IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Cytotoxic Activity Of Marine Brown Algae Sargassam Swartzii Extract By Using Mtt Assay

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ABSTRACT

The study has been undertaken to find out the effect of Sargassam swartzii extract on TT human medullary cancer cells. The method used for analysis is the MTT assay. The test extract with different concentration were applied on cells and observed cell viability. In this particular study a reduction in cell viability is observed which is dependent on the concentration of extract. This assay gives a result which shows that the cytotoxic effect at 100 µg/mL at cell viability is 68.53%. The result from this study shows that the marine brown algae Sargassam swartzii with moderate cytotoxic activity and this marine algae is cytotoxically active.

INDEX TERMS

MTT assay, Cytotoxicity, TT cell line, Cell viability

AIM

To evaluate the potential cytotoxic activity of the marine brown algae, extract on human TT medullary thyroid cancer cell line using MTT assay.

OBJECTIVES

To measure cell viability, proliferation and cytotoxicity

To calculate the percentage of viable cell measure and IC50 value

1.INTRODUCTION

Cancer is a disease which is an abnormal cell growth process, with the potential to invade and metastasize other parts of the body.[1] Algae is a group of diverse organisms, comprise a wide range of simple, typically autotrophic life forms [2]. Marine algae, such as brown and green algae, have been identified as potential sources of anticancer agents [3,4]. In vitro studies is an essential step in the preclinical evaluation of macroalgae-derived compounds for cancer treatment, serving as a fundamental step first step in identifying bioactive compounds, determining their mechanisms of action, and assessing cytotoxicity against cancer cells. Therefore, understanding the mechanisms behind their anticancer effects leads to the way for future research, potentially leading to the development of safe and effective chemotherapeutic agents derived from algae [5,6,7].

Human medullary thyroid carcinoma (MTC) is a calcitonin producing tumour develops from the parafollicular C-cells accounting for 5-10% of all thyroid tumours. MTC may forms occur radically, in a familiar form without associated endocrine disorders or as part of multiple endocrine neoplasia type 2A or 2B which follows autosomal dominant inheritance pattern [8,9]

The MTT assay is used to measure cell viability, proliferation and cytotoxicity. The present study was done in the extract of marine brown algae to investigate the cytotoxicity against TT human medullary thyroid cancer cell line using MTT assay.

Cell line and Culture

The cells were cultured in F12K (DMEM-HI media), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100 U/mL), Streptomycin (0.1 mg/mL), and Amphotericin B (0.25 µg/mL). The cell containing TC flasks (25 cm^2) were incubated at 37˚C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170, Eppendorf, Germany).

2.PROCEDURE

MTT Assay Procedure

The cells (10,000 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37˚C and 5% CO_2 environment in the incubator for 24 hours. Sample was prepared in DMSO (10mg/mL). The samples were further diluted in F12K media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 50, 50, 100µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2 hours for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was measured with micro plate reader. Three wells per plate without cells served as blank. All the experiments were done in triplicates.

Calculation of cell viability

The cell viability was expressed using the following formula:

$$Percentage\ of\ cell\ viability = \frac{Average\ absorbance\ of\ treated}{Average\ absorbance\ of\ control} \times 100$$

3.RESULTS

The cytotoxic activity of the marine brown algae was evaluated using the MTT assay and it shows a dose dependent reduction in variability.

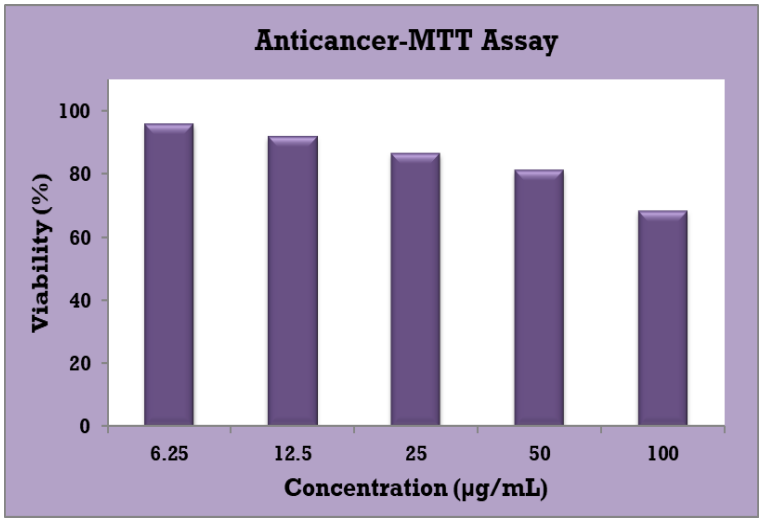
MTT assay results for varying concentration of test sample

Concentration (µg/mL)	Triplicate values			Average OD
	OD1	OD2	OD3	
Control	0.791	0.792	0.797	0.793
6.25	0.755	0.769	0.763	0.762
12.5	0.730	0.736	0.724	0.730
25	0.699	0.684	0.686	0.690
50	0.645	0.649	0.648	0.647
100	0.547	0.536	0.548	0.544

Percentage of viability for varying concentration of test sample

Concentration (µg/mL)	Percentage of viability
6.25	96.09
12.5	92.02
25	86.93
50	81.60
100	68.53
IC 50	>100 µg/mL

Graphical representation.



4.DISCUSSION

This study was evaluated the cytotoxic activity of a marine brown algae extract against TT medullary thyroid cancer cells using the MTT assay. The results shows a concentration dependent reduction in cell viability, indicates the cytotoxic potential of the algae extract. Dose dependent reduction in cell viability was observed in TT cancer cells administered with different concentrations of the sample. The maximum cytotoxicity was observed with 100µg/mL of the sample (% Viability-68.53%).

5. CONCLUSION

This study was demonstrated that the Marine brown algae Sargassam swartzii extract exhibited a cytotoxic activity against TT human medullary thyroid carcinoma cells. The observed reduction in cell viability shows that anticancer properties of algal extract.

Overall, this study findings shows that Sargassam swartzii extract could serve as a natural source for anticancer activity. However, further detailed studies and investigations are required to validate its therapeutic potential.

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