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Article

Invitro cytotoxicity assay of Fucoidan extracted from *Turbinaria conoides* against cancer cell lines MCF7, A549, and normal cell line L929

[1]Revathi Chitra Santhanam, [1]Syed Ali Mohammed Yacoob*, Anuradha Venkatraman[2]

[1]Department of Biotechnology, Mohamed Sathak College of Arts & Science, Affiliated to the University of Madras, Sholinganallur, Chennai, TamilNadu, India, [2]Department of Biochemistry, Mohamed Sathak College of Arts & Science, Affiliated to the University of Madras, Sholinganallur, Chennai, TamilNadu, India

The main aim of the study is to quantify the cytotoxic property of the Fucoidan extracted from the Turbinaria conoides using the MTT assay with the standard fucose. Fucoidan was extracted using the soaked water method and it was determined using the HPLC procedure the obtained Test sample Fucoidan extracted from the Turbinaria conoides and standard fucose was subjected to the cytotoxicity assay against the MCF7 Human breast cancer cell line, A549 lung cancer cell line, and L929 normal mouse fibroblast cell line. From the results it was found that the Test sample showed good IC50 value for MCF7 cell line then A549 with an increasing concentration 24 hours incubation at 37°C The IC50 for MCF7 was 115.21 µg/ml and A549 396.46µg/ml and the Fucoidan extract was checked for its cytotoxicity against the normal mouse fibroblast cell line L929, Fucoidan was found non-lethal to the L929 mouse fibroblast normal cell line. Standard fucose also gave a significant result towards MCF7 and against the L929. This indicates that the Fucoidan extracted from Tubinaria conoides shows better anticancer potential in it. Hence its application can be further extended in the pharmacological fields**.**

Keywords: MTT assay. MCF7 (breast cancer cell line). A549 (lung cancer cell line). Fucoidan extract. Turbinaria conoides.

INTRODUCTION

The significance of brown seaweeds is elevated day by day in the exploration domain to satisfy the medical needs without causing any side effects. Seaweeds are the boon to human health. Seaweed plays a major role in treatingseveral diseases. Seaweeds as well-balanced, harmless, natural sources with a high degree of bioavailability of trace elements are strongly advised for fast growth in children and pregnant women (Booth, 1964). Seaweeds are the rich source of polysaccharides, Fucoidan is a type of polysaccharide which can cure many diseases with the help of emerging science innovations. The inability to cure many diseases including cancer has encouraged the need for the development of new drugs from natural sources. Among isolated from marine brown algae by (Kylin, 1913) now it is named as "Fucoidan" according to IUPAC rules, but also called as fucan, fucosan or sulfated fucan. Fucoidan is considered as a cell wall-reinforcing molecule and seems to be associated with protection against the effects of desiccation when the seaweed is exposed at low tide. Fucoidan can be isolated from several species of brown seaweed and found to have different chemical compositions, for example, Fucus vesiculosus, have simple chemical compositions, mainly being composed of fucose and sulfate. But the chemical compositions of most Fucoidans are complex (Senthilkumar *et al.*, 2013). Fucoidan is viscous in very low concentrations and susceptible to breakdown by

***Correspondence:** S. A. M. Yahoob. Department of Biotechnology. Mohamed Sathak College of Arts & Science. Affiliated to the University of Madras. Sholinganallur,Chennai, TamilNadu, India. Phone: 98420 52929. E-mail: ID syedmicro555@gmail.com
Orcid: https://orcid.org/0000-0003-0233-1105

diluted acids and bases. (Holdt, Kraan, 2011). According to the World Health Organization, breast cancer is the most common cancer among women worldwide, claiming the lives of hundreds of thousands of women each year and affecting countries at all levels of modernization. Lung cancer is also responsible for the largest number of deaths (1.8 million deaths, 18.4% of the total), because of the poor prognosis for this cancer worldwide. Lung cancer is the most commonly diagnosed cancer in men (14.5% of the total cases in men and 8.4% in women) and the leading cause of cancer death in men (22.0%, i.e. about one in 5 of all cancer deaths). Over the years marine algal species offer the biological diversity for sampling in discovery-phase of new drug development (Munro *et al.,* 1999). Therefore, it is documented that, pre-clinical pharmacological research with new marine compounds continued to be extremely active in recent history (Mayer, Gustafson, 2003). The discovery of novel natural compounds with low toxicity and high selectivity for killing cancer cells is an important area in cancer research.

MATERIAL AND METHODS

Seaweed sample *Turbinaria conoides*, standard L-Fucose Extra pure-SRL, Chitosan (Low MW Extra pure SRL), Fetal Bovine Serum, MTT Reagents and D-PBS (Himedia), DMSO and Camptothecin (Sigma). All chemicals and reagents used were of analytical grade.

Cell line and culture

Cell lines MCF7 -Human Breast Cancer Cell Line, A549-Human Lung Cancer Cell Line and L929- normal mouse fibroblast cell line was purchased from NCCS Pune and cultured in the Cell culture medium: DMEM-High Glucose (Himedia).

METHODOLOGY

Sample collection and isolation of Fucoidan

Turbinaria conoides was collected from the Mandapam coast of Rameshwaram Tamilnadu. The collected sample was cleaned, shade dried in room temperature and further dried in a hot air oven at 50oC and it was blended in the mixer till it turns powder form and it was sieved. The fine powder was used for the isolation of Fucoidan.

Isolation of Fucoidan using the soaked water method

A new extraction method of Fucoidan from the soaked water of brown seaweed (Xiaolin Chen *et al*., 2012) was used for the extraction of Fucoidan from *Turbinaria conoides* with a slight modification followed by (Revathi Chitra *et al*, 2018).100gms of powdered seaweed sample was mixed with 2.4l of distilled water and it was kept in the shaker for 24hours. Then it was filtered and the filtrate was separated. From the filtrate, 150ml of the filtered solution was taken and mixed with 1% chitosan.1% chitosan was prepared in 1% acetic acid solution. The mixed solution was kept for 12 hours in a shaker for agitation. The obtained mixture was centrifuged at 1500rpm for 5 minutes. The supernatant was discarded and the pellet was dried in the hot air oven at 50°C. From the dried pellet 4gms was obtained and it was mixed with 551ml of distilled water maintained at pH6 and it was kept for 12 hours. After the period of 12 hours, the mixture was agitatedat room temperature for 2 hours and it was filtered. The filtrate was washed with ethanol for 15minutes and it was centrifuged and the pellet was removed, air-dried and it was used for further analysis.

HPLC for the detection of Fucoidan using Fucose standard

Hydrolysis of Polysaccharide

The polysaccharide was hydrolysed with 2 M Trifluoroacetic acid at 100 °C for 8 hours in a sealed tube under nitrogen. The hydrolysate was evaporated to dryness and used for monosaccharide analysis.

High-Performance Liquid Chromatography (HPLC)

Identification of the Test component Fucoidan polysaccharide component was performed on HPLC

equipped with a Refractive Index detector (HPLC RID-20A Shimadzu, Tokyo, Japan). A 250 mm long column with an outer diameter of 4 mm (Hypersil; Thermo-Scientific) using acetonitrile: water (80:20) containing 1 % (v/v) acetic acid as an eluent at a flow rate of 0.8 ml. Sugar identification was done by comparison with reference standard L.Fucose.

MTT assay background of the study

MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on the reduction of the yellow coloured water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple colour, the intensity of which is proportional to the number of viable cells and it was measured spectrophotometrically at 570nm.

PROCEDURE

200µl cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Cells were allowed to grow for about 24 hours. To that, varying concentrations (25, 50, 100, 200, 400µg/ml) of the test agent Fucoidan extracted from *Turbinaria conoides* was dissolved in the DMSO 1mg/ml. The dissolved solution was added in the ELISA Plates ELISA plate was incubated for 24 hours at 37°C in a 5% CO2 atmosphere. After the incubation period, the plates were taken from incubator and spent media was removed and MTT reagent was added to a final concentration of 0.5mg/mL of total volume then the Plates were wrapped with aluminium foil to avoid exposure to light and it was kept in the incubator for 3 hours after that MTT reagent was removed and 100µl of solubilisation solution (DMSO) was added. Gentle stirring was given in a gyratory shaker which enhanced the dissolution. Occasionally, pipetting up and down was done to completely dissolve the MTT formazan crystals. Along with this, blank (medium without cells), positive control (medium with cells and 25uM of Camptothecin standard) and negative control medium with cells but without the experimental compound was used. The same procedure was followed for the standard fucose using the MCF7 and normal cell line L929. Readings were taken in duplicates and mean value was used to calculate the IC50 value**.** Readings were taken in the spectrophotometer at 570nm to 630nm and were used as the reference wavelength. The IC50 value was determined by using a linear regression equation i.e. Y =Mx+C. Here, Y = 50, M and C values were derived from the viability graph for MCF7, A549 and normal cell line L929.

RESULTS

Isolation of Fucoidan by soaked water method gave the yield of 2gms from 100gms of the powdered extract. The presence of Fucoidan in the test sample was determined from HPLC by using the standard fucose. Standard fucose gave a single peak with the Retention time at 6.307 and the test sample Fucoidan gave the Retention at 6.293(Figure 1). Cytotoxicity assay was performed for MCF7, A549 and L929 cell line against the test compound Fucoidan extracted from *Turbinaria conoides. The* IC50 value ofMCF7 115.21 µg/ml, while the A549 gave an IC50 value of 346.49 andthe Normal fibroblast cell line gave an IC50 value of 441.80(Figure 2).MCF7 treated with and standard fucose gave IC50 value of 38.98 for and IC 50 for normal cell line treated with standard fucose resulted in 373.87(Figure 3).

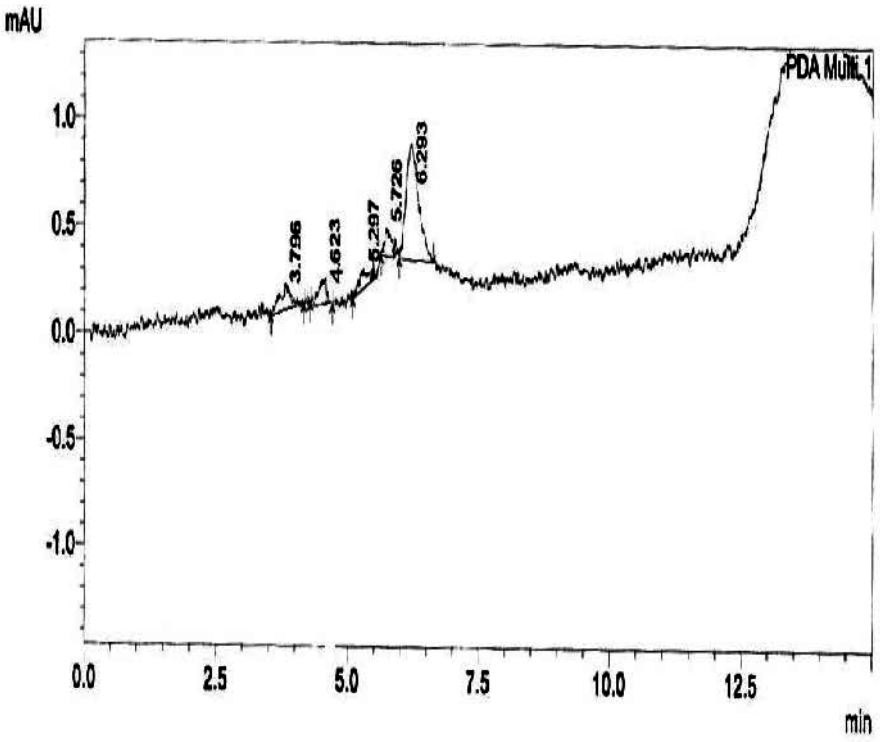

FIGURE 1 - HPLC chromatogram for the determination of Fucose in the test sample**,** the graph for the test sample Fucoidan extracted from the *Turbinaria conoide*s shows different peaks with varying Retention time on comparison with standard fucose. The peak with retention time 6.293 that covers the area percentage 46.215 indicates the presence of fucose.

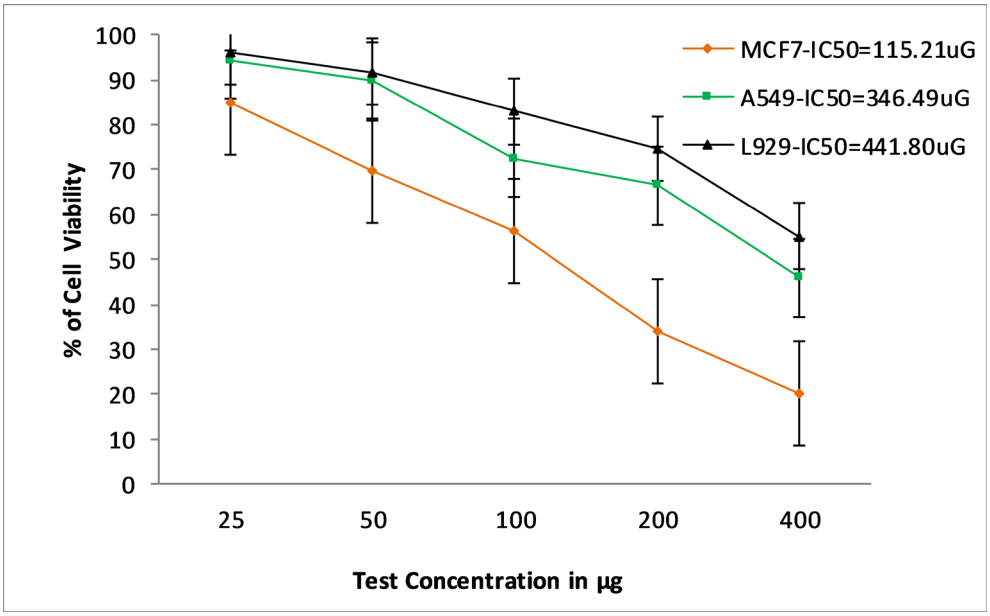

FIGURE 2 - Test sample Fucoidan extracted from *Turbinaria conoides* was treated with MCF7 (Breast Cancer Cell Line), A549 (Lung cancer cell line, and L929 (Normal fibroblast cell line) to check the viability of the cancer cell lines and the normal cell line. The concentration of the test sample Fucoidan was taken in varying concentrations like 25, 50, 100, 200, 400µg/ml against its percentage of viability. The graph represents that, the percentage of cell viability decreases as the concentration of the test sample Fucoidan increases. MCF7 cell line showed a significant decrease in the cell viability compared to A549 cell lines with the IC 50 value 115.21µg for MCF7, 346.49µg for A549 cell lines and normal mouse fibroblast cell line was viable to the test sample with the IC 50 value 441.80µg.

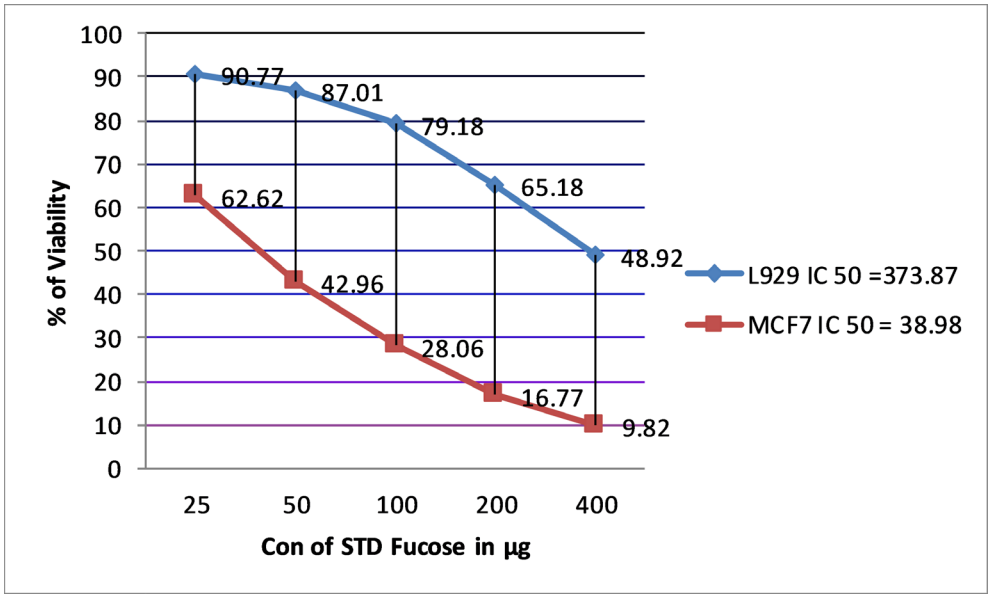

FIGURE3 -Standard fucose treated with MCF7 breast cancer cell line and normal Fibroblast cell line L929.The graph shows the concentration of standard fucose at 25, 50, 100, 200, 400 µg/ml against the percentage of viability against MCF7 cancer cell line and L929 Normal cell line for 24 hours of incubation at 37°C. The graph represents that, the percentage of cell viability of the MCF7 Breast cancer cell line decreases intensively with the increase in the concentration of the standard fucose with the IC50 value of 38.98µg and IC50 value of 373.87µg for L929 normal mouse fibroblast cell line.

DISCUSSION

From our results, it is found that the Cytotoxicity assay showed that the inhibitory effect of the MCF7 Breast cancer cell line was very effective compared to the A549 Lung cancer cell line this indicates that the fucoidan has the potential to kill the MCF7 Breast cancer cell line than the A549 lung cancer cell line. This might be due to the potential of the fucoidan to arrest MDAMB-231 breast carcinoma cell adhesion to platelets, the process which leads to the critical implications in tumour metastasis. This effect was reported by (Cumashi *et al*., 2007). It is also noted that the test sample doesn't show any inhibitory effect towards the normal cell line L929 and a drastic decrease in MCF7 cell line was found in the standard fucose, this may be because of the pure form of the standard fucose.

CONCLUSION OF THE STUDY

The results suggest that Fucoidan extracted from the *Turbinaria conoides* may be used as an alternative source by replacing the chemotherapy for treating breast cancer. This can be confirmed infurther *invivo* studies. Thus it may be used as a potential anticancer agent to overcome breast cancer in the future.

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