

ANTICANCER ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *Clerodendrum inerme* AGAINST LUNG ADENOCARCINOMA EPITHELIAL CELL LINE

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Article Info

Received 23/12/2015

Revised 16/01/2016

Accepted 19/02/2016

Key words:- Medicinal Plant, Anticancer activity, A549 and Cytotoxicity.

ABSTRACT

Recent research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of new compounds. The present research article was aimed to study the cytotoxic activity of ethanolic leaves extracts of *C.inerme* plant by *in vitro* cytotoxic assays like MTT against lung adenocarcinoma epithelial cell line A549 which exhibited anticancer activity with I_{c50} values of 15.6 $\mu\text{g/ml}$ concentration. This study creates the awareness of this plant leaves which is having potential activities against A549 which will be a boon to the mankind systematic way.

INTRODUCTION

Medicinal plants are potential source of raw materials used for manufacture of drugs and perfumery products. More than a quarter of all the medicines used in the world today contain natural compounds derived from plants that often serve lead molecules whose activities can be enhanced by manipulation through combinations with chemicals and by synthetic chemistry that can be exploited in the field of new drugs research and development [1]. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment [2].

The World Health Organization (WHO) estimates that almost 75% of world's population has therapeutic experience with herbal drugs. Cancer is one of the most dangerous disease in humans and presently there is a considerable scientific discovery of new anti cancer agents from natural products [3]. Lung cancer is a disease characterized by uncontrolled cell growth in tissues of the lung and is the commonest form of death worldwide.

Medicinal plants are considered to be the exclusive source of life saving drugs for majority of world's population. The potential of using the natural products as anti cancer drugs was recognized in 1950's by U.S Natural Cancer Institute (NCI) and major contributions have taken for the discovery of naturally occurring anti cancer drugs [4].

Clerodendrum inerme occurs predominantly in the mangrove region of coastal India, exposed to wide range of salinity fluctuations. They have an advantage over other species that lack strategies to deal with salt in the environment and thus are excellent competitors in saline environments [5]. This plant is rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids etc., which have been found to have anticancer properties *in vitro* [6]. Most of the currently used anticancer drugs are highly toxic, expensive, and resistance mechanisms pose a significant problem [7-8]. There is a continuing need to identify new drug candidates that are more effective, widely available and less toxic. Plants extracts are an important source of potentially useful compounds for the development of new anticancer drugs. Here we investigated ethanolic leaf extract of *Clerodendrum inerme* against cytotoxic activities in cancer-derived cell lines.

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MATERIALS AND METHODS

Preparation of leaves extract

The selected plant *Clerodendrum inerme* were collected from Thanjavur rural station, Tamilnadu India. Collected plant samples were washed by distilled water (DW) to remove undesirable materials and excess of water was drained off. The leaves were separated from each other and they were sliced into small pieces. The sliced leaves were shade dried for few days. The shade dried leaves were powdered separately by grinding machine and about 5g of powdered leaf of *C. inerme* was taken into clean flat-bottomed glass container and soaked with 200 ml of 95% ethanol. The containers with its contents were sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper. The obtained filtrates were rotary evaporated and this dried content was used for anticancer study.

Test chemicals

Growth medium with 10 % FCS, Trypsin (0.25 % + EDTA, 1 mM, in PBS), MTT: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-dephenyltetrazolium bromide, 5 mg/ml, filter sterilized, dissolved in PBS, Dimethyl sulfoxide (DMSO) and Dulbecco's modified eagle's medium (DMEM) were purchased from Himedia (India).

Anticancer activity

Cytotoxic potential of *C. inerme* ethanol leaf extract was tested on human lung adenocarcinoma epithelial cell line A549 was purchased from the National centre for cell science (NCCS), Pune, India. The growth medium, Minimum Essential Medium (MEM) was

removed after incubation using micropipette. The monolayer of cells was washed twice with MEM without Foetal calf serum (FCS) to remove the dead cells and excess FCS. To the washed cell sheet, 1ml of medium (without FCS) containing defined concentration of the leaf extract in respective wells was added. Each dilution of the compound ranges from 1:1 to 1:128 and they were added to the respective wells of 24 well titre plate. The control well was prepared with cells containing 1ml MEM without any added test sample. The titer plate was incubated at 37°C in 5% CO₂ environment and observed for cytotoxicity using inverted microscope as well as MTT assay.

After incubation, the medium from the wells was carefully removed for MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) assay. Each well was washed with MEM (without) FCS for 2-3 times and 200µl of MTT (5mg/ml) was added. The plate was incubated for 6 hrs in 5% CO₂ incubator for cytotoxicity. After incubation 1ml of DMSO was added in each well, mixed and left for 45sec. Viable cells present in the medium formed crystals which were dissolved by adding solubilizing reagent Dimethyl sulphoxide (DMSO) that resulted in formation of purple colour. The absorbance of the suspension was measured spectrophotometrically at 540nm by taking DMSO as a blank [9]. The percentage growth inhibition was calculated using following formula,

$$\% \text{ cell inhibition} = 100 - \left\{ \frac{(At - Ab)}{(Ac - Ab)} \right\} \times 100$$

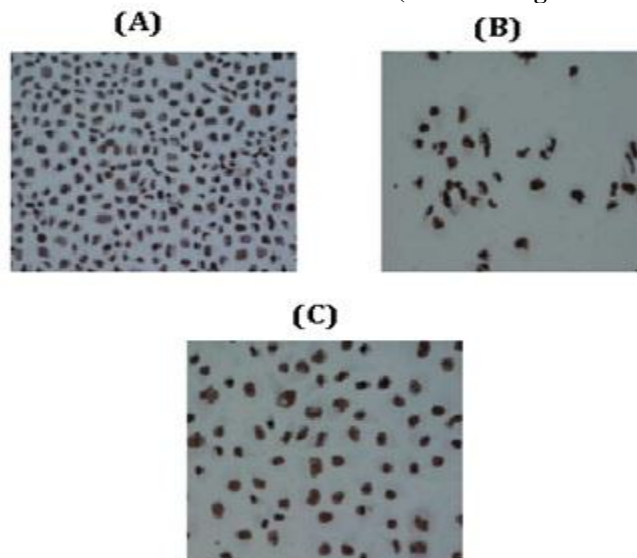
Where,

At= Absorbance value of test compound

Ab= Absorbance value of blank

Ac=Absorbance value of control

Figure 1. Effect of Ethanolic leaves extract of *C. inerme* on A549 (Human lung adenocarcinoma epithelial) cell line



A- Control B- Ethanolic leaves extract of *C. inerme* (500µg/ml) C- Ethanolic leaves extract of *C. inerme* (15.6µg/ml)

Table 1. Cytotoxic activity of ethanolic leaves extract of *Clerodendrum inerme* on A549 (Human lung adenocarcinoma epithelial) cell line

S. No.	Test sample concentration (µg/ml)	0.01% ethanol dilutions	Cell inhibition/ cytotoxicity (%)
			A549
1	500	1:1	90.4
2	250	1:2	70.04
3	125	1:4	60.10
4	62.5	1:8	57.56
5	31.3	1:16	53.83
6	15.6	1:32	50.19
7	7.8	1:64	48.09
8	3.9	1:128	47.06
9	Cell control	Neat	0.06

RESULTS AND DISCUSSION

Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value usually secondary metabolites that produce a definite physiological action on the human body [10]. Cancer is a disease that has always been a major threat and has been characterized by proliferation of abnormal cells. Though Chemotherapy is now being used as a standard treatment method, search for anticancer agents from natural products has increased. In order to annotate the mechanism of prevention of cancer and to identify new anticancer activities a number of plants have been explored [11].

In the present study the cytotoxic effect of the *C. inerme* leaf extract on A 549 cell lines was determined at different concentrations, ranging from 3.9 to 500µg/ml respectively (Table 1). The leaf extract exhibited significant inhibition of cell proliferation of A 549 cell lines in direct proportion to its concentration taken (3.9 to 500µg/ml). In the highest concentration (500µg/ml) the cell inhibition was in the mean value of 90.4%. IC_{50} value of A549 cell lines was 15.6 µg/ml of extract concentration (Fig.1) and predicted lowest cell inhibition in the lowest concentration tested (3.9 µg/ml).The extract *C. inerme* inhibited the growth of A549 cancer cells. In the same way as the above stated results, the water extract of *Rheum*

officinale exhibited significant antiproliferative activity by inducing apoptosis in MCF-7 and A549 cell lines. [12].The methanolic extracts of *Artocarpus heterophyllus* was tested for anticancer activity by MTT assay on different cell lines like HEK293, A549, HeLa and MCF-7 .The IC_{50} value was found to be 35.26 µgm / ml by MTT assay against A549 [13]. The ethanol and chloroform extracts of *Cissus quadrangularis* exhibited more cytotoxicity towards A549 and HeLa cancer cell line and less toxicity towards normal monkey kidney cell line Vero as estimated by MTT assay [14].

CONCLUSION

The ethanolic leaf extracts of *C. inerme* exhibits antiproliferative activity and showed interesting results. Further focus should be moved towards isolating the potential phytochemicals responsible for the activity. Our results provide the basis for the further investigation and potential identification of medicinal compounds of anti-cancer property. Elucidating the mechanisms by which these anti-cancer properties are derived is of crucial importance to identify, select for and optimize therapeutic compounds. From this observation, it was clear that the leaf extract of *C.inerme* showed potential anticancer activity and showed the possibilities for the herbal treatment of these deadly diseases.

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