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Evaluation of in vitro anticancer potential of *Achyranthes aspera* against EAC cell line

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Abstract

Cancer is a disease in which there is an uncontrolled multiplication and spread, within the body, of abnormal forms of the body's own cells. Assessment of *in-vitro* cytotoxicity has been recently become popular as a primary screening method for evaluating anticancer activities of various natural sources. This study was carried out to characterize anti-cancer activity of aqueous and ethanolic extracts of the whole plant of *Achyranthes aspera* L on Ehrlich Ascites carcinoma cell line [EAC] by MTT [(3- (4,5 dimethyl thiazol-2-yl) 2,5-di phenyl tetrazolium bromide] assay. Aqueous and ethanolic extracts of the whole plant of *A. aspera* have a significant anticancer effect against EAC cell line in the concentration range between 15.62ìg and 250ìg determined by using MTT assay. The minimum inhibition of aqueous extract showed 6.11% at 15.62ìg/ml and maximum inhibition 59.07% was observed at 250ìg/ml. IC_{50} value of *A. aspera* on EAC cell line was 192.64ìg/ml by MTT assay. The minimum inhibition of ethanolic extract showed 7.80% at 15.62ìg/ml and maximum inhibition 68.77% was observed at 250ìg/ml. IC_{50} value of *A. aspera* on EAC cell line was 156.51ìg/ml by MTT assay. It is concluded that the extract of *A. aspera* has potent anti-cancer activity on EAC cell line.

Keywords: Anti-cancer, MTT Assay, EAC Cell Line, *Achyranthes aspera*.

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INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the drawn of civilization (Anonymous, 1985). Medicinal plants can be important source of previously unknown chemical substances with potential therapeutic effect. The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. The World Health Organization (WHO) has estimated that over 75% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, higher safety margins and lesser costs.

Cancer

Cancer is the second leading cause of death all over the world. It is characterized by uncontrolled growth and spread of abnormal cells (Merel *et al.*, 2012). Cancer refers to a group of diseases those results from the abnormal growth of the cells. These cells divide and produce new cells in an uncontrolled way that can spread throughout the body and cause damage to essential organs. When cancer spreads to other parts of the body, this is called metastasis. Metastases can occur when cancer cells enter the bloodstream or lymph system. These systems circulate all over the body and allow the cells to travel (Sudhakar, 2009).

Cancer is derived from the Latin word cancerene for "crab". It is a multifactorial, multifaceted and multimechanistic disease requiring a multidimensional approach for its treatment, control and prevention (Jernal *et al.*, 2003). It is caused by external factors (tobacco, chemical, radiation and infectious organism) and internal factors (mutation, hormone and immune conditions) (English *et al.*, 1997).

Cancer Statistics worldwide

An estimated 12.7 million new cancer cases were diagnosed worldwide in 2008. Lung, female breast, colorectal and stomach cancers were the most commonly diagnosed cancers, accounting for more than 40% of all cases. Worldwide, an estimated 7.6 million deaths due to cancer occurred in 2008 (Merel *et al.*, 2012).

Cancer Scenario in India

In India, the International Agency for Research on Cancer has estimated that about 635, 000 people died

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due to cancer in 2008, representing about 8% of all estimated global cancer deaths and about 6% of all deaths in India. These compiled data show that the number of male, female and the total cancer patients in 2004 were 390809, 428545 and 819354 respectively. The number of male and female cancer patients increased continuously up to 2009, with 454842, 507990 and 962832 cases for male, female and total cancer patients, respectively. Similarly, 462408 male cancer patients and 517378 female cancer patients were recorded, with a total number of 979786 patients in 2010. In India, there is an increment of 10,000 new cancer patients every year and the number of total victims stands at about 25 lakh all over, according to Indian Council of Medical Research (ICMR).

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 (Uma Devi *et al.*, 2009).The present article deals with the results of the evaluation of the anticancer activity of whole plant extract of of *A. aspera*.

MATERIALS AND METHODS

Experimental plant

Achyranthes aspera is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour, branches terete or absolutely quadrangular, striate, pubescent, leaves thick, 3.8 - 6.3 × 22.5 - 4.5 cm, ovate – elliptic or obviate rounded, finely and softly pubescent on both sides, entire, petiolate, petiole 6 – 20 mm long, flowers greenish white, numerous in axillary or terminal spikes up to 75 cm long, seeds subcylindric, truncate at the apex, rounded at the base, reddish brown (Jain *et al.*, 2006).

Collection, Identification and Authentication of plant materials

The plant species namely *A. aspera* was collected from in and around Koothanallur, Thiruvarur District, Tamil Nadu, India. The plant was identified with the help of the Flora of Presidency of Madras and authenticated by Dr. S. John Britto, RAPINAT Herbarium and Centre for Molecular Systematics, St. Joseph's College, Tiruchirappalli (Voucher number of the specimen, AMTA 001) (Gamble, 1997). The plant was air dried under shade for 10-15 days. Then the dried material was ground to fine powder using an electric grinder and stored in air tight bottles. The powder matter was used for further analysis.

Preparation of the aqueous extract

The whole plant material was shade dried and coarsely powdered with electrical blender. 200g of *A. aspera* was mixed with 1200ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. Paste form of the extract obtained was subjected to preclinical screening.

Preparation of the Ethanol extract

Ethanolic extracts was prepared according to the methodology of Indian pharmacopoeia (Anonymous, 1996). The coarse powder material was subjected to Soxhlet extraction separately and successively with 210 ml ethanol and 90 ml distilled water. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature at 40 – 50 °C. The paste form of the extracts was put in an air tight container and stored in refrigerator.

In vitro anticancer activity

MTT assay

The MTT assay was analyzed by the method of Scudiero *et al.* (1988). Increasing concentrations of aqueous and ethanolic extracts of *A. aspera* were added to the cells and incubated at 37°C for 24 hrs in CO_2 incubator with 5% CO_2. The media was replaced with a fresh growth medium along with 20 µl of 3(-4, 5-dimethyl thiazol-2-yl) 2, 5 di phenyl tetrazolium bromide (MTT, sigma) and MTT reagent was added to it. Again it was incubated for 4 hrs at 37 °C. After incubation purple precipitate was clearly visible under the inverted microscope and then the growth medium was removed and 200µl of 0.1% 0.1N acidic isopropyl alcohol was added to the cells to dissolve the Formazan crystals. Then the covered plates were kept in the dark at 18-24 °C for overnight. The samples were drawn every 2 hrs and observed the reading at 570nm. Each experiment was conducted in triplicate form. The average was calculated, and compared with the control test samples. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ of Inhibition} = \frac{\text{Control OD} - \text{Treated OD}}{\text{Control OD}} \times 100$$

RESULTS AND DISCUSSION

Anticancer activity

Cancer is one of the most dreaded diseases of 20th century and spreading further continuously with increasing incidence in 21th century. It is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, growth, local tissue invasion and distant metastases (Xia *et al.*, 2004). Over the past few years, cancer has remained a major cause of the death and number of individuals affected with cancer is continuing to expand. Hence a major portion

Table 1. *In vitro* cytotoxicity effect of aqueous extract of *Achyranthes aspera* against EAC cell line (MTT Assay)

Concentration	OD-1	OD-2	OD-3	Average	% of Inhibition	IC_{50} Value (µg/ml)
Control	0.48	0.471	0.473	0.474	-	
15.62	0.451	0.445	0.441	0.445	6.11	
31.25	0.401	0.399	0.393	0.397	16.24	192.64
62.5	0.353	0.349	0.345	0.349	26.37	
125	0.287	0.281	0.279	0.282	40.5	
250	0.201	0.197	0.185	0.194	59.07	

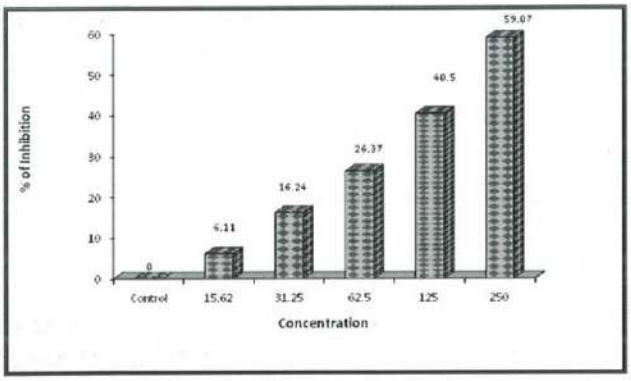

Fig. 1. *In vitro* cytotoxicity effect of aqueous extract of *Achyranthe aspera* against EAC cell line (MTT Assay)

Table 2. *In vitro* cytotoxicity effect of ethanolic extract of *Achyranthes aspera* against EAC cell line (MTT Assay)

Concentration	OD-1	OD-2	OD-3	Average	% of Inhibition	IC_{50} Value (µg/ml)
Control	0.48	0.471	0.473	0.474	-	
15.62	0.441	0.437	0.433	0.437	7.8	
31.25	0.387	0.381	0.375	0.381	19.62	156.51
62.5	0.323	0.319	0.307	0.316	33.33	
125	0.252	0.247	0.241	0.246	48.1	
250	0.153	0.147	0.145	0.148	68.77	

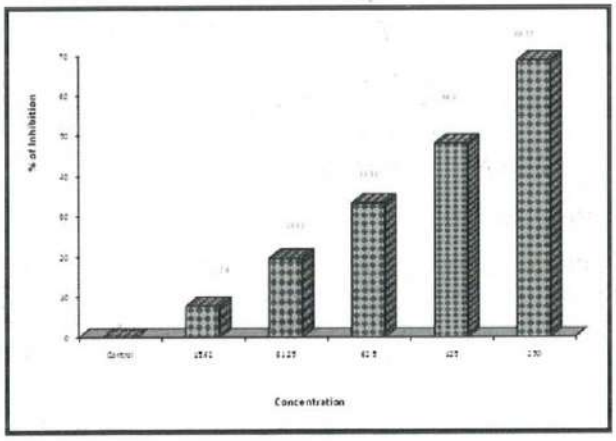

Fig. 2. *In vitro* cytotoxicity effect of ethanolic extract of *Achyranthe aspera* against EAC cell line (MTT Assay)

of current pharmacological research is developed to anticancer drug design customized to fit new molecular targets (Kim *et al.*, 2005). Due to enormous property of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom has become a potential source of chemical constituents with antitumor and cytotoxic activities. The plant kingdom represents an enormous reservoir of biologically active molecules and so far, only small fractions of plants with medicinal activity have been assayed. Nearly 50% of drugs used in medicine are of plant origin.

The whole plant of *A. aspera* is evidently a potential source of medicine as depicted in the Tables 1 and 2, and Figs. 1 and 2. The cytotoxic effect of aqueous and ethanolic extracts of *A. aspera* against EAC cell lines by *in vitro* method increases with the increase in concentration of the extracts. The aqueous extract at 15.62, 31.25, 62.50, 125 and 250ìg/ml caused mortalities of 6.11, 16.24, 26.37, 40.50 and 59.07 in EAC. Similarly, the ethanolic extract at 15.62, 31.25, 62.50, 125, 250ìg/ml caused mortalities of 7.80, 19.62, 33.33, 48.10 and 68.77 respectively in EAC.

In this study, cytotoxicity data obtained from EAC culture showed that the aqueous and ethanolic extracts of the whole plant of *A. aspera* were able to bind to EAC membrane and readily penetrate within the cells. These findings suggest that in terms of cellular injury, the above extracts evaluated have proved to possess cytotoxicity and presented more pronounced effects. It is important to stress that repeated exposure to cytotoxicity can result in chronic cell injury, compensatory cell proliferation, hyperplasia and ultimately tumor development (Mally *et al.*, 2002).

CONCLUSION

From the above findings it could be concluded that the minimum inhibition of aqueous extract showed 6.11% at 15.62µg/ml and maximum inhibition 59.07% was observed at 250µg/ml. IC_{50} value of *A. aspera* on EAC cell line was 192.64µg/ml by MTT assay. The minimum inhibition of ethanolic extract showed 7.80% at 15.62µg/ml and maximum inhibition 68.77% was observed at 250µg/ml. Further in depth studies are to be carried out to understand the molecular mechanisms of anticancer action of the methanol extract of *A. aspera* flowers coupled with animal studies and clinical trials would result in the arrival of cost effective, safe, efficacious anticancer drug which is a boon for ailing human society.

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