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Antineoplastic Screening of Some Medicinal Plants Against Ehrlich Ascites Carcinoma in Mice

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Swiss Albino mice inoculated with Ehrlich ascites carcinoma cells were treated with the extract of some medicinal plants include *Citrullus colocynthis*, *Withania somnifera*, *Bambusa arundinacea*, *Mesua ferrea*, *Acorus calamus*, *Myrica nagi*, *Calotropis procera*, *Catharanthus roseus*, *Ficus racemosa* and also with clinically highly effective drug bleomycin. Among the medicinal plants *Bambusa arundinacea* showed highest cell growth inhibition 81.9%. The tumor cell growth inhibition were found to be decreased as bleomycin>*Bambusa arundinacea* (ethylacetate extract)>*Citrullus colocynthis*>*Ficus racemosa* (methanol extract)>*Withania somnifera*>*Mesua ferrea*> *Catharanthus roseus* (methanol extract). *Acorus calamus* and *Calotropis procera* did not show any inhibition.

Key words: EAC, antineoplastic, medicinal plants

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INTRODUCTION

Human civilization and all living organisms are dependent on plant kingdom to a great extend for many of their daily necessities of life. Plants are unique in their ability to synthesize carbohydrates, fats and proteins that constitute three major food classes for human race. Besides, the plants also synthesize some compounds such as glycosides, alkaloids, sterols, toxalbumins, saponins, vitamins, essential oils, resins, tanins, coloring materials which posses medicinal values and used for the treatment of different diseases[1]. Taxol, an anticancer taxane diterpenoid derived from Western Yew tree, *Taxus brevifolia* nutt has recently (Dec. 1992) been approved in the United States for the treatment of refractory ovarian cancer[2,3]. Interesting discoveries from higher plants include vincristine vinblastine, vindesine podophylotoxin, 10-hydrxycampheein[2,4,5] which possess antitumor activity. Vincristine and vinblastine (vinca alkaloid) isolated from the plant *Vinca rosea* Linn have strong antiproliferative effect[6].

In the ancient time most of the people were dependent on plants as remedy of diseases. Still now some of the patients in the third world countries including Bangladesh take medicine in the form of Ayurvedic and Unani formulations which are derived from plants. In Bangladesh large number of plants are traditionally known to have cytotoxic and antitumor properties. Some have folkloric reputation of being used in different types of cancer[3].

In this study 9 plants were selected, some of them was proven cytotoxic and antoproliferative activity (brine shrimp lethality bioassay and potato disc bioassay) and some of which possess folkloric antitumor activity.

Acorus calamus, Mesua ferrea, Myrica nagi and *Withania somnifera* have folkloric cytotoxic and antiproliferative activity. *Ficus racemosa* have strong cytotoxic effect against brine shrimp and antitumor activity against Crown gall tumor[3]. *Citrullus colosynthis, Catharathus roseus, Calotropies procera* also possess important medicinal values[7].

In continuation of our pharmacological and phytochemical investigation of indigenous medicinal plants of Bangladesh, it is decided to investigate the antitumor activity of the cited plants against Ehrlich ascites carcinoma in mice. The present study reports the antitumor activity of the cited plants against transplantable tumor model.

MATERIALS AND METHODS

Plant materials: *Bambusa arundinacea, Citrullus colocynthis, Ficus racemosa, Withania somnifera, Catharanthus roseus, Acorus calamus* and *Calotropis procera* were collected from Natore region of Bangladesh where as *Mesua ferea, Myrica nagi* were collected from Mohini Departmental Store, Rajshahi, Bangladesh. All the plants were sun dried and pulverized, then stored as a powder in a polythene bags individually.

Extraction and preparation of the test samples: The individual plant materials (powdered, 500 g) were extracted in a Soxhlet apparatus consequently with petrolium ether (B.P.60-80°C), methanol. Methanol extract of the plant materials (except *Calotropis procera*, *Catharanthus roseus* and *Ficus racemosa*) were fractionated into ethylacetate and chloroform extract using solvent-solvent partitioning method. All these fractions were, respectively concentrated by the evaporation of their respective solvents in a vacuum rotatory evaporator. The concentrated masses were then dried in a hot plate under regulated temperature (up to 70°C). Methanol extract of *Calotropis procera*, *Catharanthus roseus* and *Ficus racemosa* were not fractionated but used after evaporation as a crude extracted sample. For our experiment 0.2 g of test samples were dissolved in 100 ml distilled water containing 0.2% DMSO to make a stock solution. Suitable dilutions were made from the stock solution before animal experimentation.

Animals: Swiss Albino male mice of 6-8 weeks of age, weighing 20-25 g each were collected from International Center for Diarrhoeal Disease Research Bangladesh. Animals were fed with standard mouse pellet collected from ICDDR'B and water was given ad libitum.

Experimental tumor model: Ehrlich ascites carcinoma cells were obtained from Indian Institute of Chemical Biology, Calcutta 700032, West Bengle, India and were maintained in our laboratory by weekly intraperitoneal (i.p.) transplantation.

Antitumor activity: Studies on *in vivo* tumor cell growth inhibition[8] were carried out with the test samples using chloroform extract of, *Citrullus colocynthis, Withania somnifera, Acorus calamus, Mesua ferea, Myrica nagi, Calotropis procera* and ethyl acetate extract of *Bambusa arundinacea*, *Catharanthus roseus* and *Ficus racemosa*.

Pure bleomycin (0.3 mg kg^{-1}) was used as standard drug. 2×10^6 EAC cells were inoculated (i.p.) into 17 groups of mice (6 in each group) on day 0. After 24 h of inoculation mice were treated with the test samples at doses 20 mg kg^{-1} i.p and bleomycin at dose 0.3 mg kg^{-1}. Control group was treated with 0.2% DMSO (vehicle). The treatment was continued for 4 days and on day 5, animals were sacrificed. Tumor cells were collected by repeated intraperitoneal washing with 0.9% saline. The viable tumor cells were counted (trypen blue test) with a

Table 1: Effect of Test Compounds on EAC Cell Growth Inhibition *(in vivo)*

Name of Plants	Used part	Extracts	Dose (mg kg^{-1})	No. of EAC cells/mouse on day 5 after tumor cell inoculation	% of cell growth inhibition
Control	-	-	-	$(5.833\pm0.33)\times10^7$	-
Bleomycin (6)	-	Antibiotic	00.3	$(0.312\pm0.037)\times10^7$	94.4**
Citrullus colocynthis (6)	Roots	$CHCl_3$	20.0	$(1.238\pm 0.07)\times10^7$	78.77**
		Et.Ac	20.0	$(6.95\pm 0.81)\times10^7$	-0.19
Withania somnifera (6)	Roots	$CHCl_3$	20.0	$(1.706\pm 0.31)\times10^7$	70.75**
		Et.Ac	20.0	$(9.42\pm 0.78)\times10^7$	-61.00
Bambusa arundinacea (6)	Roots	$CHCl_3$	20.0	$(5.675\pm 0.68)\times10^7$	2.70
		Et.Ac	20.0	$(1.053\pm 0.21)\times10^7$	81.9**
Mesua ferrea (6)	Flowers	$CHCl_3$	20.0	$(2.635\pm 0.36)\times10^7$	54.8**
		Et.Ac	20.0	$(3.4\pm 0.47)\times10^7$	41.7
Acorus colamus (6)	Stems	$CHCl_3$	20.0	$(5.468\pm 0.47)\times10^7$	6.0
		Et.Ac	20.0	$(4.70\pm 0.37)\times10^7$	-19.0
Myrica nagi (6)	Stem barks	$CHCl_3$	20.0	$(7.235\pm 0.54)\times10^7$	-24.0
		Et.Ac	20.0	$(9.0\pm 0.47)\times10^7$	-54.0
Calotropis procera (6)	Roots	Methanol	20.0	$(4.8\pm 0.20)\times10^7$	17.7
Catharanthus roseus (6)	Leaves	Methanol	20.0	$(3.145\pm 0.34)\times10^7$	46.0**
Ficus racemosa (6)	Roots	Methanol	20.0	$(1.388\pm0.33)\times10^7$	76.0**

$CHCl_3$ =Chloroform, Et.Ac.= Ethylacetate

Results shown are mean values±SEM. No. of mice per group is given in the parenthesis. **P<0.001 when compared with control (highly significant)

haemocytometer. Total number of viable cells per animal of the treated groups was compared with those of control group.

Statistical analysis: Significance of the experiments were statistically evaluated by students "t" test and significance at $p<0.05$[9].

RESULTS

Effect of the test samples and bleomycin on growth of EAC cells on day 5 after tumor transplantation is shown in Table 1. Treatment with Ethyl acetate extract of *Bambusa arundinacea* (20 mg kg^{-1} i.p.) resulted in a significant tumor growth inhibition (P<0.001) as evident from 81.9% reduction of tumor cells, which was found to be 94.12% for bleomycin (0.3 mg kg^{-1}i.p.).

Citrullus colocynthis (chloroform extract), *Ficus racemosa*(methanol extract), *Withania somnifera* (chloroform extract), *Mesua ferrea* (chloroform extract), *Catharanthus roseus*(methanol extract) showed 78.77, 76, 70.75 54.8 and 46% reduction of tumor cells, respectively.

Acorus calamus (chloroform and ethylacetate extract), *Calotropis procera* (methanol extract), *Myrica nagi* (chloroform and ethylacetate extract) did not show any activity. Chloroform extract of *Bambusa arundinacea* did not show activity. In addition ethylacetate extract of *Citrullus colocynthis, Withania somnifera, Mesua ferrea, Acorus calamus and Myrica nagi* did not show any activity against Ehrlich ascites carcinoma in mice.

DISCUSSION

Antineoplastic screening was performed using methanol, chloroform and ethylacetate extract of 9 medicinal plants and a standard drug bleomycin was also used for the comparison among them. Significant antitumor activity (~82% inhibition) was found from the crude ethyl acetate extract of *Bambusa arundinacea* whereas pure drug bleomycin showed 94.4% antitumor activity against EAC.

Otherwise a significant antitumor activity was found from *Citrullus colocynthis* (78.77%), *Ficus racemosa* (76%), *Withania somnifera* (70.75%), *Mesua ferrea* (54.6%), *Catharanthus roseus* (46%).

Literature survey shows that most of the polar compounds are biologically active e.g. alkaloids, flavanoids, terpens etc Low polar compound i.e. simple alkaloids, flavanoids and terpens would be extracted with chloroform but their oxygenated derivatives and those that contain more functional groups which determine polarity would be extracted with ethylacetate.

So if the active pure compound would be isolated by further research, it may be possible to get highly effective antitumor agent like vincristin, vinblastin etc. Therefore, plants can be appropriate starting materials for developing newer, safer, more effective and selective anticancer drugs.

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