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Research Article

IN VITRO ANTICANCER ACTIVITY OF METHANOL EXTRACTS OF *AVICENNIA MARINA* (FORSSK) VIREH AGAINST HT-29 COLON CANCER CELL LINE

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ABSTRACT

In the present study the crude methanol extracts of Avicennia marina were prepared by using soxhlet apparatus and to investigate the efficacy of the extract against HT-29 cell line. The anticancer activity of A. marina leaf extract on proliferation of HT-29 cancer cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) microculture tetrazolium viability assay. The colon cancer cell was exposed to different concentration 7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/ml. About 50% rise in cell death was seen when the concentration of A. marina was increased from 62-125 µg/mL. At the dose of 250 µg/mL 29.30% cytotoxicity was observed. The IC50 value of methanol extract of A. marina was 62.5µg/ml. Our findings infer that the potential bioactive compound of this plant was effective against colon cancer and acquire anticancer activity.

KEYWORDS: *Anti-cancer agent, Avicennia marina, MTT, Cytotoxicity, IC50.*

INTRODUCTION

Tumour is one of the primary causes of death in the planet, and it is the second driving reason for mortality after heart sickness. It is an imperative health problem in developing and developed countries. Consistently, a normal 182 for every 100000 people's experiences ill effect of growth around the world, and 102 pass on by malignancy as indicated by the World Health Organization, 14 million individuals experience the ill effects of malignancy and 8 million die by cancer around the world. The commonness rate of cancer in Iran is 7/134 per 100000 people (Kumar *et al*., 2014). Nowadays, different strategies are utilized for malignancy treatment, for example chemotherapy, yet in these strategies, due to non selectivity of medicines, a high level of healthy cells will be lost with tumour cells.

Colon disease is a standout amongst the most common place sorts of growth around the world. While chemotherapy is one of the strategy generally utilized remedial techniques against colon disease, it is likewise has a few confinements. The discovery of new medicine for use in conceivable procedures in tumour treatment is thusly profoundly alluring. Plants are viewed as exceptionally encouraging from this viewpoint, since they speak to considerable wellsprings of substances with different remedial employments. Most anticancer drugs are today created from therapeutic plants. (Rosa *et al*., 2010; Rezaie-Tavirani *et al*., 2013).

The restorative plants comprise an unquestionable store of antiproliferative compound. A few bioactive mixes having a place with a few classes of possible metabolites separated from medicinal plants indicated noteworthy antiproliferative action against disease cells. Late research confirmations insist Indian mangrove plant species have antibacterial and anticancer exercises (Arivuselvan *et al*., 2011). The capability of mangrove plants as a wellspring of new bio dynamic standards is as until now unexplored.

Avicinea marina (Forssk) Vireh (Avicenniaceae) is commonly known as a gray mangrove tree. It is a high saltiness tolerant plant and is found on the coastline. Recent research also suggests *A. marina* plant species have antibacterial and anticancer activities. *A. marina* is likewise one of the plants they have anticancer property. So far the mechanism of anticancer activity of *A. marina* extract has not been explored against colon growth cell lines. Herbal based therapeutics for colon malignancy issue have been practiced in India for a long time and popularized globally by leading pharmaceutical companies. But constrained investigations are accessible on the utilization of marine halophytes for the administration of colon illnesses. The present assessment intends to decide the anticancer and

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the cytotoxicity capability of the unrefined methanol extracts of leaf from *Avicennia marina*.

MATERIALS AND METHODS

Collection of materials:

Avicennia marina (Forssk) Vireh collected from the Pichavaram mangroves in Tamil Nadu. The plant was identified at CAS in Marine Biology, Annamalai University, Parankipettai, Tamilnadu, India. The materials were then thoroughly washed in tap water followed by distilled water to remove the salt on the surface of the leaves. For drying, washed specimens were placed on blotting paper and spread out at room temperature in the shade. The shade dried samples were grounded into fine powder using a tissue blender. The powdered samples were then stored in the refrigerator for further use.

Reagents:

MEM was purchased from Hi Media Laboratories Fetal Bovine Serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyldiphenyl- 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.

Extraction of sample:

The dried and powdered materials (5 g) were extracted successively with 250 mL of methanol by using a Soxhlet extractor for 8 hrs at a temperature not exceeding the boiling point of the solvent. The aqueous extracts were filtered by using Whatman filter paper (No: 1) and then concentrated in vacuum at the 40°C using Rotary evaporator. The residues obtained were stored in a freezer -20°C until further tests.

Cell line and cell culture:

The HT-29 cell lines were obtained from King Institute, Guindy, Chennai. The cancer cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37°C. Cells were fed with fresh cultured medium every 2–3 times per week and subcultures when 80% confluent. All cultures were free of Mycoplasma.

Evaluation Cytotoxicity activity by MTT assay (Mosmann, 1983):

Cells (1 × 10^5/well) were plated in 24-well plates and incubated in 37°C with 5% CO_2 condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µL/well (5 mg/mL) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl) -2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hrs. After incubation, 1 mL of DMSO was added in all the wells. The absorbance at 570 nm was measured with a UV-spectrophotometer using DMSO as the blank. Measurements were performed and the concentration necessary for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was intended using the following formula:

% cell viability = A570 of treated cells / A570 of control cells × 100

Graphs are plotted using the % of Cell Viability at Y-axis and the concentration of the sample in X-axis. Cell control and sample control are incorporated in each assay to compare the full cell viability evaluation.

Statistical analysis:

The data on cell viability were analyzed by using the one way ANOVA followed by the Dennett's multiple comparison tests with equal sample size by using SPSS 17.0. The difference was measured significant when $p<0.005$. All the values were expressed as mean ± standard deviation (S.D). Triplicate assays were performed for each set of test conditions.

RESULTS AND DISCUSSION

In this assessment, we have the tendency to utilize the MTT test to assess the cytotoxic impact of the methanol leaf extract of *A. marina* against on HT-29 Human colon cancer cell line. The cytotoxic action of methanol concentrates of *A. marina* against human HT-29 Human colon cancer cell line irrefutable a measure and time-subordinate inhibitory impact. The methanol concentrates of *A. marina* confirmed a potential inhibitory impact when contrasted with the cell control.

MTT assay was carried out to observe the despotic effects of methanol extracts of *A. marina* on the growth of HT-29 Human colon cancer cell line and the results are represented in Table 1; Eight completely diverse concentrations (7.8, 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/mL) of methanol extract of *A. marina* were applied. Plate.I shows the percentage of growth inhibition against the methanol extracts of *A. marina*.

Table No. 1: Effect methanol Leaf extract of *A. marina* on Human colon cancer cell line (HT-29)

S.No	Concentration (µg/mL)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.047	7.57 ± 0.010
2	500	1:1	0.116	18.67 ± 0.015
3	250	1:2	0.182	29.33 ± 0.035
4	125	1:4	0.254	40.89 ± 0.015
5	62.5	1:8	0.321	51.71 ± 0.020
6	31.2	1:16	0.394	63.43 ± 0.020
7	15.6	1:32	0.451	72.61 ± 0.010
8	7.8	1:64	0.535	86.11 ± 0.030
9	Cell control	-	0.621	100 ± 0.000
F-VALUE			7.129666	
P-VALUE			0.000	

The highest percentage (86.1.5%) of growth inhibition was revealed with the treatment using 7.8 µg/mL of methanol extract. This was followed by the treatment using methanol extracts at 15.6, 31.2, 62.5, 125, 250, 500 and 1000 µg/mL with 7.07, 15.82, 20.89, 27.92, 41.79, 53.71 and 58.98 and of growth inhibition, correspondingly. Treatments with 500 and 1000 µg/mL methanol extracts did not show any growth inhibition. The methanol extract of *A. marina* inhibited the proliferation of HT-29 Human colon cancer cell line in a dose dependent manner. The cell control showed 100% protection. Plants take part in an important role in medicine, and most anti-cancer constituents are from leaf extracts, for example, the leaves of Curcumin (Prasana *et al*., 2009), *Hibiscus sabdariffa* (Lin *et al*., 2012), C*aralluma fimbriata* (Shenai Ashwini *et al*., 2017), *Trigonella foenum* (Hatice Gumushan Aktas *et al*., 2015).

For the treatment with different grouping of methanol extracts from *A. marina,* at 1000 µg/mL showed development hindrance (7.56%) as outlined in Table 1. The accumulation of methanol extracts focuses brought about the decrement of development restraint as observed with 250 and 500 µg/mL of concentrates exhibited 63.44 and 72.62% of development interruption, separately. Similarly, antiproliferative activities of methanol extract of *Theobroma cacao* leaf displayed the strongest andti-cancer effect but moderate antioxidant activity and total phenolic content (Baharum *et al*., 2016).

The results indicated that all the methanol extracts have positive inhibition on the HT-29 Human colon cancer cell line. The results of our study exhibit that methanol extracts of *A. marina* have a cytotoxic effect against HT-29 Human colon cancer cell line in a concentration dependent manner, the methanol extracts of *A. marina* and showed a high therapeutic value, IC_{50} was 50.4 µg/mL, seen in methanol concentrates of *A. marina,* individually. Neeta *et al*., (2012) reported that extract of *Padina gymnospora* exhibited cytotoxic activity against Hep G2 cells *in vitro* with a CD50 of 250µg/mL. Similarly, Ekta *et al*., (2011) suggested the amla fruit was used to assess the inhibitory effect of the growth of colorectal cancer and the result holds good guarantee towards the fact that *E. officinalis* is a beneficial fruit to combat human colon cancer.

The morphological studies additionally confirmed that the methanol extract of *A. marina* showed potent cytotoxic effect. It had been discovered that exposure to methanol extracts of *A. marina* for 24 hrs, HT-29 Human colon cancer cell line growth was inhibited as it is clear from MTT assay and direct cell count. The morphological changes in cells clearly specify that cells undergo apoptosis at 24 hrs after incubation with the concentration of methanol extracts of *A. marina* chosen based on the MTT assays. This means that methanol extracts of *A. marina* decrease the potential of individual cells to make a colony and there by acts as an antitumour drug. Recent analysis evidences recommend Indian mangrove plant species have antibacterial and anticancer activities (Arivuselvan *et al*., 2011).

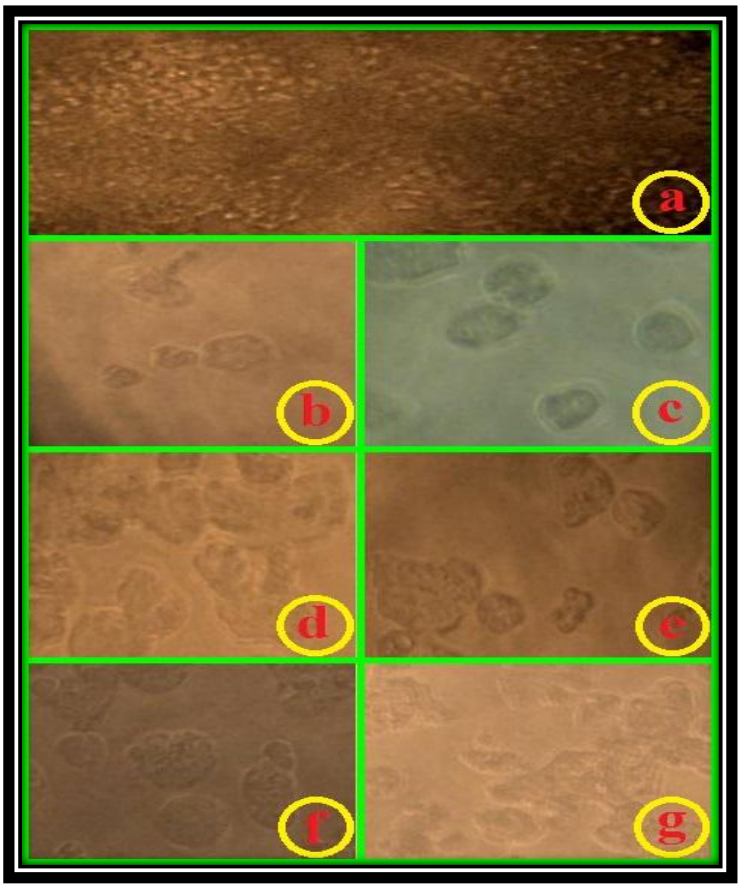

Plate I: Inhibition of cell proliferation by methanol extracts of *A. marina.*

A) Normal HT-29 cell lines Cell line; B) Methanol extract of HT-29 cell line treated with 1000 µg/mL of A. marina; C) Methanol extract of HT- 29 cell line treated with 500 µg/mL of A. marina; D) Methanol extract of HT-29 cell line treated with 250 µg/mL of A. marina; E) Methanol extract of HT-29 cell line treated with 125 mg/mL of A. marina; F) Methanol extract of HT-29 cell line treated with 62.5 µg/mL of A. marina; G) Methanol extract of HT-29 cell line treated with 7.8 µg/mL of A. marina

CONCLUSION

The present study could conclude that the cytotoxic activity of methanol leaf extract of *A. marina* be a good candidate for anticancer activity. It can be a new source as for antitumor medicine and efficiently used as a hepatoprotective agent after successful clinical trials.

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