COMPARATIVE EVALUATION OF *IN VITRO* ANTI CANCER ACTIVITY OF ETHYLACETATE AND ETHANOL EXTRACTS OF *PHYLLANTHUS SIMPLEX* Retz

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ABSTRACT

The present study was performed experimentally by in vitro method to examine the anti cancer activity of various concentration(10μgm/ml - 200μgm/ml) of ethyl acetate and ethanolic extract from roots and rhizomes of *Phyllanthus simplex* Retz. 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 200μgm/ml by both the extracts. 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: *Phyllanthus simplex* Retz, plant extracts, tryphan blue dye, Lymphoma cells.

INTRODUCTION

India is a rich source of medicinal plants and a number of plant extracts have been used in various system of medicines such as ayurveda, sidda, 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 chemopreventive 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 fueled 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.

Phyllanthus simplex Retz. is commonly called as "Kaya-an","Bhuiamali" and "Kayut-bulang" which belongs to the family Euphorbiaceae. It is a glabrous twining perennial herb, which has a taproot and its branches are compressed. Its flowers are solitary and its leaves are distichous. The siddha and ayurvedic medications text showed that the Hindus used equal parts of the fresh leaves, flowers, fruit and cumin seeds with sugar, made into an electury for the treatment of gonorrhea by taking a teaspoonful for twice a day. The fresh leaves , bruised and mixed with buttermilk, make a wash to cure itches in children .The root is used in Chota Nagpur as an external application for abscesses[5-7]. Previous reports indicate that triterpenoids and iridoid glucosides possess anticancer property[8]. To our knowledge a detailed study on the plant *phyllanthus simplex Retz.*appears scanty surveys regarding anticancer activity. So the presence study had been focused on investigating in detail about anticancer activity of the plant.

PREPARATION OF CRUDE DRUG FOR EXTRACTION

After the entire plant material was collected including the roots and rhizomes, they were washed in running tap water to remove the adhered materials. The root and rhizomes part had been isolated from the plants and shade-dried. These works 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.

PREPARATION OF BENZENE AND ETHANOL EXTRACTS

About 1kg of the powdered plant was weighed and subjected to successive soxhlet extractions with petroleum ether (60-70°C), benzene, ethanol (95%V/V) and acetone for a period of 48 hours[9]. Then remained marc was dried in oven at 20°C. The dried marc was subjected to cold maceration[10] by using ethyl acetate and hydroalcohol (50:50) for 3 consecutive days. Finally obtained extracts were filtered through muslin cloth. Then they were concentrated under reduced pressure and dried in vacuum condition to get a semisolid consistency whose yields are tabulated. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents[11] present in them.

Table 1: *In vitro* cytotoxic activity of ethylacetate extract

Type of cancer cells (1×10^5)	Concentration of extract (µg/ml)	Number of samples	Number of cells		% of dead cells
			Live	Dead	
Dalton's Ascitic Lymphoma (DAL)	200	a	53	47	
		b	69	31	44%
	100	a	62	38	
		b	66	34	36%
	50	a	74	26	
		b	82	18	22%
	25	a	88	12	
		b	93	07	9.5%
	10	a	94	08	
		b	96	04	5%
	Control 10µg/ml	a	98	02	
		b	98	02	2%
Ehrlich Ascitic Carcinoma (EAC)	200	a	58	42	
		b	66	34	38%
	100	a	61	39	
		b	69	31	35%
	50	a	68	32	
		b	74	26	29%
	25	a	85	15	
		b	91	09	12%
	10	a	92	08	
		b	96	04	6%
	Control 10µg/ml	a	97	03	
		b	98	02	2.5%

Table 2: *In vitro* cytotoxic activity of ethanol extract

Type of cancer cells (1×10^5)	Concentration of extract (µg/ml)	Number of samples	Number of cells		% of dead cells
			Live	Dead	
Dalton's Ascitic Lymphoma (DAL)	200	a	03	97	
		b	06	94	95.5%
	100	a	10	90	
		b	12	88	89%
	50	a	32	68	
		b	38	62	65%
	25	a	74	26	
		b	82	18	22%
	10	a	94	06	
		b	92	08	08%
	Control 10µg/ml	a	99	01	
		b	99	01	01%
Ehrlich Ascitic Carcinoma (EAC)	200	a	02	98	
		b	06	94	96%
	100	a	06	94	
		b	12	88	91%
	50	a	16	84	
		b	20	80	82%
	25	a	69	31	
		b	73	27	34%
	10	a	87	13	
		b	88	12	12.5%
	Control 10µg/ml	a	98	02	
		b	98	02	02%

IN VITRO ANTICANCER ACTIVITY

Using tryphan blue dye assay method[12] out the in vitro anticancer activity was carried out. Both the extracts were dissolved in suitable solvent namely ethylacetate and ethanol. Two samples from various concentrations (200, 100, 50, 25 and 10μg/ml) of both the extracts had been prepared by using a micropipette. About 100μl of each concentration were transferred in to the required number of graduated pipettes. Then phosphate buffer saline was added up to 800μl. Finally 100μl (1million cells in 1 ml) of both the Dalton's Ascitic Lymphoma (DAL) and Ehrlich Ascitic Carcinoma (EAC) were added to all the test tubes. Then all the samples were incubated at 37°C in an incubator (Olympus company). About 100μl of tryphan blue dye was added to all the test tubes. Then the number of dead cells were counted by using a haemocytometer under a compound microscope. Percentage of cytotoxicity was calculated by the following formulae,

% dead cells = Number of dead cells/sum of dead cells and living cell × 100.

RESULTS

The phytochemical evaluation shows the presence of Flavonoids, Phenolic compounds, Tannins, Glycosides, Saponins and Carbohydrates both in Benzene and Ether extract which are already evaluated. The results of in vitro anti cancer test were showed in Table no 1 and 2 respectively. The benzene 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 anticancer activity against the tested cell lines Dalton's Ascitic Lymphoma (DAL) than the Ehrlich Ascitic Carcinoma (EAC). At 200μgm, concentration of alcoholic extract has showed 98% (DLA) and 98% (EAC) of acetone extract and 40% (DLA) and 34% (EAC) of activity of alcoholic extract.

CONCLUSION

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

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