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INVESTIGATION ON *IN VITRO* CYTOTOXIC ACTIVITY OF A SELECTED WILD CUCURBITACEAE PLANT *CORALLOCARPUS EPIGAEUS* AGAINST CANCER

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ABSTRACT: Medicinal plants have been investigated for possible anti-cancer effects. *Corallocarpus epigaeus* (Roth & Willd.) cl. is an ethnobotanically important wild climber that belongs to the family Cucurbitaceae. The aim of the present study is to examine the cytotoxic activity of *Corallocarpus epigaeus* (Roth & Willd.) cl., on two tumor cell lines. The methanolic root extract of the plant *Corallocarpus epigaeus* (Roth & Willd.) cl. was subjected to cytotoxic activity study against two human cancer cell lines (MCF7, human breast adenocarcinoma and HT-29, human colon carcinoma) using the MTT assay. The concentration of methanolic root extract yields the IC₅₀ value of 183.33±0.7 µg/ml and <62.5µg/ml respectively for HT-29 and MCF-7 cell lines by MTT assay. The results showed that the root extract of *Corallocarpus epigaeus* has potential anti-cancer activity against MCF-7 and HT-29 human cancer cell lines.

INTRODUCTION: Medicinal plants are gifts of nature to cure limitless number of diseases among human beings. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value¹. The medicinal value of plants lies in some chemical substances usually secondary metabolites, which produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolics². Over the past few years, cancer has remained a major cause of death and the number of individuals affected with cancer is continuing to expand. Hence, a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets³.

The rich and diverse plant sources of India are likely to provide effective anticancer agents. One of the best approaches in the search of anticancer agents from plant sources is the selection of plants based on ethnomedical leads⁴.


Corallocarpus epigaeus is commonly known as Akasgaddah in Hindi and Akashagaruda in Tamil, and is distributed in tropical and temperate regions of India, Ceylon, Deccan and South Maratha country. The rhizome is especially useful in Syphilitic cases, old venereal complaints, chronic dysentery, cancer, anti-inflammatory and snake bite⁵.

In this paper, we report the cytotoxic activity of the methanolic root extract of *Corallocarpus epigaeus* against MCF-7 and HT-29 cell lines.

MATERIALS AND METHODS:

Plant material:

For the present study a medicinally important plant *Corallocarpus epigaeus* belonging to the family Cucurbitaceae has been selected. Root of the *C. epigaeus* was collected from different sites of

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Mysore and Chittoor districts of India in the month of September and December 2013.

Processing of plant material

The roots of *Corallocarpus epigaeus* were cleaned and shade dried. The dried material was powdered using mechanical method and resulting powder is sieved with sieve of 0.3mm aperture size and stored in the airtight container.

Extraction of plant material:

The plant materials were extracted with Methanol using soxhlet extraction apparatus continuously for 16 hours. For extraction, the dried plant material was used. Initially 50gms of material was packed in filter paper and loaded into the thimble of soxhlet apparatus. 300 ml of ethanol was poured into the flask pot and the whole apparatus was set. The soxhlet extraction was performed for 12- 16 hours until the collected solvent in siphon tube appears to be clear. Later the extracted solvent was evaporated under reduced pressure to get semi solid extract⁶.

Chemicals:

3-(4,5-dimethyl thiazol-2-yl)-5-diphenyl tetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) Trypsin Phosphate Versene Glucose (TPVG) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

Cell Lines and Culture Medium:⁷

HT-29 (Adenocarcinoma) and MCF-7 (Breast carcinoma) cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100IU/ml), streptomycin (100µg/ml) and amphotericin B (5µg/ml) in an humidified atmosphere of 5% CO₂ at 37° C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25cm² culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of Test Solutions:

For Cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1mg/ml concentration and sterilized and filtered. Serial two fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cytotoxicity by MTT Assay:

Principle: The ability of the cells to survive a toxic insult has been the basis of most Cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The principle involved is the cleavage of tetrazolium yellow coloured salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. Hence it is a colorimetric assay. The number of cells was found to be proportional to the extent of formazan production by the cells used. Therefore this assay is used for assessing cell viability⁷.

Procedure:

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10⁵ cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off. The monolayer was washed once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO₂ atmosphere, and microscopic examination was carried out and observations were noted every 24 h interval. After 72 h, the drug solutions in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in 5% CO₂ atmosphere.

The supernatant was removed and 100µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader

at a wavelength of 540nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC₅₀) values is generated from the dose-response curves for each cell line. The % growth inhibition was recorded using the following formula:

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

RESULTS AND DISCUSSION: MTT assay was performed which is the technique utilized for survival determination measurements and the concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. The effect of the samples on the proliferation of HT-29 and MCF-7 was expressed as the % cell viability. Results are tabulated in **Table1 & 2** and graphically represented in **Fig.1 & 2**. The percentage of growth inhibition of *Corallocaarpus epigaeus* was found to be increasing with increasing concentration of test compounds. From the graphs the concentration of methanolic extract of HT-29 and MCF-7 yields the value of IC₅₀ as 183.33±0.7µg/ml (78% of inhibition concentration) and <62.5µg/ml (88% of inhibition concentration) respectively (**Table1 & Fig1**) and (**Table2 & Fig 2**). So the extracts have a potent action against human colorectal adenocarcinoma and human breast cancer.

Our phytochemical screening revealed the presence of terpenoids, flavonoids, phenols, glycosides, phytosterols, tannins and alkaloids in the methanolic extracts of *Corallocaarpus epigaeus*, which could be responsible for this activity. The phytochemical constituents such as flavonoids and terpenoids are the major components that are responsible for the potential cytotoxic activity⁸. The flavonoids have reported for their cytotoxic activity due to presence of phenolic groups⁹. Terpenes have found to inhibit the growth of cancerous cells, decreases tumor size, decrease cholesterol level and also decrease micro-organism concentration¹⁰.

Our further plan is to isolate and evaluate these active principles and elucidate exact mechanism of action.

TABLE 1: CYTOTOXIC PROPERTY OF CORALLOCAARPUS EPIGAEUS ON HT-29 CELL LINE

Name of the test sample	Test Conc (µg/ml)	% Cytotoxicity	IC ₅₀ (µg/ml)
<i>Corallocaarpus epigaeus</i>	1000	78.49±1.8	183.33±0.7
	500	66.67±0.6	
	250	53.83±0.4	
	125	47.27±0.2	
	62.5	38.02±0.6	

All data is expressed as mean value ± standard deviation (SD)

TABLE 2: CYTOTOXIC PROPERTY OF CORALLOCAARPUS EPIGAEUS ON MCF-7 CELL LINE

Name of the test sample	Test Conc (µg/ml)	% Cytotoxicity	IC ₅₀ (µg/ml)
<i>Corallocaarpus epigaeus</i>	1000	88.62±0.3	<62.5
	500	86.23±0.4	
	250	74.49±0.3	
	125	71.48±0.6	
	62.5	68.98±0.4	

All data is expressed as mean value ± standard deviation (SD)

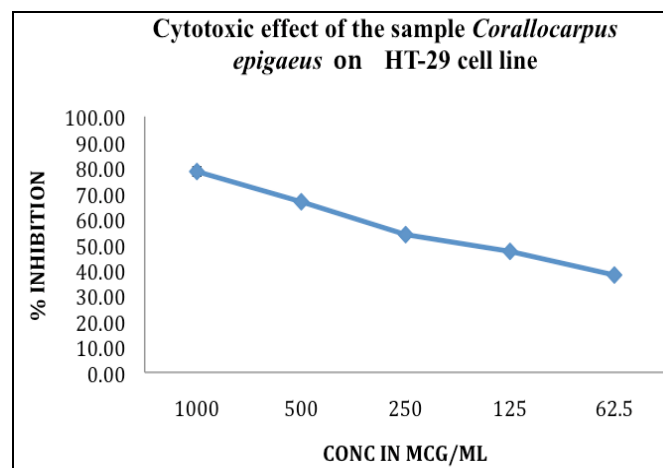


FIG 1: GRAPHICAL REPRESENTATION OF CYTOTOXIC EFFECT OF CORALLOCAARPUS EPIGAEUS ON HT-29 CELL LINE

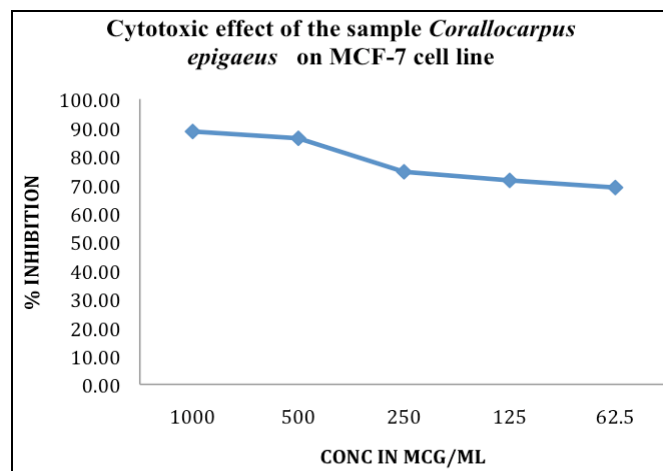


FIG 2: GRAPHICAL REPRESENTATION OF CYTOTOXIC EFFECT OF CORALLOCAARPUS EPIGAEUS ON MCF-7 CELL LINE

CONCLUSION: In this study, the root extract of *Corallocarpus epigaeus* showed cytotoxic activity against HT-29 and MCF-7 cell lines with the percentage mortality increased with an increase in concentration. However, further investigations are to be carried out to isolate and characterize the specific bioactive principles.

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CONFLICT OF INTEREST: Authors declare no conflict of interest.

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