Screening of *in vitro* cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India

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**ABSTRACT**

**Objective:** To explore the cytotoxic activity of the alcoholic extracts of some medicinal plants used traditionally to treat cancer in Chhattisgarh state, India. **Methods:** *In–vitro* cytotoxicity of alcoholic extracts of five plants *i.e.* Artocarpus heterophyllus, Alangium salvifolium, Buchanania lanzan, Sesbania grandiflora and Wrightia tinctoria was studied against human breast cancer (MCF–7) and human leukemia (HL–60) tumor cell lines, using the thiazolyl blue test (MTT) assay. **Results:** Alcoholic extract of Sesbania grandiflora exhibited a prominent inhibitory effect against MCF–7 (IC_{50} 7.00±0.08 μg/mL) and HL–60 (IC_{50} 18.50±0.60 μg/mL) under *in vitro* condition. **Conclusions:** From the result it can be found that the Sesbania grandiflora extract has potent *in vitro* cytotoxic activity.

1. Introduction

Plants have long history of use in the treatment of cancer. Several studies have been conducted on herbs under a multitude of ethnobotanical grounds. For example, Hartwell has collected data on about 3000 plants, those of which possess anticancer properties are subsequently used as potent anticancer drugs[1–3]. Plant secondary metabolites and their semi–synthetic derivatives continue to play an important role in anticancer drug therapy[4,5]. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin and paclitaxel (taxol). Several promising new agents are in clinical development based on selective activity against cancer related molecular targets, including flavopiridol and combretastatin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest. Sixty percent of currently used anticancer agents are derived in one way or another from natural sources[6].

Use of plants for medicinal remedies is an integral part of the Indian cultural life and this is unlikely to change in the years to come. Many traditional healers and herbalists in the Chhattisgarh state of India have been treating cancer patients for many years using various medicinal plant species[7,8]. Hence, an attempt has been made to screen some medicinal plants used for the prevention and treatment of cancer in Chhattisgarh state, India. It is generally known that ethnomedical data provide substantially increased chance of finding active plants relative to random approach[9,10].

Uncontrolled proliferation is a universal property of tumor cells. Investigation of the cellular growth control mechanism has contributed to the understanding of carcinogenesis and to the identification of compounds with specific antitumoral activity[11,12].

In this study, we have explored five alcoholic bark extracts from selected medicinal plants, *i.e.* Artocarpus heterophyllus (*A. heterophyllus*), Alangium salvifolium (*A. salvifolium*), Buchanania lanzan (*B. lanzan*), Sesbania grandiflora (*S. grandiflora*) and Wrightia tinctoria (*W. tinctoria*) for their cytotoxic activity on the human MCF–7 and HL–60 cell line. The selection was made on the basis of ethnobotanical information. The ethnobotanical information of the plants assayed is presented in Table 1.
2. Materials and methods

2.1. Plants material

The bark of five plants *i.e.* *A. heterophyllus*, *A. salvifolium*, *B. lanzan*, *S. grandiflora* and *W. tinctoria* was collected during the months of November and December 2008. The plants were identified and authenticated by Dr. HB Singh, Scientist, National Institute Scientific Communication and Research (NISCAIR), New Delhi (India). The voucher specimens were stored in Herbarium of SLT Institute of Pharmaceutical Sciences, Bilaspur, India (specimen No. 01/HSAH, 02/HSAS, 03/HSBL, 04/HSSG and 05/HSWT).

2.2. Cancer cell lines

Human breast cancer (MCF-7) and human leukemia (HL-60) cell lines were provided by Deshpandey Laboratory, Bhopal, India.

2.3. Chemicals

Glutamine (Jinan Jiaquan Chemical Co. Ltd. Bombay, India), gentamicin (Anhui Minmentsal Dev. Imp. & Exp. Co., Ltd. Japan), trypsin (Deyang Sinozyme Pharmaceutical Co., Ltd. China), non-essential amino acid (Archon Vitamin Corporation, Irvington, New Jersey), fetal calf serum (ZEN BIOTECH PVT, LTD. Hyderabad), dimethyl sulfoxide (DMSO) and 3-(4,5-
dimethyl-2-thiazolyl)-2,5-diphenyl-2H-
tetrazolium bromide (MTT)—reagents were obtained from HIMEDIA, Mumbai, India. All other chemicals used were of analytical grade.

2.4. Preparation of extract and phytochemical screening

The dried bark of the plants was cleaned of dirt and ground to powder, using a commercial mill. Dried powder was defatted with light petrol (60–80 °C) and filtered. The residue was extracted with 90% ethyl alcohol by using Soxhlet extraction apparatus. Then solvent was completely removed under reduced pressure and the extract was stored in vacuum desiccators. The percentage yield of the extracts was calculated. The phytochemical constituents were identified by qualitative analysis[13].

2.5. In vitro cytotoxicity

The cytotoxic effect of five alcoholic plant extracts was evaluated by MTT assay using MCF-7 and HL-60 tumor cell lines. This MTT assay was performed according to a slight modification of the procedure reported by Mosman[14]. Cells were cultured in minimum essential medium (MEM) supplemented with glutamine (0.6 g/L), gentamicin (25 mg/mL) and 10% fetal calf serum at 37 °C and in humidified 5% CO2. For experiments, cells were plated in 96-well plate (10⁵ cells/well for adherent cells or 0.3×10⁶ cells/well for suspended cells in 100 μL of medium). After 24 h, the extracts (0.01, 0.1, 1, 10 and 100 μg/mL) dissolved in DMSO (1%) was added to each well and incubated for 96 h. The control groups received the same amount of DMSO. Doxorubicin (0.01, 0.1, 1, 10 and 100 μg/mL) was used as positive control. Growth of tumoral cells was quantified by ability of living cells to reduce the yellow dye MTT to a blue formazan product. At the end of 96 h incubation, the medium in each well was replaced by fresh medium containing 0.5 mg/mL of MTT. Four hour later, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured at 550 nm. Drug effect was quantified as the percentage of control absorbance of reduced dye at 550 nm. Percentage inhibitions [100 – (absorbance of test wells/absorbance of control wells) ×100] were calculated and plotted against the concentrations used to calculate the IC50 values[15,16]. The experiments were performed in triplicate.

2.6. Statistical analysis

Data were presented as mean ± SEM. The IC50 values were obtained by nonlinear regression using the GRAPHPAD program.

3. Results
3.1. Phytochemical screening

The percentages of yielded alcoholic extract and the results of the phytochemical screening of all the plants were given in Table 2. Flavonoids, terpenoids and phenolics were identified in plants having cytotoxic activity.

3.2. In vitro cytotoxicity

In order to evaluate the cytotoxic effect of five plant extracts that are used in Chhattisgarh traditional medicine, an antiproliferative assay with two human cell lines (MCF-7 and HL-60) was performed. Table 3 showed the cytotoxic activity of the five plant extracts that are commonly used in the treatment of cancer in Chhattisgarh traditional medicine. Of the plants used to treat cancer diseases, *S. grandiflora* was active on both cell lines (IC\(_{50}\) values 7.00±0.08 \(\mu\)g/mL and 18.50±0.60 \(\mu\)g/mL). *W. tinctoria* was found active on MCF-7 (IC\(_{50}\) value 10.00±0.05 \(\mu\)g/mL) and moderately active on HL-60 (IC\(_{50}\) value 48.00±0.85 \(\mu\)g/mL) cell line, while *A. heterophyllus* was found moderately active on MCF-7 (IC\(_{50}\) value 35.00±0.72 \(\mu\)g/mL) and least active on HL-60 (IC\(_{50}\) value 86.00±0.51 \(\mu\)g/mL) cell line. On the other hand, *A. salvifolium* and *B. lanzan* extracts were not found active on both cell lines (IC\(_{50}\)>100 \(\mu\)g/mL).

4. Discussion

In the present study, the cytotoxic effect of five alcoholic plant extracts on MCF-7 and HL-60 cells was evaluated by MTT assay. MTT assay is a well-established in vitro method for cytotoxicity against cancer cell lines and non-cancer cell lines[17], and here it was utilized to determine the selective activity of the extracts. Different dilutions of extracts were treated and IC\(_{50}\) values were calculated. In our screening program, we adopted the criteria of the American National Cancer Institute to consider a crude extract promising for further purification based on the IC\(_{50}\) values lower than 30 \(\mu\)g/mL in order to discover and develop potential anticancer natural compounds[18,19]. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work[20,21]. It is of interest that the extract of the plants showed cytotoxicity against cancer cell line, and, if this also occurs in vivo, the use of these plants by traditional healer for the treatment of cancer patients would have some scientific support. Several plant species rich in flavonoids are reported having disease preventive and therapeutic properties. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits and the association of vegetable and fruit consumption with reduced cancer risk has been reported[22-24]. Cytotoxic activity recorded in the present study is in accordance with this finding, since the phytochemical evaluation indicated the presence of flavonoids in all of the three plant species with promising activity. High contents of quercetin, myricetin and kaempferol were identified in *S. grandiflora* leaf extracts[25], a novel protein fraction was isolated from the flower of *S. grandiflora* which showed potential anticancer and chemo preventive efficacy[26]. Recently nine flavonoids, artocepin, cudrallavone C, 6-prenylapigenin, kuwanon C, norartocarpin, alhanin A, cudraflavone B, brosimone I and artocepinone were identified from the methanol extract of the wood of *A. heterophyllus* which showed in vitro cytotoxic activity against B16 melanoma cells[27]. The cytotoxic activities of active plants are probably due to presence of flavonoids.

Table 2

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Yield (%)</th>
<th>Alkaloids</th>
<th>Sterols /terpenoids</th>
<th>Phenolics</th>
<th>Coumarines</th>
<th>Flavanoids</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. heterophyllus</em></td>
<td>6.2±0.4</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>A. salvifolium</em></td>
<td>8.7±0.7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. lanzan</em></td>
<td>18.5±1.1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>S. grandiflora</em></td>
<td>11.6±0.6</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>W. tinctoria</em></td>
<td>16.6±0.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+: Present; -: Absent.

Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MCF-7 cell line</th>
<th>HL-60 cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC(_{50}) (\mu)g/mL</td>
<td>Status</td>
</tr>
<tr>
<td><em>A. heterophyllus</em></td>
<td>35.00±0.72</td>
<td>Moderately active</td>
</tr>
<tr>
<td><em>A. salvifolium</em></td>
<td>97.00±0.81</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>B. lanzan</em></td>
<td>&gt;100</td>
<td>Inactive</td>
</tr>
<tr>
<td><em>S. grandiflora</em></td>
<td>7.00±0.08</td>
<td>Active</td>
</tr>
<tr>
<td><em>W. tinctoria</em></td>
<td>10.00±0.05</td>
<td>Active</td>
</tr>
</tbody>
</table>
extract has potent in vitro cytotoxic activity against both cell lines. Further studies are also in process to evaluate the most potent fraction of the active plant.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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