

ANTICANCER ACTIVITY OF OSCILLATORIA TEREBRIFORMIS CYANOBACTERIA IN
HUMAN LUNG CANCER CELL LINE A549S.Mukund¹ and V. Sivasubramanian¹Department of Plant Biology and Plant Biotechnology,, RKM Vivekananda College, Chennai, India.
E.mail: t.s.mukunth@gmail.com**ABSTRACT****Purpose:** To evaluate the anti-cancer properties of the cyanobacterial extract of *Oscillatoria terebriformis***Methods:** The extract was tested in Human lung cancer cell lines and examined for its effect on cell viability, nuclear morphology and sub-G1 formation. Cell viability was determined by micro culture tetrazolium technique (MTT), nuclear morphology investigated using 4'-6-diamidino-2-phenylindole (DAPI) staining technique, and apoptosis assay using DNA fragmentation.**Results:** The results showed decreasing cell viability in a concentration-dependent manner. Altered cell morphology after treatment with the extract demonstrated that cells experienced apoptosis.**Conclusion:** The data demonstrate that *Oscillatoria Terebriformis* extract induced apoptosis in Human lung cancer A549 cells, and therefore, has a potential as an anti-cancer agent.Keywords: MTT, Human lung cancer cells, *Oscillatoria terebriformis*, DNA fragmentation, MTT, DAPI staining, Apoptosis, LDH, GST and GR.**INTRODUCTION**

According to the World Health Organisation fact sheet, cancer is a leading cause of death, accounting for around 13% of all deaths in 2008. Lung cancer (1.37 million), Liver cancer (695,000), colon cancer (608,000), and breast cancer (458,000) cause the number cancer deaths each year. Deaths from cancer world wide are projected to continue rising, with an estimated 13.1 million deaths in 2030 (Globacan, 2010). Lung cancer, the second most common cancer in men, cancers of the colon and rectum in combination rank third in frequency among males in the United States. Anticancer drugs are used to target all rapidly proliferating cells, that is, cancer cells, and any normal rapidly dividing cells. One example of a normal cell that is most affected by such drugs is the bone marrow forming cells. The effect of these drugs on normal cells might induce the development of a second cancer. Nevertheless, the benefits of these drugs outweigh the possibility that they will induce the development of a second cancer. The cyanobacterial genus *Oscillatoria* is evenly distributed throughout the ponds having many species mainly marine in nature, rich in secondary metabolites such as flavonoids. The methanolic extract of *oscillatoria* may contain large amount of water soluble vitamins and phycocompounds. In the present study, *Oscillatoria terebriformis*, cyanobacteria was procured from Department of Algal Biotechnology, Vivekananda Institute of Algal Technology (VIAT). The primary objective of this study is to evaluate the anti proliferative potential of methanolic extracts of the cyanobacteria on A549 cells by standard anti-proliferative assays. DNA profiling was studied to investigate any change in DNA of the treated cells.

MATERIALS AND METHODS**Cyanobacterial culture**

Oscillatoria terebriformis, a thermophilic cyanobacterium were obtained from the culture collection of Vivekananda Institute of Algal technology (VIAT) Chennai. Biomass was obtained by growing algal cultures in 20L of water and 0.25g / L of NPK fertilizer was added with a facility to pump the culture with aeration pump. The algae was grown for 20 days and harvested.

Preparation of Cyanobacterial extract

0.5g of dried cyanobacterial material was extracted in 20ml of methanol kept in an orbital shaker for overnight as described by (Sivasubramanian et.al.2011). The obtained extracts were filtered with Whatman no.1 filter paper and the filtrate was collected. The solvents were removed under reduced pressure at 50°C to yield a concentrated extract (15%).

Cell Line and Culture

Human lung cancer A549 cell lines were obtained from National center for cell sciences Pune (NCCS). The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C.

Reagents.

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Caithersburg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

Cell proliferation Assay by MTT Method

The Cytotoxicity of samples on A549 cells was determined by the MTT assay (*Mosmann et al. 1983*). Cells (1×10^5 /well) were plated in 100 µl of medium/well in 96-well plates (Hi media). After 48 hours incubation the cell reaches the confluence then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4hours of incubation, 0.04M HCl/isopropanol were added. Viable cells were determined by the absorbance at 570nm with reference at 655nm. Measurements were performed in 3 times, 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 (Bio-Rad, Richmond, CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of human Lung cancer cells was expressed as the % cell viability, using the following formula:

$$\% \text{ cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100\%$$

Data analysis

The IC₅₀ values (concentration at which 50% of cells were death) are reported as mean± standard deviation of three independent experiments. The IC₅₀ values against the human cancer cell lines were calculated for the solvent extracts inhibiting at least 50% inhibition when tested at a concentration. One-way analysis of variance (ANOVA) and Student t-tests were used to compare data using Statistica version 5.0 software at a 95% confidence limit.

DNA Fragmentation Analysis

A semiquantitative method for measuring apoptosis was described by Bortner, *et. al* (1995). Fragmentation of chromatin to units of single or multiple nucleosomes that form the nucleosomal DNA ladder in agarose gel is an established hallmark of programmed cell death or apoptosis. Briefly, the cells were cultured in 100 mm dishes, treated with sardine oil for 48 h. Following this treatment, the cells were washed with PBS (pH 7.5), harvested and pelleted by centrifugation (12000 rpm) at 4 °C. The pellet was incubated with DNA lysis buffer [10 nMTris pH (7.5), 400 mM NaCl, 1mM EDTA and 1% Triton X-100] for 30 min on ice and then centrifuged at 12000 rpm. The supernatant that was obtained was incubated overnight with RNase (0.2mg/ml) at room temperature and then with proteinase K (0.1mg/ml) for 2 h at 37 °C. DNA was extracted using Phenol: chloroform: isoamylalcohol (25:24:1) mixture and precipitated with 0.1M of sodium acetate and 2 volume of absolute ethanol. Equal amount of DNA samples (20 µg) were electrophoresed on a 1.5% agarose gel in Tris-borate EDTA buffer and visualized by ethidium bromide staining.

DAPI Staining

DAPI staining was performed as described by Sandra *et al* . Briefly, the cells were seeded onto glass slides and treated with Cyanobacterial extract for 24 h. Untreated and treated cells were rinsed with phosphate buffered saline (PBS), fixed with ice-cold 10% trichloroacetic acid, and further washed with cold 70, 80, 90% and absolute ethanol. The cells were permeabilized with Triton-X (10%v/v) and stained with 1 µg/ml 4'-6-diamidino-2-phenylindole (DAPI) for 3 min. To reduce the background, the stained cells was washed with PBS, cover-slipped with 90% glycerol and observed under a Image Express Micro.

Estimation of Lactate dehydrogenase (EC 1.1.127)

The enzyme is assayed based on the method of King (1965). 1.0 ml buffer substrate and 0.1 ml sample added into each of two tubes. Added 0.2 ml water to the blank. Then to the test added 0.2 ml of NAD. Mixed and incubated at 37°C for 15 min. Exactly after 15 min, 1.0 ml of Dinitrophenyl hydrazine was added to each (test and control). Left for further 15 min. Then added 10 ml of 0.4 N Sodium hydroxide and the color developed was read immediately at 440 nm. A standard curve with Sodium pyruvate solution was taken. The enzyme activity was expressed as units/mg protein in tissues.

Determination of glutathione s-transferase activity (EC 2.5.1.18)

Inhibition of the activities of cytosolic GSTs by the cyanobacterial extract was assessed as described previously by Habig et al with slight modifications. GST mediated conjugation of 1-chloro-2, 4-Dinitrobenzene (CDNB) to glutathione (GSH) was measured using multiplate reader, 425-106 at the wavelength of 340 nm for 5 minutes. Incubation mixtures (300µL) contained 0.1 M potassium phosphate buffer pH 6.5, 30 mM CDNB, 30mM GSH, and GST enzymes (0.125 mg/mL cytosolic fraction). The plant extracts were dissolved in distilled water were tested at a concentration range of 1000µg/ml – 1.93 µg/ml. Tannic acid was used as a positive control for the *in vitro* study at a concentration range of 0.3–10 µg/mL. All assays were linear functions of protein concentration and of time for at least 5 minutes. The enzyme activities were expressed as percent specific activity over control.

Determination of Glutathione Reductase activity (EC 1.8.1.7)

The cells were treated with different concentration of Cyanobacterial extract for 48 hours incubation. After incubation the dead cells was collected and the detached cells were collected by using Trypsin EDTA solution and transferred to an eppendroff tubes. The homogenate was centrifuged for 45min at 14,000 rpm. The pellets were suspended in a small volume of 0.25Msucrose and centrifuged. The supernatants were combined with the previous centrifuge. The pooled material was adjusted to pH 5.5 with cold 0.2M acetic acid and centrifuged as described above (fraction). The level of total acid-soluble SH compound (glutathione GSH) was determined with Ellman's reagent according to DeVos *et al.*, 1992. The buffer was mixed with 630µl of 0.5 M K HPO and 25 µl of mM 5, 5'-dithiobis (2-nitrobenzoic acid) (final pH 7). The absorbance at 412 nm was read after 2 min. GSH was used as a standard. The rate of oxidation of NADPH by GSH at 300 was used as a standard measure of enzymatic activity. The reaction system of 1ml contained: 1.0mMGSSG, 0.1 mM NADPH, 0.5 mM EDTA, 0.10 M sodium phosphate buffer (pH 7.6), and a suitable amount of the glutathione reductase sample to give a change in absorbance of 0.05 to 0.03/min. The oxidation of 1 µmol of NADPH/min under these conditions is used as a unit of glutathione reductase activity. The specific activity is expressed as µM/min/mg protein.

RESULTS

Anticancer properties were studied from methanolic extract of *Oscillatoria terebriformis* Ag.VIAT010 in A549 Lung cancer cells. The assays consisted of MTT [3-(4, 5-dimethylthiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] assay, viable cell count, DNA fragmentation, Tryphan blue assay, LDH, GSH and GST,GR.etc.

MTT Assay

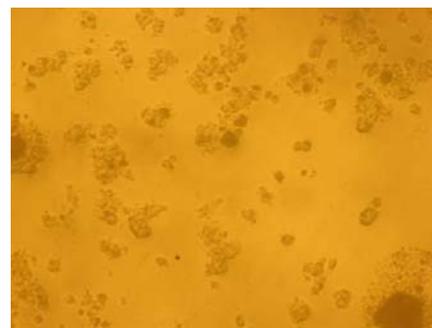
Data on the cytotoxic effects of *Oscillatoria terebriformis* extract using Human lung cancer cell lines in vitro are showed in Figure 2. *Oscillatoria terebriformis* extract has cytotoxic effects in vitro at clinical acceptable concentrations (IC₅₀ values ≤ 50 mg L⁻¹) by MTT method. The cytotoxic effect of *Oscillatoria terebriformis* extract was determined using concentrations ranging 0 to 1000 µg/ml for 48 h. After 48 h exposure, *Oscillatoria terebriformis* extract induced concentration-dependent cytotoxic effects in A549 cell lines with IC₅₀ of cell viability 14.67 ± 0.98 in A549 cells using MTT method. Differential inhibitions of compounds with incubation time could be due to their solubility, which determines their bio-accessibility.

Validation of apoptosis measurement by DNA laddering

In our results, the A549 cells were treated with cyanobacterial extract, and the DNA was directly extracted and run on agarose gel. DNA fragmentation, if presented, was seen as a stepwise ladder of DNA fragments. The data (Figure 3) shows that DNA laddering is pronounced for *Oscillatoria terebriformis* extract (31.25 µg/ml) in A549 cells. These results confirm that cyanobacterial extract can induce apoptosis of A549 cells. Apoptosis DNA Fragmentation is a key feature of programmed cell death and also occurs in certain stages of necrosis. Apoptosis is characterized by the activation of endogenous endonucleases with subsequent cleavage of chromatin DNA into internucleosomal fragments of 180 BP and multiples there of. DNA cleavage during apoptosis occurs at sites between nucleosomes, protein-containing structures that occur in chromatin at ~200-BP intervals.

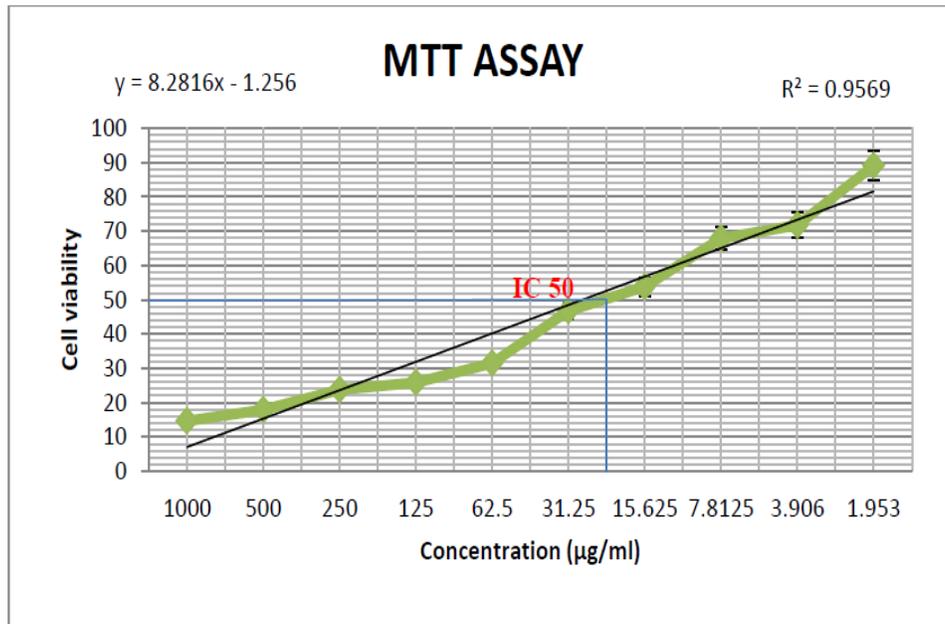
DAPI Staining

Based on the MTT assay results, DAPI staining was conducted to investigate whether *Oscillatoria terebriformis* extract induced apoptosis. Treatment of cells with *Oscillatoria terebriformis* extract at concentrations of 31.25, 62.5 and 1000 $\mu\text{g/ml}$, respectively, caused nuclear morphological changes compared to normal cells and this may be indicative of apoptosis (compare Figures A, B and C). Morphological changes observed in the treated cells included cell shrinkage, nuclei that were broken into discrete fragments and cell budding (indicated by arrow) that resulted in cells of various sizes. Higher concentrations of *Oscillatoria terebriformis* extract appeared to cause more morphological changes, indicating that apoptosis occurred in a concentration-dependent fashion

**A) Normal A 549 cells****B) Treated 1000 $\mu\text{g/ml}$** **C) 500 $\mu\text{g/ml}$** **D) 62.5 $\mu\text{g/ml}$** **E) 15.625 $\mu\text{g/ml}$**

Cytotoxicity effect of samples on lung cancer cell lines. The cells were treated with various concentrations (A:control, B: 1000 $\mu\text{g/ml}$, C: 500 $\mu\text{g/ml}$, D: 62.5 $\mu\text{g/ml}$ and E) 15.625 $\mu\text{g/ml}$ of *Oscillatoria terebriformis* for 24 h.

Figure 1: Cytotoxicity Effect on Lung Cancer Cell lines



Oscillatoria terebriformis extract inhibited proliferation of A549 cells

Figure 2: Graphical Representation of IC₅₀ inhibition in MTT Assay from Oscillatoria Terebriformis

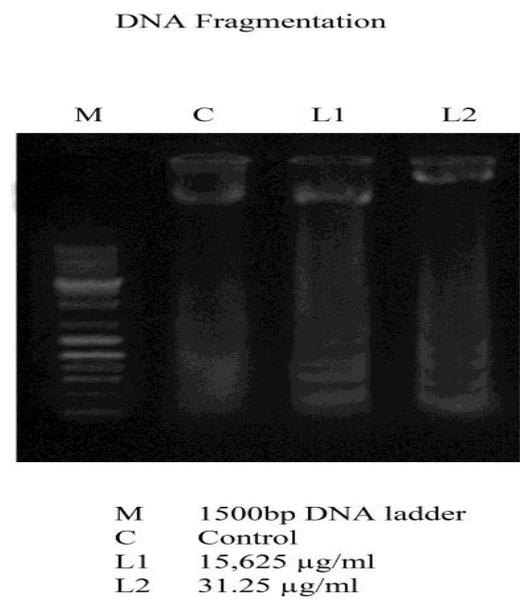


Figure 3: DNA Fragmentation Analysis on Treated Oscillatoria Terebriformis

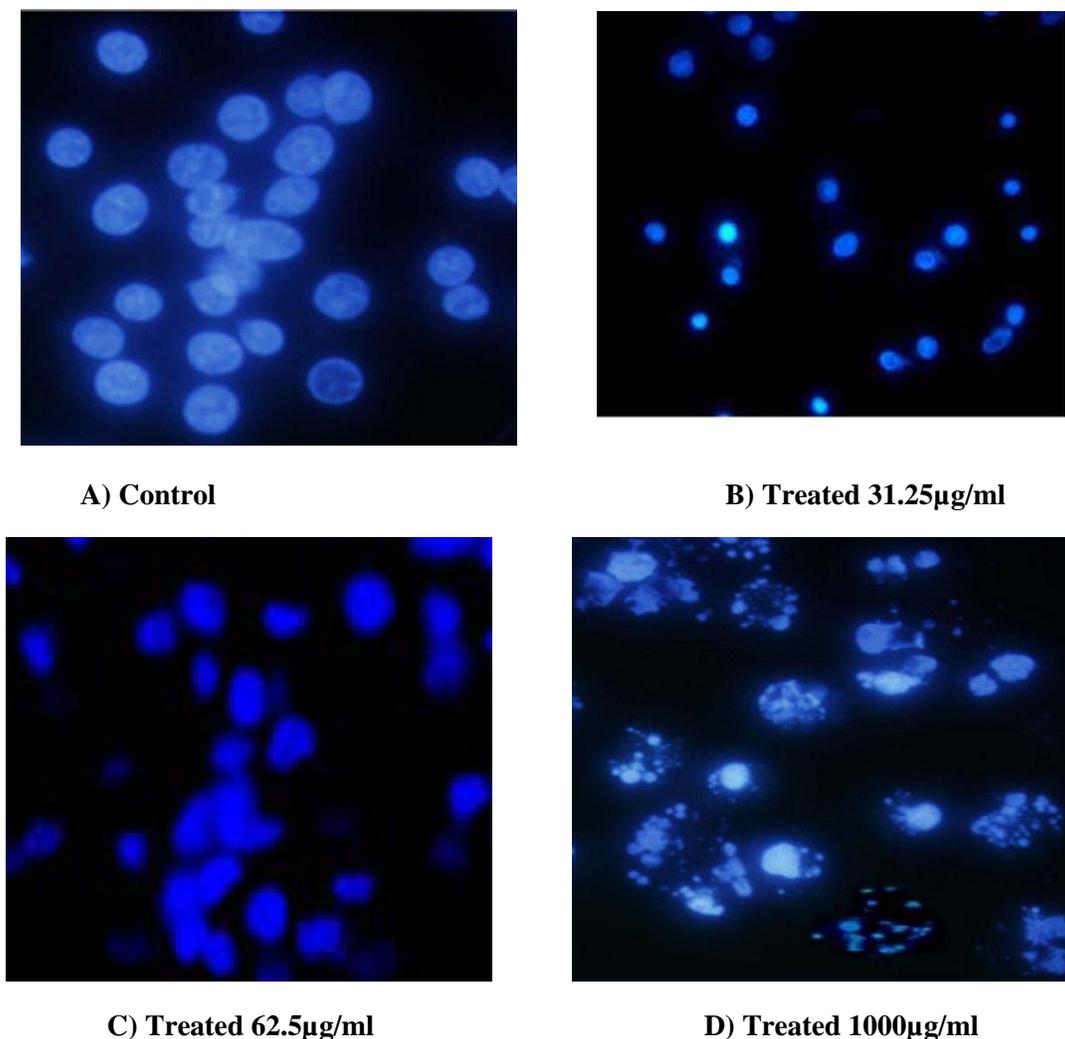


Figure 4- DAPI Staining of *Oscillatoria Terebriformis*

Lactate Dehydrogenase (LDH) release assay

The cytotoxic effect of *Oscillatoria terebriformis* was tested via the Lactate dehydrogenase (LDH) release assay, based on the extent of LDH leakage into the medium. The augmented release of LDH into the media is reflective of cellular damage. To confirm the *Oscillatoria terebriformis* induced cell death through apoptotic pathway, the LDH leakage assay was performed to assess the plasma membrane integrity. The levels of LDH released into the medium of control and *Oscillatoria terebriformis* treated (in various concentration) A549 cells are presented in Figure 6. From this table, it was observed that LDH levels were found to be significantly elevated after 48 h of exposure in the medium containing *Oscillatoria terebriformis* when compared to the control. Cells were exposed to different concentrations of extract for 48 hrs. Results were expressed as the percentage of LDH leakage was analyzed. Values are mean \pm SD (n = 3). *P < 0.05 and **p < 0.0001 represents the statistical significance between control and treated A549 cells.

Glutathione reductase

Glutathione reductase, also known as GR, is an enzyme (EC 1.8.1.7) that reduces glutathione disulfide (GSSG) to the sulfhydryl form GSH, which is an important cellular antioxidant. Glutathione reductase content was higher in treated cells compared to control treatment. GSH content was gradually increased by increasing of *Oscillatoria terebriformis* extract. Concentrations of glutathione (GR) quantified in A549 cell lines of *Oscillatoria* extracts are summarized in Figure 6. The increase in the GR activity in general, used as indication for the antitumor activity of the tested materials in A549 cells

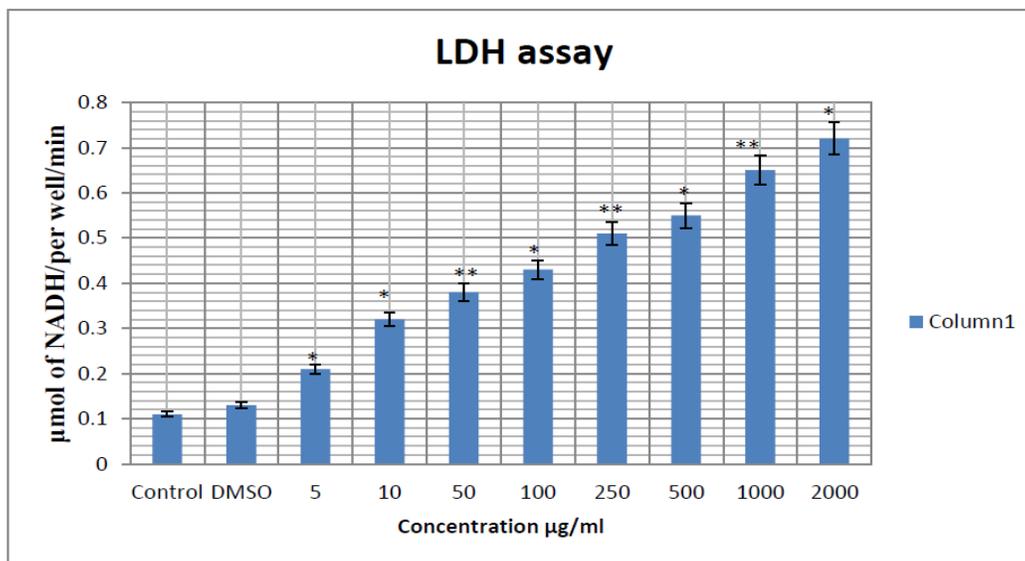


Figure- 5 Lactate Dehydrogenase (LDH) Activity of *Oscillatoria Terebriformis* in Lung Cancer Cell Lines

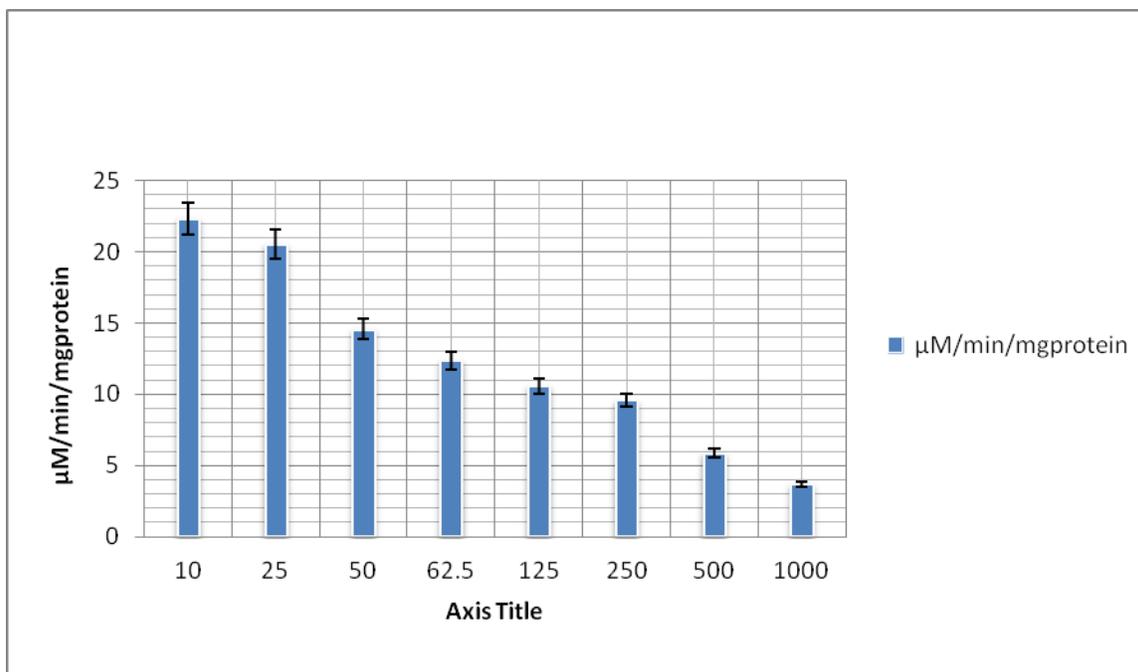


Figure 6 .Glutathione Reductase Activity of *Oscillatoria Terebriformis* in Lung Cancer Cell Lines

Glutathione-S-transferase

Glutathione-S-transferase (GST, EC 2.5.1.18) comprises an important class of biotransformation enzymes, the main function of which is the detoxification of toxic compounds. The inhibitory effect of samples on GST activity with increasing concentration of *Oscillatoria terebriformis* extract. Concentrations of glutathione (GST) quantified in the A549 cell line are summarized in Figure 5. Also the activity of GST was enhanced by incubation of tumor cells with cyanobacterial extracts. The activity of glutathione reductase is used as indicator for oxidative stress. The activity can be monitored by the NADPH consumption, with absorbance at 340 nm, or the formed GSH can be visualized by Ellman's reagent.

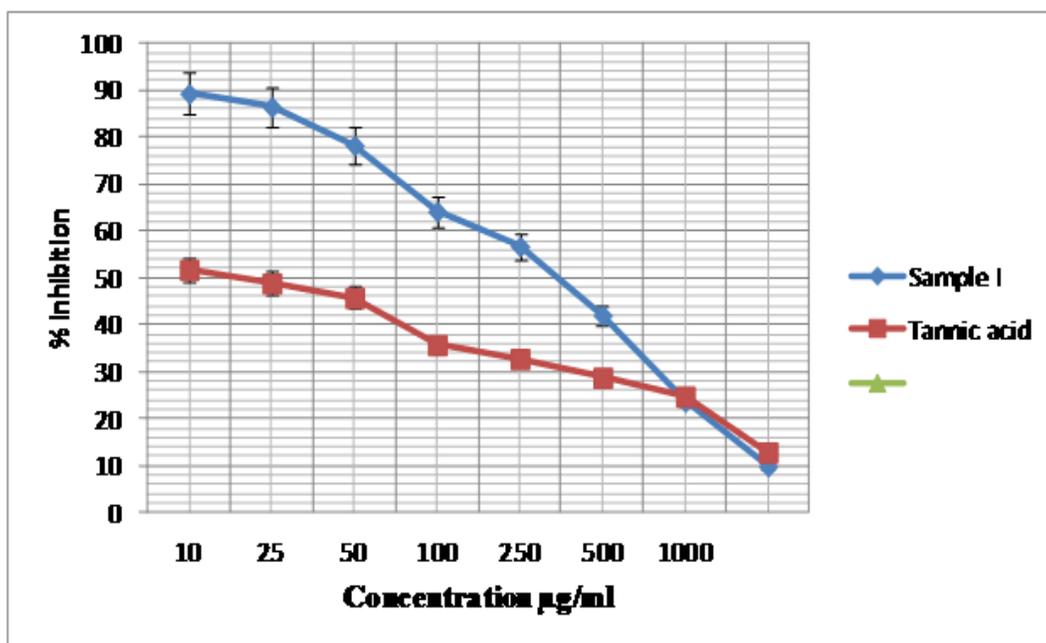


Figure -7 Glutathione S-Transferase activity of *Oscillatoria Terebriformis* in Lung Cancer Cell Lines

DISCUSSION

This study has shown that the *oscillatoria terebriformis* significantly inhibited cell proliferation and induced apoptosis in human lung carcinoma cell line A549 at low concentration. Many cyanobacteria produce compounds are generally considered to be secondary metabolites, that is, compounds that are not essential for general metabolism or growth of the organism and are present in restricted taxonomic groups. Cyanobacteria such as *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria* produce a great variety of secondary metabolites. A number of important marine cyanobacterial molecules, including dolastatin 10,, cryptophycins and curacinA, have been discovered and these were either in preclinical or clinical testing as anticancer agents (Newman *et. al* 2004).Cyanobacteria are a prolific source of nearly 800 diverse bioactive secondary metabolites, originating mainly from nonribosomal peptide synthetase (NRPS) or mixed polyketide synthesis (PKS)–NRPS biosynthesis (Welker *et.al* 2006, Tan *et. al* 2007).

MTT [3-(4, 5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] assay

In order to know the effect of secondary metabolites on Lung cancer cells, experiments were conducted using cultured human Lung carcinoma cells. Streptomycin was used as positive control for the comparison of inhibitory potentials. Dose dependent inhibition of A549 cells was observed at different concentrations (1.953-1000 µg/ml) of *Oscillatoria terebriformis* extract. Cell proliferation was highly significant at concentrations above 50 µg/ml ($P < 0.001$).Recent results have demonstrated the inhibition of proliferation of number of cancer cells by *Oscillatoria terebriformis* extract. Most of the principle components present in cyanobacterial extracts are polyphenols, beta-carotene, ascorbic acid, alpha tocopherol, chlorophyll, have shown anticarcinogenic activity and these have higher bioavailability. In the present study, the inhibition of lung cancer cells may be due to the presence of Flavonoid (Mukund *etal.*, 2013).

DNA Fragmentation of A549 cells

Fragmentation of DNA as ~200 base pairs fractions is considered as one of the hallmark in the process of apoptosis. Results of the study have shown fragmentation of DNA upon treatment of cells with extract indicating involvement of apoptosis. Analysis of DNA from the cells treated with volatile oil has shown disintegration of DNA, which partially resembles apoptotic pattern. Disintegration of DNA was observed prominently, which was not found in untreated cells (control). Assay is based on Terminal Deoxynucleotidyl Transferase (TdT)-mediated dUTP nick-end-Labeling. Anticancer drug/ cytotoxic compound breaks the DNA strands of cancerous cell to expose a large number of 3'-hydroxyl ends. Fluorescein deoxythymidine analog 5-bromo-2'-deoxyuridine 5'-tri-phosphate (BrdUTP) gets attached at the free 3'-hydroxyl ends of fragmented DNA with the help of TdT. Once incorporated into the DNA, BrdU can be detected by an anti-BrdU antibody using standard immunohistochemical techniques or fluorescence microscopy or flow cytometry (Negoescu, 1998 and 1999, Fehsel, 1991, Grasl-Kraupp, 1995 , Kishimoto, 1990)

Most of the anticancer drugs in current use have been shown to induce apoptosis in susceptible cells. German scientist Carl Vogt was first to describe the principle of apoptosis in 1842, which is a programmed cell death that may occur in multicellular organisms. Biochemical events lead to characteristic cell changes (morphology) and death. These changes include loss of cell membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. One of the later steps in apoptosis is DNA fragmentation, a process which results from the activation of calcium and magnesium dependent nucleases which degrade DNA endonucleases during the apoptotic program. These nucleases degrade the higher order chromatin structure into fragments of ~300 kb and subsequently into smaller DNA pieces of about 50 bp in length. Excessive apoptosis causes atrophy, such as in ischemic damage, whereas an insufficient amount results in uncontrolled cell proliferation, such as cancer (Kerr, 1972 and Gavrieli., 1992).

DAPI Staining

In order to determine whether the cytotoxic activity of *Oscillatoria terebriformis* extract was due to apoptosis, A549 cells were treated for 10 minutes with various concentrations of *Oscillatoria terebriformis* extract. The cells, stained with DAPI staining (10 μ M) revealed marked chromatin condensation and apoptotic body formation when examined by a fluorescence microscope. In addition, DAPI Staining revealed that the highest concentration (1000 μ g/ml) of *Oscillatoria terebriformis* extract had the effect of causing highly condensed, fragmented nuclei morphology, and nuclei shrinkage, the typical characteristics of apoptosis.

Cytotoxicity through LDH, GST and GR

Measurement of LDH and GST content in culture medium is used as an indicator for cytotoxicity in apoptosis research (Johnson and Mukhtar, 2007). Results of the current study indicated that cytotoxicity was also induced due to loss of membrane integrity by cyanobacterial extracts which may be due to high presence of flavonoids. Lactate dehydrogenase (LDH) is known to be released from cells due to damage in cell membrane, which indicated cell death either due to necrosis or apoptosis. Cancer cells are destroyed by *Oscillatoria terebriformis*. LDH has been found to be a cytosolic marker. Cell death by apoptosis leads to the release of cytoplasmic enzymes such as alkaline and acid phosphatase, glutamate-oxalacetate transaminase, glutamate pyruvate transaminase and arginosuccinate lyase. However, their use has been limited by the low amount of those enzymes present in many cells and by the elaborate kinetic assays required to measure most enzyme activities, whereas LDH is a stable cytoplasmic enzyme that is released into the culture medium following loss of membrane integrity resulting from apoptosis. LDH activity, therefore, can be used as an indicator of cell membrane integrity and serves as a general means to assess cytotoxicity resulting from chemical compounds or environmental toxic factors ((Bonfoco 1995, Legrand 1992). Lactate dehydrogenase regulates the interconversion of pyruvate to lactate, using NAD as cofactor. LDH the tetrameric protein forms the center for delicately balanced equilibrium in the metabolism of carbohydrates. LDH also takes part in the biosynthesis of carbohydrates. The high glycolytic rate is important for rapidly proliferating cancers not only as a major energy source but also to provide such cells with precursors for nucleotide and lipid biosynthesis. As a result of this high glycolytic rate, there is an elevated level of LDH during cancerous conditions (Arathi, *et al.*, 2003). Tumor cells have an increased glucose transport and this glucose is metabolized via the anaerobic glycolytic pathway to produce lactic acid. Enzyme is useful in the recognition of neoplastic disease. Malignant tumors are known to have high rates of glycolytic activity leading to high production of lactic acid (Bygrave *et al.*, 1976). Elevated serum LDH is also seen in anemia. This elevated LDH levels are due to the destruction of the abnormal red cell precursors (Elliott and Fleming, 1965). Decrease in the level of LDH activity was observed in N-acetylcysteine (NAC) treated cells infecting with induction in cell proliferation (Sridharan *et al.*, 2001). These reports suggest cyanobacterial extract treatment has inhibiting effect over the proliferating mass. Gupta *et al.*, (2004) who studied the relationship between antitumor activity and antioxidant role in anticancer activity. The increase in the GST activity in general, used as indication for the antitumor activity of the tested materials in both normal and tumor transplanted animals. Therefore, this enzyme has been used as antitumor factor (Oude-Ophuis *et al.*, 1998). In the tumor cells, the increase of cellular enzymes that regulate the cell oxidative stress such as SOD and GST and antioxidants such as GSH induced cancer regression and stimulated large number of tumor necrosis factor-alpha (TNF). Tumor necrosis factor (TNF) is one of the most important growth modulatory cytokines produced by almost all cell types of the immune system. A variety of GST inhibitors were shown to modulate drug resistance by sensitizing tumor cells to anticancer drugs (Tew *et al.*, 1988; Hall *et al.*, 1989; Ford *et al.*, 1991). The first clinical modulatory studies focused on an approved drug, ethacrynic acid (EA). EA inhibits GST-a, -m, and -p by binding directly to the substrate binding site of the isozyme, as well as by depleting its cofactor, GSH, via conjugation of the Michael addition intermediate to the thiol group of GSH (Mulder *et al.*, 1997; Oakley *et al.*, 1997).

The key role of cytosolic GST is to catalyze the nucleophilic attack of reduced glutathione (GSH) on the electrophilic center of toxic compounds of both endogenous and xenobiotic origin, the first step in the mercapturic acid pathway that leads to elimination of the toxic compounds. (Habig *et al.*, 1981)

GR also plays an essential role in the cellular defence against oxidative stress and a controlled decrease of the level of GR in human fibroblasts results in lowering the cell viability (Chavkova *et al.*, 2001). When GR activity is impaired; the ability of the cell to reduce GSSG to GSH may be devastated, leading to GSSG accumulation within the cytosol. The decrease in GR activity after extract treatment could be one of the other possible steps involved to decrease the GSH level, thus, affecting cellular antioxidant machinery, and resulting antitumor activity. The key role of cytosolic GST is to catalyze the nucleophilic attack of reduced glutathione (GSH) on the electrophilic center of toxic compounds of both endogenous and xenobiotic origin, the first step in the mercapturic acid pathway that leads to elimination of the toxic compounds (Habig *et al.*, 1981). The increase in the GST activity in general, used as indication for the antitumor activity of the tested materials. Therefore, this enzyme has been used as antitumor (as tumor factor (high amounts of GSTP-1 in tumor cells). In the tumor cells, the increase of cellular enzymes that regulate the cell oxidative stress such as GST. When cells exposed to high levels of oxidative stress, like red blood cells, up to 10% of the glucose consumption may be directed to the pentose phosphate pathway (PPP) for production of the NADPH needed for this reaction. In the case of erythrocytes, if the PPP is non-functional, then the oxidative stress in the cell will lead to cell lysis and anemia (Champe *et al.*, 2008). These results clearly indicate that *Oscillatoria terebriformis* Ag. VIAT010 can induce cytotoxic activity against A549 cells.

CONCLUSION

In this study, we found that *Oscillatoria terebriformis* extract, reduced cell viability by inducing apoptosis in Human lung cancer cell lines in vitro. This extract, therefore, has the potential of an anti-cancer agent. However, further study may still be necessary to elucidate the mechanism of apoptosis induction in A549 cells by *Oscillatoria terebriformis* extract.

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