Anti-proliferative Potential of *Erythrina indica* Leaf Aqueous Extract against Human Breast Cancer Cells

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i62A35619

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/77807

Received 20 October 2021
Accepted 26 December 2021
Published 28 December 2021

ABSTRACT

**Introduction:** Breast cancer is a type of cancer that arises in the cells of the breast. Breast cancer can develop in either the lobules or the ducts. Breast cancer might develop in the fatty tissue or fibrous connective tissue.

**Materials and Methods:** The effect of *Erythrina indica* (*E.indica*) on cell viability was measured by MTT assay. Briefly, the cells (1 x 10^5 cells/ml) were seeded in a 96 well microtiter plate with replications. Treatment was carried out for 24 with different concentrations (50-300 μg) of *E.indica*. The percentage of cell viability was calculated and plotted in graph. The cell morphological changes of *E. indica* leaf aqueous extract treated cells were observed under inverted phase contrast microscopy.

**Results:** The crude extract obtained from *E.indica* leaf greatly inhibits the cancer cell proliferation in dose dependent manner. We observed IC_{50} at 100 μg/ml of *E. indica* leaf aqueous extract treated for 24 hrs in breast cancer cells and also it induces apoptosis, which was confirmed by cell morphological changes evaluated using phase contrast microscope.
Conclusion: The results suggest that the *E. indica* leaf aqueous extract shows the potent anti-proliferative activity against breast cancer cells, and it might be a novel new anticancer drug for cancer therapy.

**Keywords:** Anticancer; sea grass; breast cancer cell line; Erythrina indica; cytotoxicity.

### 1. INTRODUCTION

*Erythrina indica* is a spiky, medium-sized deciduous tree that grows to be quite tall [1,2]. Young stems and branches are heavily armed with robust conical spines up to 8 mm long, which fall off after two to four years; occasionally, some spines remain and are kept with the corky bark [3,4]. Leaves trifoliate, 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 frequent tumours worldwide, although the pathophysiology of the disease is poorly understood. Single-cellular electrophysiological studies have shown that membrane depolarization is linked to breast cancer proliferation and metastasis [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]. *E. indica* incorporates glycosides and phenol compounds which can be capable of behaving as antifungal and anticancer, and even incorporates steroid compounds which act as antibacterial and anticancer [11]. It has been said that crude extract from *E. 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 *E. indica*. Since different sorts had been suggested to include anticancer bioactive compounds, any other studies to decide the capability of *E. 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.

### 2. MATERIALS AND METHODS

#### 2.1 Chemicals

Sigma Chemicals Co., St. Louis, USA provided the DMEM medium, 0.25 percent Trypsin-EDTA solution, sodium bicarbonate solution, bovine serum albumin (BSA), low melting agarose, and MTT. Himedia provided the foetal bovine serum (FBS) and antibiotic/antimycotic solution, DMSO. Sisco Research Laboratories (SRL) in India supplied sodium phosphate monobasic and dibasic, sodium chloride, sodium hydroxide, sodium carbonate, hydrochloric acid, and methanol.

#### 2.2 Preparation of Extract

*E. 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 cell viability assay.
2.3 Cytotoxic Assay

The cytotoxic effect of *E. indica leaf aqueous extract* 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 5x10^3/100μl and treated with different concentrations (50, 100, 150, 200, 250 and 300 μg) of *E. indica leaf aqueous extract* for 24hrs. After 24hrs incubation, 20 μl of 5 mg/ml MTT stock solution was added to each well and incubated for 4hrs 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:

\[
\text{Cell viability} = \left( \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100
\]

2.4 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.

3. RESULTS AND 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 extensively utilized to treat various kinds of cancer. Many researchers 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 [13-33]. The present study aims to identify the anti-proliferative effect of *E. indica leaf aqueous extract* 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 Figs. 1 & 2, demonstrating the bioactivity of *E. indica leaf aqueous extract* in MCF-7 cells. *E. indica leaf aqueous extract* at a concentration of 250 μg ml\(^{-1}\) hindered the growth of MCF-7 cells.

![MTT Assay](image)

Fig. 1. Represent the cytotoxic effect of *E. indica leaf aqueous extract* against breast cancer cells for 24hrs. The X axis represents different concentrations of *E. indica leaf aqueous extract* and Y-axis represents the percentage of cell viability. Green colour denotes control and blue colour represents the different concentration of *E. indica leaf aqueous extract* 50-300 μg/ml. Data are shown as means ± SD (n = 3) compared with the control-blank group, p < 0.001. At 100 μg/ml of *E. indica leaf aqueous extract* only 50% of the cells were viable, which shows the good cytotoxic activity of the herb.
Breast cell lethality level by semi polar extract was higher than polar extract, but not significantly different with cancer medicine doxorubicin. The presence of physiologically active phytoconstituents in the extract of C. serrulata is revealed. According to the chromatogram obtained by GCMS, the primary components of the ethanol extract of E. indica are palmitic acid, myristic acid, and pentadecanoic acid. They may be produced by the plant defense itself from stress as secondary metabolites [34]. These cytoprotectants demonstrated pharmacological activity comparable to synthetic drugs. Palmitic acid has been reported to have anticancer, antimicrobial, and nematicide activity [35]. Palmitic acid boosts the quantity of probiotic bacteria in the gut, which helps with intestinal growth. It is required for the production of lung lecithin, which is linked to foetal maturation, and it has been proposed that the presence of palmitic acid in Nigerian meals may contribute to the country's low respiratory disease rate. Palmitic acid has been shown to inhibit human hepatoma cell growth in a dose- and time-dependent manner. Thus, they possess anticancer. Because other types have been reported to contain anticancer bioactive compounds, more research should be conducted to determine the potential of E. indica as a source of anticancer bioactive compounds [36-53].

4. CONCLUSION

This study aimed to reveal the anti-proliferative effect of E. indica leaf aqueous extract against breast cancer cells. The results show that the E. indica leaf aqueous extract has greatly inhibited cell proliferation at 100 μg/ml (IC₅₀ value) concentrations for 24hrs. Further, morphological changes like membrane blebbing, nuclear condensation and fragmentation have been observed upon E. indica leaf aqueous extract treatment showing antitumor activity against cancer cells. These promising results suggest that E. indica as a promising source of natural ingredients, and pave the way to develop novel anticancer drugs for treating cancer, including breast cancer.

DISCLAIMER

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.

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).
ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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