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Assessment of antimicrobial, antialgal and cytotoxic activities of crude extracts from rhizospheric and freshwater cyanobacterial strains

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Abstract

Background: This study describes the evaluation of antimicrobial, antialgal and cytotoxic activities of crude extracts from cyanobacterial strains isolated from rhizospheric and freshwater environment.

Methods: Four cyanobacterial strains were isolated from freshwater and rhizospheric samples collected from various sites of University of the Punjab, Lahore, Pakistan. Selected strains were identified by 16S rDNA ribotyping as species of genera, Cyanothece (CY1), Synechococcus (CY2), Leptolyngbya (CY3) and Synechococcus (CY4). The organic extracts i.e., methanolic, ethanolic and acetonic of selected cyanobacterial strains were checked for antibacterial and cytotoxic activity. Antibacterial and antialgal activities of cyanobacterial extracts were determined against, four Gram positive and three Gram negative bacteria using Muller-Hinton (MH) agar well diffusion assay and two algal species using 96-well microtiter plate respectively. Cytotoxic activity was determined against Vero cells and Huh-7 cells.

Results: The results showed that all cyanobacterial extracts showed activities against Gram positive bacteria while some of the extracts showed activity against Gram negative bacteria. Acetonic extract of CY4 and CY2 showed moderate discoloration against *Chlamydomonas* sp. and *Chroococcus* sp. respectively. In cytotoxicity bioassay, methanolic extracts of strain CY1 and CY2 were most active with an IC50 of 0.625 mg/ml against Vero cells while acetonic extract of strain CY1 showed highest activity against Huh-7 cells ($p<0.05$).

Conclusion: The data of current study conclusively suggest that selected cyanobacteria may be an excellent source for further fractionation to obtain novel antibacterial, antialgal and anticancer substances.

Introduction

In the current age of modern technology, natural products are still playing a leading role in the discovery and development of new drugs for curing different human diseases. In early times, plants were considered as major source of biologically active natural compounds [1]. In recent times, microorganisms have gained attraction of scientists to be considered as major source of bioactive compounds. Algae, comprised of micro- and macro algae, are the most populous organisms found on surface of the Earth. They live in the most extreme environments. Due to the adaption of algae under extreme environmental conditions, the most of them produce a variety of secondary metabolites which sometimes show promising biological activities [2].

Cyanobacteria are Gram negative prokaryotes with abilities 1) to synthesize their own food by photosynthesis and 2) to inhabit on every conceivable habitat for over two billion years ago on the Earth [3]. Several factors are involved in their successful survival in such a vast range of environmental niches. Among them, the most important one is their potential to produce different types of secondary metabolites. Recent studies about the discovery of new pharmacologically active compounds have shown cyanobacteria as an untapped natural resource for new bioactive compounds. Such studies have focused on screening and identification of new bioactive compounds such as anticancer, antiviral, antibacterial, antifungal, antialgal, anti-inflammatory, anthelminthic, antiprotozoal, protease inhibiting activities and other toxic metabolites produced by cyanobacteria [4-8]. The actual role of these compounds in producer organisms is yet to be completely investigated but they may have major role in the survival of cyanobacteria in diverse range of habitats.

The first study of cyanobacteria was focused on their impact on environment and public health due to their ability to produce different types of toxins imposing lethal effects on plants, invertebrates and vertebrates, including humans [9,10]. In humans, cyanobacterial toxins such as microcystins, nodularins and cylindrospermopsin have been found to cause damage to liver, kidneys, nervous system and gastrointestinal system [11]. Recently, a number of studies have shown that cyanobacteria can produce compounds having biotechnological and pharmacological importance [5,12,13].

Secondary metabolites produced by cyanobacteria have diverse range of structures which may be due to the diversity of cyanobacteria. These metabolites have shown a range of biological activities including anticancer, antibacterial, antiviral, antifungal and antiprotozoal activities [14,15]. Marine environments, especially benthic species of cyanobacteria are among the best source of bioactive compounds. Screening for anticancer compounds has been mainly focused by the scientists now a days. Territorial species of cyanobacteria have produced interesting antiviral compounds by showing promising anticancer leads. Apart from this, a wide range of linear and cyclic peptides have also been isolated from planktonic cyanobacteria [16].

Recent studies on bioactive compounds produced by cyanobacteria are mainly focused on freshwater and marine species [17-20]. Keeping in mind the diverse range of cyanobacterial compounds, the main objective of the present study is to evaluate the antimicrobial, antialgal and cytotoxic activities of extracts obtained from rhizospheric and freshwater cyanobacteria.

Methods

Cyanobacteria and culture conditions

Different freshwater and rhizospheric samples were collected from different sites of the University of the Punjab, Lahore, Pakistan (latitude/ longitude = 31.49° N, 74.29° E) as given in Table 1. All the samples were serially diluted and 100 µl from 106 and 107 dilutions were spread on BG-11 agar medium [21]. The plates were incubated under illumination with 16 h:8 h of light: dark cycle and 200 µE m-2 s-1 light intensity at 25-28˚C for three weeks. After incubation, single and isolated colonies were obtained which were then purified on fresh prepared BG-11 medium. The process was repeated three times for getting single, isolated and purified colonies. For 16S rDNA ribotyping, four purified colonies were sent to Macrogen Sequencing Facility, Korea. The obtained sequences were aligned with already reported species of cyanobacteria in NCBI database. Selected four colonies were characterized as species of genera Cyanothece, Synechococcus, Leptolyngbya and Synechococcus. Biomass of four selected strains was produced by culturing in BG-11 medium. Cultures were grown under same conditions as described above. At the stationary phase of growth, the cultures were harvested by centrifugation (model 3K30, SIGMA laborentifugen) at 10,000×g for 15 min for obtaining high yield of biomass. The biomass was freeze-dried and stored at -20˚C until further use.

Preparation of crude extract

Extraction of cyanobacterial biomass was carried out with the solutions of acetic acid: methanol, acetic acid: ethanol and acetic acid: acetone respectively. One ml of acetic acid: methanol (10:1) solution was added to 100 mg of freeze dried biomass and sonicated with an ultra-

sound probe for 5 min (cycles: 5s on/10s off) on ice. The solution was centrifuged at 10000×g for 10 min. The supernatant was filtered, condensed and freeze-dried to obtain crude methanol extract. Stock solution (200 mg/ml) of methanol extract was prepared by dissolving 20 mg of dried methanol extract in 1 ml of dimethyl sulfoxide (DMSO). Similarly, the extraction was performed with the acetic acid solutions of ethanol and acetone respectively. The DMSO stock solutions of all extracts were filtered (0.2 µm) and stored at -20˚C until use for bioassays. The concentrations from stock solution were prepared freshly for each bioassay.

Strain	Genera	Source
CY1	*Cyanothece* sp.	Freshwater
CY2	*Synechococcus* sp.	Freshwater
CY3	*Leptolyngbya* sp.	Rhizosphere
CY4	*Synechococcus* sp.	Rhizosphere

Table 1: Cyanobacteria strains and their source of isolation used in this study.

Antimicrobial screening bioassay

Test microorganisms

Antibiotic activity of cyanobacteria extracts was tested against four Gram positive bacteria as Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228) and Micrococcus luteus (ATCC 9341), three Gram negative bacteria as Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) and Proteus mirabilis (ATCC 49565) and two algal species of genera, *Chlamydomonas* sp. and *Chlorococcus* spp.

Antibacterial sensitivity test

Agar well diffusion method was used to determine the antibacterial activity of cyanobacterial extracts. Fresh colonies of selected overnight bacterial cultures were suspended in saline solution (0.9% NaCl) and streaked on surface of Muller-Hinton (MH) agar plate [22]. Wells were prepared on MH agar surface with the help of sterile Pasteur pipette and 10 µl of methanolic, ethanolic and acetonic cyanobacterial extract (stock conc. 200 mg/ml) was transferred to each well of respective MH agar plate of test bacterial strains. Wells with DMSO were used as negative controls and with antibiotic i.e., penicillin (15 µg/well) were used as positive controls. All the plates were incubated at 37˚C for 24 h for each bacterial strain. The results were measured as zone of inhibition in millimeters. The experiment was performed in triplicates.

Antialgal sensitivity test

Antialgal assay was performed in 96-well microtiter plate [23]. Algal culture was concentrated by centrifugation at 5000×g for 10 min (SIGMA laborentifugen model 3K30) and 100 µl of suspension (105 cells/ml) was transferred to each test well. An aliquot of 10 µl of cyanobacterial extract (stock conc. 200 mg/ml) was transferred to each test well and –ve control contained only DMSO solution. The plates were incubated at 28˚C under continuous illumination (25-30 µmol photon m-2 s-1) for 48-72 h. The results were recorded as discoloration of the algal culture caused by extract.

In vitro cytotoxicity assay

Cell lines used

Two cell lines, Vero cells (derived from kidney of African green monkey) and Huh-7 cells (Human hepatocyte derived cellular carcinoma cell line) were used for cytotoxicity bioassay.

Culture conditions

For testing cytotoxicity effect of selected cyanobacterial extracts against Vero cells and Huh-7 cells, Glasgow Minimum Essential Medium (GMEM) and Dulbecco's Minimum Essential Medium (DMEM) were prepared and supplemented separately with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin. Both cell lines i.e., Vero cells and Huh-7 cells were maintained as a monolayer culture on GMEM and DMEM respectively in a humidified atmosphere of 5% CO2 at 37˚C.

Neutral red uptake assay

Selected cell lines were seeded in a 96-well microtiter plate for overnight to evaluate the cytotoxicity effect of cyanobacterial extracts. Each well of microtiter plate was filled with 100 µl of new media by replacing the old media. The wells of first row of the plate were filled with 100 µl of each extract with concentration of 5 mg/ml and then serially diluted to a conc. of 0.039 mg/ml. Culture media containing the corresponding DMSO concentration was used as a negative control for each extract. The microtitre plate was incubated at 37˚C in 5% CO2 chamber for 48-72 h. Cytotoxicity was measured using neutral red uptake assay which was designed to measure cell viability. The cells seeded in microtiter plate for appropriate period, were treated with media containing neutral red dye for 2 h. In the next step, the washing was done and then the dye was extracted from each well by adding acidified ethanol. The absorbance (O.D.) was measured using UV-VIS spectrophotometer [24].

MTT bioassay and IC50 value

The experiment was carried out for the quantitative determination of viable cells of Vero and Huh-7 by the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-terazolium bromide (MTT) (yellow color) to formazan (a blue colored product) [25]. For this experiment, the medium in each well of 96-well

microtiter plates was replaced by MTT solution (20 cell/well, 5 mg/ml in phosphate-buffered saline) and incubated for 24 h humid conditions as 5% CO_2 and 95% air at 37°C. After the formation of monolayer of actively dividing cells and then trypsinization, 105 cells/ml were seeded in the wells containing culture media and different concentrations of cyanobacterial extracts (stock conc. 200 mg/ml). Culture media containing the corresponding DMSO concentration was used as a negative control for each extract. The microtitre plate was then incubated for 48 h at optimum conditions as described above. After incubation, 20 µl (5 mg/ml) of MTT reagent was added and incubated at same conditions for 4 h. After incubation, MTT reagent was removed, and the formazan crystals produced by viable cells were dissolved in 100 DMSO and gently shaken. The absorbance at 492 nm was determined by ELISA reader. The results were recorded as % cell death caused by extract in test wells as compared to control well without extract. IC50 was calculated via through dose dependent curve and by the formula given below was applied for each dilution to calculate the growth inhibition rate.

Thin Layer Chromatography (TLC)

The experiment was performed on 0.5 mm thick preparative silica gel TLC plates (www.merck.com). An aliquot of 10 µl of methanolic extract of CY1 and Cy2 (was loaded to the bottom of plate and developed with desired solvent system i.e., CH2Cl2: MeOH (9:1 v/v). After the development, the plate was dried and observed under UV trans-illuminator.

Statistical Analysis

All the experiments were performed in triplicates and the data were expressed as mean ± SD. Analysis of variance (ANOVA) of all the data was performed using SPSS software.

Results

Antimicrobial activity

Antibacterial activity of all the extracts was determined against three Gram negative bacteria i.e., Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 27853) and Proteus mirabilis (ATCC 49565), and four Gram positive bacteria i.e., Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis (ATCC 12228) and Micrococcus luteus (ATCC 9341). The results were recorded as zones of inhibition after 18-24 h of incubation. All the extracts (methanolic, ethanolic and acetonic) of CY1 showed less activity against Gram negative bacteria as compared to Gram positive bacteria. Among Gram positive bacteria, ethanolic extract showed highest activity against M. luteus with a zone of inhibition of 16 mm followed by S. aureus with 13 mm and B. subtilis with 10 mm. Among the extracts of CY2, methanolic extract showed highest antibacterial activity against test bacteria. The extract of CY2 showed good inhibiting activity against Gram positive bacteria with zone of inhibition of 18 mm against B. subtilis, followed by S. aureus with 14 mm, M. luteus with10 mm and S. epidermidis with 10 mm. Among the extracts of CY3, the ethanolic extract showed highest activity against M. luteus with a zone of 18 mm. Other extracts of this cyanobacterial strains showed moderate activity. The extracts of CY4 were moderately active against all bacteria except ethanolic extract which showed a zone of 10 mm against M. luteus. All the extracts were mostly active against Gram positive bacteria and were less active against Gram negative bacteria. The results are summarized in Table 2.

Antialgal activity

For antialgal assay, the results were recorded as discoloration of the algal culture, *Chlorococcus* spp. and *Chlamydomonas* sp. after three days of incubation with cyanobacterial extracts. Methanolic extract of CY1 showed highest activity showing complete discoloration of the *Chlamydomonas* sp. culture after 48 h. Methanolic extracts of all other strains showed moderate antialgal activity against *Chlamydomonas* sp. Ethanolic extract of all the strains showed moderate activity against *Chlamydomonas* sp., by causing minimal discoloration of the culture after 48 hours of incubation. Acetonic extract of CY1, CY2 and CY3 showed no activity whereas CY4 showed less activity against *Chlamydomonas* sp. In case of cyanobacterial activity against *Chlorococcus* spp., the methanolic and ethanolic extracts of all four cyanobacterial strains showed moderate activity causing minimal discoloration of the culture after 48 hours of incubation. Acetonic extract of strains, CY1, CY3 and CY4 showed no activity against *Chlorococcus* spp., while only extract of CY2 showed minimal inhibitory activity. The results are given in Table 3.

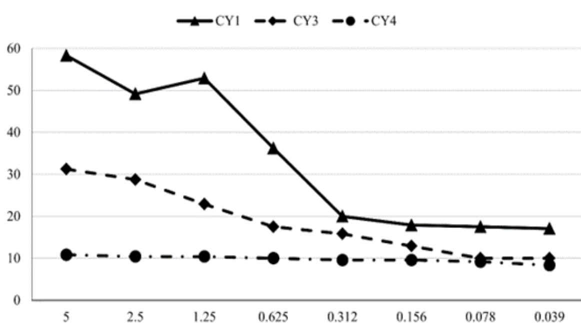
Minimum Inhibitory Concentration (MIC)

MIC (minimum inhibitory conc.) of cyanobacterial extracts was determined using broth microdilution method. Methanolic extract of all strains showed good results with MIC of 0.1 mg/ml against S. aureus MIC of ethanolic extract of CY3 was 0.1 mg/ml against B. subtilis. Acetonic extract of CY4 showed MIC of 0.1 mg/ml against B. subtilis, while extract of CY1 showed MIC of 0.1 mg/ml against S. aureus and S. epidermidis.

Cytotoxic activity

Two cell lines, African green monkey kidney cell line (Vero) and human hepatocyte derived cellular

carcinoma cell line (Huh-7) were used to determine the cytotoxicity of cyanobacterial extracts. The results of MTT assay were recorded as % cell death in test wells as compared to control. Among the acetonic extracts, the extract of strain CY1 at a conc. of 5 mg/ml showed good cytotoxic activity by causing 58% cell death. Cell death (%) was gradually decreased as the conc. of the extract was lowered. Extract of CY3 caused 31% cell death at same conc. while CY4 caused only 10% cell death at conc. 5 mg/ml as shown in Fig. 1. A significant difference was measured among the activities of different cyanobacterial extracts against Vero cells ($p<0.0014$). Methanolic extracts of all strains showed the highest activity by causing cell death. At a conc. of 5 mg/ml, CY1, CY2, CY3 and CY4 extracts caused death in 67%, 61%, 70% and 58% of cells as shown in Fig. 2. Among the ethanolic extracts, extract of CY1 was the most active causing 58% of cell death at conc. of 5 mg/ml, while CY2 caused death of 48% cells at the same concentration. Extract of strains CY3 and CY4 caused death of 12% and 16% cells, respectively at conc. of 5 mg/ml as shown in Fig. 3.

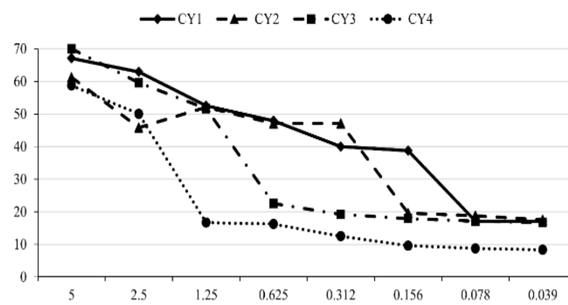


Different Concentrations of acetonic extracts (mg/ml)

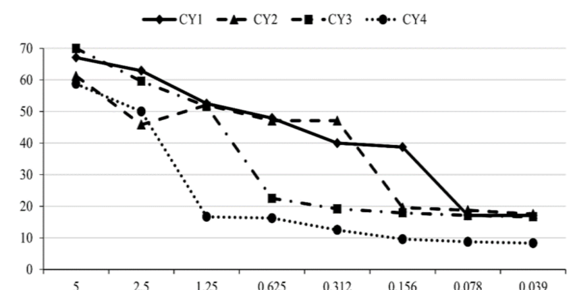
Figure 1: Cytotoxicity of different concentrations of acetonic extracts of selected cyanobacterial strains (CY1, CY3 and CY4) against Vero cells.

IC50 value of extracts

IC50 (minimum conc. causing death of 50% cells) was calculated for all the extracts. Methanolic extract of strain CY1 (Cyanothece) and CY2 (Synechococcus) were the most active with an IC50 of 0.625mg/ml as shown in Fig. 4. IC50 for CY3 and CY4 was 1.25 mg/ml and 2.5 mg/ml, respectively. Among the acetonic extracts, only CY1 showed IC50 at conc. of 2.5 mg/ml by causing death of 50% cells. IC50 was showed by ethanolic extracts of CY1 and CY2 at the conc. of 1.25 and 2.5 mg/ml respectively whereas ethanolic extract of the other strains did not show IC50. Four extracts i.e., CY1, CY4 (acetonic), CY1 and CY4 (methanolic) were assessed for their activity against Huh7 cells. Acetonic extracts of CY1 and CY4 caused 88% cell death at conc. of 5 mg/ml by showing good cytotoxic activity. By lowering the conc. of extracts, the activity was also decreased as shown in Fig. 5. Cell death was calculated as 66% and 55% by methanolic extracts of CY1 and CY2 respectively at conc. of 5 mg/ml. A gradual decrease in activity was observed with decrease in conc. of extract as shown in Fig. 6. IC50 was calculated as 0.625 mg/ml and 1.25 mg/ml for Acetonic extract of CY1 and CY4 respectively. The methanolic extracts did not show IC50 against selected cell lines.



(a) Different Concentrations of methanolic extracts (mg/ml)



(b) Different Concentrations of ethanolic extracts (mg/ml)

Figure 2a, b: Cytotoxicity of different concentrations of methanolic extracts of selected cyanobacterial strains (CY1, CY2, CY3 and CY4) against Vero cells.

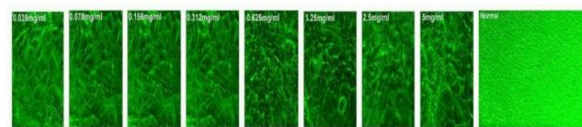
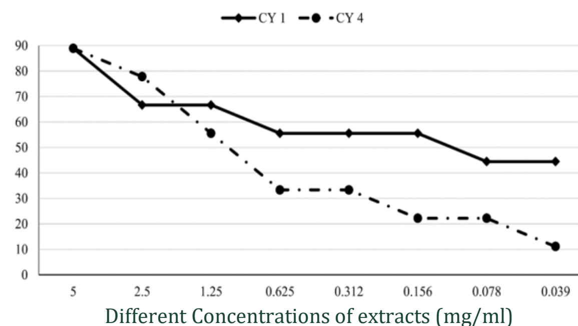


Figure 3: Cytotoxicity against Vero cells with different concentrations (IC50= 0.625mg/ml) of methanolic extract of CY2 (*Synechococcus*).



Different Concentrations of extracts (mg/ml)

Figure 4: Cytotoxicity of different concentrations of acetonic extracts of CY1 and CY2 against Huh-7 cells.

Cyanobacterial extract	Gram staining	Bacterial strains	Zone of inhibition of cyanobacterial extracts (mm)					
			CY1	CY2	CY3	CY4	-ve Cont.	+ve Cont.
Methanolic	Gram (+ve)	*M. luteus*	4 ± 0.07	10 ± 0.07	8 ± 0.14	6 ± 0.14	0	20 ± 0.08
		S. aureus	8 ± 0.14	14 ± 0.14	4 ± 0.07	0	0	19 ± 0.15
		S. epidermidis	9 ± 0.07	10 ± 0.07	4 ± 0.14	6 ± 0.21	0	19 ± 0.20
		B. subtilis	6 ± 0.21	18 ± 0.21	0	8 ± 0.07	0	20 ± 0.08
	Gram (-ve)	*P. aeruginosa*	0	0	0	0	0	20 ± 0.08
		P. mirabilis	0	0	0	0	0	19 ± 0.15
		E. coli	10±0.14	8±0.14	10±0.21	0	0	20 ± 0.08
Ethanolic	Gram (+ve)	*M. luteus*	16 ±0.21	0	18 ±0.14	10 ±0.21	0	19 ± 0.15
		S. aureus	13 ±0.14	8 ±0.21	4 ±0.21	4 ±0.14	0	19 ± 0.20
		S. epidermidis	0	8 ±0.14	0	0	0	20 ± 0.08
		B. subtilis	10 ±0.21	0	8 ±0.14	8 ±0.21	0	19 ± 0.15
	Gram (-ve)	*P. aeruginosa*	8 ±0.14	6 ±0.21	0	6 ±0.14	0	20 ± 0.08
		P. mirabilis	0	0	8 ±0.07	6 ±0.21	0	19 ± 0.15
		E. coli	0	0	7 ±0.07	6 ±0.14	0	20 ± 0.08
Acetonic	Gram (+ve)	*M. luteus*	6 ±0.21	6 ±0.21	0	0	0	20 ± 0.08
		S. aureus	7 ±0.14	0	6 ±0.07	4 ±0.14	0	19 ± 0.15
		S. epidermidis	5 ±0.21	0	4 ±0.14	4 ±0.07	0	20 ± 0.08
		B. subtilis	0	6 ±0.28	0	7 ±0.14	0	19 ± 0.15
	Gram (-ve)	*P. aeruginosa*	0	8 ±0.21	5 ±0.14	0	0	20 ± 0.08
		P. mirabilis	8 ±0.28	10 ±0.14	0	0	0	19 ± 0.15
		E. coli	0	0	0	6 ±0.07	0	20 ± 0.08

+ve Cont= Positive Control, -ve Cont= Negative Control, The results are mean of triplicates ±Standard Deviation.

Table 2: Antibacterial activity of different extracts of cyanobacteria.

Test organisms	Type of extracts	Discoloration by cyanobacterial extracts				
		CY 1	CY 2	CY 3	CY 4	-ve Cont.
Chlamydomonas sp.	Ethanolic	+	+	+	+	-
	Methanolic	+++	+	+	+	-
	Acetonic	-	-	-	+	-
Chlorococcus sp.	Ethanolic	+	+	+	+	-
	Methanolic	+	+	+	++	-
	Acetonic	-	+	-	-	-

= No activity, + = Less activity, ++ = Moderate activity, +++ = High activity, -ve Cont= Negative Control

Table 3: Antialgal activity of cyanobacterial extracts against *Chlamydomonas* sp. and *Chlorococcus* spp.

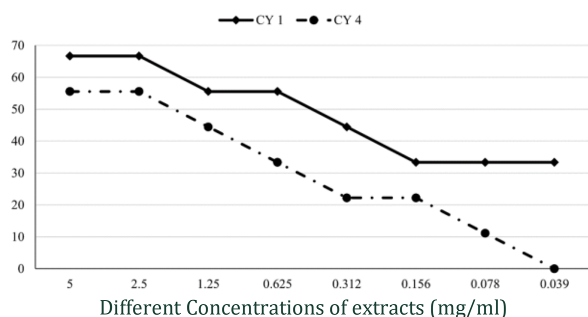


Figure 5: Cytotoxicity of different concentrations of ethanolic extracts of CY1 and CY4 against Huh-7 cells.

Figure 6: TLC of methanolic extracts of CY1 and CY2

TLC of cyanobacterial extracts

The development of different spots of extracts of CY1 and CY2 confirmed the presence of different compounds which might be involved in antibacterial, antialgal and cytotoxic activities.

Discussion

Cyanobacteria are known to produce a diverse range of bioactive compounds [5,26]. Antimicrobial compounds are produced by a range of cyanobacteria belonging to different genera [27-29]. In the perspective of antibacterial activity, the results of this study are in strong agreement with previous results showing that antibacterial activity of cyanobacteria is mostly shown against Gram positive bacteria [30-32]. The results of this study can also be related to the fact that Gram negative bacteria are resistant to chemicals due to presence of extra lipopolysaccharides layer on their outer membrane [33].

By the ecological point of view, cyanobacteria and Gram-negative bacteria have same morphology so they both produce compounds against Gram positive bacteria [19,34]. The difference in activity may also be due to difference in mechanism of action of these compounds in both cells. The results also showed that the change in activity may be due to change in permeability of both membranes towards these compounds [7]. Out of all the extracts tested, methanolic extract was the most active in inhibiting growth of all Gram-positive bacteria tested. These results give an indication that active components

of the extract were mostly soluble in methanol. Ethanolic extract was found active against all Gram-negative bacteria. It can be hypothesized that that by using different solvents, the different compounds can be extracted with activity against different type of microorganisms [35,36]. The extracts, CY1, and CY2 and CY4 of unicellular strains, Cyanothece and Synechococcus were more active respectively than the extract of filamentous strain CY3 (Leptolyngbya). It showed that the compounds produced by the unicellular strains are soluble in these solvents. It also showed that the unicellular strain has more potential than filamentous strain.

Cyanobacteria are known to produce compounds showing cytotoxicity against different cell lines. These compounds have different mechanism of action targeting different cell organelles [37]. The results of this study showed that cyanobacteria have the potential to produce different cytotoxic compounds which can be used to outcompete other organisms in late phases of growth. The results are in agreement with previous studies which also showed the cytotoxic potential of cyanobacteria [11,38-40].

The current study also showed that extracts of cyanobacterial strains inhibiting bacterial growth also possess cytotoxic activity, especially in case of CY1 and CY2. It can be concluded that these extracts can affect both eukaryotic and prokaryotic cells or the same cyanobacteria produce different compounds affecting both type of cells. Cyanobacteria is an untapped natural resource of bioactive compounds which provides an excellent opportunity for global scientists to screen them for potential new drug lead compounds. As the rate of discovery from traditional microbial drug producers like actinomycetes and hyphomycetes is decreasing so it is time to switch to cyanobacteria to fight against resistant pathogens and new emerging diseases. The current study shows the potential of cyanobacteria to produce compounds against prokaryotic and eukaryotic cells, so this fertile field of research needs more attention of global scientists.

Competing Interest

The authors declare that there is no conflict of interest in this study.

Author Contributions

WM performed the experimental work, AA wrote the manuscript and performed statistical analysis, MHK and NM provided the lab facilities for cell lines studies and MA proofread the manuscript and overall supervised this study.

References

1. Fiorito S, Preziuso F, Epifano F, Scotti L, Bucciarelli T, et al. Novel biologically active principles from spinach, goji and quinoa. Food chemistry, (2019); 276: 262-265.
2. Mashjoor S, Yousefzadi M, Esmaeili MA, Rafiee R. Cytotoxicity and antimicrobial activity of marine macro algae (Dictyotaceae and Ulvaceae) from the Persian Gulf. Cytotechnology, (2016); 68(5): 1717-1726.
3. Rastogi RP, Kumar A, Tyagi MB, Sinha RP. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. Journal of Nucleic Acids, (2010); 592980.
4. Wiegand C, Pflugmacher S. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicology and Applied Pharmacology, (2005); 203(3): 201-218.
5. Singh RK, Tiwari SP, Rai AK, Mohapatra TM. Cyanobacteria: an emerging source for drug discovery. Journal of Antibiotic Research, (2011); 64(6): 401-412.
6. Rastogi RP, Sinha RP. Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnology Advances, (2009); 27(4): 521-539.
7. Martins RF, Ramos MF, Herfindal L, Sousa JA, Skærven K, et al. Antimicrobial and cytotoxic assessment of marine cyanobacteria-Synechocystis and Synechococcus. Marine Drugs, (2008); 6(1): 1-11.
8. Farghl AA, El-Sheekh MM, Mousa AS. Extraction and characterization of antimicrobial active substance from cyanobacteria Nostoc carneum and Anabaena circinalis. Fresenius Environmental Bulletin, (2019); 28(7): 5481-5490.
9. Codd GA, Morrison LF, Metcalf JS. Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology, (2005); 203(3): 264-272.
10. Carmichael W. Cyanobacteria secondary metabolites—the cyanotoxins. Journal of Applied Bacteriology, (1992); 72(6): 445-459.
11. Costa M, Costa-Rodrigues J, Fernandes MH, Barros P, Vasconcelos V, et al. Marine cyanobacteria compounds with anticancer properties: a review on the implication of apoptosis. Marine Drugs, (2012); 10(10): 2181-2207.
12. Kumar J, Singh D, Tyagi MB, Kumar A Cyanobacteria: Applications in biotechnology. Cyanobacteria (2019). Amsterdam, The Netherlands:: Academic Press. pp. 327-346.
13. Barzkar N, Tamadoni Jahromi S, Poorsaheli HB, Vianello F. Metabolites from marine microorganisms, micro, and macroalgae: Immense scope for pharmacology. Marine Drugs, (2019); 17(8): 464-470.
14. Singh S, Kate BN, Banerjee U. Bioactive compounds from cyanobacteria and microalgae: an overview. Critical Reviews in Biotechnology, (2005); 25(3): 73-95.
15. Seddek N, Fawzy M, El-Said W, Ragaey M. Evaluation of antimicrobial, antioxidant and cytotoxic activities and characterization of bioactive substances from freshwater blue-green algae. Global NEST Journal, (2019); 21(3): 329-337.
16. Sivonen K, Börner T Bioactive compounds produced by cyanobacteria. In: Herrero A, Flores E, editors. The Cyanobacteria: Molecular Biology, Genomics and Evolution (2008). Norfolk, United Kingdom:: Caister Academic Press. pp. 159-197.
17. Dixit RB, Suseela M. Cyanobacteria: Potential candidates for drug discovery. Antonie Van Leeuwenhoek, (2013); 103(5): 947-961.
18. Tan LT. Pharmaceutical agents from filamentous marine cyanobacteria. Drug Discovery Today, (2013); 18(17): 863-871.
19. Ruiz-Güereca DA, Licea-Navarro AF, del Pilar Sánchez-Saavedra M. Evaluation of antimycobacterial activity from marine and freshwater microalgae. Revista de Biología Marina y Oceanografía, (2019); 54(1): 82-90.
20. Abobaker HM, Elsalhin HE. Antibacterial activity of Anabaena circinalis isolated from fresh water. Journal of Advanced Microbiology, (2019); 15(3): 1-7.

21. Rippka R, Deruelles J, Waterbury JB, Herdman M, Stanier RY. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. Journal of General Microbiology, (1979); 111(1): 1-61.
22. Pollock HM, Barry A, Gavan T, Fuchs P, Hansen S, et al. Selection of a reference lot of Mueller-Hinton agar. Journal of clinical microbiology, (1986); 24(1): 1-6.
23. Volk R-B, Furkert FH. Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. Microbiological Research, (2006); 161(2): 180-186.
24. Repetto G, del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature Protocols, (2008); 3(7): 1125-1131.
25. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, (1983); 65(1-2): 55-63.
26. Kumar G, Teli B, Mukherjee A, Bajpai R, Sarma B Secondary metabolites from cyanobacteria: A potential source for plant growth promotion and disease management. Secondary metabolites of plant growth promoting rhizomicroorganisms (2019). Singapore: Springer. pp. 239-252.
27. Kreitlow S, Mundt S, Lindequist U. Cyanobacteria—a potential source of new biologically active substances. Journal of Biotechnology, (1999); 70(1): 61-63.
28. Soltani N, Khavari-Nejad R, Tabatabaei Yazdi M, Shokravi S, Fernandez-Valiente E. Screening of soil cyanobacteria for antifungal and antibacterial activity. Pharmaceutical Biology, (2005); 43(5): 455-459.
29. Madhumathi V, Deepa P, Jeyachandran S, Manoharan C, Vijayakumar S. Antimicrobial activity of cyanobacteria isolated from freshwater lake. International Journal of Microbiology Research, (2011); 2(3): 213-216.
30. Mian P, Heilmann J, Bürgi H-R, Sticher O. Biological screening of terrestrial and freshwater cyanobacteria for antimicrobial activity, brine shrimp lethality, and cytotoxicity. Pharmaceutical Biology, (2003); 41(4): 243-247.
31. Taton A, Grubisic S, Ertz D, Hodgson DA, Piccardi R, et al. Polyphasic study of antarctic cyanobacterial strains. Journal of Phycology, (2006); 42(6): 1257-1270.
32. Skočibušić M, Lacić S, Rašić Z. Evaluation of Antimicrobial Potential of the Marine Cyanobacterium, Rivularia mesenterica. Journal of Advanced Microbiology, (2019); 16(4): 1-11.
33. Dixon R, Al-Nazawi M, Alderson G. Permeabilising effects of sub-inhibitory concentrations of microcystin on the growth of Escherichia coli. FEMS Microbiology Letters, (2004); 230(2): 167-170.
34. Piccardi R, Frosini A, Tredici MR, Margheri MC. Bioactivity in free-living and symbiotic cyanobacteria of the genus Nostoc. Journal of Applied Phycology, (2000); 12(3): 543-547.
35. Sethubathi GVB, Prabu VA. Antibacterial activity of cyanobacterial species from adirampattinam coast, southeast coast of palk bay. Current Research Journal in Biological Sciences, (2010); 2(1): 24-26.
36. Cao J, Chen L, Li M, Cao F, Zhao L, et al. Two-phase systems developed with hydrophilic and hydrophobic deep eutectic solvents for simultaneously extracting various bioactive compounds with different polarities. Green chemistry, (2018); 20(8): 1879-1886.
37. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria - a prolific source of natural products. Tetrahedron, (2001); 57(46): 9347-9377.
38. Medina RA, Goeger DE, Hills P, Mooberry SL, Huang N, et al. Coibamide A, a potent antiproliferative cyclic depsipeptide from the Panamanian marine cyanobacterium Leptolyngbya sp. Journal of the American Chemical Society, (2008); 130(20): 6324-6325.
39. Caires TA, da Silva AM, Vasconcelos VM, Affe HM, de Souza Neta LC, et al. Biotechnological potential of Neolyngbya (Cyanobacteria), a new marine benthic filamentous genus from Brazil. Algal research, (2018); 361-9.
40. Karan T, Aydin A. Anticancer potential and cytotoxic effect of some freshwater cyanobacteria. Tropical Journal in Pharmaceutical Research, (2018); 17(11): 2183-2188.

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