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RESEARCH ARTICLE

# Antitumor Activity of Erythrina Indica Leaf Extract in Human Lung Cancer Cells

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## ABSTRACT

Over the few decades, lung cancer has appeared to be a global threat as globalisation improved worldwide. There are various risk factors which keep increasing, making the incidence and pathogenesis of lung cancer more complex. This study focuses on erythrina Indica lam. extract which has been believed to have various pharmaceutical effects based on their numerous phytochemical constituents. The aim of the study is to determine the antitumor activity of erythrina Indica lam. Leaf extract in human lung cancer cells. The effect of Erythrina Indica lam. From the cell viability experiment, the erythrina indica lam extract showed 50% cell growth inhibitory effect at 100µg/ ml (IC50) against the human lung cancer cells. Thus, there exists a significant antitumor activity of erythrina Indica lam. Extract, precisely at 100µg/ml, shows maximum potential inhibiting 50% of human lung cancer cells. Further studies should be done focusing the antitumor activity of the erythrina leaf extract, with any possibilities of side effects caused to make it as a natural alternative medication.

KEYWORDS:
Antitumor; Erythrina Indica Lung Cancer; Leaf Cancer; Leaf Extract; MTT Assay; Innovative Technology.

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## INTRODUCTION

Lung cancer has changed from a rare disease to a global threat due to the new urbanisation, industrialism growing up in all parts of the world. The etiological factors have increased in complexions which has brought the world's attention in controlling the rate of lung cancer. (1). It was at a rate of 1.6million as of 2016 and is expected to be up to 3million by 2035. It is also believed to be increased two fold with leading men's incidence closely followed by females. The most increase is expected to be in African and western Mediterranean regions. The incidence of lung cancer in developed countries is mostly due to ageing and in developing countries is due to tobacco usage (2,3). Lung cancer is the leading cancer in men and second leading in women. The incidence rates keep increasing and decreasing in different demographic and geographic situations.The incidence keeps increasing in low and middle socioeconomic countries (4). The etiologies and risk factors also keep changing for different demographic and geographic locations. There are genetic factors like genes, polymorphism, others like tobacco, alcohol, diet , occupational hazards, rediations, etc. which keeps gradually increasing in today's world (4,5).

Understanding the importance of lung cancer, they are broadly classified as small cell and non small cell lung cancer. The same way the treatments are broadly divided as chemotherapy, radiotherapy, surgery, targeted therapy. (6-9)Development of biomarkers, novel therapies, prognostic markers, etc. helps treat early small cell lung cancer. Non small cell lung cancer can be treated with surgery, primary

radiotherapy followed up by chemotherapy(10) (11) (12) (13). The late stages involve various lines of intense chemotherapy (2,3,14). There are developments of new targeted therapies like EGFR mutations, ALK rearrangements, etc. which seems to be promising and immunotherapies by checkpoint inhibitions, tumour suppressions, etc. these treatments help meaningful options and reduce normal cell destructions as liabilities (15). The side effects of the chemotherapy, the major treatment option of this carcinoma has been studied which reveals various diverse side effects in which common ones are high grade fever, vomiting, diarrhoea, anemia, etc. (16,17).

Erythrina Indica lam. is a plant with many phytoconstituents which constitutes various therapeutic and pharmaceutical characteristics. Studies were done to concentrate on its effects. It's anti ulcer activity was studied in comparison with ranitidine like standard drug and was proved there exist significant reduction in ulcer scores in dose dependent manner (18). The diuretic activity was also supervised in comparison with standard drugs and proved to increase urine volume and ions concentration (19). There are also several effects of these extracts which help treat and maintain various ill effects and conditions. It is universally accepted that sometimes treatments cause more trauma, both physically and emotionally than the illness itself which has brought an era where natural extracts were concentrated and its effect studied to replace them with synthetic and harmful treatments. (6–9,20)

Our team has extensive knowledge and research experience that has translate into high quality publications(21–25),(26),(27),(28),(29),(30),(31),((23,32,33),(34–38),(39),(40). This present study focuses on evaluating the antitumor activity of erythrina Indica lam. Leaf extract in human lung cancer cells.

## MATERIALS AND METHODS

### Chemicals

DMEM medium( Dulbeccos Minimal Essential Media), 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 Herbal Extract

Leaf powder of erythrina indica lam. was commercially obtained from IMPCOPS (Chennai, India) was used for conducting the present study. 150g of the erythrina Indica lam. Extract was taken and immersed in 500ml of 95% ethanol and stored for 3 consecutive days, undisturbed. The solution prepared was then filtered using crude filter paper, whatman paper. The filtered extract was concentrated using a rotary evaporator and 3g of the material was obtained from a hot air oven overnight dry. The samples were immediately stored at 4 ℃ for further analysis.

### Cell Culture Reagents Dmem

The DMEM available contains 7.5% sodium bicarbonate solution. 500ml of DMEM, 5ml of penicillin/streptomycin solution and 0.5ml of amphotericin B solution was added. Then this medium was then sterilised by filtering (0.22µ) inside the hood. The medium finally was transferred into sterile containers and stored at 4°C.

Growth Medium (DMEM with 10% FBS)

10ml of FBS was diluted to about 100ml using DMEM. It was then transferred and stored in a sterile container in cool and sterile condition.

Phosphate Buffered Saline (PBS; pH 7.4)

0.63 g of sodium phosphate monobasic ($NaH_2PO_4$), 0.17 g of sodium phosphate dibasic ($Na_2HPO_4$) and 4.5 g of sodium chloride (NaCl) was added to 500 ml of double autoclaved milliQ water. Their pH was adjusted to 7.4 by adding adequate 1 N HCl and 1 N NaOH, sterile filtered (0.22 µ) and then transferred to a sterile container.

Trypsin-EDTA versus Glucose Solution

Trypsin purchased as 1 x with EDTA (0.5% trypsin, 5.3 mM EDTA sodium salt).

0.89% Physiological Saline and 890 mg of sodium chloride were added to 100 ml of double autoclaved milliQ water.

### Cell Line

Human lung adenocarcinoma-A549 cell line was procured from the National Centre for Cell Science (NCCS, Pune), India. The cells are grown in T25 culture flasks which contain DMEM medium supplemented with 10% FBS. On reaching 90% confluence, cells were expanded using 0.25% trypsin- EDTA solution for further experiments.

### Testing Viability of Cells

The viability of A549 cells was assessed by trypan blue exclusion test Perry et al., (1997).

Reagents used were:

1. Trypan blue solution: (0.5% trypan blue (w/v) in physiological saline).

Procedure done:

100L of trypan blue solution were mixed with 10µl of cells present in the medium and incubated at 37℃ for 5minutes. The stained cells were washed thrice with a 1X phosphate buffer saline then resuspended in 100µl of 1x PBS, from which 10µl was kept in a haemocytometer, viewed under a microscope. The unstained cells present show the viable cells while the damaged cells get stained. The count of stained and unstained cells and percentage of viable cells were calculated using this formula:

$$\% \text{ of viability} = \frac{\text{No. of unstained cells}}{\text{Total no. of cells}} \times 100$$

The viability of the cells is found to be between 90-95%.

### MTT ASSAY

The anticancer activity of ethanolic extract of erythrina indica lam. At different concentrations was assessed via MTT assay(3-(4,5-dimethyl thiazol-2 yl)-2,5-diphenyl tetrazolium bromide) assay to determine the antitumor activity by the method explained in (6–8). Initially, the cells were placed on 96-well plates at a density of 5x103/100µl. The cells were incubated for 24 hours at different ethanolic extract concentrations like 50, 100,150,200,250,300 µg/ml. The serum free extract free well negative controls used were wells with extract After the incubation for 24hours at 37oC after which 10microlitre of MTT reagent was added and incubated again for 4hours at 37oC under dark conditions. 100µl of Sorensen's glycine buffer (0.1M glycine, 0.1M NaCl, pH 10.5,0.1N NaOH) was then added which turns the formazan crystals soluble. The absorbance value was measured at 570 nm subsequently. The same experiment was done thrice and each concentration was tested in triples. The formula for calculating cell viability:

$$\text{Cell viability}(\%) = \frac{\text{absorbance of sample X 100}}{\text{Absorbance of control}} \text{ @ OD of 570 nm}$$

inhibition(%)= 100- cell viability(%).

### Statistical Analysis

All data obtained were analyzed using Student's-t-test using MS-Excel, expressed as mean ± SD for six animals in each group. The results are then computed statistically (SPSS/10 Software Package; SPSS Inc., Chicago, IL, USA) via one-way ANOVA. Post-hoc testing was then performed for inter comparisons using the LSD. In all these tests, the level of statistical significance was given at $p<0.05$.

## RESULTS

In the present study, the anti tumor activity of the ethanolic extract of erythrina Indica lam was studied by assessing the percentage of cell viability at different extract concentration via MTT assay. It was found that at 100micrograms/ ml extract concentration, the cell viability was 50% affirming this concentration as therapeutic index value.

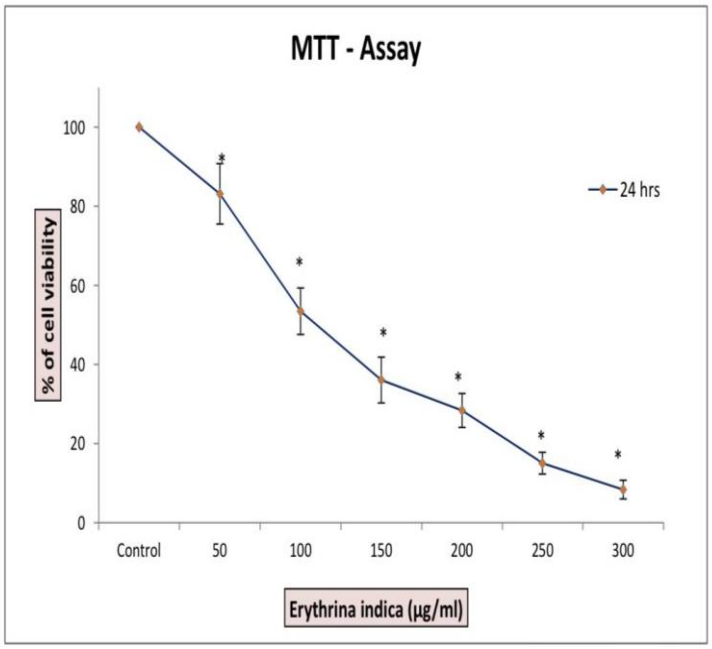


**Fig.1:** The antitumor activity of erythrina Indica leaf extract via MTT assay at a regular interval of 24 hours (Fig.1). The X axis represents the different concentrations of erythrina Indica leaf extract in (µg/ ml) while the Y axis represents the percentage of cell viability (in numbers). There is a decrease in cell viability on increase in extract concentration in a non regular manner. Data are shown as means ± SD (n = 3). * compared with the control-blank group, $p < 0.001$.

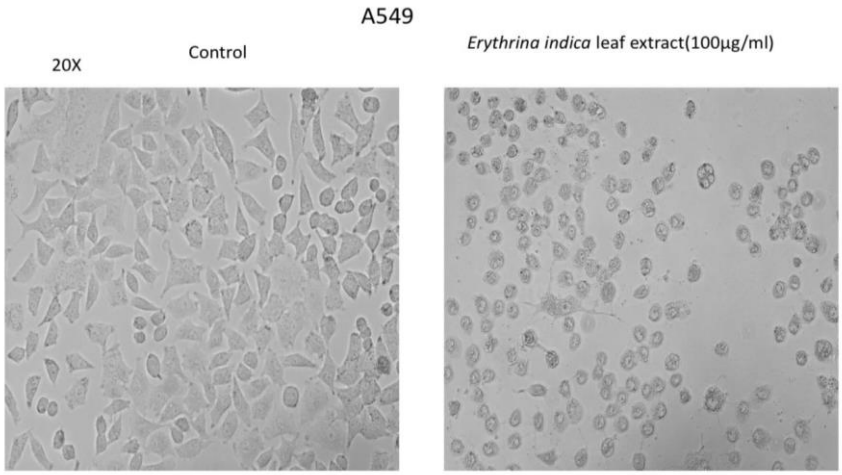


**Fig.2:** Morphological changes upon erythrina Indica leaf extract (100 µg/ml) treatment compared with control cells (Fig.2) were observed under phase contrast microscope with 20x magnification.

## DISCUSSION

Erythrina indica lam. extract was believed to have anti cancer activity as one of their pharmaceutical effects due to their phytochemical constituents. The present study was done to determine the antitumor activity against human lung cancer cells. The anti-cancer potential was determined through MTT assay at different extract concentrations( µg/ml) at a 24hours interval against human lung cancer cells viability. In figure1, the antitumor activity of erythrina indica leaf extracts after different concentrations against cell viability percentage at 24hours interval, where it was found that there was an irregular manner of decrease in cell viability on increase in the concentration of the leaf extract. In figure 2, the comparison was made between the antitumor activity of control and erythrina indica leaf extract treatment against A549 human lung cancer cells where the morphological changes were recorded using phase contrast microscope at 20x magnification. It was found that at 100 µg/ml, the extract has been reported to inhibit 50% of human lung cancer cells. Thus proving that erythrina indica lam. extract having significant antitumor potential against cancer cell lines(41) (42) (43) (44) (44,45) (46) (47) (48) (49) (50) (51) (52) (53) (54) (55) (56) (57)

Human lung cancer has changed from being a rare disease to a leading cause of cancer related death in both women and men worldwide. This cancer has been broadly classified as small cell lung cancer and non-small cell lung cancer, pathologically which is critical to be differentiated biologically and on molecular basis. (6–9,20,58)On focusing the molecular biology of lung cancer, there are various categories involved like tumor suppressor associated with cancer syndromes, chromosome 3p deletion, cell lines, epigenetic gene inactivation, autocrine growth factors, proteomic analysis, epidermal growth factor receptor tyrosine kinase inhibitors (19,59). Apoptosis is a simple yet important biological process which involves morphological changes like nuclear fragmentation, chromatin condensation and biochemical changes like activation of caspases, proteolytic changes, membrane changes of phagocytic cells.(60) There are previous studies proving the effect of apoptosis on malignant cells and their potential role in carcinogenesis by reduction or malfunction. Reduced activation of caspases, imbalance between pro-apoptotic and anti-apoptotic proteins and impaired cell signalling. Knowing the importance of apoptosis in cancer progression, the new targeted therapies focus on apoptosis inorder to prevent the occurrence and progression of cancer to get precisely and avoid side effects (6)

There are various treatment options available for lung cancer and its progression. There are traditional methods like surgery, radiotherapy, chemotherapy. But due to their side effects like hair loss, chemo brain, lymphedema, dental and bone problem, weight loss, blood in urine and stools, there is an urge for minimally invasive and much less harmful treatment for treating cancer. Nanotechnologies have improved and have been playing a huge role in diagnosis, prevention and treatment of cancer.(61) There are various magnetic nanoparticles used like co-polymer, gold magnetic, iron oxide, silver magnetic, etc. which seems to be suitable as they are biodegradable, safe, enhances bioavailability, etc. but there also exists disadvantages like renal failure, nonspecific targeting, toxicity due to ROS production. These statements help us understand the requirement of new treatment options with less brutal side effects and tumor resistance (6,7).

There are various previous studies done to study the effect of different types of extracts of various natural herbs at different concentrations against various cancer cell lines. A study was done to determine the anti tumor activity of erythrina variegata L. against liver cancer cells via MTT assay. On analysing proliferation rates, inhibition rates, growth curves, the antitumor effect was studied and has been found to have significant potential to increase in its concentration. (2)The present study focuses on the antitumor activity of erythrina indica lam. Leaf ethanolic extract which has been proved to have significant potential against human lung cancer cells which gives a start to study the plant along with other parts, extract from different medium, performing various tests to add assurance towards the present studies findings and move towards replacing the synthetic drugs with hugely impacting side effects into natural plant extracts with significant potential on comparison with the contro(37) (62) (63) (64) (65)

(66) (67) (68) (69) (70) (71) (72) (73) (74) (75)

The limitation of the study is that only 2 studies are done to assess the antitumor activity and is an in vitro study as the side effects of the trial cant be noticed and studied. Further studies should be focused on studying the effects of various natural product extracts to replace them for the synthetic drugs which cause more brutal side effects and trauma than the disease itself.

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## CONFLICT OF INTEREST

The author declares that there was no conflict of interest in the present study.

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## CONCLUSION

Our study concluded that the ethanolic extract of erythrina indica lam has significant antitumor potential was determined by MTT assay against human lung cancer cells. Further studies must be done to replace these natural extracts from synthetic drugs as treatment for various cancer and other diseases.

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