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In-vitro Anticancer Activity of Cinnamomum iners Reinw. against DAL and EAC cell lines.

KEYWORDS: Acetone extract; Ethanolic extract; Cinnamomum iners Reinw; anticancer;

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ABSTRACT *Cinnamomum iners Reinw. has been evaluated for its in-vitro anti cancer activity against DAL and EAC cell lines by Trypan blue exclusion method. Both the acetone and ethanolic extracts showed remarkable cytotoxic activity against DAL and EAC cell lines as judged from cellular death in a dose-related fashion, the strongest effect being observed at higher concentrations. The results suggested that the anticancer effects of acetone and ethanolic extracts of stem bark of Cinnamomum iners Reinw may be related to their content of flavonoids. This study validates the traditional use of the plant in management of Cancer.*

INTRODUCTION

Plants have been used as remedies and botanical literature has described the usage of plant extracts. Medicinal plants are used in various countries in the treatment and prevention of cancer(Madhuri *et al.*,2009). Over the years, researchers have focused on the anticancer activity of plants (Saluja M.., 2011; Muthuraman *et al.,* 2008; Sowemimo *et al.,* 2007). Medicinal plants have been known to be good sources of effective anticancer drugs (Cragg *et al.,* 2005) such as Taxol, vincristine and camptothecin. Despite the development of new drugs, cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives every year (Abdullaev *et al.,* 2000). Hence, the need to search for new drugs that could prolong the life span of patients. Researchers have recently focused on the use of Ehrlich ascites carcinoma cells in the investigation of plants reported to cure cancer locally (Gupta *et al.,*2004; Zumrutdal *et al.,* 2008; Bromberg *et al.,* 2010).

Cinnamomum iners Reinw known as Tejpat or bay leaves in trade, is a promising medicinal plant species. It is a Moderate ever green tree found in tropic and subtropical and Himalayan region. Its leaves are widely used as spice throughout the world since ancient times. It is used in Indian system of traditional medicines in various Ayurvedic formulations. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhea, nausea and vomiting. The essential oil of the leaves called tejpat oil is medicinally used as carminative, antiflatulent, diuretic, and in cardiac disorders. The medicinal properties of the plants have been investigated in the recent scientific developments throughout the world, due to their broad spectrum of activity, no side effects and economic viability. Anticancer activity of the plant is mainly due to the presence of flavonoid compounds. The importance of medicinal plants and the contribution of phytomedicine to the well- being of a significant number of the world's population have attracted interest from a variety of disciplines. *Cinnamomum iners* has been reported for its stimulant, carminative, hypoglycemic (Swanston-Flatt *et al.,* 1987) antioxidant (Devi *et al.,* 2007) and antidiarrhoeal (Rao *et al.,* 2008) properties.

A review of literature afforded no information on the *in-vitro* anticancer activity against DAL and EAC cell lines of this plant. So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its *in-vitro* anticancer activity against DAL and EAC cell lines by Trypan Blue exclusion method.

MATERIALS AND METHODS

Plant material

The stem bark of the *Cinnamomum iners* were collected from the foothill of Yercaud, Salem, in the month of August 2014 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist and a voucher specimen (CIK-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of stem bark of the *Cinnamomum iners* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water. After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

In-vitro cytotoxic activity against DAL and EAC cell lines.

In-vitro cell culture

Ten days after inoculation of cell lines in the abdominal cavity of mice, Dalton's ascetic lymphoma (DAL)/Ehrlich ascites carcinoma (EAC) cells were isolated by needle as-

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piration from tumour bearing animals. Isolated cells were washed 2-3 times using phosphate buffer saline (PBS) pH 7.4 followed by centrifugation (1000 rpm). The clear supernatant was removed carefully. 100 µl of cells were taken and made up to 900 µl using PBS. To this 100 µl of trypan blue solution was added. Mixed well and the cells were loaded on haemocytometer and checked for viability. (2-5 dead cells are negligible). The no. of cells/Quadrant of haemocytometer were counted. The no. of cells should be 100 (110-120) in each quadrant to get 1million cells/ml in the stock. If the cell concentration >100, it was diluted using PBS, till the desired concentration was obtained.

Trypan Blue exclusion method

Different concentrations (200, 100, 50, 20 & 10 µg) of the sample solution (10 µl) were pipetted out and the volume in all the tubes were made up to 800µl with PBS pH 7.4. Then 100 µl of DAL/EAC (1million cells/ml) were added to all the test tubes. A control having solvent alone was also prepared (10 µl). The test tubes were incubated at 37°C for three hours. 100 µl of trypan blue was added to all test tubes and the numbers of cells were counted by using the haemocytometer with the help of compound microscope. The percentage of cytotoxicity was calculated by No. of dead cells/total 100 cells. The results were shown in table no.1

RESULTS AND DISCUSSION

The stem bark of the *Cinnamomum iners* Reinw. was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of acetone and ethanolic extracts of *Cinnamomum iners* was found to be 5.16 and 3.89% w/w respectively. The phytochemical evaluation showed the presence of flavonoids, phenolic compounds, tannins, glycosides, saponins and carbohydrates in the acetone and ethanolic extracts of the plant.

It is evident from the results in Table 1 that the cytotoxic effect of acetone and ethanolic extracts of *Cinnamomum iners* against EAC and DAL tumour cells *in-vitro* increases with the increase in concentration of the extracts. The acetone extract at 10, 20, 50, 100, 200 µg/ml caused mortalities of 4.5, 26, 90, 94 and 96% respectively in DAL and 7, 26, 93.6, 96 and 98.5% respectively in EAC. Similarly, The ethanolic extract at 10, 20, 50, 100, 200 µg/ml caused mortalities of 2.5, 13, 30, 58 and 80% respectively in DAL and 5.5, 15, 36.5, 60 and 88% respectively in EAC.

The trypan blue exclusion test can be used to indicate cytotoxicity. Dead cells take up the blue stain of trypan blue, whereas live cells have yellow nuclei. The principle behind the tryphan blue assay is simple. This dye is negatively charged and will bind to positively charged proteins in the cytosal only if the plasma membrane is ruptured (Freshney *et al.*,1987).

In this study, cytotoxicity data obtained from DAL and EAC cultures demonstrated that the extracts were able to produce cellular death in a dose-related fashion, the strongest effect being observed at higher concentrations. Thus, it may be hypothesized that both the acetone and ethanolic extracts of stem barks of *Cinnamomum iners* evaluated were able to bind to DAL/ EAC membrane and readily penetrate within the cells. These findings suggest that, in terms of cellular injury, the above extracts evaluated were potent cytotoxicants, and presented more pronounced effects. It is important to stress that repeated exposure to cytotoxicants can result in chronic cell injury, compensatory cell proliferation, hyperplasia and ultimately tumor development (Mally *et al.,* 2002).

CONCLUSION

From the results of the above investigation, it was concluded that Both the acetone and ethanolic extracts of stem barks of *Cinnamomum iners* possess good invitro cytotoxic activity agaist EAC and DAL cell lines. However, further invivo studies are required to evaluate its effect in tumour induced animal models and its possible mechanism of action.

Table No.1.Invitro cytotoxic activity of acetone and ethanolic extracts of *Cinnamomum iners* against DAL and EAC by Trypan Blue exclusion method.

Samples	Concentration (µg)	% activity	
		DAL	EAC
Acetone extract of *Cinnamomum iners*	200	96	98.5
	100	94	96
	50	90	93.6
	20	26	26
	10	4.5	7
Ethanolic extract of *Cinnamomum iners*	200	80	88
	100	58	60
	50	30	36
	20	13	15
	10	2.5	5.5

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