Available online at www.elixirpublishers.com (Elixir International Journal)

Applied Botany

Elixir Appl. Botany 72C (2014) 25537-25544

ISSN: 2229-712X

Growth and biochemical parameters of selective cultured cyanobacteria and exploiting antibacterial potency against human bacterial pathogens

Riyazulla Azeez, Dhanalakshmi. P. K and Surendirakumar. K Thangaraju Nallamuthu*

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Guindy, Chennai – 600 025.

ARTICLE INFO

Article history:

Received: 28 May 2014;

Received in revised form:

19 June 2014;

Accepted: 3 July 2014;

Keywords

Cyanobacteria,

Bacterial,

Pathogens,

Oscillatoria.

ABSTRACT

The increasing emergence of drug resistant pathogens has prompted research into novel treatments to encounter the prevalence of persistent infections. Cyanobacteria of freshwater habitats have rarely been explored for their antibacterial and antioxidant potential. Therefore in the present investigation we had selected eight different cyanobacterial strains for their active antimicrobial and antioxidant activity. All the cyanobacteria used for the research were showed very good growth and biomass production. All the isolates were showed maximum metabolite production on 20th day of growth period. The most significant increase was observed in young clusters. Maximum metabolite production was observed in *Lyngbya* sp. *Oscillatoria* sp. *Phormidium sp*, *Calothrix* sp followed by *Nostoc* sp. *Anabaena* sp. produced meager metabolite and also for Phytochemical analysis. The biosynthetic information on the chemical structures unique to these organisms will be very valuable for gene manipulation aimed at creating new therapeutic agents, and in the near future they will achieve the same position as Streptomyces and other Actinomycetes we have today.

Introduction

Natural products are outstanding in the diversity of their chemical structures and biological activities. In contrast, the chemical diversity produced by the pharmaceutical industry using methods such as synthetic combinatorial chemistry, seldom shows as potent or diverse biological activities (Constantino *et al*., 2004; Berdy, 2005). The exploitation of biologically active secondary metabolites for useful applications, including therapeutic drugs, is far from new. Since ancient times, nature has been recognized as an important source of potential drugs; examples of early uses and benefits of natural products for human can be found in most major civilizations (Newman *et al*., 2000; Constantino *et al*., 2004).

Cyanobacteria are nature's unique gift to mankind, as they possess several innate properties that make them ideal organisms with potential for multifaceted biotechnological applications. They are large and morphologically diverse group of unique photosynthetic organisms of great importance because of their very long existence for well over 3.5 billion years and cosmopolitan distribution in terrestrial, freshwater and marine habitats.

Infectious disease is the main cause of death in developing countries worldwide and they hold the second position after heart diseases. Gram-negative bacterial pathogens are a common cause of infection in human beings and the prevalence and rates of resistance among these pathogens to existing antimicrobial agents are increasing. High-level resistance attributable to β-lactamase expression alone or in combination with other mechanisms is becoming increasingly prevalent among Enterobacteriaceae and gram-negative non-fermenting organisms. 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.

To date, many chemically unique compounds of fresh water origin with various biological activities have been isolated, and some of them are under investigation. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Parekh J *et al*., 2007). With this knowledge the present study was aimed to screen the antibacterial potential of cyanobacteria against human pathogens. We investigated the growth study by observing biochemical parameters and screened the cyanobacterial extracts with organic solvents for their biological activity against various gram negative bacterial human pathogens. It is expected that *in vitro* antibacterial screening will permit the selection of extracts and compounds with potentially useful properties to be used for further chemical and pharmacological evaluation. The comparative study may facilitate the selection of strains with a relatively high level of potency and/or with wider range of cytotoxic components.

Materials and Methods

Cyanobacterial culture collection

The cyanobacterial cultures such as *Nostoc* sp., *Anabaena* sp., *Chroococcus* sp., *Oscillatoria* sp., *Calothrix* sp., *Synechococcus* sp., *Phormidium* sp., *Lyngbya* sp. were obtained from Algal Culture Laboratory, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Guindy, Chennai - 600 025. As cyanobacteria are autotrophic organisms, they require simple nutrient media for growth. Many media designed for this purpose, among them BG11 medium is suitable for cyanobacteria and easy to prepare. In this work all cultures were grown in BG11 medium (Rippka *et al.*, 1979). The cultures were maintained at 25 ± 1ºC under light illumination of 30 µEm^{2}s^{-1} units, with 16/8 h light and dark condition. Only liquid cultures were used throughout the investigation. Clumping of cells was reduced by gently shaking the flasks manually day by day.

Tele:+91 99403 96478; +91 9789430467, +91 8883095789

E-mail addresses: nthangam@gmail.com*; riyazscientist@gmail.com

Mass culture of cyanobacterial cultures

The mass culture of collected cyanobacterial strains were grown under laboratory condition for the period of 30 days. The experiment was carried out in 10 liters of BG11 medium. This medium was distributed to twenty 2000mL conical flask. Each flask contains 1000mL of BG11 medium inoculated with 1000mL of optimally grown culture of Cyanobacterial Cultures. This culture was analyzed by different biochemical parameters during the growth period.

Biochemical parameters

The present study was carried out for a period of 30 days for samples under laboratory conditions. On every 4th day, 5 mL of the sample was withdrawn and centrifuged at 5000 rpm for 10 minutes and the pigments namely, Chlorophyll-*a*, Carotenoid (Lichtenthaler, 1987), Phycocyanin and Allophycocyanin (Bennet and Bogarad (1973)) and then Total Carbohydrate (Dubois *et al*., 1956), Total Lipid (Folch *et al*., 1956), Total Protein (Bradford, 1976) and were extracted and estimated.

Dry Weight

Ten milliliters of the sample was taken and filtered through pre-weighed Whatmann No: 1 filter paper. The filter paper along with the Cyanobacteria sample was kept in oven at 60º C for 24 hours and the dry weight of the sample was calculated and recorded.

Estimation of Total Phenolic constituents

Total phenolic content was estimated by Folin–Ciocalteu method (Singleton & Rossi, 1965).

Antioxidant methods

The antioxidant activities of the extracts were observed for Thiobarbituric acid (TBA) test (Kikuzaki and Nakatani, 1993), Total reducing power (Prieto *et al*., (1999) and DPPH radical scavenging activity (Nenedis and Tsimidou, 2002).

Preparation of crude extracts

Grown cyanobacterial samples were centrifuged at 2500 rpm for 10 minutes to remove the water content. 25 g of fresh biomass was ground and sonicated for 10 min, then extracted with 50 mL of organic solvents such as ethyl acetate and methanol. Extracts were dried in freeze drier and the weight was calculated.

Antimicrobial activity

Stock solution preparation

All the extracts were dissolved in their respective solvents at the concentration of 10 mg/mL. The antibacterial activity was performed by well diffusion method. The respective bacterial culture swabbed on the Muller Hinton agar plates for uniform distribution of microorganisms. Using sterile well puncture 3 mm wide well was made on each agar plates. Various concentrations of organic crude extracts (500 -2000 µg/well) from the stock were poured into each well using a sterile micropipette. Streptomycin (30µg/well) was used as positive control and their respective used as negative control. The plates were incubated for 24 hours at 37° C. At the end of incubation period, the zone of inhibition was measured.

Bacterial inoculums

The test bacterial reference strains such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio alginolyticus, Shigella boydii, Shigella flexneri, Proteus vulgaris, Shigella dysenteriae, Enterococcus faecalis, Vibrio cholerae, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* (ATCC 27853) were collected from reference laboratory and maintain as per the laboratory standards.

Preparation of inoculum

The inoculum was prepared from a 24 h old culture on Muller Hinton Agar (MHA). With a sterile loop, the tops of 4-5 colonies were transferred to a tube containing 5 mL of nutrient broth. The tube was then incubated at 37˚C for 18 - 24 h. The turbidity of the culture suspension was adjusted with broth on a sterile saline solution (0.85 – 0.9%). The density of this culture was adjusted with 0.5 McFarland standards and finally made the inoculum size approximately of $5x10^5$ CFU/mL.

Agar well diffusion assay

The agar well diffusion method was employed to determine the antimicrobial activities of the tested gram negative bacterial pathogens. This assay was found to be a simple, cheap and reproducible practical method (Maidment *et al*., 2006). A suspension of each sample tested micro-organism diluted prior to 10^{-1}, 10^{-2} and 10^{-3} (1 ml of 10^8 cells/ml) was spread on a solid agar medium in Petri dishes (Mueller-Hinton agar). Well is made in inoculated agar (5 mm in diameter) different concentration of extract were loaded into the well separately and allowed for 15 min for the diffusion of compound, then incubated at 37°C for 24 h. The diameters of the inhibition zones were measured in millimeter.

Results

Cyanobacterial cultures

In the present study, eight different Cyanobacterial cultures such as *Nostoc* sp., *Calothrix* sp., *Chroococcus* sp., *Phormidium* sp., *Lyngbya* sp., *Synechococcus* sp., *Anabaena* sp., *Oscillatoria* sp. were grown in BG11 medium. The grown cultures were morphologically identified using bright field microscopy under 100X objective.

The identified strains were mass cultured in BG11 medium for the period of 30 days in the controlled temperature of 24° C under light of 30 μEm^2s^{-1} units. The salinity and contamination of the culture were checked periodically.

Growth study

Growth rate of cyanobacteria was accessed by estimating chlorophyll a, total carotenoid, phycocyanin, allophycocyanin, total carbohydrate, total protein, total lipid and dry weight. Growth was estimated at an interval of 4 days up to 30 days.

Chlorophyll a

The maximum level of chlorophyll a was recorded on 20th day as 12.9 µg/mL in *Lyngbya* sp., 12.36 µg/mL in *Oscillatoria* sp., 11.22 µg/mL in *Anabaena* sp., 9.8 µg/mL in *Nostoc* sp., 9.6 µg/mL in *Phormidium* sp., 9.5 and 9.3 µg/mL, for *Chroococcus* sp. and *Synechococcus* sp., 6.5 µg/mL in *Calothrix* sp. (Fig 1.1).

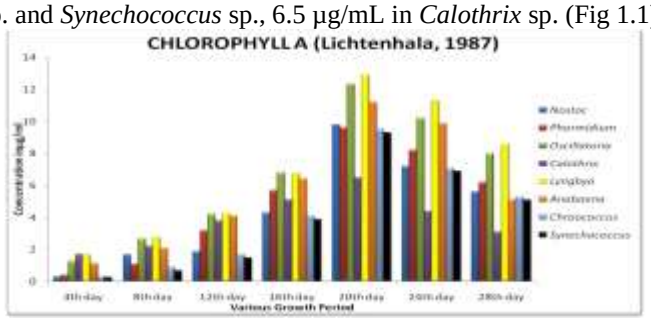

Fig 1.1 Estimation of Carotenoids for various Cyanobacterial extracts

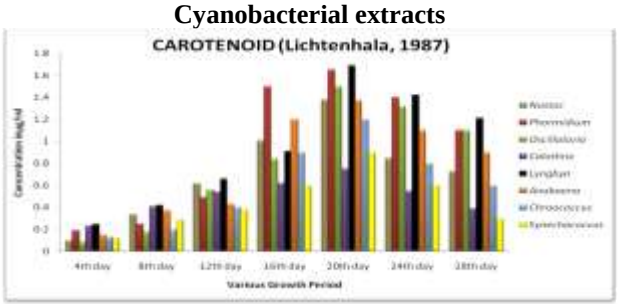

Fig 1.2 Estimation of Carotenoids for various Cyanobacterial extracts

Total Carotenoid

The maximum amount of total carotenoid was recorded on 20th day as 1.69 µg/mL in *Lyngbya* sp. followed by, 1.65 µg/mL in *Phormidium* sp., 1.5 µg/mL in *Oscillatoria* sp., 1.38 µg/mL in *Nostoc* sp., 1.37 µg/mL in *Anabaena* sp., 1.2 µg/mL in *Chroococcus* sp., 0.91 µg/mL in *Synechococcus* sp., 0.95µg/mL in *Calothrix* sp. (Fig 1.2).

Phycobilin Pigments

The maximum level of phycocyanin was recorded on 20th day as 0.86 µg/mL in *Lyngbya* sp. followed by, 0.83 µg/mL in *Oscillatoria* sp., 0.75 µg/mL in *Phormidium* sp., 0.62 µg/mL in *Nostoc* sp., 0.61 µg/mL in *Anabaena* sp., least concentration of 0.56, 0.57, 0.52 µg/mL in *Chroococcus* sp., *Synechococcus* sp. and *Calothrix* sp., respectively (Fig. 5). The maximum level of allophycocyanin was recorded on 20th day as 0.82 µg/mL in *Lyngbya* sp., 0.8 µg/mL in *Oscillatoria* sp., 0.71 followed by *Phormidium* sp., *Anabaena* sp. least concentration 0.46 for *Synechococcus* sp. obtained on 20th day (Fig 1.3 & 1.4).

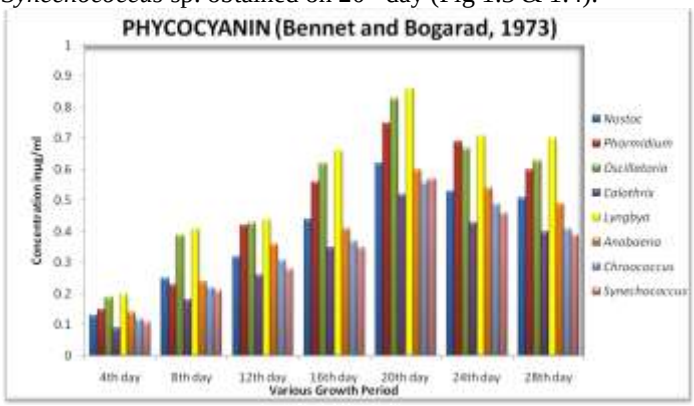

Fig 1.3. Estimation of Phycocyanin for various Cyanobacterial extracts

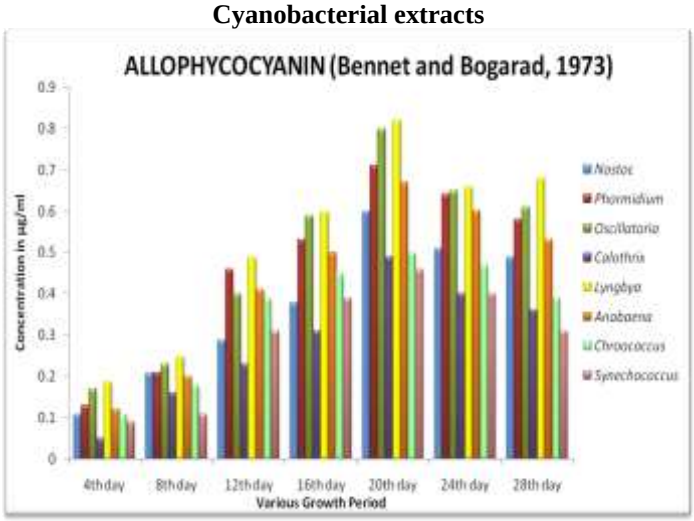

Fig 1.4. Estimation of allophycocyanin for various Cyanobacterial extracts

Total carbohydrate

Maximum total carbohydrate content was recorded on 24th day as 13.31 µg/mL in *Lyngbya* sp. followed by 12.36 and 12.26 µg/mL in *Oscillatoria* sp. and *Anabaena* sp. on 20th day. 9.8 µg/mL in *Nostoc* sp., 9.6 µg/mL in *Phormidium* sp., 9.7 and 9.6 µg/mL in *Chroococcus* sp.,and *Synechococcus* sp. respectively. Least concentration of 6.5 µg/mL was obtained in *Calothrix* sp. (Fig 1.5).

Total protein

A steady increase in protein concentration form 1st day to 24th day and decreasing concentration of protein was obtained on 28th day. Maximum total protein content was recorded on 24th day as 3.1 µg/mL in *Lyngbya* sp. *and Anabaena* sp. followed by an average of 2.5 µg/mL in other strains (Fig 1.6).

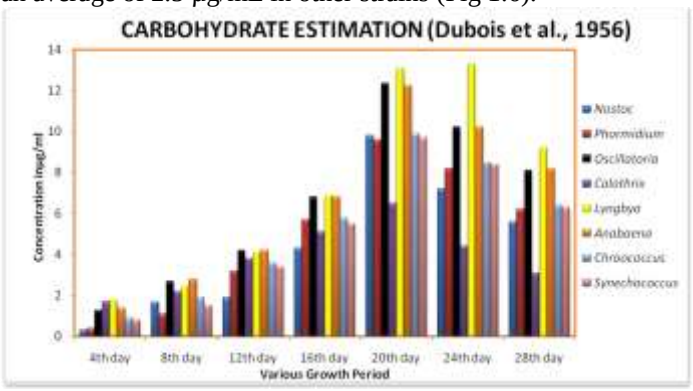

Fig 1.5. Estimation of carbohydrates for various Cyanobacterial extracts

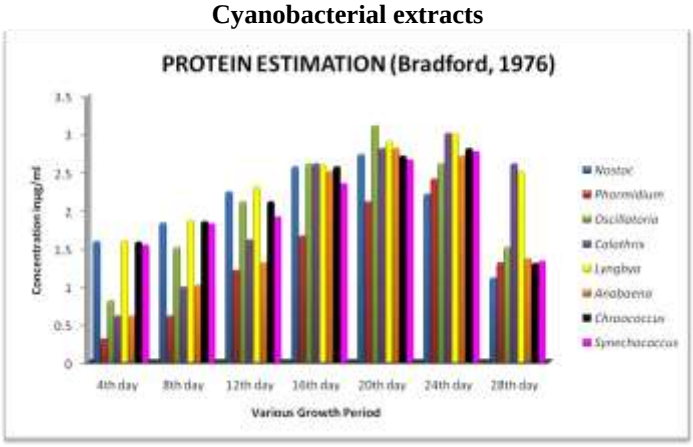

Fig 1.6. Estimation of proteins for various Cyanobacterial extracts

Total lipid

The maximum amount of total lipid was recorded on 24th day as 15.1 µg/mL in *Lyngbya* sp. followed by 14.9 and 14.6 µg/mL in *Oscillatoria* sp., *Phormidium* sp., and all the other cultures obtained at an average of 7.1 – to 8.0 µg/mL. Of these *Calothrix* sp. showed least concentration (Fig 1.7).

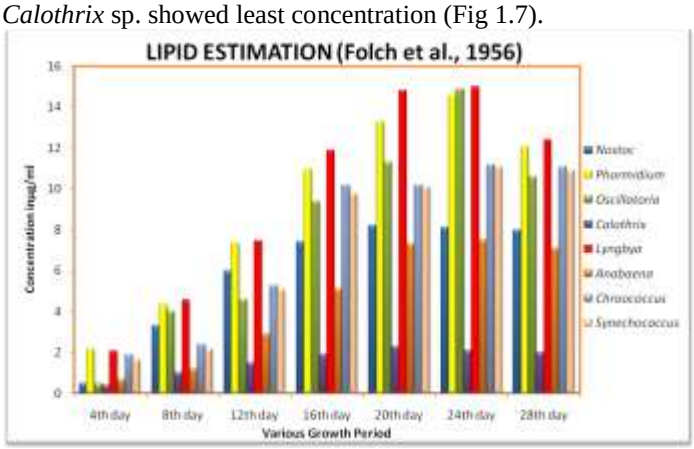

Fig 1.7 Estimation of lipids for various Cyanobacterial extracts

By comparing all these parameters, *Lyngbya* sp. shows maximum growth when compared with other Cyanobacteria followed by *Oscillatoria* sp. and *Phormidium* sp. it was identified using biochemical analysis.

Preparation of crude extract

The maximum cyanobacterial biomass of 36.5 g/L in *Lyngbya* sp., followed by 20.4 g/L in *Calothrix* sp., 21.4 g/L in *Nostoc* sp., 17.1 g/L in *Oscillatoria* sp., 16.9g/L in *Phormidium* sp., 15.1 g/L *Anabaena* sp. 9.7 and 9.0 g/L *Chroococcus* sp. and *Synechococcus* sp. respectively were collected and freeze-dried

at -20ºC for 18 h. The freeze-dried biomass was extracted by using different polar solvents such as methanol, ethyl acetate. The crude extracts were stored in vial at 4^{o} C.

***In vitro* Antioxidant activity**

Thiobarbituric acid (TBA) test

During the oxidation process, peroxides are gradually decomposed to lower molecular weight compounds, like malonaldehyde, which can be measured by TBS method on the final day of the incubation period. The antioxidant activity of α-tocopherol was high followed by methanolic extracts of *Nostoc* sp., *Calothrix* sp., *Chroococcus* sp., *Phormidium* sp., *Lyngbya* sp., *Synechococcus* sp., *Anabaena* sp., *Oscillatoria* sp., on 7^{th} day of incubation (Fig.1.9).

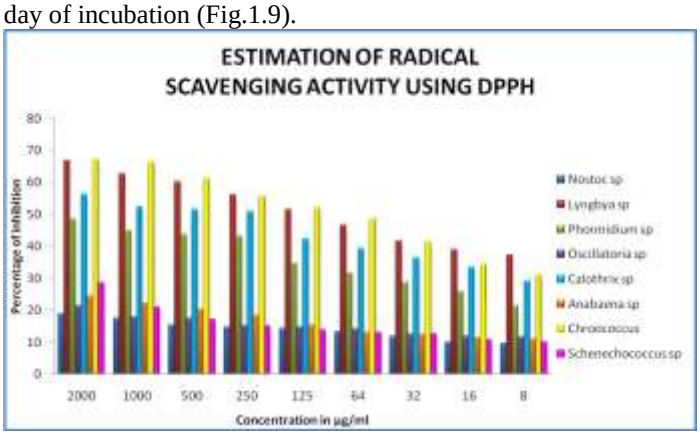

Fig 1.8 Estimation of radical scavenging activity using DPPH for various Cyanobacterial extracts

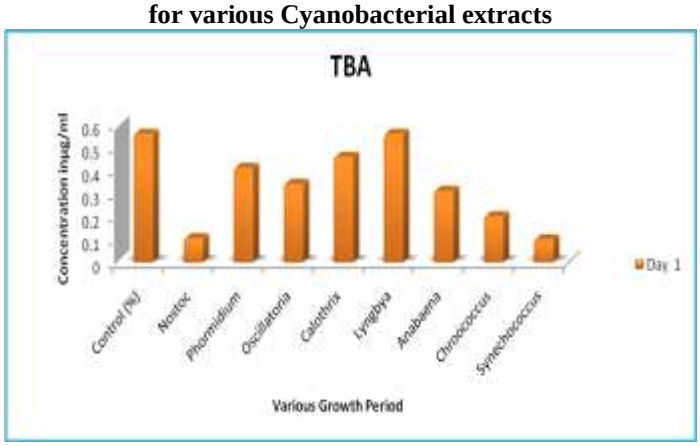

Fig 1.9 Estimation of TBA for various Cyanobacterial extracts

DPPH radical scavenging activity

The methanolic crude extract of *Chroococcus* sp., and *Lyngbya* sp. showed maximum hydrogen donar activity. The maximum DPPH radical activity was recorded as 66.8% at 1 mg/mL in *Chroococcus* sp., and *Lyngbya* sp., followed by 56.25% in *Calothrix* sp., 48.7% in, *Phormidium* sp., 21.5% in *Oscillatoria* sp. and 19% in *Nostoc* sp. (Fig 1.8).

Estimation of Phenol

The phenolic content of methanolic fractions varied from 49.9 GAE/mg in *Lyngbya* sp., followed by *Oscillatoria* sp. (47.6 GAE/mg), *Calothrix* sp. (37.6 GAE/mg), *Phormidium* sp. (28.3) and *Nostoc* sp. (29.3 GAE/mg), *Anabaena* sp. (29.3 GAE/mg), *Chroococcus* sp. (15.3 GAE/mg), *Synechococcus* sp. (13.4 GAE/mg) (Fig 1.10).

Antimicrobial activity

All the test bacteria showed resistance to cyanobacterial crude extracts (methanol, ethyl acetate) up to 250 µg concentration. An increasing concentration from 500 -2000 µg/ml showed moderate sensitivity against some of the test bacterial pathogens.

Among them, *Lyngya* sp. showed in moderate sensitivity to most of the tested bacterial pathogens such as *Pseudomonas aeruginosa*, *Vibrio alginolytica*, *Shigella boydii*, *Shigella dysentriae*, *Pseudomonas* ATCC culture and showed resistant pattern to *Klebsiella pneumoniae*, *Shigella flexineri*, *Proteus vulgaris*, *Shigella dysentriae*, and *Escherichia coli*. Maximum zone of inhibition was obtained in ethyl acetate extract of *Vibrio alginolytica*. This results indicate that *Lyngbya* sp. have the very high antibacterial potential compounds which are moderately effective to the tested gram negative pathogens.

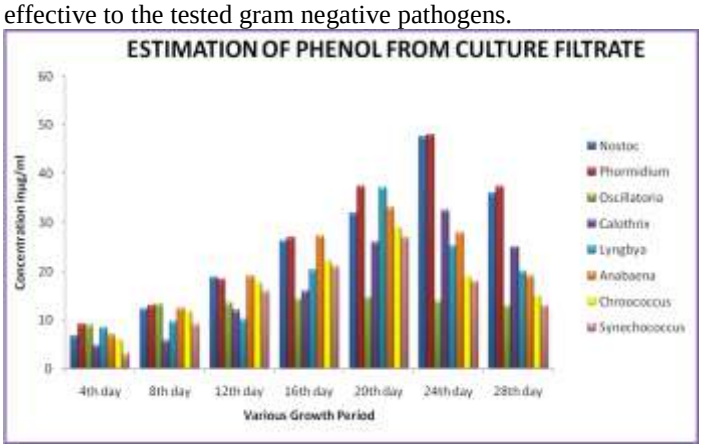

Fig 1.10. Estimation of phenol for various Cyanobacterial extracts

Nostoc sp. showed a very good moderate sensitivity to most of the tested bacterial pathogens and showed the maximum zone of 15mm for the methanolic extracts. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Vibrio alginolytica*, *Shigella boydii*, *Shigella flexinerii*, *Proteus vulgaris* against methanolic extract showed sensitive organisms. All the other tested stains showed resistant pattern.

A *Chroococcus* sp. also showed moderate sensitivity to some of the enteric gram negative pathogens. Of these methanolic extract of *Chroococcus* sp. showed strong sensitivity to *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Vibrio alginolytica*, *Shigella boydii*, all the other strains resistant to all the other strains tested.

Synechococcus sp. extracts (ethyl acetate and methanol) also showed moderate sensitivity to *Pseudomonas aeruginosa*, *Vibrio alginolytica*, *Shigella boydii*, *Salmonella typhi*, *Shigella dysentriae*, *Enterococcus faecalis* and showed resistant against *Klebsiella* sp., *Shigella flexineri*, *Proteus vulgaris*, *Vibrio cholerae and Escherichia coli*.

Anabaena sp extracts showed (Ethyl acetate and methanol) showed only less sensitivity to *Vibrio alginolytica* and *Enterococcus faecalis* and showed resistance all other tested pathogens.

Calothrix sp. showed moderate sensitivity to *Pseudomonas aeruginosa* and *Escherichia coli* at 2mg/ml concentration. All the other pathogens showed resistant to both extracts.

Oscillatoria sp. and *Phormidium* sp. showed only very little sensitivity *Escherichia coli* and showed resistant to all the other bacterial pathogens (Table 1.0).

The present antimicrobial studies reveals that cyanobacteria have the potential antibacterial activity against gram negative enteric pathogens further research in search of new drugs in the field of Cyanobacteria will leads to the new compound discovery.

Discussion

The untapped potential of cyanobacteria for their scientific exploitation has been realized only in recent years. It needs an extensive screening, which is expected to result in the discovery of better cyanobacterial strains of immense industrial interest.

The acquisition of fundamental knowledge of these versatile organisms is necessary for further progress. Evaluation of their physiological as well as biochemical characteristics leads to the selection of more prospective strains. It is, therefore, very essential to prepare an excellent database by determining the biochemical composition of cyanobacteria which can be used for commercial application.

All the eight cyanobacterial cultures were grown well in BG 11 medium at an average biomass of $5g/L^{-1}$ fresh weight and optimized temperature and light 16/8 dark condition. The maximum biomass was obtained in filamentous cyanobacteria than unicellular forms. Biochemical parameters were observed during the growth period shows the effective production of pigments such as chlorophyll and carotenoids. The carbohydrates and proteins were also documented to be high in this organism. This finding also confirms the use of organism as a food for humans and also as a feed for aquaculture developments. The presence of markable amount lipid shows that this organism may be effectively used for biofuel production.

The antioxidant compounds are mostly phenolic or poly phenolic in nature, e.g., α-tocopherols, and flavonoids. In cyanobacteria, polyunsaturated fatty acids present in thylakoid membranes of cyanobacteria are susceptible to oxidative damage (He and Hader, 2002). DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997). The reduction capacity of DPPH radicals was determined by decrease in its absorbance at 517 nm, which is induced by antioxidants. The antioxidant activity of *Lyngbya* sp. was maximum may be due to the presence of increase in the amount of phenol and phycobilin pigments.

The total phenolic content in every organism tested showed valuable result. The production of particle compound can be increased by strain improvements, since cyanobacteria require sun light and simple nutrients which can be easily grown in industries. The great impact apart from the food and oil is reduction of global warming which is a serious threaten nowadays.

Enteric bacteria are bacteria in the family Enterobacteriaceae. These bacteria reside normally in the guts of many animals, including humans, and some are pathogenic, causing disease in certain animal species. At least 40 genera have been identified in this family, including *Salmonella* sp., *Proteus* sp., *Serratia* sp., *Enterobacter* sp., *Citrobacter* sp., *Pseudomonas* sp., and *Klebsiella* sp. People usually become infected with enteric bacteria as a result of poor hygiene and contact with people who have existing infections. These pathogens are widespread in nature, inhabiting soil, water, plants, and animals (including humans). *Pseudomonas aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia. Pseudomonal infections are complicated and can be life threatening. The current antibacterial assay against our cyanobacterial extracts produced moderate to high activity to ethyl acetate extracts of *Chroococcus* sp., *Nostoc* sp., *Calothrix* sp., *Lyngbya* sp. upto 4mg/ml concentration. Hence these extracts have the effective bioactive compounds used to treat against Pneumonia and Urinary Tract infections *Klebsiella* cause a variety of clinical syndromes. Common klebsiellae infections in humans include community-acquired pneumonia, UTI, nosocomial infection, rhinoscleroma and ozena, and colonization. Of the various tested cyanobacterial extracts *Nostoc* sp. and *Chroococcus* sp. showed the remarkable sensitivity against *Klebsiella pneumoniae* in methanolic extracts.

Shigella sp. organisms are a group of gram-negative, facultative intracellular pathogens. They were recognized as the etiologic agents of bacillary dysentery or shigellosis. Of the eight cyanobacterial extracts tested only *Chroococcus* sp. showed high sensitivity to *Shigella boydii*, and moderate sensitivity to *Nostoc* sp. extracts to *Shigella boydii* and *Shigella flexineri* and not active to *Shigella dysentriae* strains.

Proteus sp. is part of the Enterobacteriaceae family of gram-negative bacilli. *Proteus* organisms are implicated as serious causes of infections in humans, along with *Escherichia* sp., *Klebsiella* sp., *Enterobacter* sp., and *Serratia* sp. methanolic extract of *Nostoc* sp. showed moderate activity to *Proteus mirabilis*. *Salmonella* sp., are gram-negative facultative intracellular anaerobes that cause gastroenteritis, enteric fever (caused by typhoid and paratyphoid serotypes), bacteremia, focal infections, to a convalescent lifetime carrier state. The antimicrobial assay had shown only meager sensitivity against *Lyngbya* sp., extracts. None showed sensitivity against *Salmonella* strain.

The genus *Enterococcus* includes more than 17 species, but only a few cause clinical infections in humans. With increasing antibiotic resistance, *Enterococci* sp., are recognized as feared nosocomial pathogens that can be challenging to treat. Only ethyl acetate extract of *Synechococcus* sp. showed moderate activity against *Enterococcus* sp. *Escherichia coli* is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatal meningitis and pneumonia. *Calothrix* sp., extract only showed high sensitivity against *E. coli.*

V. alginolyticus is a halophilic Vibrio first recognized as being pathogenic in humans in 1973. Their clinical syndromes reported in association with *V. alginolyticus* infection include chronic diarrhea in a patient with AIDS, conjunctivitis, and post-traumatic intracranial infection. Resistance to tetracycline and chloramphenicol has been reported in a few isolates of *V. alginolyticus*. Sensitivity pattern against the fresh water cyanobacterial extracts results in a high activity to *Chroococcus* sp., *Synechococcus* sp., and *Lyngbya* sp. ethyl acetate extracts. Other cyanobacterial extracts showed weekly active to *V. alginolyticus.* This cyanobacterial extracts proved to be effective against infection caused by this test pathogen.

Among eight cyanobacterial extracts tested, all the crude extracts exhibited antibacterial activity against at least one pathogen. These results showed that this new microbial group has enough potential to produce antibacterial substances against highly virulent gram enteric pathogens. Our results are in good agreement with the observations by Jaki *et al*., (1999).

The antibacterial activities of extracts prepared with different solvents in our study indicate that methanol seems to be suitable solvent for extraction of antimicrobial compounds from cyanobacterial biomass. The methanol extract of *Lyngbya* sp. was found to be active against most of tested bacteria. These results are contradictory with the findings of other studies. Kreitlow *et al*., (1999) assessed hydrophilic and lipophilic extracts of eleven freshwater cyanobacterial strains but none of them showed activity against Gram-negative bacteria. Martin *et al*., (2008) observed that out of 36 extracts prepared from nine cyanobacterial strains, no one was active against Gram negative bacteria

Table 1.0 Antibacterial activity of ethyl acetate, methanol extracts of cyanobacterial species against enteric gram negative pathogens

Cyanobacterial Species			Gram Negative Enteric pathogens											
			1	2	3	4	5	6	7	8	9	10	11	12
Chroococcus sp.	EA	10	-	-	-	***	-	-	-	-	-	-	-	**
		20	-	-	-	***	-	-	-	-	-	-	-	**
		30	-	-	-	***	-	-	-	-	-	-	-	-
		40	-	-	-	***	-	-	-	-	-	-	-	-
	M	10	-	**	-	**	-	-	-	-	-	-	-	-
		20	*	**	*	**	-	-	-	-	-	-	-	-
		30	**	***	***	***	-	-	-	-	-	-	-	-
		40	**	***	***	***	-	-	-	-	-	-	-	-
Oscillatoria sp.	EA	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	-	*
	M	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	-	-
P*hormidium* sp.	EA	10	-	-	-	-	-	-	-	-	-	-	*	-
		20	-	-	-	-	-	-	-	-	-	-	*	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	**	-
	M	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	-	-
Nostoc sp.	EA	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	*
		40	-	-	-	-	-	-	-	-	-	-	*	**
	M	10	**	*	*	**	*	**	-	-	-	-	-	-
		20	**	*	*	**	**	*	-	-	-	-	-	-
		30	*	**	*	**	*	*	-	-	-	-	-	-
		40	*	**	*	**	*	**	-	-	-	-	-	-
Calothrix sp.	EA	10	*	-	-	-	-	-	-	-	-	-	-	-
		20	*	-	-	-	-	-	-	-	-	-	-	-
		30	*	-	-	-	-	-	-	-	-	-	-	-
		40	*	-	-	-	-	-	-	-	-	-	-	-
	M	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	***	-
Anabaena sp.	EA	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	*	-	-	-	-	-	-	-	-	-
		40	-	-	*	-	-	-	-	-	*	-	-	-
	M	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	-	-	-	-	-	-	-	-	-	-
		40	-	-	-	-	-	-	-	-	**	-	-	-
Synechococcus sp.	EA	10	-	-	-	-	-	-	-	-	**	-	-	-
		20	-	-	*	-	-	-	-	*	*	-	-	-
		30	*	-	**	-	-	-	-	*	**	-	-	-
		40	**	*	**	-	-	-	-	*	**	-	-	-
	M	10	-	-	-	-	-	-	-	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	-	-	-
		30	-	-	*	*	-	-	*	-	-	-	-	-
		40	-	-	*	*	-	-	*	-	-	-	-	-
Lyngbya sp.	EA	10	*	-	*	*	-	-	*	-	-	-	-	-
		20	*	-	-	*	-	-	*	-	*	-	-	-
		30	**	-	**	*	-	-	*	-	-	-	-	*
		40	*	-	**	*	-	-	*	-	*	-	-	**
	M	10												
		20	-	-	-	-	-	-	*	-	*	-	-	-
		30	-	-	-	-	-	-	-	-	*	-	-	-
		40	-	-	-	-	-	-	-	-	-	-	-	**
			-	-	-	-	-	-	*	-	-	*	-	**

(-) No activity, (***) strong activity < 17mm, (**) moderate activity between 11-16mm, (*) week activity > 10mm, 1- *Pseudomonas aeruginosa*, 2- *Klebsiella pneumoniae*, 3- *Vbrio alginolyticus*, 4- *Shigella boydii*, 5- *Shigella flexineriri*, 6- *Proteus vulgaris*, 7- *Salmonella typhi*, 8- *Shigella dysentriae*, 9- *Enterococcus faecalis*, 10- *Vibrio cholerae*, 11- *Escherichia coli*, 12- *Pseudomonas aeruginosa* ATCC 27853

Cyanobacteria use various systems, particularly non-ribosomal peptide synthetase (NRPS) and polyketide synthatase (PKS) systems to produce novel natural products with therapeutic potential (Ehernich *et al.,* 2005). They may be a preferred source of antibiotics due to their economical cultivation (Jaspars *et al*., 1998). Several studies reporting the antibacterial activity of cyanobacterial extracts have been performed during last decade (Qstenswik *et al.,* 1998, Skullberg *et al.,* 2000, Biondi *et al*., 2008). In different studies, various crude extracts from *Nostoc* sp. (Singh *et al*., 2010), *N. muscorum* (Caire *et al.,* 1990), *Phormidium* sp. (Fish & Codd, 1994), *Lyngbya majuscule* (Sethubathi and Ashok Prabu, 2010) were evaluated for their antimicrobial effects on pathogenic microorganisms.

They have also reported that the extracts prepared in different solvents were effective against both Gram-positive and Gram-negative organisms. In the present study, ethyl acetate extract of cyanobacteria was found to be active against most of the test human pathogens. The sensitivity pattern also reveals the active bioactive compound in freshwater cyanobacteria can be used for the treatment against most of the enteric diseases.

Concluding Remarks

Cyanobacteria are a promising but still unexplored natural resource offering a wealth of chemicals for lead compounds discovery and new drugs. Of the new drugs approved between 1983 and 1994, up to 80% of antibacterial and anticancer drugs were derived from natural products. Traditional microbial drug producers like Actinomycetes and Hyphomycetes have been in the focus of pharmaceutical research for decades. Now that the rate of discovery of interesting compounds in these classical source organisms is decreasing, it is the time to turn to cyanobacteria and exploit their potential. Despite, the complexity of the algal genome, the mechanism behind their enormous chemical diversity is being slowly unfolded. The biosynthetic information on the chemical structures unique to these organisms will be very valuable for gene manipulation aimed at creating new therapeutic agents, and in the near future they will achieve the same position as Streptomyces and other Actinomycetes we have today.

Acknowledgement

I would like to thank our beloved Prof. R. Rengasamy, Director, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Guindy, Chennai for providing the opportunity to carry over the research.

Reference

Benett, A and Bogorad, L, (1973). Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell Biol*, **58**: 419.

Berdy, J, (2005). Bioactive Microbial Metabolites. *J. Antibiot* , **58**: 1-26.

Biondi, N., Tredici, M. R., Taton, A., Wilmotte, A., Hodgson, D. A., Losi, D, (2008). Cyanobacteria from benthic mats of Antarctic lakes as a new source of bioactivities. *J. Appl. Microbiol*, **105**: 105-115.

Bradford, M. M., (1976). A dye binding assay for protein. *Anal. Biochem*, **72**: 248-254.

Caire, G. Z., De M. S., De Cano S., De Mule M. C. Z. and De Halperin D. R., (1990). Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani*. *Phyton*, **51**: 1- 4.

Constantino, V., Fattorusso, E., Menna, M., Taglilatela-Scafati, O., (2004). Chemical diversity of bioactive marine natural products: an illustrative study. *Curr. Med. Chem.,* **11**: 1671-1692.

Dubois, M. K., T. Gillers, R. A. Hamilton, Robeus and F. Smith, (1956). Calorimetric methods for determination of sugars and related substance. *Anal. Chem.*, **28**: 305-365.

Ehernich I. M, Waterbury J. B, Webb E. A, (2005). Distribution and diversity of natural product genes in marine and fresh water cyanobacterial cultures and genomes. *App. Env. Microbiol*., **71**: 7401-7403.

Fish, S. A., and G. A. Codd, (1994). Bioactive compound production by thermophilic and thermotolerant cyanobacteria (blue-green algae*). World J. Microb. Biotech*, **10**: 338-347.

Folch, J., M. Lees and G. H. S. Stanley, (1956). A simple method for the isolation and purification of total lipides from animal tissues. J. *Biol. Chem.*, **226**: 497-509.

He, Y.Y., Hader, D.P., (2002), Reactive oxygen species and UV-B: effort on cyanobacteria. *Photochem. Photobiol. sci.*, **1**: 729-736.

Jaspars M, Lawton L. A, (1998). Cyanobacteria- a novel source for pharmaceuticals. *Current Opinion in Drug Dis. Dev*, 77- 84.

Jaki, B., Helimann, J., & Sticher, O., (2000). New antimicrobial metabolites from the cyanobacterium *Nostoc Commune*. *Journal of Natural products*. **63**: 1283-1285.

Kikuzaki, H., and Nakatani, N., (1993). An effect of some ginger constituents. *J. Food. Chem.*, **57**: 2144-2155.

Kreitlow, S., Mundt, S., Lindequist, U., (1999). Cyanobacteria—a potential source of new biologically active substances. *J. Biotech.*, **70**: 61–63.

Lichtenthaler, H. K., (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.* **148**: 350-382.

Maidment, C., Dyson, A., Haysom, I ., (2006). A study into the antimicrobial effects of cloves (*Syzgium aromaticum*) and cinnamon (*Cinnamomum zeylanicum*) using disc-diffusion assay. *Nutr. Food Sci*., 36 **(4)**: 225 – 230.

Martins, R.F., Ramos, M. F., Herfindal, L., Sousa J. A., Skarven, K., and Vasconcelos V. M., (2008). Antimicrobial and cytotoxic assessment of marine cyanobacteria - *Synechocystis* and *Synechococcus*. *Mar. Drugs*, **6**: 1–11.

Nenadis, N., Wang, L. F., Tsimidou, M., & Zhang, H. Y. (2004). Estimation of scavenging activity of phenolic compounds using the ABTS assay. *Journal of Agricultural and Food Chemistry*, **52**: 4665–4669.

Newman, D. J., Cragg, G. M., Snader, K. M., (2000). The influence of natural products upon drug discovery. *Nat. Prod. Rep.*, **17**: 215-234.

Parekh, J., Chanda, V. S. In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk. J. Biol*. 2007; **31**: 53-58.

Prieto, P., Pineda, M., and Aguilar, M, (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*. **269**: 337-341.

Qstenswik, O., Skulberg, O. M., Underdal, B., Hormazabal, V., (1998). Antibacterial properties of extracts from selected planktonic freshwater cyanobacteria – a comparative study of bacterial bioassays. *J. App. Microbiol*., **84**: 1117–1124.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y., (1979), Genetic assignments Robak J, Marcinkiewicz E (1995). Scavenging of reactive oxygen species as the mechanism of drug action. *Pol. J. Pharmacol*. **47**: 89-98.

Sethubathi, G., and Ashok Prabu, V., (2010), Antibacterial activity of Cyanobacterial species from Adirampattinam Coast, Southeast Coast of Palk Bay. *C. Res. J. Biol. Scie.,* 2**(1)**: 24-26.

Singh, S. R. K., Upadhyay, S., Tiwari, S. P., Rai, A. K., and Mohapatra1, T. M., (2010), Screening of cyanobacterial extracts against bacteria causing nosocomial infections. *Journal of Pharmacy Research,* 3**(9)**: 2096-2098.

Singleton, V. L., and Rossi, J. A., (1965), Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic*, **16**:144-158.

Skulberg, O. M., (2000), Microalgae as a source of bioactive molecules; experience from cyanophyte research. *J. App. Phycol*, **12**: 341-348.

World Health Organization, (2005), WHO model list of essential medicines.
http://whqlibdoc.who.int/hq/2005/a87017_eng.pdf.