

BIOACTIVE POTENCY OF CYANOBACTERIA *OSCILLATORIA SPP.*

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ABSTRACT

Objective: The microalgae regarded as the cyanobacteria makes the bioactive metabolites useful as antibiotics because of the presence of antagonistic compounds and above all it eradicates cancer cells. The cyanobacteria called *Oscillatoria Spp.* namely *Oscillatoria boryana*, posses these antagonistic nature due to the presence of the secondary metabolites they produce.

Method: In this study the *Oscillatoria spp* were isolated and the nature were studied. The mass cultivation of these cyanobacteria were done and crude extract was obtained by extraction methods. The crude concentration of the extract was screened against breast cancer cell line(MCF-7) using MTT assay.

Result: The results emerged prove the cytotoxic effect of the cyanobacteria and the effectiveness of the bioactive components in the cyanobacterial spp.

Conclusion: The crude extract of cyanobacteria *Oscillatoria boryana* proved to be cytotoxic against cancer cells. As these metabolites are obtained from the natural cyanobacterial source there is an added advantage of being eco friendly.

Keywords: Cyanobacteria, Bioactive components, Cytotoxicity.

INTRODUCTION

Cyanobacteria are a group of photosynthetic prokaryotic organism found in fresh water and marine water habitat. Their structure resembles gram negative bacteria and they are photoautotrophic. The presence of antagonistic compound make them antibiotic. The origin of the organism is dated back three or four billion years. Although they are truly prokaryotic, Cyanobacteria have an elaborate and highly organized system of internal membranes which function in photosynthesis. [1] Found in almost every conceivable habitats, from oceans to fresh water to bare rock to soil, cyanobacteria produce compounds responsible for "earthy" odors we detect in soil and some bodies of water.

Oscillatoria is a genus of filamentous cyanobacterium which is named after its oscillation movement. They reproduce by fragmentation. They are already a subject of research into the natural production of butylated hydroxytoluene (BHT), an antioxidant, food additive and industrial chemical. *Oscillatoria spp.* also have known to produce vitamins, minerals, viridamines and anti protozoal activity. [2]

The present work was aimed to find the anti cancer properties of the cyanobacteria *Oscillatoria spp.* as this would be a novel way of approach as they are truly ecological. Regardless of the other chemical side effects created by the chemical compounds used in the cancer treatment this would give a blissful effect in the field of science.

Presently 50% of the drugs used in the cancer treatment comprises of the natural sources like bacteria, actinomycetes, fungi sponges, Plants and animals. Another alternate source could be cyanobacteria from which effective anti cancer drug have been isolated and reported. [3]

These findings may pave a way for many other cyanobacteria with an effect on tumor cells and widen the scope of treatment.

MATERIALS AND METHODS

Isolation of cyanobacteria

Oscillatoria spp., a marine cyanobacteria, autotrophic filamentous photosynthetic organism was chosen for this study. It was collected from the kovalam beach from three different places. The obtained culture was grown in the cyanobacterial medium. [4] The growth characteristics were studied and the biochemical nature were

compared with the cultures obtained from National facility of marine cyanobacteria (NFM) tiruchirappally.

Culture media

The *oscillatoria spp.* were cultured in BG11 broth and enhanced with a nitrogen source like urea. This was provided with a light source and incubated for $25 \pm 2^\circ\text{C}$ in 1,500 lux with 12 hrs day/night cycle and was allowed to grow for 15-20 days.

Harvest

After the duration of 15-20 days, the mass was harvested using a sieve and was washed many times with tap water followed by distilled water to remove salts. The fresh weight of the mass was obtained using an electronic balance (Precisa 125A, Switzerland). This wet mass was used for the preparation of extract.

Extraction

The weighed wet mass was grounded in a pestle and mortar with 100% alcohol (distilled). The ground material was centrifuged at 10,000 rpm for 10 minutes at 4°C (Remi cooling centrifuge C24) and the supernatant was separated and collected. This process was repeated till the pellet turned grey or the supernatant turned colorless. The supernatant was pooled and filtered through crude filter paper, followed by Whatmann No.1 filter paper and then it was concentrated using a speed vacuum evaporator. Weight of this crude extract was determined. This crude extract was used for the further analysis.

Cytotoxic activity (MTT assay)

Cytotoxicity of extracts at various concentrations (12.5- 1000 $\mu\text{g/ml}$) was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) (Sigma) assay. Human breast cancer MCF-7(GD055) Human adeno carcinoma cell lines obtained from National centre for cell sciences pune (NCCS). The cells were maintained in RPMI 1640 supplemented with 10% FBS, penicillin, (100U/ml), and streptomycin (100 $\mu\text{g/ml}$) in a humidified atmosphere of 50 $\mu\text{g/ml}$ CO₂ at 37°C . Assay plates were read using a spectrophotometer, and viable cells were determined by absorbance at 570nm with reference at 655nm. Measurements were performed in 3 times for each sample and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The absorbance at 570 nm was measured with a

microplate reader using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability. Cytotoxic activity was expressed as the mean IC₅₀ (\pm standard deviation) of three independent experiments. One way analysis of variance (ANOVA) and the Student t-tests were used to compare data using statistica version 5.0 at a 95% confidence limit.

RESULTS AND DISCUSSION

The results of the present study clearly showed that the given sample of *Oscillatoria boryana* showed anti cancer activity against human breast cancer cell lines. The presence of the bioactive compounds present in the crude extract of these samples may possess the anti cancer activity against the breast cancer cell lines (MCF-7). The sample showed varying inhibition of viability (IC₅₀) *Oscillatoria boryana* with 10.45 μ g/ml (Table 1 and Fig.1). Quercetin was used as the standard solvent. No inhibition was seen with the cell control and the viability of the cells were marked as 100% in which the crude extract was not added. To treat the diseases like cancer the world is looking for biological sources, as the already existing chemotherapeutic agent may cause side effects like fatigue, irritation of oesophagus that can cause difficulty in swallowing and inflammation of lungs. It may also cause vomiting, neutropenia, anemia, another infectious complications.[5] Streptavidin-a bacterial derived protein showed anticancer activity against breast cancer cells *in vitro*. Discodermolide, a metabolite from a rare marine sponge metabolite possess anti tumor activity.[6] Another alternate

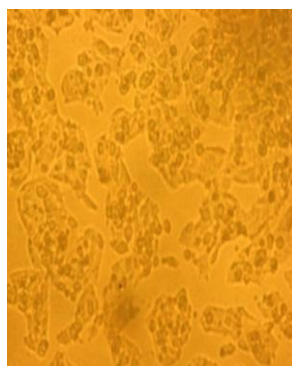
source could be cyanobacteria from which effective anti cancer compounds have been isolated and reported. The cyanobacteria *Oscillatoria spp.* are a group of organisms which plays a major role in photoproduction of biofuels, ammonia, various metabolites, vitamins, toxins, therapeutic substances, aqua or animal feed.[7] Cyanobacteria are also used as energy source and biofertilizers. They have been flourished today and begun to reap the benefits of molecular biology to enhance their performance.[8] From MTT assay it is clear that the sample possess anti cancer activity. Further research is necessary for successful separation, purification and characterization of bioactive compounds using chromatographic methods and spectroscopic techniques.

Table 1: Cytotoxicity effect on MCF7 Cell line

S. No.	Concentration (μ g/ml)	% cell viability	
1	1000	3.27 \pm 0.91	2.34 \pm 1.88
2	500	10.98 \pm 0.78	4.27 \pm 0.91
3	250	15.27 \pm 1.27	8.98 \pm 0.72
4	125	20.78 \pm 1.89	14.52 \pm 0.55
5	100	25.27 \pm 1.54	18.90 \pm 0.78
6	50	34.43 \pm 1.54	23.52 \pm 1.32
7	25	39.80 \pm 1.43	28.79 \pm 1.78
8	12.5	45.27 \pm 1.89	35.43 \pm 0.27
9	2.25	54.32 \pm 1.54	47.25 \pm 0.97
10	3.125	58.98 \pm 0.9	51.27 \pm 0.25
11	Cell control	100	100



Treated 500 μ g/ml



Treated 100 μ g/ml



Treated 12.5 μ g/ml



Breast cancer (cell Control)

CONCLUSION

To summarize we screened cyanobacteria *Oscillatoria spp* with potent anticancerous activity. The results from the MTT assay widen the scope of the study and pave a way for further research analysis. Resorcinolic acid a natural amphiphilic phenol having bioactivity have been demonstrated in several algae and cyanobacteria.[9] As it is obtained from a novel natural resource steps for drug development can be taken into consideration. The cyanobacteria *Oscillatoria spp.* has many species exhibiting its biodiversity, with most of the species actively possessing bioactive compounds.[10] With the obtained results we can progress for the structural activity in relationship of the bioactive compounds.

REFERENCES

- Mundt S, Kreilow S, Nowotny A, Effmert U (2001). Biochemical and pharmacological Investigation of selected cyanobacteria. Int.J.Hyg.Environ.Health;203:327-334.
- Babu B, Wu J. T. Production of natural butylated hydroxyl toluene as an anti oxidant by fresh water phytoplankton. Journal of phycology 2008;44(6):1447-1454.
- Hoppe HA Marine algae and their products and their constituents in pharmacy. In: Marine algae In Pharmaceuticals. Hoppe HA, Levring T, Tanka Y. Water de Gruyter, Berlin 1979;25-119
- R. Sugumar et al. Diversity of marine cyanobacteria from cape comorin coast of tamilnadu. Journal of phycology. ISSN:2075-6240.
- Henry JB. Clinical diagnosis and management by laboratory methods. Philadelphia: W.B Saunders Co., 1996.
- Smith CD, Zhang X. Mechanism and action of cryptophycin interaction with the Vinca Alkaloid domain of tubulin. J. Biol. Chem. 1996;271(11):6192-6198.
- Mohanty P. Cyanobacterial photosynthesis: Concepts and applications. J. Sci. and Indus. Research. 1996;55:553-554.
- Tirunalasundari T, Subramanian G. Bioactive potentials of Marine Cyanobacteria. Algological Research in India. 2002. 293-303.
- Kozubek A, Arnowski R, Stasiuk M, Gubernater J. Natural amphiphilic phenols as bioactive Compounds. Cellular and Molecular Biol. Letters. 2001;6(2A)351-355.
- Sabeen Naz et al. Biodiversity of *Oscillatoria (Nostophyceae, Cyanophyta)* from northern Areas of Pakistan. Pak J. Bot., 2004;36(3):503-530.