Screening of Some Rubiaceous Plants for Cytotoxic Activity Against Cervix Carcinoma (KB-3-1) Cell Line

การคัดกรองความเป็นพิษต่อเซลล์มะเร็งปากมดลูก ชนิด KB–3–1 ของพืชบางชนิดในวงศ์ RUBIACEAE

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Abstract

Twenty plants in the family Rubiaceae collected from Northern Thailand were extracted and compared for their cytotoxic activities against a human cervix carcinoma cell line (KB-3-1). MTT assay was the technique utilized for cell survival determination. These plants were classified into three groups as active, moderately active, and inactive plants, according to their cytotoxic activity. Gardenia obtusifolia and Gardenia sootepensis were the species possessing the highest activity (IC₅₀ ≤ 4 µg/ml) against the KB-3-1 cell line when compared to the other rubiaceous species. Other three plants categorized as the moderately active species (20 µg/ml < IC₅₀ < 100 µg/ml) were Ixora cibdela, Mussaenda pava and Psychotria ophioxyloides. The results from this study revealed 25% success rate in discovering plants with high and moderate cytotoxic activity. The usefulness of taxonomy in plant selection was confirmed in this study.

Key words

Screening, Cytotoxicity, Rubiaceae, Cervix carcinoma (KB-3-1)

Introduction

Cancer is one of the leading causes of death in Thailand. In 2001, over forty-two thousand cancer-related deaths were reported, which was the highest number among all causes of death (1). In every 100,000 people, approximately 80 men and 56 women had different types of cancer or malignant neoplasm as the causes of death (2). For specific types of cancer occurred only in women, breast and cervix cancers were the two highest causes of death in Thai women (2). Chemotherapy plays a prominent role in cancer treatment, besides surgery, radio-therapy, and immunotherapy. The sources of chemotherapeutic agents could be from synthesis, partial synthesis, and natural products (3, 4).
However, many chemical lead compounds and several invaluable chemotherapeutic agents are derived from plants, such as paclitaxel from Taxus brevifolia for breast cancer treatment and vincristine from Catharanthus roseus for treatment of leukemia (5). Thailand is a tropical country with high biodiversity of plants, especially in tropical forests. Although Thai government continuously supports the discovery of medicinal agents from natural sources, the searching is still under low-throughput format and fragmented (6). In this regard, our goal of searching for plants in the same family bearing cytotoxic activity against the same cancer cell line was to achieve a systematic screening of potential plant candidates. In this study, twenty plants from the family Rubiaceae collected from northern region of Thailand were screened for their cytotoxic activity against cervix carcinoma cell line (KB-3-1). The reports on cytotoxic activity of plants in several rubiaceous genera, e.g. Canthium, Gardenia, Ixora and Morinda had led to the selection of plants in this family for our study (7-11).

Materials and Methods

Plant materials

Whole parts of herbs and leaves of woody plants of 20 rubiaceous plants were collected from Doi Suthep-Pui National Park, Chiang Mai Province and Doi Khuntan National Park, Amphur Maetha, Lampoon Province, Thailand from April to October, 2000. Voucher specimens were collected for each plant, identified by J. F. Maxwell (12), and deposited in the Chiang Mai University (CMU) Herbarium, Biology Department, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand.

Extraction

Powder of samples (approximately 4 gm) was macerated three times during a period of one week with 95% ethanol. Crude ethanol extracts were used for cytotoxic activity tests. The extracts were evaporated and dissolved in dimethylsulfoxide (DMSO) at the concentration of 1 mg/ml. The stock solutions of the extracts were kept frozen (-20°C) before working solutions were prepared in a culture medium at the concentrations of 100, 50, and 5 µg/ml, respectively.

Cell line and culture medium

Human cervix carcinoma cell line (KB-3-1) (13) was used in this study. This cell line was obtained from Dr. Michael M. Gottesman, National Institute of Health, National Cancer Institute (NCI), Bethesda, Maryland (USA). The culture medium Dulbecco’s Modified Eagle Medium (DMEM, Gibco® BRL) was supplemented with 10% fetal calf serum (FCS, Biochrom KG), and 100 IU/100 µg/ml penicillin/streptomycin. KB-3-1 cells, at 3,000 cells/well (100 µl volume in 96-well tissue culture plate), were incubated in a humidified incubator with an atmosphere of 95% air and 5% CO2 at 37°C for 1 day before subjecting to cytotoxicity test.

Determination of cytotoxic activity

The cytotoxic activity was expressed as a concentration required for inhibiting cell growth by 50% (IC50 value). The American NCI (14) defined plants which give the extracts with the IC50 values of ≤ 20 µg/ml and ≤ 4 µg/ml for pure compounds as the plants with cytotoxic activity. The screening method by MTT assay has been adapted from Su et al. (2000) (15) and Mosmann (1983) (16). Briefly, the cells in 96 well plates were incubated with the extracts for 48 hours before the addition of a chromogenic dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The dye was converted by the mitochondrial succinate dehydrogenase in viable cells. Then, the culture plate was incubated at 37°C in a humidified incubator with an atmosphere of 95% air and 5% CO2. After 4 hours of incubation, the medium in the 96-well plates was removed and 200 µl of DMSO was added to each well, and mixed thoroughly to dissolve the formazan crystals. Absorbance was measured by using an ELISA plate reader at 540 nm with a reference wavelength of 630 nm. Each extract was tested in triplicate (n=3) and compared to the control well (1% DMSO as a vehicle control with no extracts). The fractional absorbance was calculated by the following formula: % Cell survival = (mean absorbance in test wells)/(mean absorbance in control well) x 100. The IC50 value
was determined from a dose–response curve, vinblastine sulphate, which was included in every test plate.

**Table 1.** Cytotoxic activity of rubiaceous plants against KB-3-1 cells

<table>
<thead>
<tr>
<th>Categories</th>
<th>Chemical or Botanical name (25)</th>
<th>Thai name (25)</th>
<th>IC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive control drug</td>
<td>Vinblastine sulfate</td>
<td>-</td>
<td>7.4 ± 0.8 ng/ml</td>
</tr>
<tr>
<td>active (IC₅₀ ≤ 20 µg/ml)</td>
<td><em>Gardenia obtusifolia</em> Roxb. ex Kurz</td>
<td>พุดนา, คำออมกลิ้ง, กระนอน</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td><em>Gardenia sooteponsis</em> Hutch.</td>
<td>ใส่ยาง, คำออมกลิ้ง, คำออมกลิ้ง</td>
<td>1.0</td>
</tr>
<tr>
<td>moderately active (20 µg/ml &lt; IC₅₀ &lt; 100 µg/ml)</td>
<td><em>Ixora cibdela</em> Craib var. <em>puberula</em> Craib</td>
<td>เข็มแดง, เข็มดำใต้, เข็มผัก</td>
<td>70.0</td>
</tr>
<tr>
<td></td>
<td><em>Mussaenda pava</em> Wall. ex G. Don</td>
<td>แก้มยิ้ม</td>
<td>62.5</td>
</tr>
<tr>
<td></td>
<td><em>Psychotria ophioxyloides</em> Wall.</td>
<td>เดาเนินยี้</td>
<td>69.5</td>
</tr>
<tr>
<td>inactive (IC₅₀ ≥ 100 µg/ml)</td>
<td><em>Haldina cordifolia</em> (Roxb.) Ridsdale</td>
<td>กระทุ้นเข้า, กระทุ้นแดง, ขว้าง</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Hymenodictyon orixense</em> (Roxb.) Mabb.</td>
<td>ลาชา, ลุ้น, สีมัน</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Ixora stricta</em> Roxb. (red)</td>
<td>เข็มหญ้าภูเขา, เข็มแดง</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td><em>Dioecrescis erythroclada</em> (Kurz) Tirveng. (Gardenia erythroclada Kurz)</td>
<td>จิ๋งกิ่ง, จิ๋งกิ่งขาว, จิ๋งกิ่งแดง, ผักดองแดง</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Lasianthus kurzii</em> Hook. f.</td>
<td>ป่าيعา</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Mitragyna hirsuta</em> Havil.</td>
<td>กระทุ้นเข้า, กระทุ้นแดง, ขว้าง</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Paederia pilifera</em> Hook. f. var. <em>kurzii</em></td>
<td>ต่ำทุ่งตอน, ตุ่มทุ่ง, ตุ่มสีมัน</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Pavetta tomentosa</em> Roxb. ex Sm.</td>
<td>ป่าสาวาสาวา, บึ้มเพื่า</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Gardenia angusta</em> (L.) Merr. (G. jasminoides J.Ellis.)</td>
<td>เตาทุ่ง, ตุ่มทุ่ง, ปากดิบ, ปากดิบสีมัน</td>
<td>&gt;100</td>
</tr>
<tr>
<td></td>
<td><em>Spermacoce latifolia</em> Aubl. (Borreria alata DC.)</td>
<td>กระดุมใบใหญ่</td>
<td>.*</td>
</tr>
<tr>
<td></td>
<td><em>Spermacoce laevis</em> Roxb. (Borreria laevis Griseb)</td>
<td>ปากผ่าปลายนิ้ว, ปากผ่าปลายนิ้วสีมัน</td>
<td>.*</td>
</tr>
<tr>
<td></td>
<td><em>Canthium glabrum</em> Blume</td>
<td>เตาการ, คำย่างตั้ง, หยดสี</td>
<td>.*</td>
</tr>
<tr>
<td></td>
<td><em>Catunaregam spathulifolia</em> Tirveng.</td>
<td>หนามบงกช, หนามบงกชสีมัน</td>
<td>.*</td>
</tr>
<tr>
<td></td>
<td><em>Tarennoidea wallichii</em> (Hook. f.) Tirveng. &amp; Sastre</td>
<td>เทือกสีเข้ม, เทือกสีเข้ม</td>
<td>.*</td>
</tr>
<tr>
<td></td>
<td><em>Uncaria macrophylla</em> Wall.</td>
<td>เตาอาการแม่ดอย, ตาอาการแม่ดอย</td>
<td>.*</td>
</tr>
</tbody>
</table>

*- = inactive and unable to specify the IC₅₀ from dose-response curve
Bold Thai name = official name in Thai
Results and discussion

To date, the cytotoxic activity among the species in the family Rubiaceae has not been compared. Therefore, this research had focused on the discovery of rubiaceous plants in Northern Thailand with cytotoxic activity against human cervix carcinoma cell line (KB-3-1). Twenty rubiaceous plant extracts were tested for their cytotoxic activity against KB-3-1 cells. In plant selection, two recognized strategies: the taxonomic and random approaches were our main methods. Plants in the same genus with the previously reported species were screened for their potential cytotoxic activity against this cervix carcinoma cell line. Whole parts of herbs and leaves of trees or shrubs were used in this preliminary screening protocol due to an economical reason and conservation of precious resources, since if different parts of a plant (roots, leaves, bark, twig, flowers, fruits, seeds, latex, etc.) were to be included, hundreds of samples would be required in the assay. The results of the cytotoxic activity test are shown in Table 1. In order to ensure the precision of the cytotoxic activity test, a positive control substance i.e., vinblastine sulfate, was included in every test plate. The IC50 of vinblastine sulfate against KB-3-1 cells was 7.4 ± 0.8 ng/ml (n=6). The cytotoxicity of these plants was classified into 3 levels: active, moderately active, and inactive. The crude extracts from the leaves of Gardenia obtusifolia and G. sootepensis exhibited high cytotoxicity (according to NCI, USA criteria) against KB-3-1 cell line, while G. erythroclada and G. jasminoides showed no activity. This finding supports the elegant works of Silva et al. (11) and Tuchinda et al. (17), in which the cytotoxic components against a panel of cell lines were isolated and identified from G. sootepensis and G. obtusifolia, respectively. How-ever, the positive test against a human cervix carcinoma (KB-3-1) cell line of these plants was first reported here. Other three extracts from the leaves of Ixora cibdela, Mussaenda pava, and Psychotria ophioxyloides exhibited moderate cytotoxic activity (IC50 = 70, 62.5 and 69.5 µg/ml, respectively). Several articles have reported the cytotoxic and/or antitumor activity against a variety of cell lines and tumors of plants in genus Ixora (18-21), but there was no report on the activity of Ixora cibdela, which was included in this study. A previous report on Mussaenda glabra, a plant closely related to Mussaenda pava, showed an equivocal antitumor activity against Yoshida sarcoma cells when administered intraperitonially to the rats (22). The cytotoxic activity of two Psychotria species (Psychotria forsteriana and Psychotria rostrata Bl.) was also previously reported (23-24). These plants were known for the existence of alkaloids which exhibited cytotoxic activity. Specific alkaloid extraction of Psychotria ophioxyloides leaves, which exhibited only moderate activity in this study, could result in higher activity of this plant. The other 15 plants selected for this study showed no activity against the KB-3-1 cell line. This could be because the plants have no inherent activity or the active components existed in other parts of the plants or the extraction process did not extract or concentrate enough active components into the crude extracts. The results from this preliminary study revealed some rubiaceous plants with potential cytotoxic activity against cervix carcinoma cells. The plants with high and moderate activity should be further investigated in details.

Conclusion

Twenty plants in the family Rubiaceae collected from Northern Thailand were extracted and compared for their cytotoxic activity against a human cervix carcinoma cell line (KB-3-1). Crude extracts from the leaves of Gardenia obtusifolia and Gardenia sootepensis exhibited the highest activity (IC50 ≤ 4 µg/ml) against the KB-3-1 cell line compared with the extracts from other rubiaceous species. Other three plants categorized as moderately active (20 µg/ml < IC50 < 100 µg/ml) were Ixora cibdela, Mussaenda pava and Psychotria ophioxyloides. To obtain the active component, bioassay-guided fractionation should be conducted. The results from this study revealed 25% success rate in discovering plants with high and moderate cytotoxic activity. The usefulness of
taxonomy in plant selection was confirmed in this study.

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References


