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ANTICANCER ACTIVITY OF *EXCOECARIA AGALLOCHA* LEAF EXTRACT IN CELL LINE MODEL

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ABSTRACT

***Objective**: To identify the anti-cancer activity of Excoecaria agallocha (Euphorbiaceae) is a Mangrove Plant used in folklore medicine for the treatment of several diseases. The present study was aimed at using leaf crude extract as anti cancer agent. The methanol extract was treated with under various concentrations and similarly treated with chloroform extract. The results are clearly indicated the anti cancer cell line activity of E. agallocha. **Method**: To analyze the in vitro studies using the crude extract. The leaf extract of E. agallocha was used for this study. **Result:** The cell viability was maximum at the lower concentration (µg/ml) when compare to higher concentration. At 3.906 (µg/ml) concentrations, the cell viability of 85.32 and 81.96 were found to occur at 1:128 dilution of Methanol and Chloroform extracts respectively. At the same concentration of 31.25 and at the dilution of 1.16, cell viability was observed 65.55 in methanol and 45.55 in chloroform extracts. At the higher concentration the cell viability of 22.35 and 8.12 was recorded in the Methanol and Chloroform extracts.*

KEYWORDS

Mangrove; Exchocaeria; Anticancer; Cell line

1. INTRODUCTION

The Mangrove forest is distributed in two sub divisions like tropical and subtropical coast of the world. In the world, mangrove forests spreads over 30 countries and covers a total area of 99,300 sq Km. The world's largest mangrove forest exist in Indonesia and decreasingly exits in Brazil, Australia, India and Nigeria. The developing country loses acres of Mangrove forest in the last three decades. The mangrove forest exists in Indian coastal region and in Islands of India. As per the data collected during the year 1987, mangrove forest extended to 6740 sq Km .The largest mangrove forest occurs in West Bengal spreading over in 4200 sq Km and followed by Andaman and Nicobar Islands and in small levels mangrove forest exists in Gujarat, Maharashtra, Andhra Pradesh , Goa ,Orissa, and Tamil Nadu. In Tamil Nadu, the mangrove forest got well developed in Pitchavaram area, Muthupet. In the mangrove plants of Pitchavaram area more than 45 species are available. To contain the degradation of mangrove forest, research foundation of M. S. Swaminathan, a non government organization made trenches in some areas to enhance flushing of tidal water. During early period of the December 2004, Tsunami level of exploitation of Pitchavaram areas mangrove far exceeded in sustainable level [1,7].

In ancient times, Natural product of plant has been used for several years to treat various diseases in countries like Egypt, China, India and Greece. They practiced and used to prepare medicine from plants in the ancient time and an impressive number of modern drug have been developed in the developing country. About 120

million people world over are silently suffering from anti cancer disease. The present study was treating the mangrove plant "*Excoecaria agallocha*"extract as anti cancer agent.

2. MATERIALS AND METHOD

- Monolayer culture bottle of Hep 2 cells
- 5ml, 10ml serological pipette
- Minimal essential media (MEM) with 10%, 2% foetal calf serum
- TPVG (Trypsin, PBS, versene, glucose)
- Discarding jar, inverted microscope, dessicator
- Gloves, spirit, cotton, label pad, marker pen

2.1. Maintenance of cell line

Maintenance of cells involves the following operations:

1. Dispersion and Sub culturing (seeding) of cells.
2. Preservation of cells in repository.
3. Revival of cells from repository.

2.3. Sub culturing and maintenance of cell line

1. Bring the medium and TPVG to room temperature for thawing.
2. Observe the tissue culture bottles for growth, cell degeneration, pH and turbidity by seeing in inverted microscope.
3. If the cells become 80% confluent it goes for sub culturing process
4. Wipe the mouth of the bottle with cotton soaked in spirit to remove the adhering particles.
5. Discard the growth medium in a discarding jar keep distance between the jar and the flask.
6. Then add 4 – 5 ml of MEM without FCS and gently rinsed with tilting. The dead cells and excess FCS are washed out and then discard the medium.
7. TPVG was added over the cells. And incubate at 37º C for 5 minutes for dis aggregation. The cells become individual and it's present as suspension.
8. Add 5ml of 10% MEM with FCS by using serological pipette.
9. Gently give passaging by using serological pipette. If any clumps are present then repeat the process.
10. After passaging split the cells into 1:2 and 1:3 ratios for cytotoxic studies for plating method.

2.4. Seeding of cells

After homogenize take one ml of suspension and pour in to 24 well plates. In each well add 1ml of the suspension and kept in a desiccators in 5% CO_2 atmosphere. After 2 days incubation observes the cells in inverted microscope, if the cells became 80% confluent [3].

2.5. Performance of drug cytotoxic assay

Cytotoxic is the toxicity or damage caused to the cells on addition of drug. After the addition of the drug, cell viability is estimated by staining techniques, where by cells are treated with Trypan blue. Trypan blue is excluded by live cells, but stains dead cells blue. The results are confirmed by additional metabolic intervention experiments such as MTT assays.

2.6. Materials Required

1. Monolayer cultures in log phase.
2. Drug extract (different concentrations)
3. MEM without FCS
4. 0.45µ filter
5. 5mL sterile storage vial
6. Tissue paper, Marker pen, Spirit, Cotton and Gloves
7. 1 mL, 2 mL pipettes, Micropipette and tips
8. Discarding jar with 1 % Hypochlorite solution

2.7. Stock drug concentration

10 mg of drug is dissolved in 10 ml of serum free MEM giving a concentration of 1mg / 1 ml. The stock is prepared fresh and filtered through 0.45 □ filter before each assay. Working concentrations of drug ranging from 1mg/ml to 7.8125 mg/ml are prepared as follow

2.8. Preparation of working stock of 1 mg /ml

To 4.5 ml MEM add 0.5 ml of stock to give a working concentration of 1mg/mL. Drug concentration can be prepared from the working stock in MEM without FCS. Prepare required volume of test sample for each concentration.

1. 48hr monolayer culture of Hep2 cells at a concentration of one lakh /well seeded in 24 well titer plates.
2. The plates were microscopically examined for confluent monolayer, turbidity and toxicity if the cells become confluent.
3. The growth medium (MEM) was removed using micropipette. Care was taken so that the tip of the pipette did not touch the cell sheet.
4. The monolayer of cells was washed twice with MEM without FCS to remove the dead cells and excess FCS.
5. To the washed cell sheet, add 1ml of medium (without FCS) containing defined concentration of the drug in respective wells.
6. Each dilution of the drug ranges from 1:1 to 1:64 and they were added to the respective wells of the 24 well titer plates.
7. To the cell control wells add 1ml MEM (w/o) FCS.
8. The plates were incubated at 37ºc in 5% CO_2 environment and observed for cytotoxicity using inverted microscope.

2.9. MTT assay

MTT assay is called as (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide. MTT assay was first proposed by Mossman in 1982.[8]

Procedure

- After incubation, remove the medium from the wells carefully for MTT assay.
- In each well wash with MEM (w/o) FCS for 2 – 3 times. And add 200µl of MTT conc of (5mg/ml).
- And incubate for 6-7hrs in 5% CO2 incubator for Cytotoxicity.
- After incubation add 1ml of DMSO in each well and mix by pipette and leave for 45sec
- If any viable cells present formazan crystals after adding solublizing reagent (DMSO) it shows the purple color formation.
- The suspension is transferred in to the cuvette of spectrophotometer and an O.D values is read at 595nm by taking DMSO as a blank.
  - Cell viability (%) = × 100

3. RESULT AND DISCUSSION

In the present investigation, the anti-cancer activity of *Exchocaria agollocha* was screened from 3.906 to 1000 µg/ml of concentrations with the dilution leads to 1:1 to 1:128 using Methanol and Chloroform extracts. The anti-cancer activity of *Exchocaria agollocha* , the cell viability was maximum at the lower concentration (µg/ml) when compare to higher concentration.. At 3.906 (µg/ml) concentrations, the cell viability of 85.32 and 81.96 were found to occur at 1:128 dilution of Methanol and Chloroform extracts respectively. At the same concentration of 31.25 and at the dilution of 1.16, cell viability was observed 65.55 in methanol and 45.55 in chloroform extracts. At the higher concentration the cell viability of 22.35 and 8.12 was recorded in the Methanol and Chloroform extracts. In general, the cell viability was more in Methanol when compare to Chloroform extracts at higher concentration in particular.

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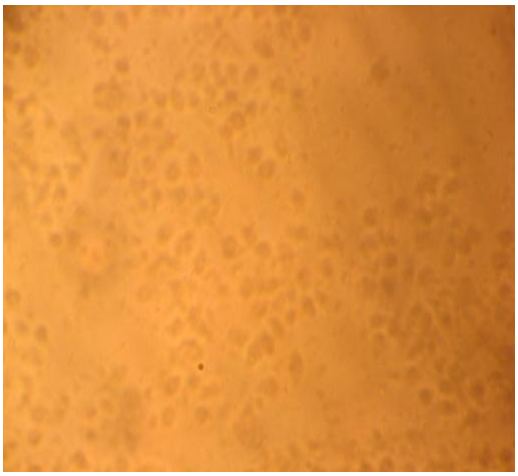
Tabe-1 Cytotoxicity effect of *Excoecaria agallocha* (Methanol Extract)

S. no.	Concentration (µg/ml)	Dilutions	Cell viability
1	1000	Neat	22.35
2	500	1:1	29.63
3	250	1:2	48.56
4	125	1:4	54.32
5	62.5	1:8	61.05
6	31.25	1:16	65.55
7	15.625	1:32	75.23
8	7.8125	1:64	78.90
9	3.906	1:128	85.32
10	Cell control	-	100

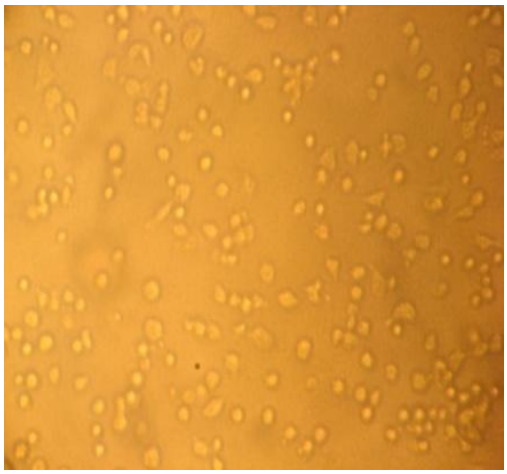
Table- 2 Cytotoxicity effect of *Excoecaria agallocha* (Chloroform Extract)

S. no.	Concentration (µg/ml)	Dilutions	Cell viability
1	1000	Neat	8.12
2	500	1:1	14.22
3	250	1:2	19.63
4	125	1:4	21.85
5	62.5	1:8	29.63
6	31.25	1:16	45.55
7	15.625	1:32	53.62
8	7.8125	1:64	75.28
9	3.906	1:128	81.96
10	Cell control	-	100

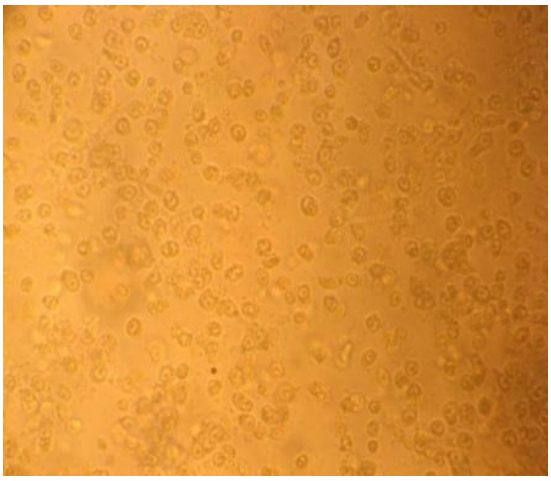
Figure: 1 Cytotoxicity effect of *Excoecaria agallocha* (Methanol Extract)



Toxicity 1 (1000µg/ml)

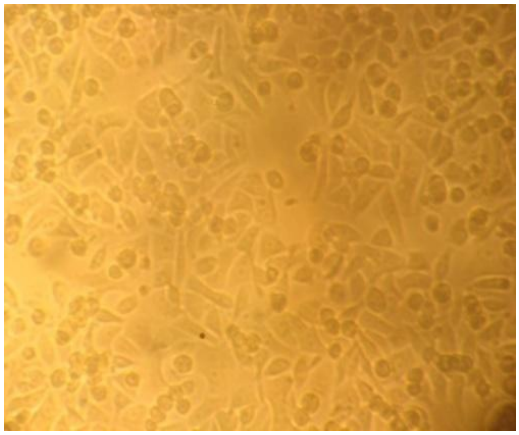


Toxicity 2 (62.5µg/ml)

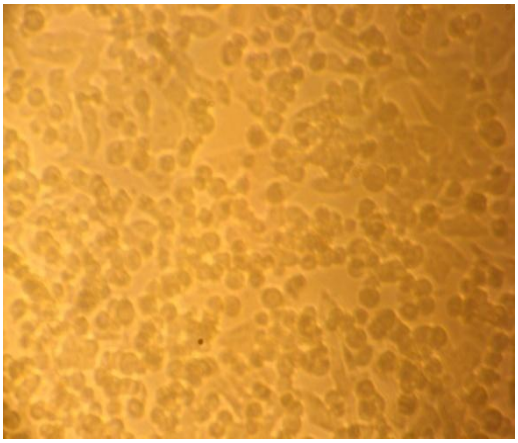


(31.25µg/ml)

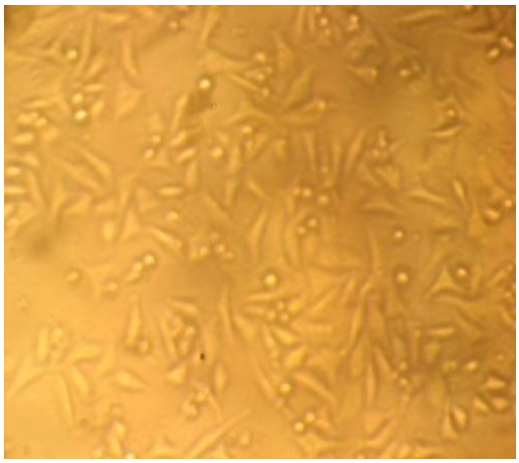
Figure: 2 Cytotoxicity effect of *Excoecaria agallocha* (Chloroform Extract)



Toxicity 1 (1000µg/ml)



Toxicity 2 (500µg/ml)



Toxicity 2 (62.5µg/ml)

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