

Potent Cancer Suppressing Boon from Natures Marine Algal Beds

Shalini Nair, and Valentin Bhimba

Abstract— Cancer being a dreadful disease is still finding its cure through various researches. Recent research prove that there are various natural resources in the nature which has the potency to eradicate the disease. One such novel natural resource is the bioactive metabolites found in the micro organisms such as micro algae or cyanobacteria. The microalgae regarded as the cyanobacteria makes them useful as antibiotics because of the presence of antagonistic compounds and above all it eradicates cancer cells. The cyanobacteria called *Oscillatoria Spp.* namely *Oscillatoria laetrivirans* posses these antagonistic nature due to the presence of the secondary metabolites they produce. 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. The results emerged prove the cytotoxic effect of the cyanobacteria.

Keywords— Cyanobacteria, Bioactive metabolites.

I. INTRODUCTION

CYANOBACTERIA, the blue green algae belonging to the family cyanophyceae is so named because of its resemblance with the bacterial characteristics. Previously they were known as myxophyceae. Apart from the main feature that the cells lack true nuclei they show other features which, with the bacteria distinguish them from the eukaryote¹. The bacteria and the cyanophyceae lack mitochondria, true vacuoles and endoplasmic reticula. Sterols are not detected in bacteria and the absence of sterols in cyanobacteria was an accepted fact. In some forms of cyanobacteria especially in *oscillatoria spp* pseudovacuoles may be formed, these contribute towards their buoyancy by virtue of the gas that they contain. The reproduction is mainly by fragmentation. 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 photosynthesis². There is no membrane bound chloroplast and in the blue green algae the photosynthetic lamellae are usually distributed in the peripheral cytoplasm. *Oscillatoria* is a genus of filamentous cyanobacterium which is

named after its oscillation movement. 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 activity³. 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. 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 reported⁴.

II. MATERIALS AND METHODS

A. 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 medium⁵. The growth characteristics were studied and the biochemical nature were compared with the cultures obtained from National facility of marine cyanobacteria (NFMC) tiruchirapally. The sample was confirmed as *Oscillatoria laetrivirans*.

B. Culture media

The *oscillatoriaspp.* 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.

C. 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.

D. 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

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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 concentrator. Weight of this crude extract was determined. This crude extract was used for the further analysis.

E. Cytotoxic activity (MTT assay)

Cytotoxicity of extracts at various concentrations (12.5-1000 µg/ml) was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazoliumbromide (MTT) (Sigma) assay. Human breast cancer MCF-7(GD055) Human adenocarcinoma 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(100µg/ml) in a humidified atmosphere of 50µg/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 the 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 (± 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.

III. RESULTS AND DISCUSSION

The results of the present study clearly showed that the given sample of *Oscillatoria laetrivans* 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 laetrivans* with 10.45µg/ml 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 complications⁶. Streptavidin-a bacterial derived protein showed anticancer activity against breast cancer cells invitro. Discodermolide, a metabolite from a rare marine sponge metabolite possess anti tumor activity⁷. 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 feed⁸. 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 performance⁹. 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 I
MTT ASSAY AND CELL VIABILITY

S.No	Concentration (µg/ml)	% cell viability sample	Quercetin
1	1000	21.45±1.25	2.34±1.88
2	500	23.45±0.78	4.27±0.91
3	250	28.89±1.77	8.98±0.72
4	125	32.21±1.87	14.52±0.55
5	100	35.78±1.89	18.90±0.78
6	50	39.78±1.32	23.52±1.32
7	25	45.52±1.27	28.79±1.78
8	12.5	48.90±1.45	35.43±0.27
9	2.25	51.23±1.08	47.25±0.97
10	3.125	54.25±0.91	51.27±0.25
11	Cell control	100	100

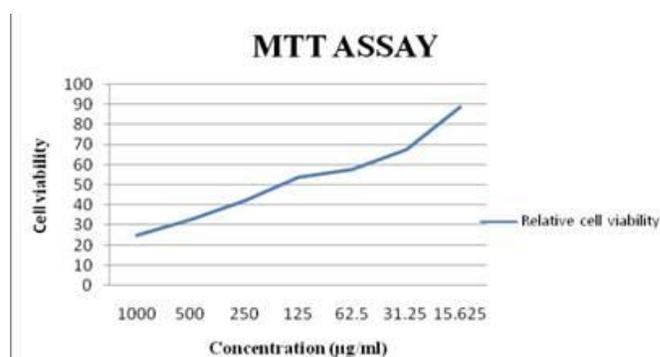
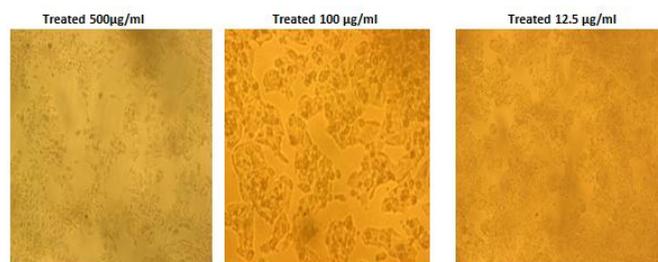


Fig. 1 MTT assay and cell viability

In vitro Cytotoxicity effect of sample on Breast cancer cell lines



IV. CONCLUSION

To summarise screening of cyanobacteria *oscillatoria spp.* with potent anticancerous properties was done. 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 cyanobacteria¹⁰. 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 compounds¹¹. With the obtained results we can progress for the structural activity in relationship of the bioactive compounds. This provides a new zest towards obtaining natural resources in the dreadful attack of diseases like cancer.

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