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Anticancer Activity of *Alangium salvifolium* Flower in Ehrlich Ascites Carcinoma Bearing Mice

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ABSTRACT

The research study was conducted to determine the antitumor effect of the flower of *Alangium salvifolium* (crude extract and diethylether fractions) against Ehrlich Ascites Carcinoma (EAC) in mice at the doses of 10 mg kg⁻¹ body weight intraperitoneally. Extract/fractions was administered for nine consecutive days. Twenty-four hours of last dose and 18 h of fasting, the mice were sacrificed and antitumor effect was assessed by evaluating tumor volume, viable and nonviable tumor cell count, tumor weight and hematological parameters of EAC bearing host. Significant (p<0.001) increases of survival times 30±0.96 and 25±0.40 days for crude extract and diethylether fraction of the *A. salvifolium* (10 mg kg⁻¹) treated tumor bearing mice, respectively were confirmed with respect to the control group (20±0.13 days). The extract/fraction also decreased the body weight of the EAC tumor bearing mice. Hematological studies reveal that the hemoglobin (Hb) content was decreased in EAC treated mice whereas restoration to near normal levels was observed in extract treated animals. There was a significant (p<0.001) decrease in RBC count and increase in WBC counts in extract/fraction treated animals when compared to EAC treated animals. From the result it was showed that the extract has significant anticancer activity and that is comparable to that of Bleomycin.

Key words: *Alangium salvifolium*, ehrlich ascites carcinoma, anticancer, mean survival time, hematological profile

INTRODUCTION

Cancer, the second leading cause of death worldwide next to cardiovascular diseases, is a group of more than 100 different diseases, characterized by uncontrolled cellular growth, local tissue invasion and distant metastases (Dashora *et al.*, 2010). Cancer is caused by internal factors (tobacco, chemicals, radiations and infectious organisms) and external factors (mutation, hormones

and immune conditions) (Kuper *et al.*, 2002) and can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy. Chemotherapy is still a major challenge to the cancer patients because such highly potent drug can be toxic and less than 1% of injected drug molecules can reach their target cells whereas the rest may damage healthy cells and tissue especially bone marrow, epithelial tissues, reticulo-endothelial system and gonads (Kathiriya *et al.*, 2010). Multidisciplinary scientific investigations are making best efforts to combat this disease but the sure-short, perfect cure is yet to be brought into world medicine. Moreover, the rate of increase of cancer incidence and lack of anticancer drugs has forced scientists to pharmacological and chemical investigation of anticancer agents from medicinal plants (Koduru *et al.*, 2006). The worldwide upsurge use of the herbal preparation and medicinal plants with its isolated active compounds has provided one of the most importance sources for pharmaceutical industry for lead compound. Furthermore, over a 100 new products are in clinical development, particularly as anti-cancer agents and anit-infectives (Hafidh *et al.*, 2009). Emerging evidence suggests that a number of plants are known to be the source of useful drugs in modern medicine (Sadiq *et al.*, 2009) and have been accepted currently as one of the main source of cancer chemoprevention drug discovery and development (Gonzales and Valerio, 2006) due to their diverse pharmacological properties including cytotoxic and cancer chemopreventive effects (Gupta *et al.*, 2004; Dahiru and Obidoa, 2007). Although, the mechanism of interaction between phytochemicals and cancer cells has been studied extensively and augmented the interest of pharmacological evaluation of various plants used in Bangladeshi traditional systems of medicine (Kumar *et al.*, 2007).

Alangium salviifolium wang is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae. This family consist one genus with twenty two species, out of which *Alangium salviifolium* wang is the only species used medicinally in Bangladesh, India, China and Phillipines (Jubie *et al.*, 2008). The different parts of this plant are used for a wide range of diseases. Root is used in diarrhoea, paralysis, piles and vomiting (Pandey and Raval, 2005). It is used as single drug in Ayurveda for the treatment of rabies and antidote for other poisonous bites including snake bites. Root is useful for external application in acute case of rheumatism, leprosy and inflammation (Anjaria *et al.*, 2002). Antibacterial compound was isolated from the flower of *Alangium salviifolium* (Anjum *et al.*, 2002). Fruits are sweet, used to treat burning sensation, constipation and haemorrhage. The leaves are used as poultice in rheumatism, stem barks exerts a biphasic action on the blood pressure in cats at lower doses and marked hypotension in higher doses. The plant has been reported for its anti-tubercular, anti-spasmodic and anti-cholinesterase activity (Warrier *et al.*, 2005). Anti-Fertility activity of the stem bark of *Alangium salviifolium* (Linn. F) Wang in Wistar female rats has also been reported (Murugan *et al.*, 2000). Previous phytochemical investigation revealed that it is a rich source of alkaloids including ipecac alkaloid and benzopyridoquinolizidine alkaloids. It is also known to produce alangiside, a tetrahydroisoquinoline monoterpene glucoside (Itoh *et al.*, 1992). Recent phytochemical studies of this plant resulted in the isolation of several flavanoid, phenolic compound, irridoid glycosides and oxyoglucoside of some alcohol (Ramni *et al.*, 2003). New alkaloid, ankorine was isolated from leaves (Jain *et al.*, 2002). Plant is also rich in tetrahydroisoquinoline monoterpene glycoside. e.g., alangiside-1 or ipecoside- 2 whose structures are closely related to the ipecac alkaloid (Itoh *et al.*, 1994). There are no such studies are revealed for its activity against treatment for pandemic cancer and hence the present study was carried out to evaluate the antitumor activity of the methanolic extract and its organic fractions of the flowers of *Alangium salviifolium* against Ehrlich Ascites Carcinoma (EAC) in mice.

MATERIALS AND METHODS

Plant materials: The flowers of the *Alangium salvifolium* were collected from the adjoining area of Rajshahi University Campus, Bangladesh during February 2007 and were identified by Taxonomist, Department of Botany and University of Rajshahi, Bangladesh where a voucher specimen number (Voucher No. 105) has been deposited.

Chemicals: The chemicals used were sodium chloride, propylene glycol, trypan blue, methyl violet, sodium sulphate, methylene blue, Bleomycin (Merck Limited, Mumbai, India). All other chemicals and reagents used were of highest analytical grade.

Preparation of extracts: The flower material was shade-dried with occasional shifting and then powdered with a mechanical grinder, passing through sieve #40 and stored in a tight container. The powdered flower (750 g) was taken in large glass bottle and extracted with Ethyl acetate: MeOH (1:1) for 7 days. The procedure was repeated twice using same solvent system for next 3 days. The extract was decanted first through a cotton plug and finally filtered through filter paper to get clear filtrate. The filtrate obtained by repeated maceration was evaporated under reduced pressure at 40°C using rotary evaporator. It renders a gummy concentrate and air evaporated to solid mass. The net weight of dry extract was 5 g. The dry plant extract (5 g) was suspended in water and fractionated in a conical flask using diethylether solvent system. Each fraction further evaporates using rotary evaporator and then air dried to solid mass (250 g).

Preliminary phytochemical investigation: The extract/fractions was subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins and tri-terpenoids (Yarnalkar, 1991).

Animal: Albino mice (25-30 g) and Wistar rats (175-250 g) of both sexes were used for assessing biological activity. The animals were maintained under standard laboratory conditions and had free access to food and water *ad libitum*. The animals were allowed to acclimatize to the environment for 7 days prior to experimental session. The animals were divided into different groups, each consisting of five animals which were fasted overnight prior to the experiments. Experiments on animals were performed in accordance with guidelines of the Institutional Animal Ethics Committee, Atish Dipankar University of Science and Technology, Dhaka, Bangladesh.

Acute toxicity: The acute oral toxicity of plant in male Swiss albino mice was studied as per reported method (Lorke, 1983).

Transplantation of tumor: Ehrlich Ascites Carcinoma (EAC) cells were obtained from Indian Institute of Chemical Biology (IICB), Calcutta, India. The EAC cells were maintained *in vivo* in Swiss albino mice by intraperitoneal transplantation of 2×10^6 cells per mouse after every 10 days. Ascitic fluid was drawn out from EAC tumor bearing mouse at the log phase (days 7-8 of tumor bearing) of the tumor cells. Each animal received 0.1 ml of tumor cell suspension containing 2×10^6 tumor cells intraperitoneally.

Treatment schedule: Sixty Swiss albino mice were divided into five groups (n = 12) and given food and water *ad libitum*. All the animals in each groups except Group-I received EAC cells (2×10^6 cells/mouse i.p.). This was taken as day '0'. Group-I served as normal saline control (5 mL kg⁻¹ i.p.)

and Group-II served as EAC control. Twenty four hour after EAC transplantation, Group-III and IV received crude extract and diethylether fractions of *Alangium salvifolium* flower at a dose of 10 mg kg⁻¹ i.p., for nine consecutive days, respectively. Group-V received reference drug Bleomycin (0.3 mg kg⁻¹ i.p.) for nine consecutive days (Mazumder *et al.*, 1997). Twenty-four hours of last dose and 18 h of fasting, 6 animals of each group were sacrificed by cervical dislocation to measure antitumor and hematological parameters and the rest were kept with food and water *ad libitum* to check percentage increase in life span of the tumor host. The antitumor activity of the extracts/fractions of *Alangium salvifolium* flower was measured in EAC animals with respect to the following parameters.

Determination of tumor volume and weight: The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. The volume was measured by taking it in a graduated centrifuge tube and weight immediately.

Tumor cell count: The ascitic fluid was taken in a WBC pipette and diluted 100 times. Then a drop of the diluted cell suspension was placed on the Neubauer's counting chamber and the numbers of cells in the 64 small squares were counted.

Viable/nonviable tumor cell count: The viability and nonviability of the cell were checked by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and nonviable cells were counted:

$$\text{Cell count} = \frac{\text{Number of cells} \times \text{Dilution factor}}{\text{Area} \times \text{Thickness of liquid film}}$$

Determination of median survival time and percentage increase in life span: The mortality was monitored by recording percentage increase in life span (% ILS) and Median Survival Time (MST) (Sur and Ganguly, 1994).

Body weight: Body weights of the experimental mice were recorded both in the treated and control group at the beginning of the experiment (day 0) and sequentially on every 5th day during the treatment period.

Hematological parameters: Collected blood was used for the estimation of hemoglobin (Hb) content, red blood cell (RBC) and white blood cell count (Armour *et al.*, 1965).

Statistical analysis: All data are expressed as Mean±SEM (n = 6 mice per groups). Statistical significance (p) calculated by Student's t test and computed using GraphPad Prism 4 (Graphpad). p<0.001 and <0.05 were considered to be statistically significant

RESULTS

Phytochemical screening: The phytoconstituents were identified by various chemical tests which showed the presence of alkaloids, tannins, phenolic and flavonoid compounds and steroid in crude extract of *Alangium salvifolium* (Table 1).

Table 1: Result of chemical group tests of the crude extract of *Alangium salvifolium* flower

Extract	Carbohydrate	Tannin	Flavonoid	Saponin	Phenol	Steroid	Alkaloid
<i>Alangium salvifolium</i>	-	++	++	-	++	+	+++

ME: Methanolic extract, +: Present, -: Absent; +++: Reaction intensity is high, ++: Reaction intensity is medium, +: Reaction intensity is normal

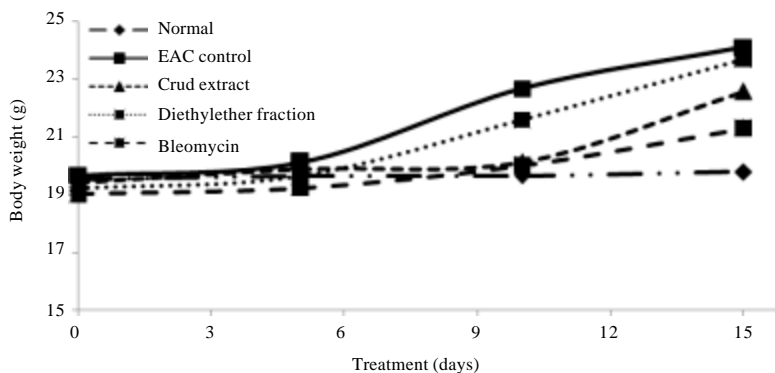


Fig. 1: Antitumor effect of extract/fraction of *Alangium salvifolium* flower on body weight of the EAC bearing mice

Acute toxicity studies: The acute toxicity studies mainly aims at establishing the therapeutic index, i.e., the ratio between the pharmacologically effective dose and the lethal dose on the same strain and species. The extract/fractions of *A. salvifolium* were safe up to a dose of 1500 mg kg⁻¹ (p.o.) body weight. Behavior of the animals was closely observed for the first 3 h then at an interval of every 4 h during the next 48 h. All extract/fractions did not cause mortality in mice and rats during 48 h observation but little behavioral changes, locomotor ataxia, diarrhea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Tumor growth and survival parameters: Antitumor activity of extract/fractions against EAC tumor bearing mice was assessed by the parameters such as tumor volume, tumor weight, cell count (viable and non viable), mean survival time and % increase of life span. The results are shown in Table 2. The tumor volume, tumor weight and viable cell count were found to be significantly (p<0.001) increased and non-viable cell count was significantly (p<0.001) low in EAC control animals when compared with normal control animals. Administration of crude extract and diethylether fraction at a dose of 10 mg kg⁻¹ significantly (p<0.05) decreased the tumor volume, tumor weight and viable cell count. Furthermore, the median survival time was increase to 30±0.96 (% ILS = 45.6) and 25±0.40 (% ILS = 18.9) on administration of crude extract and diethylether fractions at a dose of 10 mg kg⁻¹, respectively. Finally, the change in body weight of the animals (Fig. 1) suggest the tumor growth inhibiting property of *A. salvifolium* flower. All these results clearly indicate that the crude extract has a remarkable capacity to inhibit the growth of solid tumor induced by EAC cell line than the diethylether fractions in experimental animals.

Hematological parameters: Hematological parameters (Table 3) of tumor bearing mice on 14 day were found to be significantly altered compared to the normal group. The total WBC count

Table 2: Effect of the crude extract and diethylether fraction of *Alangium salvifolium* flower on tumor volume, tumor weight, mean survival time (MST), percentage increase life span (% ILS), viable and non-viable tumor cell count in EAC bearing mice

Parameter	EAC control	Crude extract	Diethylether fraction	Bleomycin
Tumor volume (mL)	3.10±0.21	0.69±0.22 [*]	1.69±0.34 [*]	0.51±0.21 [*]
Tumor weight (g)	3.90±0.24	0.74±0.54 [*]	1.42±0.21 [*]	0.61±0.11 [*]
MST (days)	20.00±0.12	30.00±0.96	25.00±0.40	42.60±0.12
% ILS	0.00	45.60	18.90	98.81
Viable cell (×10 ⁷ cell/ml)	8.10±0.22	1.30±0.0 [*]	2.70±0.05 [*]	0.50±0.05 [*]
Non-viable cell (×10 ⁷ cell/ml)	0.50±0.24	3.10±0.05 [*]	1.90±0.54 [*]	3.30±0.05 [*]
Total cell (×10 ⁷ cell mL ⁻¹)	8.60±0.15	4.40±0.25 [*]	4.60±0.21 [*]	3.80±0.05 [*]
Viable (%)	94.18	29.54	58.59	13.15
Non-viable (%)	5.82	70.46	41.31	86.85

Each point represent the Mean±SEM (n = 6 mice per group), ^{*}p<0.05 Statistically significant when compared with EAC control group

Table 3: Effect of the crude extract and diethylether fraction of *Alangium salvifolium* flower on hematological parameter in EAC bearing mice

Treatment	RBC (cell ×10 ³ mm ⁻³)	WBC (cell ×10 ³ mm ⁻³)	Hemoglobin (g %)
Normal control	5.39±0.12	3.92±0.32	13.90±3.1
EAC control	3.91±0.80 [*]	5.84±0.52 [*]	4.95±1.80 [*]
Crude extract (10 mg kg ⁻¹)	4.97±0.05 ^{**}	3.12±0.19 ^{**}	12.12±2.09 ^{**}
Diethylether fractions (10mg kg ⁻¹)	4.13±0.66 ^{**}	4.39±0.32 ^{**}	8.23±1.62 ^{**}
Bleomycin (10 mg kg ⁻¹)	5.18±0.12 ^{**}	3.15±0.83 ^{**}	12.89±2.93 ^{**}

Each point represent the Mean±SEM (n = 6 mice per group), ^{*}p<0.001 Statistically significant when compared with control group,

^{**}p<0.005 Statistically significant when compared with EAC control group

was found to be increased with a reduction of Hb content of RBC. The total number of RBC showed a modest change. At the same time interval on crude extract at a dose of 10 mg kg⁻¹ restored all the altered hematological parameters to almost near normal. Diethylether 10 mg kg⁻¹ treated also recovered these altered depleted parameters towards normal though crude 10 mg kg⁻¹ treatment was found to be more effective.

DISCUSSION

The ehrlich tumor was initially described as a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in almost all strains of mice. In ascetic form it has been used as a transplantable tumor model to investigate the antitumor effects of several substances (Segura *et al.*, 2000).

In EAC tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed. Ascetic fluid is the direct nutritional source for tumor cells and a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells (Prasad and Giri, 1994). Treatment with crude extract of *A. salvifolium* reduced the intraperitoneal tumor burden, thereby reducing the tumor volume, tumor weight, viable tumor cell count and increased the life span of the tumor bearing mice. The steadfast criteria for judging the potency of any anticancer drug are prolongation of life span of animals (Clarkson and Burchenal, 1965). It can therefore, be inferred that crude extract increased the life span of EAC bearing mice may be due to decrease the nutritional fluid volume and delay the cell division (Sur *et al.*, 1997). This hypothesis is strongly supported by previous study, in which *Aristolochia indica* increase the life span 47% at a dose of 50 mg kg⁻¹ body weight (Rana and Khanam, 2002).

Reduction in viable cell count and increased non viable cell count towards normal in tumor host suggest antitumor effect against EAC cell in mice. In this study, crude extract increase the non viable cell count upto 70.46% at a dose of 10 mg kg⁻¹ which agree with our previous study (Khatune *et al.*, 2003) and suggested that crude extract have direct relationship with tumor cells as these tumor cells are absorbed the anticancer drug by direct absorption in peritoneal cavity and this anticancer agent lysis the cells by direct cytotoxic mechanism (Kennedy *et al.*, 2001). Anemia and myelosuppression have been frequently observed in ascites carcinoma (Hogland, 1982). Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by haemolytic or myelopathic conditions which finally lead to reduced RBC number (Gupta *et al.*, 2007). Treatment with crude extract brought back the hemoglobin content, RBC and WBC count more or less to normal levels, thus supporting its haematopoietic protecting activity without inducing myelotoxicity, the most common side effects of cancer chemotherapy.

Preliminary phytochemical study indicated the presence of alkaloid, steroids, tannins, phenolic and flavonoid compounds and glycosides in crude extract of *Alangium salvifolium*. Literature survey shows that most of the polar compounds i.e., alkaloids, flavonoids etc. are biologically active. Low polar compound like simple alkaloids, flavonoids, terpenes and their oxygenated derivatives as well as more functional group containing compounds would be successfully extracted through ethylacetate. Rana *et al.* (2004) agreed with present recent study in which crude extract showed better activity than the diethylether fractions. A number of scientific reports indicate certain terpenoids, steroids and phenolic compounds such as tannins, coumarins and flavonoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis (Blois, 1958). Furthermore, flavonoids such as quercetin, kaempferol and their glycosides have been shown to possess antimutagenic and antimalignant effect (Fotsis *et al.*, 1997). Phytosterols are also able to incorporated into the cell membrane, alter membrane fluidity and the activity of membrane bound enzymes. They also alter signal transduction in pathways leading to tumor growth and stimulate apoptosis in tumor cell lines. They have also been shown to enhance *in vitro* human peripheral blood lymphocyte and T-cell proliferation *in vitro* which suggests a possible stimulation of the immune system function (Jones and AbuMweis, 2009). The anticancer activities of crude extract of *A. salvifolium* are probably due to the presence of alkaloid, phenolic compounds, flavonoids as well as terpenoids.

CONCLUSION

In present study, it was noted that crude extract of *A. salvifolium* significantly reduced tumor growth, viability of tumor cells, normalized the hematological profiles, raising life span as compared with those of EAC control mice. Therefore, it can be concluded that the crude extract of *A. salvifolium* flower demonstrated remarkable antitumor effect in Ehrlich ascites carcinoma bearing Swiss mice.

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