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Research Article

In vitro evaluation of Antimicrobial activity of some selected Cyanobacterial extracts against human pathogens

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ABSTRACT

The idea of antimicrobial compound from natural sources has mainly concentrated in recent year for the treatment of human diseases. In view of that, the present investigation was made to screen the bioactive substances from marine cyanobacteria. Four different cyanobacteria were isolated from Mallipattinam, Southeast coast of India. All the four cyanobacterial species were extracted by using chloroform. These cyanobacterial extracts were examined by *in vitro* analysis for the assessment of antimicrobial effects against four pathogenic bacteria and two fungi species by using disk diffusion method. Among the four cyanobacterial extract, *Lyngbya* sp. showed considerable inhibition effect with all the bacterial and fungal pathogens. It shows the highest inhibition zone (10mm) against *E. coli* strain and the least inhibition activity with *P. aeruginosa* (7.5mm). The antimicrobial bioassay of the extracts was further examined by determining the minimum inhibitory concentration (MIC) with all the pathogenic bacteria and fungi. The MIC result showed that the test extract had significant influence on the microbial pathogens.

Key Words: Cyanobacteria, pathogens, extract, MIC.

1. INTRODUCTION

Antibiotics are natural or synthetic chemical compounds that can suppress the growth and destroy the microorganism [1,2]. The search for novel antimicrobial agents with clinically important pathogens is significant since many clinical pathogens such as *Mycobacterium tuberculosis*, *Enteroccus*, *Pseudomonas* sp., *Streptococcus pneumoniae* and *Staphylococcus aureus* are developing resistance to routinely used antimicrobials [3]. Development of antibiotic resistance is a challengeable for the majority of the pathogens, which highlights the demand for new antibacterial product development.

Microorganism is the best source of antibiotic particularly; marine is the major source for antibiotic production[4]. In the microbial population, cyanobacteria are rapidly proving to be an extremely important source of biologically active secondary metabolites [5, 6], excellent sources for antiviral, antibacterial, antifungal and anticancer compounds [7]. Cyanobacteria considered being one of the potential organisms, which constitute a versatile group of microorganisms, occur in diverse habitats ranging from alkaline hot springs to permanent snowfields in the poles which can be useful to mankind in various ways. A number of significant advances have occurred in cyanobacterial biotechnology in the recent years. The use of cyanobacteria in the field of medical industries for its secondary metabolites such as vitamins, toxins, enzymes, pharmaceuticals and

pharmacological probes is become popular worldwide and also used in food, fuel, fertilizer, and other industries[8].

Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetic importance. The properties of secondary metabolites in nature are not thoroughly distinguished [9-11]. Secondary metabolites from cyanobacteria are combined with toxic, hormonal, antineoplastic and antimicrobial reflex [12,13]. The antimicrobial substances screened from cyanobacteria are being tested against various kinds of microorganisms. Nowadays, there has been an increasing attention in cyanobacteria as a potential source for important drugs [14-17]. Marine organism particularly cyanobacteria are proved to be one of the richest sources for novel bioactive compounds [18,5,19,8]. India is one among the mega diversified country with abundant natural resources, the proper utilization of these resources is very much important. Hence, the present investigation is focused to develop and apply antimicrobial effects of four cyanobacteria extracts.

2. MATERIALS AND METHOD

2.1. *Study area*

In order to study the antimicrobial efficiency of marine cyanobacteria, samples were collected from Mallipattinam, which is a small fishing village located at (Lat: 11° 29'' N Long: 79°46''), Southeast coast of India. The shallow coastal region of Mallipattinam (Palk Bay) is very fertile, having dense seagrass bed and rich in biodiversity since this area was selected for the present research work.

2.2. Sample collection and of Isolation marine cyanobacteria

Sediment samples were aseptically collected in sterile polythene bags and immediately brought to the laboratory in insulated ice box for further processing. For the isolation and maintaining the marine cyanobacteria, ASN3 media were used and congenial environmental conditions were maintained. ASN3 media contains Nitrate, phosphate, magnesium and calcium which is generally required by cyanobacteria. The trace metal mixture was prepared in separate conical flask. Then 1ml of trace metal mix was added to the 1000ml ASN-3 media. The pH of the medium was adjusted to 7.5 by adding 0.1N NaoH or 1N HCl and incubated at 25±2^{0}C under continuous illumination (2000-3000 lux). Since they are mostly adapted to diffused light, good growth was obtained at 1500 Lux light intensity. The optimal temperature required for their growth was 27±2 ^{0}C.

2.3. Purification and culture technique

From the mixed cyanobacterial culture individual types of cyanobacteria was isolated and purified by streaking or pour plating techniques. Dilution technique was also performed for the physical separation of individual strain of *Cyanobacterium* sp from the mixed culture. Morphologically distinct colonies were picked up for pure cultures technique. The identification of cyanobacterial strains were performed by special key [20-22]. Four different species of cyanobacteria, *Lyngbya* sp, *Nostoc* sp, *Phormidum* sp, and *Calothrix* sp were individually transferred into the medium of separate flask and special precaution was taken for maintenance of purity of the culture. After inoculation, the flasks were kept in the culture room for 25 days at the temperature of 27±2^{0}C for incubation under continuous illumination (2000-3000 lux) in order to avoid the contamination through condensation.

2.4. Extraction procedure

All the species of cyanobacteria cultures *Lyngbya* sp, *Nostoc* sp, *Phormidium* sp, *Calothrix* were selected for the antimicrobial activity assessment. The ability for production of microbial substances was tested against bacterial and fungal pathogens. The lipophilic compounds were extracted from all the four species as per the procedure given by Pesando [23]. Pre-weighed dried powder of cyanobacteria was suspended in 50ml of solvent mixture with and left in dark condition for 24hrs at room temperature. After 24hrs, the extract was filtered and the residue was resuspended in 50ml of solvent mixture and kept in the dark room for another 24hrs. The procedure was repeated several times until colorless filtrate was obtained with full extractions in solvent mixture which facilitated the recovery of all the lipophilic compounds present in the filtrates. To an equivalent amount of filtrates, a mixture of chloroform and water in the ratio 1:1 was added. This mixture was shaken well and kept in the separatory funnel overnight for the separation of the aqueous phase and the chloroform phase. After such separation the chloroform phase was taken out and evaporated in a vacuum evaporator. The residue was washed well with distilled water and again dried in a vacuum evaporator. These dry extracts were used for antimicrobial activity.

2.5. Test organism

The following test microorganisms were used for antimicrobial activity of the cyanobacterial extracts: *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Eschericia coli, Aspergillus niger* and *Penicillium* sp. All the cultures were obtained from Raja Sir Muthaiyah Medical College, Annamalai University, Chidambaram.

2.6. Preparation of inoculum

Bacterial inoculums were prepared by cultured in MHB (Muller Hinton agar Broth (Himedia, Mumbai)) and kept under incubation at 37^0C, for 24 hours. These cell suspensions were diluted with sterilized MHB to provide initial cell counts of about 10^4CFU/ml. The fungi were grown on Potato Dextrose Agar (PDA) slants at 28^0C for 10 days and the spores were collected using sterile doubled distilled water and homogenized.

2.7. Antimicrobial activity

Antimicrobial activity of cyanobacterial extracts were assayed by disc diffusion method [24]. The pathogenic organisms were grown individually in the sterile nutrient broth for 24 hr at 37°C in the case of bacteria and 28°C for 72h in the case of fungi. For antimicrobial assay, Mueller Hinton Agar (MHA) for bacteria and Sabouraud Agar for fungi were prepared. Both the media were poured aseptically into Petri plates and allowed for solidification. Then the test organisms were grown in the nutrient broth was swabbed into the sterile MH agar plates using sterile cotton buds. Then 0.5mg of the lipophilic fraction, negative and positive control was loaded on to the paper disc and was placed on the agar plates. The respective solvent was used as negative and antibiotic such as ampicillin (50µg/disc) and nystatin (25µg/disc) were used as a positive control for bacteria and fungi respectively. The petridishes were incubated at 8–10°C for 16 hours for the diffusion of the bioactive compound. Then the plates were kept incubated for 24 hours at 37°C for bacterial growth. After incubation, the plates were examined for the antimicrobial activity. Zones of inhibition were recorded in millimeters and the experiment was repeated thrice for concordant results.

2.7. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by using the method of NCCLS, [25]. The MIC determination is mainly to find out the least concentration of individual extract that inhibit the growth of human pathogens. The different concentrations (10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml and 0.312 mg/ml) of cyanobacterial extract were dissolved in Dimethyl sulfoxide (DMSO 4%). The antibacterial (ampicillin 20µg/disc) and antifungal (nystatin 10µg/disc) agent were included in the assays as positive controls. All strains were grown in MH Broth. The plates were kept in an incubator for the period of 72 h at 28^oC for fungi and for bacteria 24 h at 37^oC. The MIC for bacteria was determined as the least possible concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. After that, the diameter of the zone of inhibition was measured. All the measurements were completed in triplicates and the values were averaged.

3. RESULTS

Cyanobacteria were isolated and identified up to genus level from the Mallipattinam coastal sediment samples. Four distinct cyanobacterial species were identified (Fig. 1a-1d) which includes *Lyngbya* sp., *Nostoc* sp., *Phormidium* sp. and *Calothrix* sp.

3.1. Effect of cyanobacteria on bacterial and fungal pathogens

All the four different cyanobacterial strains were extracted and tested against four human pathogenic bacterial strains and two fungal strains for antimicrobial activities. The inhibition zones of extracts against the specific test organisms were measured. The effects of lipophilic extract of cyanobacteria on the growth of pathogens are presented in Fig. 2 and 3.

The extracts of all the four cyanobacterial species have significant inhibitory bioassay on the bacterial pathogens except *Phormidium* sp. which showed inhibition only on two strains *S. aureus* and *P. aeruginosa* no inhibition activity were seen against *B. subtilis* and *E. coli.* Among the four species cyanobacteria *Lyngbya* sp. showed considerable inhibition effect with all the bacterial and fungal pathogens. It showed the highest inhibition zone (10mm) against *E. coli* strain and the least inhibition activity with *P. aeruginosa* (7.5mm). Next to *Lyngbya* sp. the strain *Nostoc* sp. showed the significant activity with *P. aeruginosa* (7.5mm) and minimum activity with *E. coli* (5.8mm). In fungi also the strain *Lyngbya* sp. exhibited the maximum inhibition (9mm) against *A. niger* and *Penicillin* sp. (7.8mm) followed by the strain *Phormidium* sp. which showed the higher activity (6.8mm) against both *A. niger* and *Penicillin* sp. whereas in *Calothrix* sp. extract no assay was seen against fungal pathogens.

3.2. Minimum inhibitory concentration (MIC)

The MIC was estimated for all the cyanobacterial extracts with the pathogenic bacteria and fungi given in Table 1 and 2. The MIC values of the tested marine cyanobacteria extract against pathogenic bacteria were ranged between 0.312-2.5 mg/ml. The excellent antibacterial activity and low MIC value was observed with crude extract of *Lyngbya* sp. against *E. coli* (0.312 mg/ml), *S. aureus* (0.625 mg/ml), *B. subtilis* (0.625 mg/ml) and *P.*

aeruginosa(1.25 mg/ml). The species of *Callothrix* and *Nostoc* extracts showed the moderate MIC activity towards the bacterial and fungal pathogens. No inhibition was observed against *P. aeruginosa* and *E. coli* with *Phormidium* sp. Except *Callothrix* all the other three species showed excellent inhibition activity against pathogenic fungi.

4. DISCUSSION

Microorganisms from marine environment are promising as excellent resources for the innovation of active secondary metabolites. Among them, marine cyanobacteria are important recourses for novel antibiotics. In the present study, four marine cyanobacteria were isolated from Mallipattinam coastal areas and their extracts were tested against human pathogens. The results observed in the present study revealed that the marine cyanobacteria are the excellent sources for antimicrobial property. All the extracts showed the inhibitory effect on the test organisms. The genus *Lyngbya* sp. expressed the maximum inhibition assay with bacteria and fungi in *E. coli* the zone of inhibition was 10 mm and in *A. niger* it was 9 mm. In the earlier report *Lyngbya* sp isolated from soil, and marine waters Malvan coast also shown the excellent antibacterial activity [26]. Next to *Lyngbya* sp., the genus *Nostoc* sp. and *Phormidium* sp. extracts also observed with antimicrobial activity against pathogenic bacteria and fungi [27, 28.]

The major factors affecting the size of the inhibition zone are the chemical and physical properties of the growth medium [29]. The antimicrobial activity of marine epiphytic bacteria of intertidal seaweeds had been observed by Lemos *et al.*, [30]. Li zheng *et al.*, [31] isolated 341 strains from sea water, sediments and marine organism from different coastal areas of China, 12% of them showed antimicrobial activity and most of the active bacteria were associated with marine invertebrates and seaweeds.

Previously some of the works have been made on the antimicrobial activity against cyanobacterial species. It proved that the extracts of cyanobacteria may present diverse bioactive compounds responsible for the antibacterial activity [32,27]. Furthermore, many bioactive compounds may be excreted into marine environment due to stress to survival of cyanobacteria [33-35]. Thummajitsakul *et al.*, [36] selected eighteen cyanobacteria from the natural area of Thailand among them, two genera of cyanobacteria (NCI1 and NCI4) which resembles *Phormidium* sp. and *Microcoleus* sp., respectively which shown positive antibacterial activities. It was concluded that the extracts of *Phormidium* and *Microcoleus* species indicated the potential antibacterial activity against *S. enteritidis* and *E. coli.* Rania and Abedin hala, [37] made the comparative study on antibacterial and antifungal activity of cyanobacteria and green microalgae extracts and they concluded that the cyanobacterial extract have more efficient inhibition activity than that of green microalgae. The other cyanobacteria species, *Anabaena* sp. had also been reported for antibacterial properties against *S. aurseus*, *E. coli*, *P. aeruginosa*, *S. typhi* and *K. pneumoniae* [38], Helen Diana *et al.*, [39] investigated for *in vitro* antibacterial activity Cyanobacteria extract isolated from salt plan against *Escheriachia coli, Streptococcus aureus, Salmonella typhi, Klebsiella pneumonia, Streptococcus facecalis* and *Bacillus subtilis* using agar diffusion and Minimum inhibition concentration. They stated that *Spirulina major, Oscillatoria salina* and *Plectonema terebram* showed significant antibacterial activity.

The present investigation indicated that marine cyanobacteria remains an interesting source for new antimicrobial metabolites with better inhibition activity and also suggest that *Lyngbya* sp. and *Nostoc* sp. could be a potential sources for secondary metabolites with strong antibacterial and antifungal activity.

5. CONCLUSION

Marine cyanobacteria are the substantial resources for natural bioactive substances with, potential use in the pharmacological industry. In this present study, we investigated the antimicrobial activity of some crude cyanobacterial extracts which showed very good results against human pathogens. Since, further study must be necessary for compound identification and chemical characterization. It will provide an approach for discovery of novel bioactive compound from cyanobacteria.

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Table 1
MIC value against bacterial pathogen

S.No	Cyanobacteria	MIC	*S. aureus*	*P. aeruginosa*	*B. subtillis*	*E. coli*
1	*Lyngbya* sp.	mg/ml	0.625	1.25	0.625	0.312
2	*Nostoc* sp.	mg/ml	2.5	1.25	1.25	2.5
3	*Phormidium* sp.	mg/ml	1.25	-	1.25	-
4	*Callothrix* sp.	mg/ml	1.25	1.25	0.625	1.25
5	Ampicillin (DMSO 4%)	20µg/ml	0.625	0.625	0.312	0.625

Table 2
MIC value against fungal pathogen

S.No	Cyanobacteria	MIC	*Aspergillus niger*	*Penicillium* sp.
1	*Lyngbya* sp.	mg/ml	0.312	0.312
2	*Nostoc* sp.	mg/ml	0.312	0.625
3	*Phormidium* sp.	mg/ml	0.625	0.625
4	*Callothrix* sp.	mg/ml	-	-
5	Nystatin (DMSO 4%)	10µg/ml	0. 625	1.25

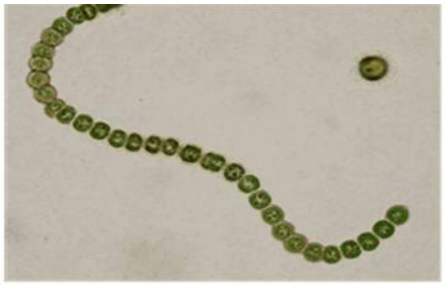

Fig (1a)- *Lyngbya* sp.

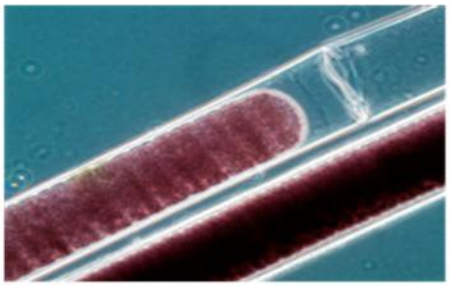

Fig (1b)- *Nostoc* sp.

Fig (1c)- *Phormidium* sp.

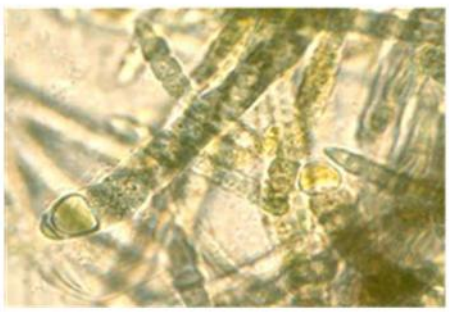

Fig (1d)- *Callothrix* sp.

Fig 1 (1a-1d).
Cyanobacterial strain isolated from Mallipattinam coastal waters.

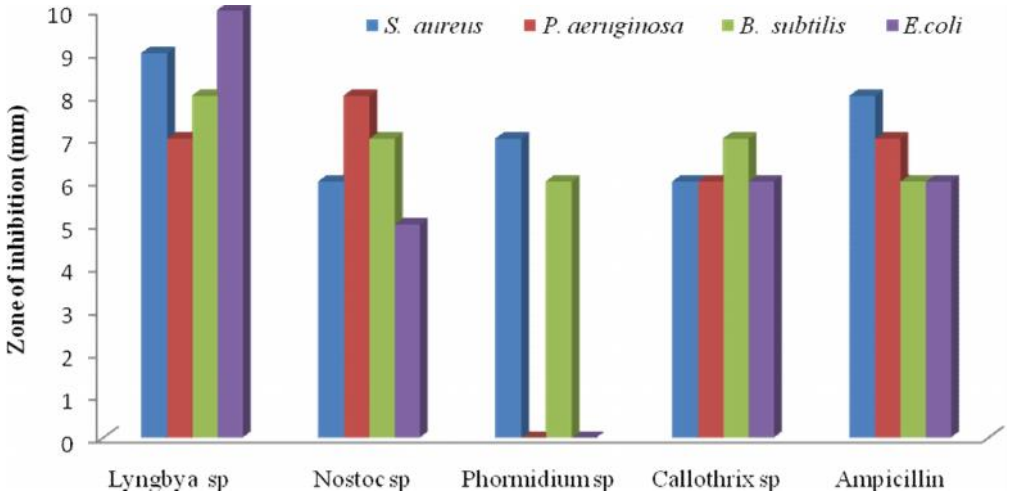

Fig 2
Effect of Cyanobacteria on bacterial pathogen

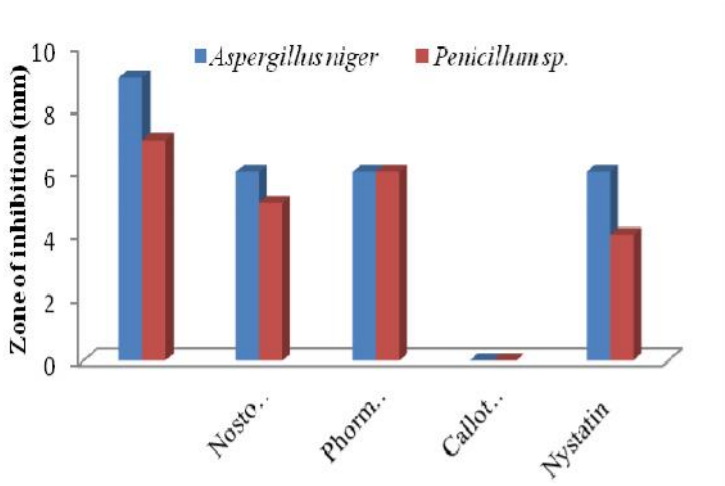

Fig 3
Effect of Cyanobacteria on fungal pathogen

7. REFERENCES

1. DeLong F, Microbial population genomics and ecology. Curr. Opin. Micro. 2002; 5: 520–524.
2. Martinez A, Kolvek S, Yip C, Hopke J, Brown A, MacNeil A, Osburne M, Genetically modified bacterial strains and novel bacterial artificial chromosome shuttle vectors for constructing environmental libraries and detecting heterologous natural products in multiple expression hosts. Appl. Environ. Microbiol. 2004b; 70: 2452-2463.
3. Anlezark G, Vaughan T, Fashola-Stone E, Murdoch T, Sims M, Stubbs S, Wigleyc S, and Minton N, Bacillus amyloliquefaciens ywrO encodes a nitroreductase enzyme which activates the prodrug CB 1954. Microbiol. 2002; 148: 297-306.
4. Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, and Prinsep MR, Review Marine natural products. Nat. Prod. Rep. 2009; 26(2): 170-244.

5. Tan LT, Bioactive natural products from marine cyanobacteria for drug discovery. Phytochem. 2007; 68(7): 954–979.
6. Gademann K, Portmann C, Secondary metabolites from cyanobacteria: complex structures and powerful bioactivity. Curr. Org. Chem. 2008; 12(4): 326-341.
7. Ruiz B, Chávez A, Forero A, García-Huante Y, Romero A, Sánchez M, Rocha D, Sánchez B, Rodríguez-Sanoja R, Sánchez S, Langley E, Production of microbial secondary metabolites: regulation by the carbon source. Crit. Rev. Microbiol. 2010; 36(2): 146–67.
8. Thajuddin N, Subramanian G. Cyanobacterial biodiversity and potential applications in biotechnology. Curr. Sci. 2005; 89(1): 47- 57.
9. Metting B and Pyne JW. Biologically active compounds from microalgae. Enzyme. Microb. Technol. 1986; 8: 386-394.
10. Inderjit and Dakshini KMM. Effect of cultivation on allelopathic interference success of Pluchea lanceolata. J. Chem. Ecol. 1994; 20(5): 1179-1188.
11. Rastogi RP, Sinha RP. Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnology Advances. 2009; (27): 521–539.
12. Carmichael WW. A review: cyanobacteria secondary metabolites-the cyanotoxin. J. Appl. Bacterial. 1992; 72(6): 445-459.
13. Patterson GML, Parker DL, and Bolis CM. Fungal cell wall polyaccaride elicit an antifungal 2 metabolite (phytoalexin) in the cyanobacterium Scytonema ocellatona. J.Phycol. 1994; 33: 54-60.
14. Glombitza KW, Koch M. Secondary metabolites of pharmaceutical potential. In Algal and cyanobacterial Biotechnology ed. R.C. Cresswell, T.A.V Rees, N. and Shah, 1989; 161-238.
15. Kulik MM. The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. Eur. J. Plant Path. 1995; 101(6): 585-599.
16. Kreitlow S, Mundt S, Lindequist U. Cyanobacteria-A potential source of new biologically active substances. J. Biotech. 1999; 70: 61-63.
17. Dvornyk V, Nevo E. Genetic polymorphism1 of cyanobacteria under permanent stress: A lessonfrom the “Evolution Canyons. Res. Microbiol. 2003; 154(2): 79–84.
18. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG and Wright PC, Marine cyanobacteria-a prolific source of natural products. Tetrahedron. 2001; 57(46): 9347-9377.
19. Gerwick WH, Coates RC, Engene N, Gerwick L, Grindberg RV, Jones AC and Sorrels CM. Giant marine cyanobacteria produce exciting potential pharmaceuticals. Microbe. 2008(6); 3: 277-284.
20. Taylor FJR. Symbiosis in marine microplankton. Ann. Inst. Oceanogr. Paris. 1982; 58: 61–90.
21. Desikachary TV. Cyanophyta. Indian Council of Agricultural Research, New Delhi. 1959; 686.
22. Drouet F. Revision of the Nostocaceae with cylindrical trichomes. Hafner Revision of the Nostocaceae with Cylindrical Trichomes. Hafner Press, (Macmillan), New York. 1979; 292.
23. Pesando D. Antibacterial and antifungal activities of marine algae. In; (I.Akatsuka, ed) Introduction to applied phy PB Accology Sademic Publishing bv, The Hague. 1990; 3-27.
24. Sujatha Peela, Bapiraju Kurada VVSN and Ramana Terli. Studies on antagonistic marine actinomycetes form Bay of Bengal. World. J. microbial. Biotech. 2005; 21: 583-585.
25. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: Proposed standard. NCCLS document M38-P. Wayne, Pa, USA, 1998.
26. Kinsalin VA, Prabhadevi D, Duraipandiyan V, and Ignacimuthu S. Isolation, characterization and antimicrobial properties of marine bacteria and fungi from the surface of Cyanobacterium Lyngbya sp. International Journal of Pharmaceutical and Clinical Science 2014; 4(1): 4-10.
27. Ploutno A, Carmeli S. Nostocyclyne A, a Novel Antimicrobial Cyclophane from the Cyanobacterium Nostoc sp. J. Nat. Prod. 2000; 63(11): 1524-1526.
28. Meizoso R, Jaime L, Santoyo S, Cifuentes A, Reina GG-B, Senorans FJ, Ibanez E. Pressurized Fluid Extraction of Bioactive Compounds from Phormidium Species. J. Agr. Food. 2008; 56: 3517–3523.

29. Glombitza MJ. Node sur la frequence de la production d'antibiotiques lipidiqes chez les algues planctoniues. Rev. Intern. Oceanogr. Med. LVIII. 1980 ; 41-44.
30. Lemos ML, Toranzo AE and Barja JL. Antibiotic activity of epiphytic bacteria isolated from intertidal seaweeds. Microb. Ecol. 1985; 11: 149-163.
31. Li Zheng, Xiaotian han, Haimin Chen, Wei lin, Xiaojun yan. Marine bacteria associated with marine macroorganisms: the potential antimicrobial resources. Ann Microbiol. 2005; 55(2): 119-124.
32. Kempf B, Bremer E. Uptake and synthesis of compatible solutes as microbial stress responses to high osmolarity environments. Arch. Microbiol. 1998; 170: 319–330.
33. Martin H, Maris P. Antiseptic and antibiotic resistance of 310 Gram-positive strains isolated from udders after use of post-milking teat germicides. Vet. Res. 1995; 26(1): 43-56.
34. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P. Resistance of Bacillus endospores to extreme terrestrial and extraterrestrial environments. Microbiol. Mol. Biol. Rev. 2000; 64(3): 548-572.
35. Soltani N, Khavari-Nejad RA, Tabatabaei Yazdi M, Shokravi SH, Fernández- Valiente E. Variation of nitrogenase activity, photosynthesis and pigmentation of the cyanobacterium Fischerella ambigua strain FS18 under different irradiance and pH values. World. J. Microbiol. Biotechnol. 2006; 22: 571-576.
36. Thummajitsakul S, Silprasit K, Sittipraneed S. Antibacterial activity of crude extracts of cyanobacteria Phormidium and Microcoleus species. Afr. J. Micro. Res. 2012; 6(10): 2574-2579.
37. Rania MA, Abedin hala MT. Antibacterial and antifungal activity of cyanobacteria and green microalgae evaluation of medium components by Plackett-Burman designs for Antimicrobial activity of Spirulina platensis. Glob. Jour. Biotech. Biochem. 2008; 3(1): 22-31.
38. Kaushik P, Chauhan A, Chauhan G, Goyal P. Antibacterial Potential and UV-HPLC Analysis of Laboratory-Grown Culture of Anabaena variabilis. Internet J. Food Saf. 2009; 11: 11-18.
39. Helen Diana Y, Reginald Appavoob M. and Parthipana B. Antibiotic activity of Cyanobacteria isolated from salt pans of Kanyakumari District (South India) against human pathogenic bacteria. Int. J. Curr. Sci. 2014; 11: 32-39.