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 (ethanol extract)>Citrullus colocynthis>Ficus racemosa (methanolic extract)>Withania somnifera>Mesua ferrea>Catharanthus roseus (methanolic extract). Acorus calamus and Calotropis procera did not show any inhibition.

Key words: EAC, antineoplastic, medicinal plants
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 possess medicinal values and used for the treatment of different diseases. Taxol, an anticancer taxane diterpenoid derived from Western Yew tree, Taxus brevifolia has recently been approved in the United States for the treatment of refractory ovarian cancer. Interesting discoveries from higher plants include vincristine vinblastine, vindesine podophytoxin, 10-hydryxycamptothecin which possess antitumor activity. Vincristine and vinblastine (vina alkaloid) isolated from the plant Vinca rosea Linn have strong antiproliferative effect.

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.

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

Acorus calamus, Mesua ferrea, Myrrica nagi and Withania sommifera have folkloric cytotoxic and antiproliferative activity. Ficus racemosa have strong cytotoxic effect against brine shrimp and antitumor activity against Crown gall tumor. Citrullus colocynthis, Catharanthus roseus, Calotropis procera also possess important medicinal values.

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 sommifera, Catharanthus roseus, Acorus calamus and Calotropis procera were collected from Natore region of Bangladesh where as Mesua ferrea, Myrrica 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 petroleum ether (B.P.60-80°C), methanol. Methanol extract of the planted 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 Bengal, India and were maintained in our laboratory by weekly intraperitoneal (i.p.) transplantation.

Antitumor activity: Studies on in vivo tumor cell growth inhibition were carried out with the test samples using chloroform extract of, Citrullus colocynthis, Withania sommifera, Acorus calamus, Mesua ferrea, Myrrica 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. 2×10⁶ 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)

<table>
<thead>
<tr>
<th>Name of Plants</th>
<th>Used part</th>
<th>Extracts</th>
<th>Dose (mg kg⁻¹)</th>
<th>No. of EAC cells/mouse on day 5 after tumor cell inoculation</th>
<th>% of cell growth inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(5.83±0.35) x 10⁴</td>
<td>94.4**</td>
</tr>
<tr>
<td>Bleomycin (6)</td>
<td>-</td>
<td>Antibiotic</td>
<td>0.3</td>
<td>(6.31±0.27) x 10⁴</td>
<td>78.77**</td>
</tr>
<tr>
<td>Citrusulus colocynthis (6)</td>
<td>Roots</td>
<td>CHCl₃</td>
<td>2.0</td>
<td>(1.39±0.37) x 10⁴</td>
<td>81.9**</td>
</tr>
<tr>
<td>Withania somnifera (6)</td>
<td>Roots</td>
<td>EL.Ac</td>
<td>2.0</td>
<td>(1.70±0.41) x 10⁴</td>
<td>79.9**</td>
</tr>
<tr>
<td>Bambusa arundinacea (6)</td>
<td>Roots</td>
<td>CHCl₃</td>
<td>2.0</td>
<td>(9.7±0.78) x 10⁴</td>
<td>61.00</td>
</tr>
<tr>
<td>Messua ferrea (6)</td>
<td>Flowers</td>
<td>CHCl₃</td>
<td>2.0</td>
<td>(5.67±0.60) x 10⁴</td>
<td>54.13**</td>
</tr>
<tr>
<td>Acorus calamus (6)</td>
<td>Stems</td>
<td>EL.Ac</td>
<td>2.0</td>
<td>(3.4±0.67) x 10⁴</td>
<td>41.7</td>
</tr>
<tr>
<td>Myrica nagi (6)</td>
<td>Stem bark</td>
<td>CHCl₃</td>
<td>2.0</td>
<td>(3.4±0.67) x 10⁴</td>
<td>60.0</td>
</tr>
<tr>
<td>Calotropis procera (6)</td>
<td>Roots</td>
<td>Methanol</td>
<td>2.0</td>
<td>(4.4±0.54) x 10⁴</td>
<td>24.0</td>
</tr>
<tr>
<td>Catharanthus roseus (6)</td>
<td>Leaves</td>
<td>Methanol</td>
<td>2.0</td>
<td>(2.35±0.47) x 10⁴</td>
<td>24.0</td>
</tr>
<tr>
<td>Ficus racemosa (6)</td>
<td>Roots</td>
<td>Methanol</td>
<td>2.0</td>
<td>(4.4±0.33) x 10⁴</td>
<td>76.0**</td>
</tr>
</tbody>
</table>

Results shown are mean values±SEM. No. of mice per group is given in the parenthesis. **P<0.01 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[7].

**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.).

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

*Ascorus 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 *Citrusulus colocynthis*, *Withania somnifera*, *Messua ferrea*, *Ascorus 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 *Citrusulus colocynthis* (78.77%), *Ficus racemosa* (76%), *Withania somnifera* (70.75%), *Messua ferrea* (54.6%), *Catharanthus roseus* (46%).

Literature survey shows that most of the polar compounds are biologically active e.g. alkaldoids, flavonoids, terpenes etc. Low polar compound i.e. simple alkaldoids, flavonoids and terpenes 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.

**REFERENCES**


