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Screening ,Charecterisation of Anticancerous Activity of Bioactive Components from Marine Cyanobacteria

Shalini Nair and Valentin Bhimba

Department of Biotechnology,Sathyabama University,Chennai - 600119

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Most medicines come from natural resources and scientists are still exploring the organisms of tropical rain forest for potentially valuable medicinal products. 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 salina* 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. Further thin layer chromatography was performed to separate and purify the components present. The results emerged prove the cytotoxic effect of the cyanobacteria .

Key words: Cyanobacteria, Bioactive metabolites.

Cyanobacteria are a group of extraordinary diverse prokaryotes originated 3.5 billion years ago. They grow in wide variety in of habitats such as fresh water,sea water,soil,rock,wall,sewage and tree trunks. More than 2000 species are recorded presently with many new species still being discovered. Like the bacteria and unlike the algae, they lack the characteristic nuclear and mitochondrial membranes and hence there are no distinct nuclei and mitochondria, although the nuclear material does appear to be localized. In some forms of cyanobacteria especially in *oscillatoria spp* psedovacuoles may be formed, these contribute towards their buoyancy by virtue of the gas that they contain .The reproduction is mainly by fragmentation.[1] They are photoautotrophic. The presence of antagonistic compound make them antibiotic . Athough they are truly prokaryotic, cyanobacteria have an elaborate and highly organized system of internal membranes which function in photosynthesis.[2]

Oscillatoria is a genus of filamentous cyanobacterium which is named after its oscillation movement. Oscillatoria spp. also have known to produce vitamins, minerals, viridamines and anti protozoal activity.[3] Cyanobacteria produce a variety of remarkable compounds that have shown potential application in major disease management such as cancer, asthma, diabetes, etc.[4] Some species of cyanobacteria are known to produce

* To whom all correspondence should be adressed. Email:nair_3112@yahoo.co.in,8939905184

antimicrobial substances. [5]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. After screening of cancer cells with the crude extract thin layer chromatography is performed for further separation and purification of the bioactive components. Presently 50% of the drugs used in the cancer treatment comprises of the natural sources like bacteria, actinomycetes, fungi sponges, plants and animals.

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. The growth characteristics were studied and the biochemical nature were compared with the cultures obtained from National facility of marine cyanobacteria (NFMC) tiruchirapally.

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±2p 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 4p 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 vaccum concentrator. 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 ìg/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 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 37p 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($IC_{50)}$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 IC_{50} (± standard deviation) of three independent experiments. One way analysis of variance (ANOVA)and the Student t-tests were used to compare data using statistical version 5.0 at a 95% confidence limit.

Extraction of the bioactive compounds

Thin layer chromatography(tlc):

Preparation of thin layer plate:

The stationery phase (silica gel) was prepared on slurry with water or buffer at 1:2 ratio. It was applied to the glass plate or an inert plastic or aluminium sheet (as thin as a glass rod or pipette) using TLC applicator of 0.25mM thickness for analytical separation and 2.5mM thickness for reparation preparation.

Calcium sulpahte $CaSo_4$.½ H_2O gypsum(10.15%) is incorporated to the adsorbent as a binder, since it facilitates the incorporated adhesion of the adsorbent to the plate. After the application of the adsorbent, the plates are air dried for 10-15 minutes. This process also known as activation of the adsorbent. The plates can be used immediately or stored in the dessicator.

Sample preparation:

Phenols

Two grams of blue green algae (BGA) cultures were lixivated in methanol on rotary shaker(180 thaws/min) for 24 hours. Then the extract was filtered by using whatman.no.1 filter paper. The condensed filtrate was used for TLC.[6]

Amino acids

Two grams of blue green algae(BGA) cultures was extracted with 70% ethanol in waterbath (80ºc/15 mins).The condensed filtrate is used for TLC.

Sterols

Two grams of blue green algae (BGA) extracted with 10ml of methanol in waterbath(80ºc/ 15 mins).The condensed filtrate is used for TLC.

Saponins

Two grams of blue green cultures (BGA)was extracted with 10ml of 70% ethanol by refluxing for 10 minutes. Then this extract was filtered by using whatman no.1 filter paper. The filter is condensed enriched with n-butanol, and thoroughly mixed. The butanol was retained condensed and used for thin layer chromatography.

Sample application

A line was drawn lightly with pencil, about 1.5 to 2.0 cm from the bottom. A scale was placed at the bottom and spotted at a distance of 1.5cm.The order was noted. The samples were spotted using capillary tubes at 1.5cm distance between them; for preparing TLC, the sample is applied as a banal across the layer than as a spot.

Solvent peparation

Phenols

The phenols were separated using chloroform and methanol(27:03) solvent mixture.

Aminoacids

The amino acids were separated by using butanol , acetic acid and water (80:20:20) solvent mixture.

Sterols

The sterols were separated by using acetone, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture.

Saponins

The saponins were separated using choloroform, glacial acetic acid, methanol and water (64:34:12:8)solvent mixture.

Running of sample in TLC:

The chromatography tank is filled with developing to a depth of 1.5cm and equilibrated for about 5hours.The thin layer plate is placed gently in the tank and allowed to stand for about 60 minutes. It was ensured that the spots did not touch the solvent directly because capillary action can cause the solvent to display as seen in paper chromatography and the separation of the compounds takes place as the front of the solvent reaches about 1.2cm from the top of the plate. The plate is removed, the front of the solvent is marked with a pencil immediately and allowed to air dry, placing the plate upside down.

Compound detection

Several methods were available to detect the separated compounds.

Phenols

The presence of phenols in the developed chromatogram was detected by spraying folin ciocalteau reagent. After the plates were heated at 80ºc for 10 minutes, there was a positive reaction to the formation of blue colour spot.(Wagner and Bladt(1996))[7]

Aminoacids

The presence of aminoacids in the developed chromatogram was detected by spraying the 0.1% ninhydrin in acetone. After the plates were heated at 80ºc for 10 minutes, there was a positive reaction to the formation of pink or purple spots.(Wagner and Bladt(1996))

Sterols

The presence of sterols in the developed chromatogram was detected by spraying folin ciocalteau reagent. After the plates were heated at 80ºc for 10 minutes a positive reaction to the formation of blue colour spots was seen.(Wagner and Bladt(1996))

Saponins

The presence of saponins in the developed chromatogram was detected by iodine vapours, and possible reaction to the formation of yellow colour spots was seen.(Wagner and Bladt(1996))

Detection of R_f value

The R_f values of the various bioactive compounds were calculated using the following formula.

R_f = **DISTANCE TRAVELLED BY THE SOLUTE**(Measured to the centre of the spot)
DISTANCE TRAVELLED BY THE SOLVENT

RESULTS AND DISCUSSION

The results of the present study clearly showed that the given sample of *Oscillatoria salina* 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 posses the anti cancer activity against the breast cancer cell lines (MCF-7). The sample showed varying inhibition of viability(IC50) *Oscillatoria salina* with8.97µg/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. Thin layer chromatography was done to separate the bioactive components,in which the separated compounds were found to be phenols, amino acids, saponins and sterols.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. Discodermolide, a metabolite from a rare marine sponge metabolite possess anti tumor activity.[8] Another alternate source could be cyanobacteria from which effective anti cancer compounds have been isolated and reported. They have been flourished today and begun to reap the benefits of molecular biology to enhance their performance. [9]From MTT assay it is clear that the sample possess anti cancer activity.[10] Further research is necessary for successful separation, purification and characterization of bioactive compounds

Table 1. MTT assay and cell viability

S. No	Concentration (µg/ml)	% cell viability sample	Quercetin
1	1000	12.78 ±0.98	2.34±1.88
2	500	23.56±1.23	4.27±0.91
3	250	28.98±0.78	8.98±0.72
4	125	32.56±1.23	14.52±0.55
5	100	43.66±0.67	18.90±0.78
6	50	48.90±0.87	23.52±1.32
7	25	52.34±0.54	28.79±1.78
8	12.5	59.88±0.66	35.43±0.27
9	2.25	65.14±1.45	47.25±0.97
10	3.125	72.34±	51.27±0.25
11	Cell control	100	100

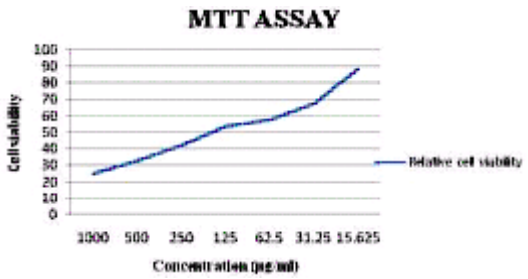

Fig. 1.

Table 2. Bio Active Compound Analysis From *Oscillatoria* Spp

Bioactive Compound	Result	R_f Value
Amino acid	+	0.57
Phenol	+	0.42
Sterol	+	0.40
Saponins	+	0.54

Fig. 2.

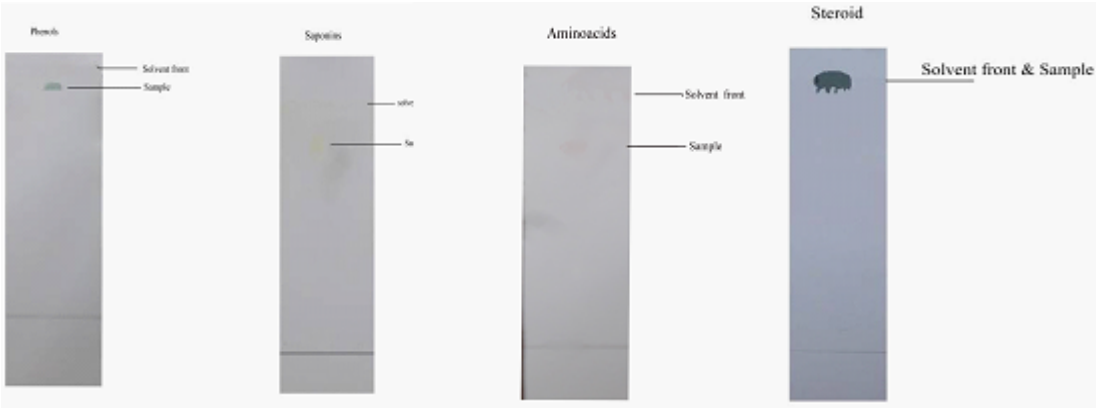

Fig. 3.

using chromatographic methods and spectroscopic techniques.

To summaries 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. Thin layer chromatography shows the presence of various compounds responsible for bioactivity, which may be proved with further study. The cyanobacteria *Oscillatoria spp.* has many species exhibiting its biodiversity, with most of the species actively possessing bioactive compounds.[11] The medicinal qualities of cyanobacteria were first appreciated as early as 1500 B.C. Cyanobacteria are one of the richest source of known and novel bioactive compounds with wide pharmaceutical applications.[12] 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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