Short communication

Cytotoxic effect of *Anthocephalus cadamba* Miq. leaves on human cancer cell lines

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**ABSTRACT**

**Introduction:** *Anthocephalus cadamba* Miq. (Rubiaceae) is used as folk medicine in the treatment of fever, anemia, leprosy, dysentery, blood and skin diseases.

**Methods:** Powdered leaves of *A. cadamba* were extracted sequentially with hexane, chloroform, ethanol, ethanol:water (50:50) and water by maceration, and evaluated for cytotoxic potential using SRB assay against four human cancer cell lines lung (A-549), ovary (IGR-OV-1), prostate (PC-3) and CNS (SF-295).

**Results:** Chloroform extract exerts potent cytotoxic effect against human lung (A-549), ovary (IGR-OV-1), prostate (PC-3) and CNS (SF-295) cancer cell lines IC<sub>50</sub> of 8, 57, 49 and 39 mg/ml respectively. Ethanol extract was found to be active only against one cell line CNS (SF-295).

**Conclusion:** The present study demonstrates the cytotoxic potential of *A. cadamba* leaves extract (chloroform) against different human cancer cell lines.

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1. Introduction

*Anthocephalus cadamba* Miq. (Rubiaceae) commonly known as kadam, is a large deciduous tree found all over the India on the slopes of evergreen forests up to 500 m. It has been used in Indian traditional medicine for treatment of different ailments and conditions such as fever, anemia, uterine complaints, snake bite, menorrhagia, blood and skin diseases, diarrhea, colitis, dysentery and in improvement of semen quality. The root extract was found beneficial in different urinary problems such as dysuria, calculi and glycosuria and also reported to possess antimicrobial, anthelmintic, hypolipidemic and antioxidant activities. *A. cadamba* was screened for hepatoprotective activity against carbon tetrachloride induced liver injury. The stem bark has been reported to have anti-inflammatory, antipyretic, diuretic and laxative activities. To the best of our knowledge, no previous cytotoxic study of this plant has been reported. In this communication, we report the in vitro cytotoxic potential of *A. cadamba* leaves extracts on four different human cancer cell lines A-549 (lung), IGR-OV-1 (ovary), PC-3 (Prostate) and SF-295 (CNS).

2. Material and methods

2.1. Plant material

The leaves were collected from botanical garden of Guru Nanak Dev University, Amritsar and authenticated by Dr. Adarsh Pal Vig, Department of Botanical and Environmental Sciences, GNDU. A specimen has been deposited at the herbarium of same department (voucher no, 0480).

2.2. Preparation of leaf extract

The air-dried powdered leaves of *A. cadamba* (550 g) were extracted by maceration using hexane, chloroform, ethanol, ethanol:water (50:50) and water. The crude extracts were obtained by evaporating the solvent using vacuum rotary evaporator (Fig. 1).

2.3. Cell lines

The human cancer cell lines procured from National Cancer Institute, Frederick, U. S. A. were used in present study. Cells were grown in tissue culture flasks in complete growth medium (RPMI-1640 medium with 2 mM glucose, pH 7.4 supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin) in a carbon dioxide incubator (37 °C, 5% CO<sub>2</sub>, 90% RH). The cells at subconfluent stage were harvested from the flask by
3. Results

The different extracts (ACH, ACC, ACE, ACA and ACA) of A. cadamba leaves were evaluated for their cytotoxic potential against four human cancer cell lines viz. lung (A-549), ovary (IGR-OV-1), Prostate (PC-3) and CNS (SF-295) using the SRB assay. The results of cytotoxic studies of the different extracts against human cancer cell lines were presented in Table 1. Paclitaxel has been used as standard in case of lung (A-549), ovary (IGR-OV-1), Mitomycin-C in case of Prostate (PC-3) while Adriamycin in case of CNS (SF-295). Among the different extracts of A. cadamba leaves, only the chloroform extract was able to inhibit the growth of different cancer cells as revealed by the value of IC50 (Table 1) and especially against Lung (A-549) cancer cell line 8 μg/ml which is comparable to standard drug Paclitaxel (IC50 4.1 μg/ml) used. The ethanolic extract bears moderate cytotoxicity only against the CNS (SF-295) cancer cell line, the other tested cell line were found resistant. The hexane (ACH), alc: aq (50:50) (ACAA) and aqueous (ACA) extracts were not found to be active against human cancer cell lines (Table 1).

4. Discussion

It is well established that plants have been a useful source of clinically important antitumor compounds and still there have been worldwide efforts to discover new anticancer agents from plants. The examples of anticancer agents developed from plants include antileukemic alkaloids vinblastine and vincristine, isolated from the Madagascar periwinkle (Vinca rosea), taxane derivative paclitaxel (isolated from Taxus brevifolia), later known as taxol and the alkaloid camptothecin (from the Chinese tree Camptotheca acuminata).9 Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product-derived with another 8% natural product mimics.10

A. cadamba is a member of cinchonoideae, a subfamily of Rubiaceae, the largest flowering plant family which comprises more than 13,000 species. Some Rubiaceae plants are found to have antitumor activity. Morinda citrifolia fruit juice has been reported to possess antitumor activity in the Lewis lung peritoneal carcinoma model.11 The dichloromethane fraction of Gardenia jasminoides was found to be capable of inducing apoptotic cell death by DNA topoisomerase 1 inhibition in KB cells.12 The role of indole based alkaloids vinblastin and vinblastin as antineoplastic drugs has been well established. The phytochemical studies of leaves and stem bark of A. Cadamba have revealed the presence of cadamine, isocadamine,13 cadambine, 3α-dihydrocadambine,14 3 β-isodihydrocadambine, 3 β-dihydrocadambine,15 cadambaginic acid,16 triterpenoids saponins and glycoside17,18. The widespread use of A. cadamba in traditional medicine and presence of indole based phytoconstituents

Fig. 1. Extraction of Anthocephalus cadamba leaves in increasing order of solvent polarity.

<table>
<thead>
<tr>
<th>Solvent Combination</th>
<th>Extractions</th>
<th>Residue</th>
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</thead>
<tbody>
<tr>
<td>Hexane (thrice)</td>
<td>Extraction</td>
<td>Hex extract</td>
</tr>
<tr>
<td>Chloroform (thrice)</td>
<td>Extraction</td>
<td>Chloroform extract</td>
</tr>
<tr>
<td>Ethanol (thrice)</td>
<td>Extraction</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>Ethanol + water (50:50) (thrice)</td>
<td>Extraction</td>
<td>Ethanol + water (50:50)</td>
</tr>
<tr>
<td>Water (thrice)</td>
<td>Extraction</td>
<td>Water extract</td>
</tr>
<tr>
<td>Ethanol + water</td>
<td>Extraction</td>
<td>Water extract</td>
</tr>
<tr>
<td>Acetic acid + water</td>
<td>Extraction</td>
<td>Water extract</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Extract/standard</th>
<th>Cell line type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lung</td>
</tr>
<tr>
<td>A-549</td>
<td>(ACH)</td>
</tr>
<tr>
<td></td>
<td>&gt;100</td>
</tr>
<tr>
<td>IGR-OV-1</td>
<td>&gt;100</td>
</tr>
<tr>
<td>PC-3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>SF-295</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>4.1</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>–</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>–</td>
</tr>
</tbody>
</table>

* ACH (hexane), ACC (chloroform), ACE (alcoholic), ACA (alc:aq (50:50)) and ACA (aqueous).
encouraged us to explore the cytotoxic potential. The compounds which have been previously isolated from the A. cadamba contains indole nucleus in the present study, cytotoxic action against cancer cells may be attributed to these active ingredients of A. cadamba extract, which can induce anti-proliferation pathway leading to cancer cell death. The result of the present study is the first report of cytotoxic activity of A. cadamba and for the cytotoxic activity, each cell line responded differently to the treatment with the tested extracts. Chloroform extract exerts potent cytotoxic effect against all tested human cancer cell lines particularly Lung (A-549) and CNS (SF-295). Further analysis and purification of the active principles of A. cadamba appears worthwhile in order to clarify the chemical nature and mode of action of the bioactive components responsible for cytotoxic potential.

Conflicts of interest

All authors have none to declare.

References