In Vitro Anti-Proliferative Potential of Leaves of Costus Igneus

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Abstract - In this study the anticancer activity of the plant Costus igneus is evaluated for the foremost time in India, it is full-fledged in gardens, especially in Kerala, where the fresh leaves are eaten raw by diabetic people. Medicinal plants have been used as a source of medicine and in widespread use of herbal remedies and healthcare preparations. Our literature study recommended that the methanol extract of Costus pictus leaves (MECpL) is able to induce apoptosis of Bone cancer cells. The current study is aimed at evaluating the anti-proliferative and apoptotic of Methanolic extract of Costus igneus (MECiL) on in vitro MCF 7 Breast cancer cell line, and also evaluating its safety to customary human lymphocytes. Our current data indicated that (MECiL) reduces tumor size without affecting the Normal cells.

Key words: Costus igneus, MCF 7 Breast cancer cell line , Apoptosis, Anti-proliferative

I. INTRODUCTION

1.1.1 CANCER

Cancer is a class of disease in which a faction of cells display uncontrolled growth, invasion and sometimes metastasis. These three malignant properties of cancers differentiate them from benign tumors, which are self-limited, do not invade or metastasis. Most cancers form a tumor but some, like leukemia, do not. The branch of medicine fretful with the study, diagnosis, treatment and prevention of cancers is oncology. Tumor suppressor genes are then inactivated in cancer cells, resulting in the loss of normal functions in those cells, such as precise DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system.

1.1.2 MCF-7

MCF-7 is a breast cancer cell line secluded in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, This cell line retained quite a few characteristics of differentiated mammary epithelium, counting the ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.

1.1.3 INSULIN PLANT

Insulin plant *(Costus igneus*) is indigenous to Southeast Asia, especially on the Greater Sunda Islands in Indonesia. It is a relatively new entrant to Kerala and India. The plant is characterized by large fleshy looking leaves. The undersides of these large, smooth, dark green leaves have light purple shade. The leaves are spirally arranged around the stem, forming attractive, arching clumps arising from underground rootstocks.

Costus igneus plant grows very swiftly. Propagation of this plant is by stem cutting. It desires sunshine but it also grows in vaguely shady areas. *Costus* does not have a problem with pests and diseases. Outdoor plants might be chewed by caterpillars, and in indoors plants might be pretentious by red spider mite.

1.1.3.1 MEDICINAL USE

In Ayurvedic treatment diabetes patients are advised to chew down the Insulin plants leaves for a month. The patient has to seize two leaves per day in the morning and evening for one week. The leaves must be chewed well before swallowing. After one week the patient should take one leaf each in the morning and evening. This

dosage should be continued for 30 days. Allopathic doctors too urge it and it is found to be effective in bringing blood sugar levels utterly under control. There is also dried and ground powder of the leaves now available in the market. In Traditional Medicine it is also used to Promotes longevity, Treats rash, Reduces fever, Treats asthma , Treats bronchitis and to Eliminates intestinal worms.

1.1.4 LEAF EXTRACTION

The extracts of *Costus igneus* leaves possess anti-tumor activity to curb the growth of many types of cancer counting breast cancer. The crude extract of *Costus igneus* leaves (MECiL) have invitro growth inhibitory effects on numerous human cancers including colon cancer, lung cancer ,breast cancer, hepatoma , and skin cancer. Moreover, our preceding research found that MECiL have antitumor activities both in vitro and in vivo. Modern pharmacological studies have showed that *Costus igneus* leaves has the possessions of antibacterial, anticancer,antidiabetic as well as antioxidative and so on.

II. REVIEW OF LITERATURE

2.1.1 AIM

To cram the anticancer activity of methanolic leaf extract of *Costus igneus* in breast cancer cell line mcf7 by invitro method.

2.1.2 OBJECTIVE

The objectives of this studies are

- To scrutinize the phytochemical components of *Costus igneus*.
- To appraise the anti cancer activity of methanolic extract of *Costus igneus.*
- To evaluate the normal cell line and breast cancer cell line by using *in vitro* method.

2.2 MATERIALS AND METHODS

2.2.1 COLLECTION AND DRYING OF PLANT MATERIALS

- Mature leaves of *Costus igneus* were serene from ABS,Botanical garden,Salem in Tamil Nadu. The leaves of *Costus igneus* were washed meticulously three times with water and once with distilled water. The plant materials were air desiccated and powdered. The powdered samples were hermetically sealed in separate polythene bags until the time of extraction.

2.2.2 PREPARATION OF PLANT EXTRACT

- 10 g of powdered leaves were extracted consecutively with 100 ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting ashen form extracts were stored in a refrigerator at 4°C for future use

2.2.3 PHYTOCHEMICAL ANALYSIS

- Phytochemical tests were done to uncover the presence of the vigorous chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins by the subsequent course of action.

2.2.4 IN VITRO CYTOTOXIC ACTIVITY OF EXTRACT:

- The beyond certain extract was subjected for *In vitro* anti cancer screening using MCF-7 cell line.

2.2.4.1 *Principle*

- This Colorimetric assay is based on the facility of Mitochondria succinate dehydrogenase enzymes in living cells to diminish the yellow water soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into an insoluble, colored formazan product which is measured spectrophotometrically23-24. Since reduction of MTT can only crop up in metabolically active cells, the level of activity is a measure of the viability of the cells.

2.2.4.2 Cell proliferation kit

- MTT (Roche applied sciences, Cat. No. 11465 007 001)

2.2.4.3 Media

- DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310).

2.2.4.4 Glasswares and plastic wares

- 96-well micro titer plate, Tissue culture flasks, Falcon tubes, Reagent bottles

2.2.4.5 Equipments

- Fluorescence inverted microscope (Leica DM IL), Biosafety cabinet classII (Esco), cytotoxic safety cabinet (Esco), CO2 incubator (RS Biotech, mini galaxy A), Sciences; Veer Narmad South Gujarat University, Surat by Dr. Minoobhai Parabia, Dr. Ritesh Vaidh.

2.2.5 Cell line used for cytotoxicity screening

- Breast cancer cell line (MCF-7) was brought from amala cancer institute Thrissur, Deep freezer, ELISA plate reader (Thermo), Micropipettes (Eppendorff), RO water system (Millipore)

2.2.6 Procedure

- The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakhcells/ml using medium containing 10% newborn calf serum. To each well of 96 well microtitre plates, 0.1ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of different test compounds were supplementary to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37oC in 5% CO2 incubator. The supernatant was removed, 50 µl of Propanol was added, and the plates were soothingly shaken to solubilize the formed formazan. The absorbance was deliberate using a microplate reader at a wavelength of 490 nm25. The percentage growth inhibition was calculated using the formula below:
- The percentage growth inhibition was premeditated using following formula,
  - %cell inhibition= 100-{(At-Ab)/(Ac- Ab)}x100
- Where,
- At= Absorbance value of test compound
- Ab= Absorbance value of blank
- Ac=Absorbance value of control
- Data interpretation
- Absorbance values that are subordinate than the control cells indicate a reduction in the tempo of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. Rarely, an increase in proliferation may be offset by cell death; evidence of cell death may be inferred from morphological changes.
  - %cell survival= {(At-Ab)/ (Ac-Ab)} x100
  - % cell inhibition= 100-cell survival

III. RESULTS AND DISCUSSION

3.1.1 Phytochemical Analysis

The Methanolic leaf Extract of *Costus igneus* was found to have the following active chemical components :Alkaloid, Glycosides, Terpenoids and Steroids, Flavonoids, Reducing sugars, Triterpenes, Phenolic compounds, Tannins, Saponins

3.1.2 Evaluation of Cytotoxicity and Cell Viability for given extract on L6 using MTT assay

The given extract does not showed any cytotoxicity not in favor of the normal L6 cell lines (Rat skeletal muscle cell line). It showed IC_{50} Value of 2000 µg/ml. at very high concentration only the extract showed cytotoxicity aligned with the normal cell lines, but it does not detrimental to the normal cell lines.

Table 1: 3.1.2 Evaluation of Cytotoxicity and Cell Viability for given extract on L6 using MTT assay

S.No	Concentration (µg/ml)	% cell viability	%cytotoxicity	IC_{50} value(µg/mg)
1	control	110.62±0.16	0.48±0.25	
2	15	99.52±0.18	2.49±0.59	
3	30	97.51±0.27	4.68±0.35	
4	60	95.35±0.26	15.39±0.75	
5	120	84.61±0.25	22.78±0.65	5000
6	240	77.22±0.68	24.56±0.32	
7	500	75.44±0.95	28.62±0.42	
8	1000	71.32±0.15	32.49±0.25	
9	1500	67.65±0.15	38.29±0.46	
10	2000	62.44±0.25	42.39±0.35	

Graph 1 : Normal cell line – Concentration vs % cell viability

X-axis : Concentration Y-axis : %cell viability

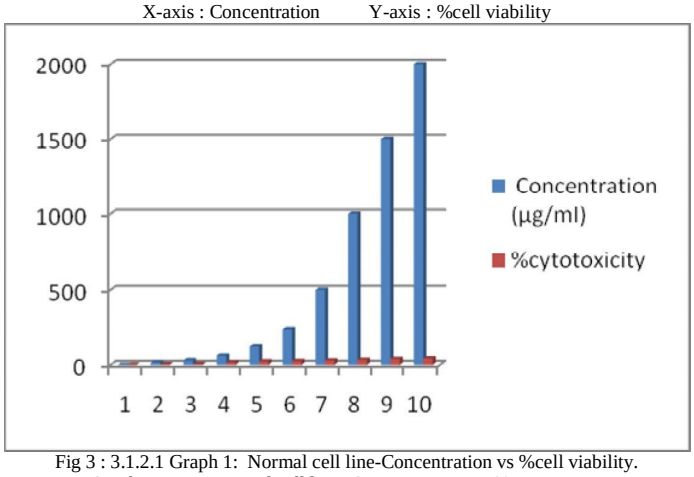

Fig 3 : 3.1.2.1 Graph 1: Normal cell line-Concentration vs %cell viability.

Graph 2 : Normal cell line- Concentration vs %cytotoxicity

X-axis: Concentration Y-axis: %cytotoxicity

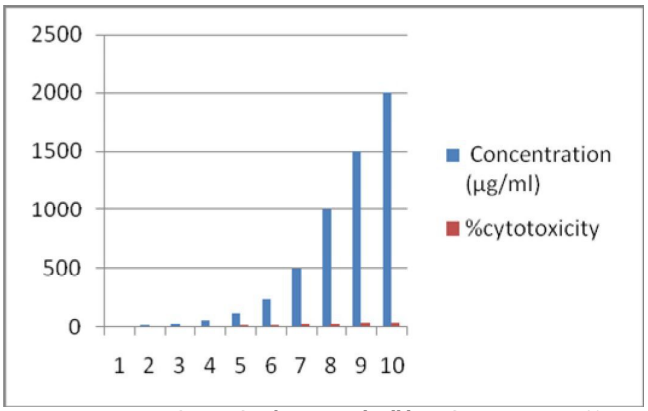

Fig 4: 3.1.2.2 Graph 2: Normal cell line- Concentration vs %cytotoxicity

3.1.3 Evaluation of Cytotoxicity and Cell Viability of given extract On MCF-7 cell lines using MTT assay

The given extract showed potent cytotoxicity against the MCF-7 cell line when compared to the control group. The control faction showed 110.52±0.10 percentage of cell viability. At the dose of 2000 µg/ml the extract showed potent anti cancer activity. At the maximum dose it showed 97.46±0.74 percentage of Cytotoxicity. The IC50 value was found to be 950 µg/ml. in wrapping up the extract has dose reliant cytotoxicity against the MCF-7 cell line.

Table 2: 3.1.3 Evaluation of Cytotoxicity and Cell Viability of given extract On MCF-7 cell lines using MTT assay

s.no	concentration (µg/mg)	% cell viability	% cytotoxicity	IC_{50} value (µg/mg)
1	control	110.52±0.10	0.98±0.25	
2	15	96.52±0.18	6.49±0.59	
3	30	93.51±0.27	10.68±0.35	
4	60	90.35±0.26	19.39±0.75	
5	120	86.61±0.25	28.78±0.65	950
6	240	78.22±0.68	34.56±0.32	

7	500	72.44±0.95	48.62±0.42	
8	1000	61.32±0.15	52.49±0.25	
9	1500	57.65±0.15	68.29±0.46	
10	2000	42.44±0.25	72.39±0.35	

Graph 1: MCF7 Cell lines – Concentration vs %cell viability
X-axis : Concentration Y-axis: % cell viability

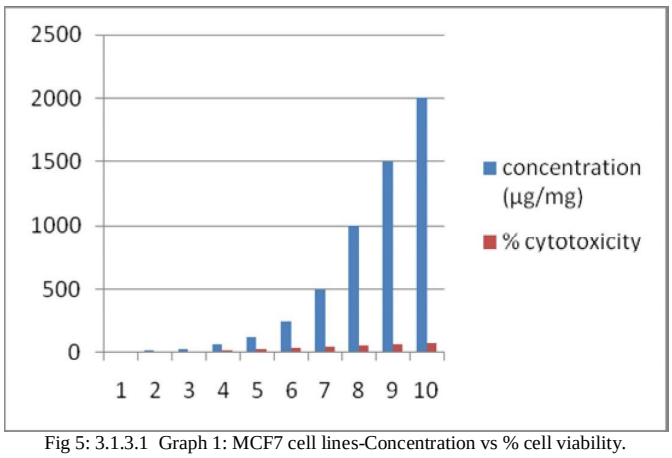

Fig 5: 3.1.3.1 Graph 1: MCF7 cell lines-Concentration vs % cell viability.

Graph 2: MCF7 Cell lines- Concentration vs %cytotoxicity
X-axis: Concentration Y-axis: Cytotoxicity

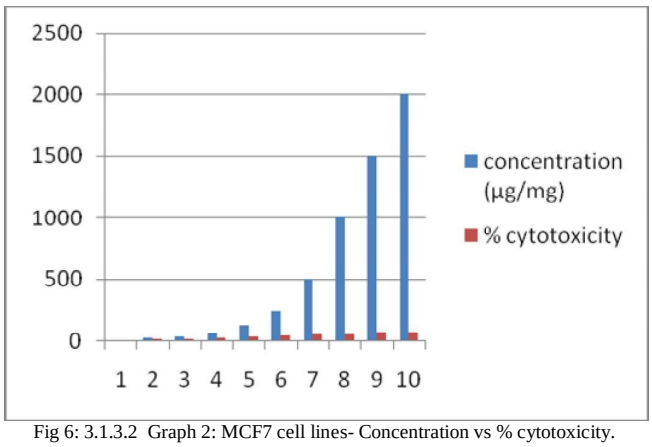

Fig 6: 3.1.3.2 Graph 2: MCF7 cell lines- Concentration vs % cytotoxicity.

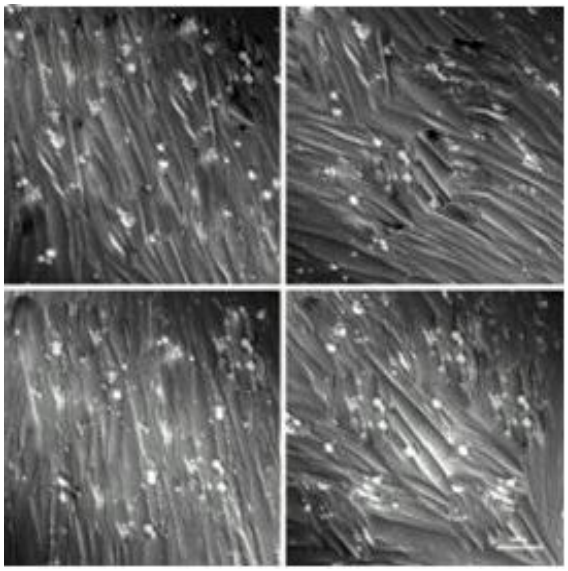

Fig 7: 3.1.2.3 Evaluation of Cytotoxicity and Cell Viability for given extract on L6 using MTT assay
a)Control b)1000µg/ml c)2000µg/ml d)4000µg/ml

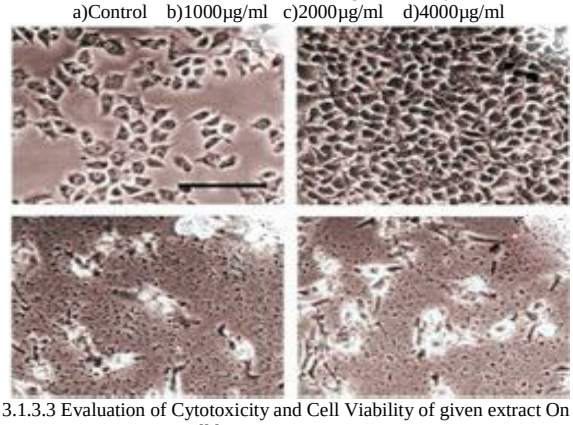

Fig 8: 3.1.3.3 Evaluation of Cytotoxicity and Cell Viability of given extract On MCF-7 cell lines using MTT assay.
a)Control b)1000µg/ml c)2000µg/ml d)4000µg/ml

IV. CONCLUSION

The plant *Costus pictus* was establish to have anti-oxidant and anti-cancer activity .So auxiliary we expanded our studies on anti-cancer activity of *Costus igneus* which belongs to the family Costaceae. Finally the given extract showed potent cytotoxicity against the MCF-7 cell line when compared to the control group. We concluded that the extract showed, it does not impinge on the normal cell lines. The IC50 value was found to be 750 µg/ml. in conclusion the extract has dose dependent cytotoxicity against the MCF-7 cell line.

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