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# *IN VITRO* ANTICANCER ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *Acampe praemorsa* (Roxb.)

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## ABSTRACT

Orchidaceae is one of the largest families among angiosperms. Recently there has been tremendous progress in medicinal plant research however orchids have not been exploited fully for their medicinal application. The main objective of the present investigation is to evaluate the *in vitro* cytotoxic activity of the crude ethanolic leaf extracts of *Acampe praemorsa* (Roxb.) A549 (lung carcinoma) cell lines using MTT assay. The IC50 value was determined as 14.63µg/ml. The concentration of ethanolic leaf extracts of *Acampe praemorsa* (Roxb.) of 6.25µg/ml inhibit the growth of 85.9%, 1.25 µg/ml inhibit the growth of 73.9%, 25µg/ml inhibit the growth of 57.9%, 50µg/ml inhibit the growth of 49.0% and 100µg/ml inhibit the growth of 34.3% The obtained results showed antitumor and cytotoxic effect of the extract against A549 (lung carcinoma) cell line support the ethnomedical use of *Acampe praemorsa* (Roxb.).

**KEYWORDS:** *Acampe praemorsa*, cytotoxic, epiphyte, lung carcinoma, MTT Assay.

## INTRODUCTION

*Acampe praemorsa* (Roxb.) is an epiphytic orchid with stout stem, 16 cm long, covered by sheathing bases of leaves with persisting old inflorescence axis and long stout aerial roots among the leaves. Leaves alternate distichous, large and coriaceous, 8-17cm oblong, channeled, unequally deeply cleft at apex.[1] Plants have been used for medical purposes since

the beginning of human history are the basis of modern medicine. Most chemotherapeutic drugs for cancer treatment are molecules identified and isolated from plants or their synthetic derivatives. *Acampe praemorsa* (Roxb) is used in traditional medicine for the treatment of bone fractures, antityphoid properties.[2,3] and anti-inflammatory activity.[4] Orchids are important in reducing fever, serving as anti-impotence aids, increasing the white blood cell count, treating fatigue, headache and most importantly functioning as anti-cancer agents.[5] Orchidaceae is a highly evolved and widely distributed monocotyledonous family with large number of terrestrial, saprophytic and epiphytic species. Orchids are well known for their medicinal value a total of 365 plants, including several orchids, are listed in the earliest known Chinese Materia Medica.[6] Cancer is one of the leading causes of death worldwide a total of 1,660,290 new cancer cases and 580,350 deaths from cancer were predicted in United States.[7]

## MATERIALS AND METHODS

*Acampe praemorsa.* (Roxb.) was collected from the hill stations of Pechipari at an altitude of about 500 to 1500 feet of Kanyakumari District, the southernmost end of the peninsular India lies between 8º-20º north of the equator and between 70º-85º in longitude**.** The collected leaves (10g) were washed to remove the adhering dirt's then it was cut into small pieces and shade dried.

### Determination of *In-vitro* anticancer activity by MTT assay

A549 (lung carcinoma) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (Gibco, Invitrogen). The cell lines were cultured in 25 $cm^2$ tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% $CO_2$ incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

### Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^4$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% $CO_2$ incubator.

**Preparation of plant extracts and compound stock**

1 mg of each plant extract or compound was added to 1ml of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

**Cytotoxicity Evaluation**

After 24 hours the growth medium was removed and plant extract was added to a final concentration of 50µg/ml to induce toxicity and freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% $CO_2$ incubator.

**Cytotoxicity Assay by Direct Microscopic Observation**

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

**Cytotoxicity Assay by MTT Method**

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% $CO_2$ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm.[8]

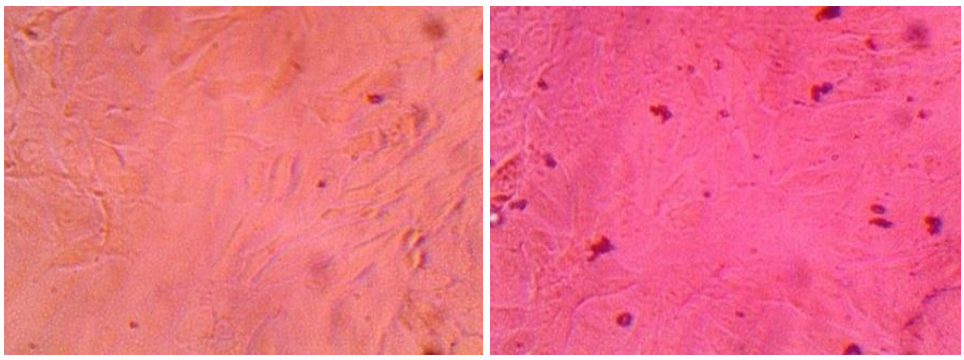
The percentage of growth inhibition was calculated using the formula

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x 100}}{\text{Mean OD of control group}}$$

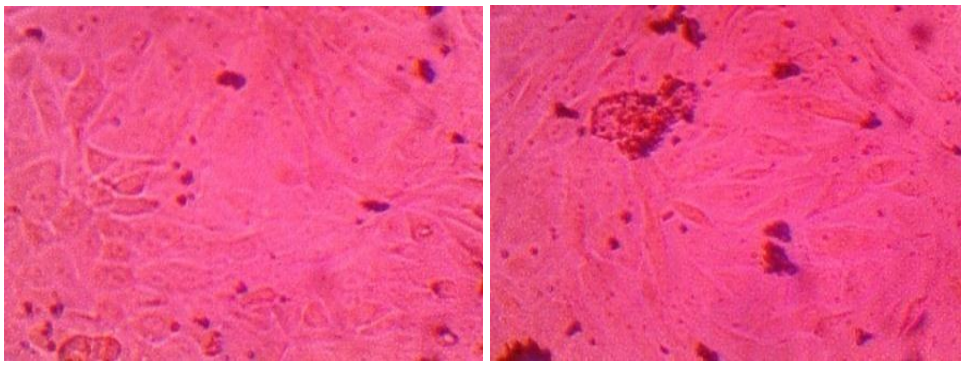
**Table1. Percentage of cell growth inhibition of ethanolic leaf extract of *Acampe praemorsa* (Roxb) on A549 cell lines.**

| Sample Concentration (µg/ml) | Average OD at 540nm | Percentage Viability |
|---|---|---|
| **Control** | **1.7274** | **100** |
| 6.25 | 1.4841 | 85.9 |
| 12.5 | 1.2774 | 73.9 |
| 25 | 1.0013 | 57.9 |
| 50 | 0.8473 | 49.0 |
| 100 | 0.5928 | 34.3 |

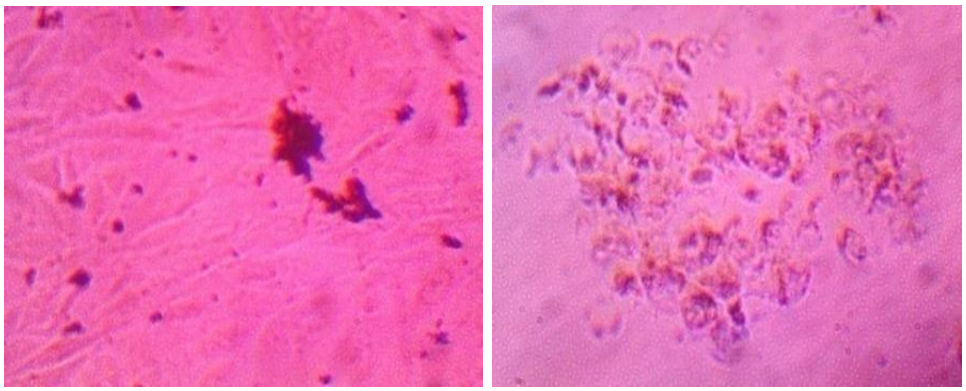
**Plate: 1 Cytotoxicity of *Acampe praemorsa* (Roxb) ethanolic leaf extract on A549 cell lines**



**Control** **6.25mg/ml**



**12.5mg/ml** **25mg/ml**



**50mg/ml** **100mg/ml**

## RESULTS AND DISCUSSIONS

### *In vitro* anticancer activity

MTT assay is used to determine the antiproliferative activity. The ethanolic leaf extract of *Acampe praemorsa.* (Roxb.) is preliminarily screened and the percentage of viability treated on A549 lung carcinoma cells and control cells was assessed in a dose-dependent manner (Plate: 1). The concentration of 6.25µg/ml inhibit the growth of 85.9%, 1.25 µg/ml inhibit the growth of 73.9%, 25µg/ml inhibit the growth of 57.9%, 50µg/ml inhibit the growth of 49.0% and 100µg/ml inhibit the growth of 34.3% (Table1). The IC50 value is determined as 14.63µg/ml using Graph Pad Prism software. The increased concentration showed less cell growth inhibition 34.3% at 100 µg/ml and similar results were obtained in the earlier investigation.[9] The obtained result showed that the ethanolic extract of *Acampe praemorsa* (Roxb.) leaf showed moderate anticancer activity.

The earlier studies regarding active profile of three most active extracts tested on HCT-118 showed the survival fraction (% of control) of *Ceropegia candelabrum* of 10µg/ml at 0.5869nm with a viability of 93.02%, 50g/ml at 0.4548nm showed a viability of 72.08% and 100µg/ml at 0.4200nm showed a viability of 48.40%. The ethanolic extract of *Ceropegia spiralis* effectively reduced the cell viability.10µg of *C. spiralis* showed 47.15% of cell death which can be considered as significant effect[10].

## CONCLUSION

The orchid components still requires proper study with full experimental trials which will lead to its acceptance as medical recommendations though the cytotoxic effect of the ethanolic leaf extract of *Acampe praemorsa* (Roxb.) is assessed to be less effective. In conclusion the highly potent activities exhibited by *Acampe praemorsa* (Roxb.) leaf even at low concentration (100µg/ml) suggest that these compounds could be developed further as anticancer drugs.

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