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ANTICANCER ACTIVITY AND ANT DIABETIC ACTIVITY OF ASPERGILLUS STEREUS AF1

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ABSTRACT

Microorganisms produce secondary metabolites which may have shown antimicrobial activity acting against certain pathogenic microorganisms. *Streptomyces* are saprophytic gram positive bacteria which have the ability to produce vast number of structurally and functionally. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The in vitro cytotoxicity assay on human cervical cancer cell line (HeLa) revealed that the secondary metabolite had the strongest cytotoxicity with IC50 of 4.9 µg/ml.

KEYWORDS: secondary metabolites, Streptomyces, MTT Assay and Heal cells.

1. ANTICANCER ACTIVITY

1. INTRODUCTION

Some microorganisms produce secondary metabolites which may have shown antimicrobial activity acting against certain pathogenic microorganisms. These metabolites, otherwise known as bioactive substances, are profoundly used as antibiotics and may be effective against infectious diseases such as HIV-1(Cragg, G.M et al:, 2001) conditions of multiple bacterial infections (penicillin, cephalosporines, streptomycin, and vancomycin); or neural tube defects and neuropsychiatric sequelae (Finglas P.M et al;, 2003, Berdy, J. 2005). Microorganisms have been reported to produce around 23,000 bioactive secondary

metabolites and over 10,000 of these compounds are produced by actinomycetes, representing 45% of all bioactive microbial metabolites discovered (Shin HJ;, 2013). *Streptomyces* are saprophytic gram positive bacteria which have the ability to produce vast number of structurally and functionally wide variety of antibiotics with clinical and industrial importance and are responsible for the production of about half of the discovered bioactive compounds (Chaudhary HS et al;, 2013). Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability.

1.2. MATERIALS AND METHODS

DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from eppendorf India.

1.2.1 Maintenance of Cell Line

The MCF-7 breast adenocarcinoma cancer cell line were purchased from NCCS, Pune and the cells were maintained in MEM supplemented with 10% FBS and the antibiotics penicillin/streptomycin (0.5 mL^{-1}), in atmosphere of 5% CO_2/95% air at 37^0C.

1.2.2 Preparation of Test Compound

For MTT assay, Each Test compounds were weighed separately and dissolved in DMSO. With media make up the final concentration to 1 mg/ ml and the cells were treated with series of concentrations from 10 to 100 µg/ ml.

1.2.3. MCF-7 CELL VIABILITY BY MTT ASSAY

Principle

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and on the assumption that dead cells or their products do not reduce tetrazolium. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple coloured

formazan crystals. The cells are then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

Procedure

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. MCF- 7 cells were trypsinized and preform the thetryphan blue assay to know viable cells in cell suspension. Cells were counted by haemocytometer and seeded at density of 5.0 X 10^3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37^0C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compound in represntive wells in 96 plates. After 48 hrs., Discard the drug solution and add the fresh medic with MTT solution (0.5 mg / $mL^{-1)}$ was added to each well and plates were incubated at 37^0C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophore formazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% values is generated from the dose-response curves for each cell line using with origin software. Medium without any chemicals was used as a negative control. Fractions of viable cells were measured with MTT assay (El-Sersy NA et al ;,2012).

$$\% \text{ Inhibition} = \frac{100 \ (\text{Control} - \text{Treatment})}{\text{Control}}$$

RESULTS

1. MCF 7 Cell line: Aspergillus Stereus AF1

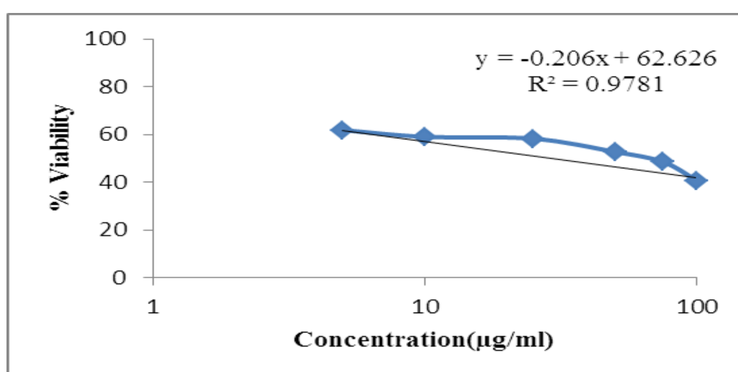

Figure 1.1: Cytotoxic effect of the *Aspergillus Stereus* AF1 on MCF 7 Cell Line.

Table 1.1: Cytotoxic Properties of *Aspergillus Stereus* AF1 on MCF 7 Cell Line.

Concentration(μg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (μg/ml)
100	0.838	0.841	0.843	0.84	0.835	40.711	61.262
75	0.998	1.002	1.004	1.001	0.996	48.561	
50	1.085	1.087	1.089	1.087	1.082	52.754	
25	1.198	1.2	1.202	1.2	1.195	58.264	
10	1.214	1.217	1.219	1.216	1.211	59.044	
5	1.271	1.273	1.275	1.273	1.268	61.823	
Untreated	2.056	2.057	2.056	2.056	2.051	100	
Blank	0.005	0.006	0.005	0.005	0		

2. Hela Cell line: *Aspergillus Stereus* AF1

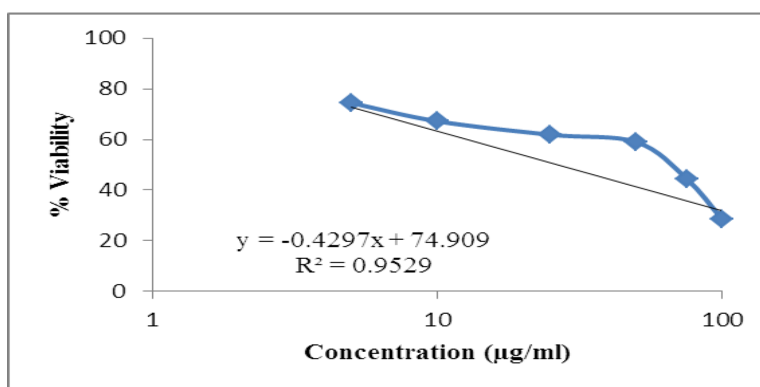

Figure 1.2: Cytotoxic effect of the *Aspergillus Stereus* AF1 Hela Cell Line.

Table 1.2: Cytotoxic Properties of *Aspergillus Stereus* AF1 HeLa cell Line.

Concentration(μg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (μg/ml)
100	0.597	0.599	0.601	0.599	0.596	28.585	58.042
75	0.93	0.932	0.934	0.932	0.929	44.556	
50	1.231	1.233	1.235	1.233	1.23	58.992	
25	1.291	1.293	1.295	1.293	1.29	61.87	
10	1.402	1.404	1.406	1.404	1.401	67.194	
5	1.552	1.554	1.556	1.554	1.551	74.388	
Untreated	2.088	2.089	2.088	2.088	2.085	100	
Blank	0.003	0.004	0.003	0.003	0	0	

3. HT -29 Cell line: *Aspergillus Stereus* AF1

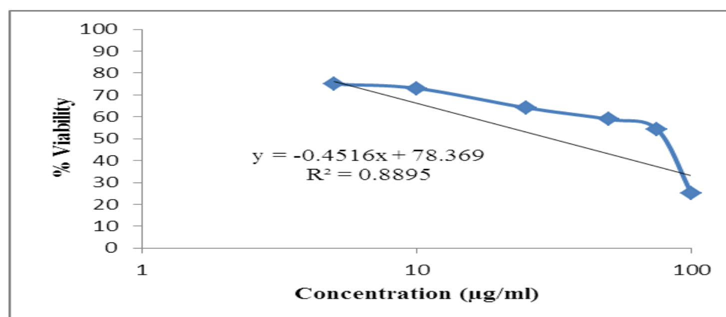

Figure 1.3: Cytotoxic effect of the *Aspergillus Stereus* AF1 HT 29 Cell Line.

Table 1.3: Cytotoxic Properties of *Aspergillus Stereus* AF1 HT – 29 Cell Line.

Concentration(µg/ml)	Absorbance at 570nm			Average	Average-Blank	% Viability	IC_{50} (µg/ml)
100	0.515	0.517	0.519	0.517	0.513	25.147	62.882
75	1.11	1.112	1.114	1.112	1.108	54.313	
50	1.204	1.206	1.208	1.206	1.202	58.921	
25	1.312	1.314	1.315	1.313	1.309	64.166	
10	1.492	1.494	1.496	1.494	1.49	73.039	
5	1.531	1.533	1.535	1.533	1.529	74.95	
Untreated	2.044	2.043	2.045	2.044	2.04	100	
Blank	0.004	0.005	0.004	0.004	0		

II. ANTI DIABETIC ACTIVITY

HepG2, Human Liver cancer cells were cultured in DMEM containing 4.5 g/L D-glucose with 10% heat-inactivated FBS at 37°C, 5% CO2 atmosphere. The cells were seeded into 96-well plate with six wells left as blank wells and let growing to confluence; then cells were fully differentiated in DMEM with 2% FBS for 5 days. Before tests, the medium was replaced by RPMI1640 (2 g/L glucose) supplemented with 0.2% BSA. The medium was removed after 2 h, and the same medium containing *Aspergillus Stereus* (0.5, 5 and 10 µg/mL), GA (0.05, 0.5 and 5 µM), metformin (0.01 mM) and DMSO in absence or presence of insulin (1 µmol/L) was added to all wells including the blank. The glucose in the medium was determined by the glucose-oxidase method after 48 h treatment. The amount of glucose uptake by muscle cells was calculated by using the following formula:

Glucose uptake = [Glucose concentration of blank wells] – [Glucose concentration of cell plated wells].

RESULTS

Table: Glucose uptake in HepG2 cells after 48 h incubation in media with glucose (2 g/L).

Treatment	Concentration	Glucose consumption (mg/100 mL) Absence of insulin	Presence of insulin (1µmol/L)
Vehicle control	0.1% DMSO	2.95 ± 0.07	7.17 ± 0.03
Metformin	0.01 mM	12.80 ± 0.06	14.96 ± 0.03
	0.1 mM	10.84 ± 0.09	12.68 ± 0.13
Aspergillus Stereus	0.5 µg/mL	24.77 ± 0.07	32.69 ± 0.06
	5 µg/mL	26.05 ± 0.08	34.79 ± 0.05
	10 µg/mL	23.98 ± 0.07	33.96 ± 0.04
Gallic acid	0.05 µM	18.04 ± 0.05	22.96 ± 0.09
	0.5 µM	15.96 ± 0.07	21.87 ± 0.10
	5 µM	16.58 ± 0.14	20.83 ± 0.09

DISCUSSION

This study evaluated the antibacterial activities and cytotoxicity of the secondary metabolites of actinomycetes. The secondary metabolites from our actinomycetes shown antibacterial activity against some bacterial pathogens isolated from the human fluid samples. These results correlated with the previous findings in which the antimicrobial activity of *Streptomyces* isolated from soil (Sahin N et al;, 2003). Earlier some marine actinomycetes isolated from Bay of Bengal (Coast of Inida) were screened for antagonistic and antimicrobial activity against pathogenic bacterial and fungi (Gandhimathi R et al;, 2008). Antimicrobial activities of mairne bacteria from the water of South East India were also studied (Anand et al;, 2006). The in vitro cytotoxicity of secondary metabolites showed a significant antiproliferative activity on HeLa (human cervical cancer) cell line and a dose dependent effect was observed (IC50 - 4.9 µg/ml). Some Bioactive compounds were isolated and found selectively cytotoxic against lung and colon cancer cell lines as well as melanoma. Interestingly, the compound exerts preferential antiproliferative effects in colon cancer cell lines with defective p53 systems (E. Erba et al;, 1999). In recent studies of the biological activity compounds revealed that only one was strains (*Aspergillus Stereus* AF1) had anticancer activity and antidieabetic activity.

CONCLUSIONS

The marine environment is a good source for valued species that need to be explored. The aim of the present study was to explore our natural environments, searching for actinomycetes producing bioactive compounds. The findings of the present study suggested that the antagonistic marine *Aspergillus Stereus* isolates, the antibacterial compounds

produced by it, could be used as antibiotics and antifoulants, which might have future applications in the aquaculture systems. In addition, the *Aspergillus Stereus* compounds may be applied as anticancer agents. Future studies are required for the identification of the product.

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