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# ANTICANCER ACTIVITY AND ANTI DIABETIC ACTIVITY OF STREPTOMYCES SPECIES JKCM1

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## ABSTRACT

The Actinomycetales, especially the genus Streptomyces, produce a number of antibiotics and other bioactive natural products. Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. 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. 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.

**KEYWORDS:** Actinomycetales, cell viability, colorimetric assay and MTT Assay.

## INTRODUCTION

The members of the order Actinomycetales, especially the genus Streptomyces, produce a number of antibiotics and other bioactive natural products. The genomic analysis of some Streptomyces strains revealed the presence of biosynthetic gene clusters for about 30 secondary metabolites (Bentley et al., 2002; Ikeda et al., 2003; Ohnishi et al., 2008).

Metabolic disorders (i.e., any of the diseases or disorders that disrupt normal metabolism) are common pathologies, and especially diabete (Kaur J;,2014). In 2013, it was estimated that over 382 million people throughout the world have diabetes and this number is expected to increase up to 500 million in 2030 (Barde S.R et al;,2015) when it is expected that this disease will be the 7th leading cause of death (Mathers C.D et al;, 2006).

## ANTI CANCER ACTIVITY

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.

## 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.

### 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 $^0$ C.

### 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.

## 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 $37^{0}$C. 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 $37^{0}$C 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.

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

**1. Hela Cell line: *Streptomyces species* JKCM1.**

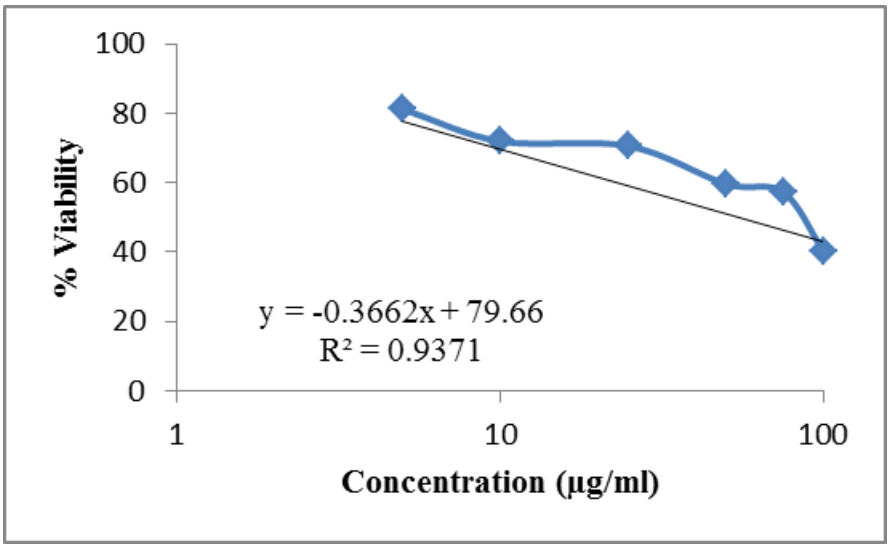


**Figure 1: Cytotoxic effect of the Streptomyces species JKCM1 Hela Cell Line.**

**Table 1: Cytotoxic Properties of Streptomyces species JKCM1 on Hela Cell Line.**

| Concentration(µg/ml) | Absorbance at 570nm | | | Average | Average-Blank | % Viability | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| 100 | 0.841 | 0.843 | 0.845 | 0.843 | 0.84 | 40.287 | 81.038 |
| 75 | 1.196 | 1.198 | 1.201 | 1.198 | 1.195 | 57.314 | |
| 50 | 1.244 | 1.246 | 1.248 | 1.246 | 1.243 | 59.616 | |
| 25 | 1.472 | 1.474 | 1.478 | 1.474 | 1.471 | 70.551 | |
| 10 | 1.501 | 1.503 | 1.505 | 1.503 | 1.5 | 71.942 | |
| 5 | 1.693 | 1.696 | 1.699 | 1.696 | 1.693 | 81.199 | |
| Untreated | 2.088 | 2.089 | 2.088 | 2.088 | 2.085 | 100 | |
| Blank | 0.003 | 0.004 | 0.003 | 0.003 | 0 | 0 | |

**2. HT - 29 Cell line: Streptomyces species JKCM1.**

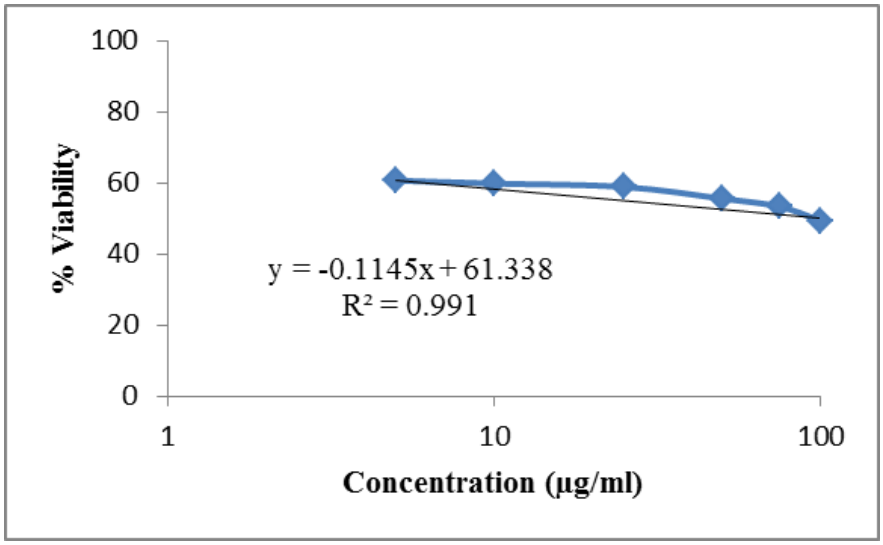


**Figure 2: Cytotoxic effect of the *Streptomyces* species** JKCM1 **HT29 Cell Line.**

**Table 2: Cytotoxic Properties of** *Streptomyces species* JKCM1 **on HT29 Cell Line.**

| Concentration(µg/ml) | Absorbance at 570nm | | | Average | Average-Blank | % Viability | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|---|---|---|
| 100 | 0.998 | 1.01 | 1.03 | 1.012 | 1.008 | 49.411 | 99.386 |
| 75 | 1.09 | 1.092 | 1.094 | 1.092 | 1.088 | 53.333 | |
| 50 | 1.135 | 1.139 | 1.14 | 1.138 | 1.134 | 55.588 | |
| 25 | 1.204 | 1.206 | 1.207 | 1.205 | 1.201 | 58.872 | |
| 10 | 1.223 | 1.225 | 1.227 | 1.225 | 1.221 | 59.852 | |
| 5 | 1.239 | 1.241 | 1.243 | 1.241 | 1.237 | 60.637 | |
| Untreated | 2.044 | 2.043 | 2.045 | 2.044 | 2.04 | 100 | |
| Blank | 0.004 | 0.005 | 0.004 | 0.004 | 0 | | |

**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 *Streptomyces species* JKCM1 (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 |
| *Streptomyces species* JKCM1 | 0.5 µg/mL | 20.77 ± 0.07 | 28.69 ± 0.06 |
| | 5 µg/mL | 21.05 ± 0.08 | 29.79 ± 0.05 |
| | 10 µg/mL | 22.98 ± 0.07 | 30.96 ± 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 |

## CONCLUSION

The anticancer drugs are available for the treatment of cancer but new anticancer drugs are urgently needed due to harmful adverse effects of the existing drugs. In our study we reported that our crude metabolite obtained from co-culture of two marine *Streptomyces species* JKCM1 has excellent anticancer activity and anti Diabetic Activity. This extract might be used in infectious and cancer diseases and further research will be done for the isolation of pure compounds.

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