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***In-Vitro* Anti Cancer Activity of Ethanolic extract of the leaves of *Achyranthes bidentata* (Blume)**

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ABSTRACT

The present study was performed experimentally by in vitro method to examine the anti cancer activity of various concentration (10 µg/ml - 200 µg/ml) of ethanolic extract of the leaves of *Achyranthes bidentata* Blume. against DAL and EAC cell lines using tryphan blue dye assay method. The report on to the experiment reveals a significant anti cancer activity at 200 µg/ml by ethanolic extract. But alcoholic extract showed a remarkable increase in percentage of DAL (95.5%) and EAC (96%) cancer dead cells than the ethyl acetate extract.

Keywords: *Achyranthes bidentata* Plant extract, Tryphan blue dye, Lymphoma cells.

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INTRODUCTION

India is a rich source of medicinal plants and a number of plant extracts have been used in various systems of medicines such as ayurveda, siddha, unani, etc. to cure various diseases. Only a few of them have been scientifically explored. Plant derived natural products such as flavonoids, terpenes, alkaloids [1], etc have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxic and cancer chemo-preventive effects [2]. Cancer is a disorder developed due to some molecular changes within the cell. It becomes the second major cause of death in the human after cardiovascular disease [3]. About 7.6 million people died due to cancer in the world during 2007(American cancer society, Published in May 2007) Hence there is an urgent at present need for developing new approaches and drugs to prevent as well as cure this devastating disease.

Within the scientific community, interest in natural compounds is increasing now a day, which is fuelled partly by well-documented limits and adverse effects of current chemotherapy drugs, as well as the ongoing search for better ways to fight the disease. Scientists are now developing newer drugs by using the natural basic skeleton of an isolated component that targets the unique makeup mechanism of cancer cells. A number of natural products have been studied still now for anti cancer activity on various experimental models. This has resulted in the availability of nearly 30 effective anticancer drugs [4] that is explored.

To our knowledge a detailed study on the plant *Achyranthes bidentata* Blume. appears scanty surveys regarding anticancer activity. So the presence study had been focused on investigating in detail about anticancer activity of the plant.

COLLECTION AND AUTHENTICATION OF PLANT MATERIALS

The plants *Achyranthes aspera* Linn and *Achyranthes bidentata* Blume (fam: Amaranthaceae) are widely found throughout India up to a height of 1200m. In Tamilnadu, these are found in Nilgiri district and in Erode district. Were identified by Prof. Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Chennai, a botanist who authenticated the plant with available literature.

PREPARATION OF THE EXTRACT

The leaf part had been isolated from the plants and shade-dried. The work had been carried out during the month of September. Then they were powdered by using a pulveriser and sieved with 40mesh size. Then they were stored in an airtight container for extraction.

The plant material which was powdered and stored was used for extraction. A weighed quantity of each of the plant powdered material was extracted by cold maceration with 50% ethanol for 72 hr with intermediate heating at 40°C one time in a day. The extract was filtered

by using Whatmann filter paper and then the filtrate was concentrated under reduced pressure and controlled temperature (40°-50°C). The marc was dried and weighted. The marc was again extracted with water by cold maceration for 72 hrs to yield aqueous extract [5].

The ethanolic extract of *Achyranthes bidentata* Blume contained carbohydrates, alkaloids, glycosides, phyto-steroids, flavonoids, terpenoids, saponins, and fixed oil.

IN-VITRO ANTICANCER ACTIVITY

Recent studies of tumor-inhibiting compounds of plant origin have yielded an impressive array of novel structures. Herbal medicines have a vital role in the prevention and treatment of cancer and medicinal herbs are commonly available and comparatively economical. A great deal of pharmaceutical research done in technologically advanced countries like USA, Germany, France, Japan and China has considerably improved quality of the herbal medicines used in the treatment of cancer. Some herbs protect the body from cancer by enhancing detoxification functions of the body. Certain biological response modifiers derived from herbs are known to inhibit growth of cancer by modulating the activity reduce toxic side effects. The mechanism of cancer therapy. Inhibiting cancer cell proliferation directly by stimulating macrophage phagocytosis, enhancing natural killer cell activity, Promoting apoptosis of cancer cells by increasing production of interferon, interleukin-2 immunoglobulin and complement in blood serum, Enforcing the necrosis of tumor and inhibiting its translocation and spread by blocking the blood source of tumor tissue, Enhancing the number of leukocytes and platelets by stimulating the hemopoietic function, Promoting the reverse transformation from tumor cells into normal cells, Promoting metabolism and preventing carcinogenesis of normal cells and Stimulating appetite, improving quality of sleep, relieving pain, thus benefiting patients health [6].

IN-VITRO CYTOTOXIC STUDY:

Dalton's ascites lymphoma (DAL) and Ehrlich ascites carcinoma (EAC) cells were obtained through the courtesy of Amala Cancer Research Center. Thrissur, Kerala. They were maintained weekly intraperitoneal inoculation of 10^6 cells/mouse. The *Achyranthes bidentata* Blume extract were choose for the invitro cytotoxic activity and the stock solution of 10mg/ml was prepared with DMSO. From this 10µg-200µg (1 - 20µl) of drug was taken for the study [8]. Dalton's ascites Lymphoma cells and Ehrlich ascites carcinoma cells are being grown in the peritoneal cavity of mice by serial transplantation. For the experiment, the cells were aspirated from the peritoneal cavity and pelleted by centrifugation. The cells were washed with phosphate buffer saline and made up to a concentration of 10 million/ml by counting. The cells (1 million) were incubated with different concentration of drugs in a total volume of 1ml with PBS. Cells were incubated at 37°C for 3hrs. After incubation 0.1 ml of trypan blue (1%) was added and the cytotoxicity was determined by counting live and dead cells using haemocytometer [7-9].

Percentage of cytotoxicity was calculated by the following formulae,

$$\% \text{ cytotoxicity} = \frac{\text{No of dead cells}}{\text{No of dead cells} + \text{No of live cells}} \times 100$$

Table 1 : In-vitro cytotoxicity activity of ethanolic extract of *Achyranthes bidentata* Blume on different cell lines

S.NO	Drug Concentration (µg/ml)	% Cytotoxicity Cells (1x10 ⁶)	
		Dalton's ascites lymphoma	Ehrlich ascites carcinoma
1.	200	27	15
2.	100	20	9
3.	50	16	7
4.	25	12	5
5	10	6	2

Fig 1: Graphical representation of results in In-Vitro cytotoxic properties of *Achyranthes bidentata* Blume on Dalton's Ascites Lymphoma cell line

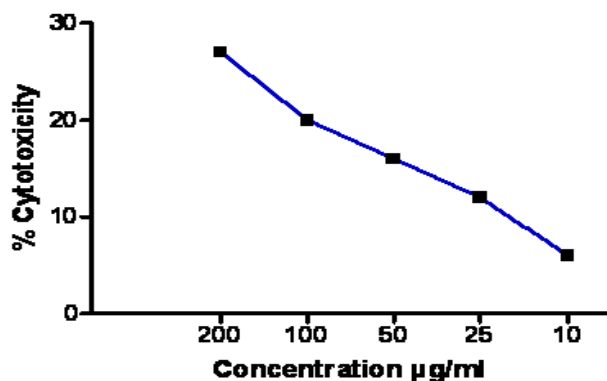
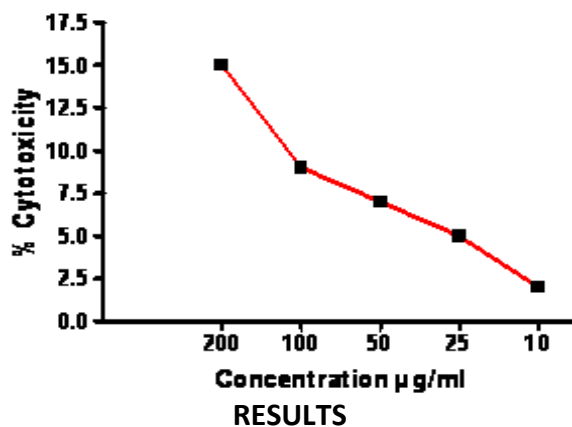


Fig 2: Graphical representation of results in In-Vitro cytotoxic properties of *Achyranthes bidentata* Blume on Ehrlich Ascites Carcinoma cell line.



RESULTS



The phytochemical evaluation shows the presence of Flavonoids, Phenolic compounds, Tannins, Glycosides, Saponins and Carbohydrates in ethanolic extract was evaluated. The results of *In-vitro* anti cancer test was showed in Table no. 1. The ethanol extract has shown remarkable anti cancer activity against the test cells namely Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascitic Carcinoma (EAC). Alcoholic extract also shows significant anticancer activity against the tested cell lines of Dalton's Ascitic Lymphoma (DAL) than the Ehrlich Ascitic Carcinoma (EAC).

CONCLUSION

The present study concluded that the ethanolic extract has shown a remarkable anticancer activity against the experimental cells namely Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascitic Carcinoma (EAC). This holds great promise for future research in human beings. The anticancer properties *Achyranthes bidentata* will provide an useful information in the possible application in the treatment of neoplastic therapy and prevention.

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