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In-Vitro Cytotoxic Effect of Apium *leptophyllum* Against EAC Cell Lines

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Abstract
Cancer is an uncontrolled cell proliferation with the potential to invade every part of the body. The treatment methods available today such as chemotherapy have not led to a significant reduction in the mortality rates. Hence it is evident that different methodologies are to be adopted to combat the dreaded disease**.** Medicinal plants have been used from time immemorial to treat various illnesses. The medicinal plants have bioactive molecules that have been developed into drugs. Many such molecules are now being utilised as anticancer drugs.
The present study aims at investigating anticancer potential of (EEAL & AEAL) on Ehrlich Ascites Carcinoma cell lines. In vitro cytotoxic assay such as Trypan Blue exclusion and MTT assay were carried out against EAC cell lines. The result revealed the significant cytotoxic effect of the extract against EAC cells. From the study it was observed that the ethanolic and aqueous extract of *Apium leptophyllum* pers showed potent anticancer activity against EAC cell line.

Keywords
Ehrlich Ascites Carcinoma, Aqueous extract of *Apium leptophyllum* (AEAL), Ethanolic extract of *Apium leptophyllum* (EEAL), MTT and Trypan blue.

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INTRODUCTION
The increasing occurrence of cancer has created a need for the development of medical solutions for fighting it. The allopathic medicine offers many methods to fight the disease. But it is evident that the healthy cells are also damaged during the treatment. Hence it is always better to refer to our traditional systems of medicine for the newer medications that can arrest menacing disease. Traditional medicine uses plant or animal-based properties as remedies for various ailments. Research to develop anticancer drugs from plants began from 1950s [1].

Factors that contribute to the onset of the disease include tobacco consumption (25-30%), infections (15-20%), diet and obesity (30-35%), radiation (ionizing and non –ionizing radiation up to 10%) lack of physical activity, stress and environment pollutions [2]. Chemoprevention is a major method adopted to prevent the cancer growth which utilize natural and synthetic interventions [3].Chemotherapy using synthetic drugs can produce severe toxic side effects and hence its usage has been restricted [4]. Chemotherapy is considered as the most important and inevitable method of treatment for the control of advanced stages of malignancies.

All the treatment models have their own side effects [5].

Vinblastine and Vincristine were the first plant-based drugs used clinically for the treatment of cancer. These compounds are primarily used in combination with other cancer chemotherapeutic drugs for the treatment for a variety of cancers, including leukemia, lymphomas, and breast and lung cancer [6]. The recent research reports that the metabolites derived from plants possess potential to inhibit and delay the multistage process of tumor growth[7].In modern medicine the secondary metabolites isolated from plants are evaluated for their anticancer efficacy and resulted in the discovery of 30 effective anticancer drugs[8].The essential advantages of plant based medicines are their safety efficacy and affordability [9].

Apium leptophyllum contains essential oil comprising thymol, thymoquinol used as carminative. Hence in the present study *Apium leptophyllum* a common plant of apiaceae was selected to assess its anticancer potential employing various *In-Vitro* methods.

MATERIALS AND METHODS

Preparation of Plant Extract

The collected seed of *Apium leptophyllum* were cleaned and powdered coarsely with a blender. The extraction was carried out using Soxhlet.

Aqueous extraction -weighed 10 gm of powdered sample and extracted with 100 ml boiling water by maceration for 48 hours.

Ethanol Extraction - 10 gm of powdered sample was extracted with 150ml of ethanol in a shaker at room temperature for 48 hours.

The respective extracts were filtered through Whatman No-1 paper, concentrated and dried under vacuum. The extracted samples were stored in airtight containers at -20 C until use.

TUMOR CELLS

Ehrlich Ascites Carcinoma (EAC) cells were obtained from Amala Cancer Research Centre, Trissur, Kerala, India and they were maintained by weekly intra – peritoneal inoculation of $1X10^6$ cells / mouse [10].

ANTICANCER ACTIVITY

IN-VITRO CYTOTOXICITY:

The cytotoxic effect of the aqueous and ethanolic extracts of the plant was evaluated against EAC cell lines using Trypan Blue Exclusion method [11] and MTT assay [12].

MTT ASSAY

EAC cell lines were cultured in 96 well plates with growth medium RPMI (Roswell Park Memorial Institute) 1640 and 10% FCS (Fetal Calf Serum). Increasing concentrations of EEAL was added to the cells and incubated at 37 ºC for 14 hrs in CO_2 incubator with 5% CO_2. The media was replaced with a fresh growth medium along with 20µl of 3,4,5 dimethyl thiazol-2yl 2,5 di phenyl tetrazolium bromide (MTT).It was then incubated for4 hours at 37°C. A purple precipitate was clearly visible under the inverted microscope then the growth medium was removed and 200 ml of 0.1% 0.1 N acidic isopropyl alcohol was added to the cells to dissolve the MTT, formazan crystals. Then the covered plates were kept in the dark at 18-24 º C overnight. The samples colour was read at 570 nm. Experiments were repeated at thrice. The average was calculated and compared with the control test samples. The percentage growth inhibition was calculated using the following formula [13].

TRYPAN BLUE EXCLUSION METHOD

The tumor cells were aspirated from peritoneal cavity of tumor bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and the cell number was determined using a haemocytometer and adjusted to 1X 10 cells / ml. For the cytotoxicity assay, different concentrations of the extracts (50-1000µg/ml) were added to each tube and the volume was adjusted to one ml with normal saline. Control tubes were maintained with the saline and tumor cells. All the tubes were incubated at 37º C for 3 hrs. 0.1 ml of test sample and 0.1ml of 0.2% Trypan blue dye in isotonic saline were mixed and the number of viable cells were counted using haemocytometer.

RESULTS AND DISCUSSION

Cytotoxic Effect of EEAL and AEAL on EAC cells (MTT ASSAY)

The results revealed that EEAL was more cytotoxic against Ehrlich Ascites Carcinoma. The results of the MTT assay and the Trypan Blue Exclusion test are depicted in Table 1&2 respectively.

Table -1 showed that 250µg/ml concentration of EEAL and AEAL produced 84.47% and 76.33% cytotoxicity with IC 50 value 133µg and 120µg. The results also showed that the cytotoxic effect of the extracts was dose dependent.

S.No	Conc of Plant Extract µg/ml	Percentage of cytotoxicity Aqueous Extract	Percentage of cytotoxicity Ethanol Extract
1	25	17.69	38.42
2	50	35.38	51.83
3	100	46.76	64.87
4	150	56.39	74.17
5	200	71.18	82.30
6	250	76.33	84.47

Cytotoxic Effect of EEAL and AEAL on EAC cells (Trypan Blue method)

Table 2- It is evident that the percentage of death cells rate of Ehrlich Ascites Carcinoma cells increased with increase in concentration of extracts of *Apium leptophyllum* pers.

Table 2: *In vitro* Cytotoxicity- Trypan Blue Assay *of* Aqueous and Ethanolic extracts of Apium *leptophyllum pers*

S.No	Conc of Plant Extract µg/ml	Percentage of Death cells (Non-viable cell) Aqueous Extract	Percentage of Death cells (Non-viable cell) Ethanol Extract
1	Control	5.12	7.25
2	50	31.61	53.46
3	100	46.54	61.07
4	200	67.00	77.79
5	500	77.69	86.41
6	1000	87.32	98.87

The percentage of viable cells decreases with increase in concentration of the plant extract. The Aqueous extract showed 87.32% and Ethanolic extract showed 98.87% of cytotoxicity (1000µg)

DISCUSSION

Cancer is often associated with high risk of death and the toxic side effects caused by the medication. Today people are turning towards complementary and alternative methods of medical treatment such as usage of phytomedicine [14].

The yellow colour tetrazolium MTT (3- (4,5 dimethyl thiazolium-2)- 2, 5 di phenyl tetrazolium bromide) is reduced metabolically by the action of mitochondrial dehydrogenase to generate reducing equivalents such as NADH and NADPH. The intracellular purple formation can be solubilized and quantified by spectroscopic method. The plant extract (EEAL and AEAL) might have induced apoptosis in the EAC cells resulting in the loss of mitochondrial function which is evident from the decreased production of formazon salt [15].

The trypan blue dye exclusion method based on the principle that death cell accepts the dye and stain with blue colour. The cytotoxic effect of aqueous and ethanolic extract of EAC cell lines was tabulated. (Table-2). The percentage of dead cells increased with increasing concentrations of the extract [16] .

CONCLUSION

The current study revealed that the Ethanol and Aqueous extract of plant *Apium leptophyllum* pers possess a potent anticancer activity against EAC cells by In-Vitro methods. The present work clearly depicted that EEAL possess comparatively better activity than AEAL.

DISCLOSURE

This research did not receive any specific grant from funding agencies in the public, commercial or non-profit sector.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

REFERENCE

[1] Radhika J, Sridharan G, Siva ganesh M and Pradeep V, Plants as Potential Cancer Therapeutics: A Review Journal of Cell and Tissue Research .17(2): 6085-6094, (2017)

[2]Tolar J and Negli JP, Tran placental and other routes of cancer transmission between individuals, J. Pediatr. Hematol.Oncol .25 (6): 403-404, (2003).

[3]Sporn MB, Liby KT. Cancer chemoprevention: scientific promise, clinical uncertainty, Nat Clin Pract Oncol. 2: 518-525, (2005).

[4]. Ito N, Fukushima, S. Tsuda, H Carcinogenicity and modification of the carcinogenic response by BHA, BHT and other antioxidants Crit Rev Toxicol. 15: 109-150, (1985).

[5]. Madhuri S and Pandey G, Some anticancer medicinal plants of foreign origin. Current Science. 6: 779-783, (1996).

[6] Cragg GM, Newman DJ, Snader KM. Natural products in drug discovery and development. J Nat Prod. 60:52-60, (1997).

[7]SurhYJ, LeeE, Lee JM. Chemo preventive properties of some pungent ingredients present in red pepper and ginger. Mutation Res. 402: 259-267, (1998).

[8] Lamartimere CA, Murrill WB, Manzalillo PA, Zhangt JX, Barnes S, Zhang X,WelH, Brown NM, Genistein alters the ontogency of mammary gland development and protects against chemically induced mammary cancer in rats. ProcSocExpBiol Med. 217:358-364. (1998).

[9] Siddiqui HH. Safety of herbal drugs – an overview. Drugs News and views. 1:7-10, (1993).

[10] Gothoskar SV and Ranadive KJ, Anticancer screening of SAN –AB, An extract of marking nut Semicarpusana cardium. Indian J ExpBiol.2 :58-62, (1971).

[11] Sheeja KR, Kuttan G and Kuttan R, Cytotoxic and antitumor activity of Berberin, Amala Res Bull, 17: 73-76, (1997).

[12] Sridharan G, Brindha P, Raja R and AmuthaPriya R, In-Vitro and In-Vivo Cytotoxic effect of *Salvia leucantha Cav* against EAC cell lines, Int J Pharm PharmSci . 4 (2),143-146, (2012).

[13] Scudiero DA, Shoemaker RH, Paul KD, Evaluation of soluble tetrazoliumformazan assay for cell growth and drug sensitivity in clusters using human and other tumor cell lines. Cancer Res: 48: 4827-4833, (1988).

[14] Kim J, Park EJ. Cytotoxic anticancer candidates from natural resources.Curr Med Chem Anticancer Agents 2002; 2 (4) : 485 -537.

[15] Christopher SP, The significance of spontaneous and induced apoptosis in gastrointestinal tract of mice Cancer Metstat Rev. 11:179-195, (1992).

[16] Sridharan.G ,Kadhiravan . M, Pavitra. R and Agnel Arul John. N, Cytotoxic and antioxidant activity of aqueous extract of *Neriumindicum* Linn. By In- vitro method, International journal of Research Instinct4 (1) 79-85, (2017)