

ANTIPROLIFERATIVE POTENTIAL OF ERYTHRINA INDICA LEAF AQUEOUS EXTRACT AGAINST HUMAN BREAST CANCER CELLS

Running title: Effect of Erythrina Indica leaf extract on cancer cells.

ABSTRACT

Introduction: Breast cancer is cancer that develops in breast cells. The cancer forms in either the lobules or the ducts of the breast. Cancer can also occur in the fatty tissue or the fibrous connective tissue within your breast.

MATERIALS AND METHODS:

In vitro cytotoxicity ~~assay~~assays ~~The~~the effect of Erythrina indica on cell viability was measured by MTT assay following the method by Mosmann. Briefly, the cells (1×10^5 cells per ml) were seeded in a 96 well microtiter ~~plate~~(plate (100 μ l per well) with replications. Treatment was conducted for 24 with different concentrations (0, 5, 7.5, 15, 30, 45, 60, 75 and 100 μ g) of Erythrina indica. After incubation, 20 μ l of 5 mg/ml MTT stock solution was added to each well and

Results: The crude extract obtained from *Erythrina indica* greatly inhibits the cancer cell proliferation in different time and dose manner, respectively. At 100 μ g/ml of *E. indica* for 24 hrs incubation disrupting the cancer cells undergoing apoptosis mediated cell death. Which was confirmed by morphology evaluation using phase contrast microscope.

Conclusion: The results suggest that the *E. indica* extract shows the potent antiproliferative activity against breast cancer cells, and it might be a novel new anticancer drug for cancer therapy.

Key words: Anticancer, Sea grass, Breast cancer cell line, Erythrina indica, innovative technique, eco friendly.

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INTRODUCTION:

Erythrina indica is a medium-sized, spiny, deciduous tree usually developing to 6-nine m (once in a while 28 m) tall and 60 cm dbh.(1,2) Young stems and branches are thickly armed with stout conical spines up to eight mm lengthy, which fall off after 2-four years; rarely, some spines persist and are retained with the corky bark. Bark easy and inexperienced while young, exfoliating in papery flakes, turning into thick, corky and deeply fissured with age(3,4). Leaves trifoliolate, alternate, shiny emerald -inexperienced, on lengthy petioles 6-15 cm, rachis 5-30 cm lengthy, prickly; leaflets easy, shiny, broader than lengthy, eight-20 with the aid of using 5-15 cm, ovate to acuminate with an obtusely pointed end(5)(6). Leaf petiole and rachis are spiny. Flowers in shiny red to scarlet erect terminal racemes 15-20 cm lengthy; stamens barely sticking out from the flower.(7,8) Fruit a cylindrical torulose pod, inexperienced, turning black and wrinkly as they ripen, thin-walled and constricted across the seeds. There are 1-eight easy, oblong, darkish pink to nearly black seeds consistent with pod.

Breast cancer is one of the most common styles of cancers internationally and yet, its pathophysiology is poorly understood. Single-mobileular electrophysiological research has furnished proof that membrane depolarization is implicated withinside the proliferation and metastasis of breast most cancers(9). However, metastatic breast most cancers cells are exceedingly dynamic microscopic structures with complexities past a single-molecular level. There is a pressing need for electrophysiological research and technology able to decipher the intercellular signaling pathways and networks that manage proliferation and metastasis, especially at a populace level. Hence, we gift for the primary time non-invasive in vitro electric recordings of strongly metastatic MDA-MB-231 and weakly/non-metastatic MCF-7 breast most cancers lines.(10) *Erythrina indica* incorporates glycosides and phenol compounds which can be capable of behaving as antifungal and anticancer, and even incorporates steroid compounds

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which act as antibacterial and anticancer(11). It has been said that crude extract from *Erythrina indica* had excessive phenolic content material. Moreover, suggested the cytotoxicity of crude extract from *E. indica*. The maximum phenolic content material is at the leaves part. One that may be located in tidal coastal regions in Indonesia is *Erythrina indica*. Since different sorts had been suggested to include anticancer bioactive compounds, any other studies to decide the capability of *Erythrina indica* as a supply of anticancer bioactive compounds ought to additionally be conducted.(1) The purpose of these studies was to decide the capability of *E. indica* leaves extract as an anticancer agent.

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MATERIALS AND METHODS

CHEMICALS:

SDMEM medium, 0.25% Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, MTT from Sigma Chemicals Co., St. Louis, USA. fetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO were from Himedia, Sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid and methanol were purchased from Sisco Research Laboratories (SRL) India.

PREPARATION OF EXTRACT:

Erythrina indica herbal powder commercially purchased IMPCOPS - Chennai (Indian Medical Practitioners Co-operative Pharmacy and Stores Limited). 200g of sample was soaked in double distilled water and kept for 3 days at 37°C temperature in continuous intervals of shaking the flask. Further, the solution was filtered and placed in a rotary vacuum evaporator to concentrate fine filtered samples and leftover solvent was evaporated to dryness in a hot air oven. 2 grammes of material was obtained and immediately sorted at 4°C, for further experiments.

The required quantity of the herbal extract was weighed and dissolved in DMSO with concentration of 1mg/ml as a stock solution. This solution was subsequently diluted to a series of concentrations ranging from 50 to 300 µg/ ~~ml~~ for ml for cell viability assay.

CYTOTOXIC ASSAY:

The cytotoxic effect of *Erythrina indica* on MCF-7, were measured with MTT (3-(4, 5-dimethyl thiazol-2 yl)-2, 5-diphenyl tetrazolium bromide) assay by Alam (12) Cells were seeded in 96-well plates at the density of $5 \times 10^3/100\mu\text{l}$ and treated with different concentrations (0, 20, 40, 80, 100, 200 and 300 μg) of *S. virginianum* for 24hrs. After 24hrs incubation, 20 μl of 5 mg/ml MTT stock solution was added to each well and incubated for 4 h at 37 °C. The obtained formazan crystals were solubilized with DMSO and the absorbance was measured at 570 nm using a microplate reader (SpectraMax M5, Molecular Devices, USA). Cell viability (%) has been shown as a ratio of absorbance (A570) in treated cells to absorbance (A570) in control cells (0.1 % DMSO). The IC_{50} was calculated as the concentration of sample needed to reduce 50 % of the absorbance in comparison to the DMSO-treated control. Percent cell viability was calculated following the equation:

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

$$\text{inhibition (\%)} = 100 - \text{cell viability (\%)}$$

STATISTICAL ANALYSIS:

All data obtained were analyzed and computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. In all tests, the level of statistical significance was set at $p < 0.05$

RESULT AND ~~DISCUSSION~~ DISCUSSION:

During the recent decades, a number of anticancer compounds derived from natural sources, such as vincristine, vinblastine, taxol, and bleomycin, have been identified and are now

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extensively utilised to treat various kinds of cancer. Many researchers ~~reports~~report, phenolic compounds have anti-carcinogenic action and alter the bioenergetic processes of MCF-7 breast cancer cells. Edible plant material includes a large number of micro-constituents, all of which are active in biological systems (13). The present study aims to identify the antiproliferative effect of *Erythrina indica* for breast cancer therapy. The results showed potential cytotoxic effects by MTT assay and morphometric analysis using phase contrast microscopy in Breast cancer cell lines are presented in figure 1 & 2, demonstrating the bioactivity of *E. indica* extract in MCF-7 cells. *E. indica* at a concentration of 250 $\mu\text{g ml}^{-1}$ hindered the growth of MCF-7 cells. (14–18),(19),(20),(21),(22),(23),(24),(16,25,26),(27–31)–,(32),(33)

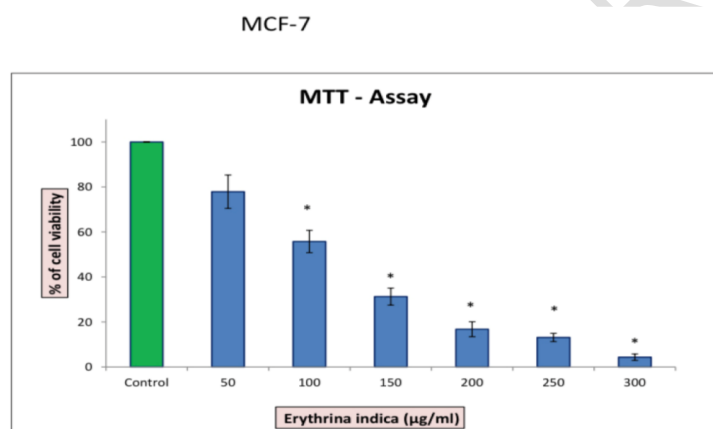


Fig.1 Represent the cytotoxic effect of *Erythrina indica* against breast cancer cells for 24hrs. The X axis represents different concentrations of *E. indica* and Y- axis represents the percentage of cell viability. Green colour denotes control and blue color represents the different levels of *E. indica* in $\mu\text{g/ml}$. Data are shown as means $\pm$ SD (n = 3) compared with the control-blank group, $p < 0.001$. At 100 $\mu\text{g/ml}$ of *E. indica* only 50 % of the cells were viable, which shows the good cytotoxic activity of the herb.

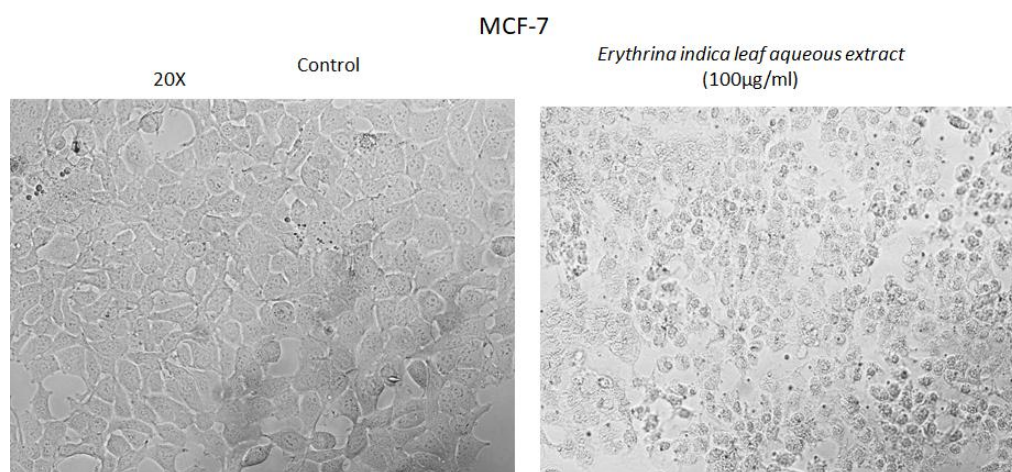


Fig.2. *E. indica*'s antiproliferative activity was evaluated by morphological changes with control and treated (100µg/ml) breast cancer cells. Cellular characteristics were disrupted upon herbal treated cells with membrane blebbing, nuclear condensation, fragmentation were observed under phase contrast microscopy 20x magnification,

Breast cell lethality level by semi polar extract was higher than polar extract, but not significantly different with cancer medicine doxorubicin. The extract of *C. serrulata* reveals the presence of phytochemicals that are biologically active. According to the chromatogram obtained by GCMS ethanol extract of *Erythrina indica* consists of palmitic acid, myristic acid, and pentadecanoic acid as a major component. They may be produced by the plant defense itself from stress as secondary metabolites (34). These cytoprotectants proved to possess pharmacological activity in a similar way as synthetic drugs. The palmitic acid reported possessing anticancer activity, antimicrobial, and nematocidal activity (35). The palmitic acid increases the number of probiotic bacteria in the gut; thus, they are involved in the development of the intestine. It is required in the biosynthesis of lung lecithin, which is related to fetal maturation as well as it has been reported that presence of palmitic acid in the Nigerian meal can

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partly be related to the low incidence of respiratory disease. Palmitic acid reported inhibiting human hepatoma cell growth in a dose dependent and time-dependent manner. Thus, they possess anticancer. Since other types were reported to contain anticancer bioactive compounds, another research to determine the potential of *Erythrina indica* as a source of anticancer bioactive compounds should also be conducted (36).(37) (38) (39) (40) (40,41) (42) (43) (44) (45) (46) (47) (48) (49) (50) (51) (52) (53)

CONCLUSION:

This study aimed to reveal the antiproliferative effect of *Erythrina indica* aqueous extract against breast cancer cells. The results show that the herbal extract has greatly inhibited cell proliferation at 100 µg/ml (IC₅₀ Value) concentration for 24hrs. Further, morphological changes like membrane blebbing, nuclear condensation and fragmentation have been observed upon *E. indica* treatment showing antitumor activity against cancer cells(30) (54) (55) (56) (57) (58) (59) (60) (61) (62) (63) (64) (65) (66) (67). These promising results suggest that *Erythrina indica* as a promising source of natural ingredients, and pave the way to develop novel anticancer drugs for treating cancer, including breast cancer.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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