did not show any inhibition zone at any concentrations selected in this study.

Table-1: Diameter of zone of inhibition on human and plant pathogens using different cyanobacteria extract (0.05 g/ml)

Cyanobacteria	Pathogen	MIZ (mm)
Synechococcus sp.	*Escherichia coli* ATCC 35218	7.583333
	Escherichia coli ATCC 25922	6.628571
	*Pseudomonas aeruginosa*ATCC27853	7.278571
	Klebsiellapneumoniae	7.209375
	*Entarococusfaecalis*ATCC 29212	6.067857
	Colletotricummusae	5.629167
	Colletotricumcoccodes	5.525
*Oscillatoria*sp.	*Escherichia coli* ATCC 35218	5.946875
	Escherichia coli ATCC 25922	5.6875
	*Staphylococcusaureus*ATCC 25923	7.771429
	Klebsiellapneumoniae	6.671429
	Colletotricummusae	7.783333
	Colletotricumcoccodes	6.835
Nostoc sp.	*Escherichia coli* ATCC 35218	6.034375
	Escherichia coli ATCC 25922	5.914286
	*Staphylococcusaureus*ATCC 25923	8.995833
	Klebsiellapneumoniae	6.1125
	*Entarococusfaecalis*ATCC 29212	6.733333
Microcystis sp.	*Escherichia coli* ATCC 35218	6.5875
	Escherichia coli ATCC 25922	6.378571
	Klebsiellapneumoniae	6.85
*Chrococcus*sp.	*Escherichia coli* ATCC 35218	7.125
	Escherichia coli ATCC 25922	7.588889
	Klebsiellapneumoniae	6.928571

After increasing the cyanobacterial extract concentration the zone of inhibition was also increased for all extracts (Figure 1, Figure 2, and Figure 3). The extract of cyanobacteria isolate *Nostoc*sp. showed highest inhibition against the pathogen *Staphylococcusaureus* ATCC 25923 at both 0.05 g/ml and 0.1 g/ml concentrations (Figure 1).

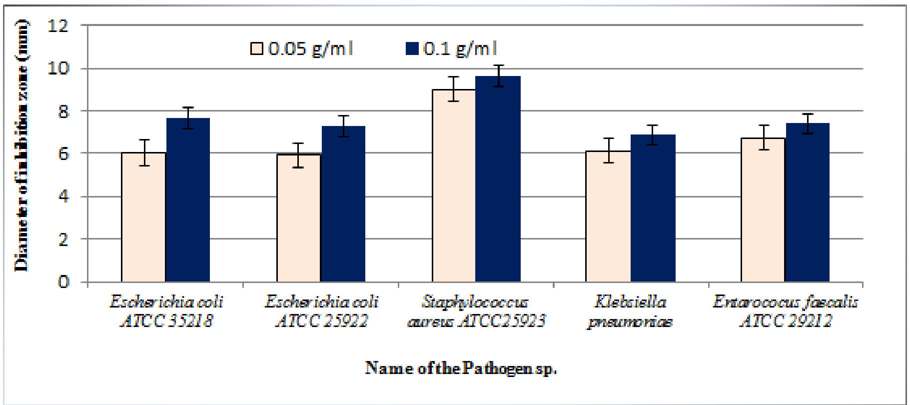

Fig-1: Zone of inhibition against different pathogens at different extracts concentrations of *Nostoc*sp

On the other hand, the extract of cyanobacteria isolate *Oscillatoria*sp. showed highest inhibition against the pathogen *Staphylococcus aureus* ATCC 25923 and *Colletorticummusae* at the extract concentration of 0.05 g/ml (Figure 2). But, after increasing the extract concentration to 0.1 g/ml, *Colletotricumcoccodes* showed the highest zone of inhibition for the extract of cyanobacteria isolate *Oscillatoria*sp. (Figure 2).

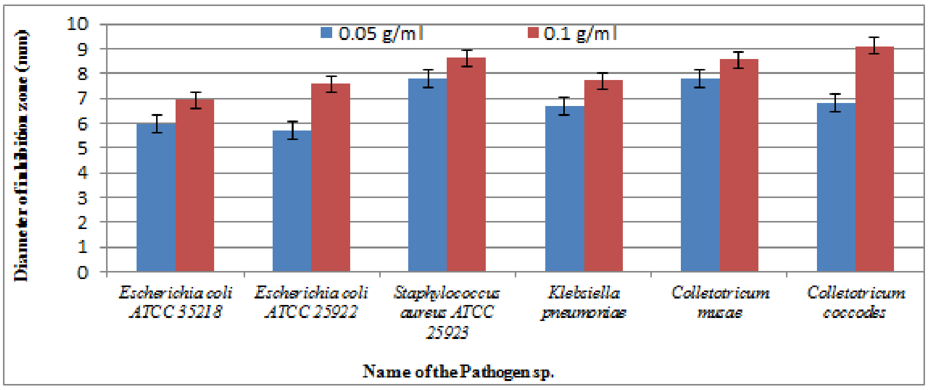

Fig-2: Zone of inhibition against different pathogens at different concentrations of *Oscillatoria*sp. extracts

However, the extract of cyanobacteria isolate *Synechococcus*sp. showed highest inhibition against the pathogen *Escherichia coli* ATCC 35218 at the extract concentration of 0.05 g/ml (Figure 3). After increasing the extract concentration to 0.1 g/ml, the zone of inhibition was increased and *Escherichia coli* ATCC 35218 showed highest inhibition zone for the extract of cyanobacteria isolate *Oscillatoria*sp. (Figure 3).

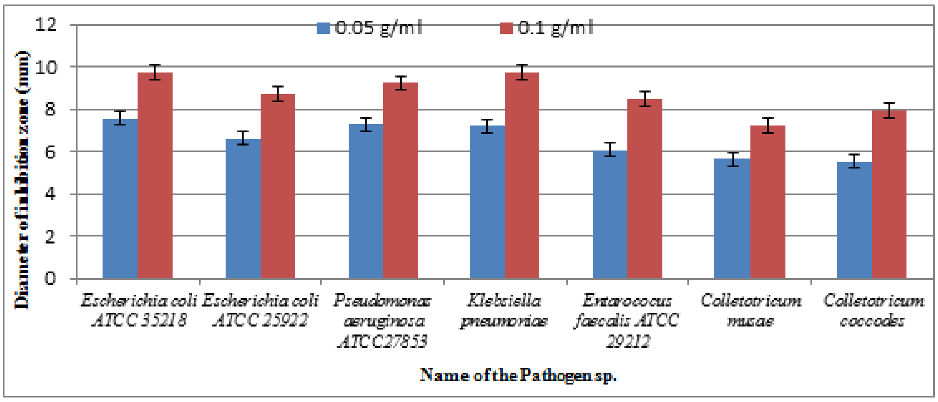

Fig-3: Zone of inhibition against different pathogens at two different concentrations of *Synechococcus*sp. extracts

A previous study on cyanobacteria cultured in terrestrial and freshwater reported that, 54.5% cyanobacterial extract had activity against gram positive bacteria and 9.1% had antifungal activity. However no extracts was active against gram negative bacteria [16]. Different studies carried out so far have reported the potential biological and therapeutic effects of Spirulina spp., Lyngbya spp., Oscillatoria spp. and Phormidiumsp [17]. But the present study reported on antipathogenic activities of Oscillatoria sp., Synechococcus sp., Dermocarpa sp., Chroococcus sp., Nostoc sp. and Microcystis sp.

The previous studies also reported that cyanobacteria Nostocsp are rich in various antibacterial compounds such as Comnostins, Muscoride A, Noscomin, Carbamidocyclophanes etc. At the same time Nostoc sp. are rich in various antifungal compounds such as Nostofungicidine, Amino-6-hydroxy stearic acid, Microviridins, Nostopeptolides, Nostocyclopeptidesetc [18].

A previous study carried out by Hornsey and Hide [19] reported on 151 species of British marine algae and found that, although antibacterial activity was more evident in some taxonomic groups, it also varied seasonally.

CONCLUSION

The ethanolic extract of cyanobacteria at the rate of 0.01 g/ml and 0.025 g/ml did not show any zone of inhibition against any pathogenic fungi and bacteria. But the extract of all cyanobacteria except the extract of Dermocarpa sp. at the concentration of 0.05 g/ml showed zone of inhibition against all pathogens. Once the concentration was increased to 0.1 g/ml the zone of inhibition also increased for all pathogens. However, Dermocarpa sp. did not show any inhibition zone at any concentrations selected in this study. The extract of Synechococcus sp. at the concentration of 0.05 g/ml showed the inhibition zone against highest seven pathogens. The extract of Oscillatoria sp. showed inhibition against six pathogens followed by Nostoc sp. against five pathogens at the same concentration.

At the same time cyanobacteria *Chrococcus*sp. and *Microcystis* sp. showed zone of inhibition against three pathogens each. Further studies are required to test the best activities of different cyanobacterial isolates related to different seasons.

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