Cytotoxic against lung cancer cells (CHAGO) by the Thai rejuvenating herbal plants

ABSTRACT

In this study, we focused on cytotoxic assay of the 10 Thai rejuvenating herbal plants including *Suregada multiflorum*, *Fagraea fragrans*, *Phyllanthus emblica*, *Tinospora crispa*, *Melia azedarach*, *Anaxagorea luzonensis*, *Streblus asper*, *Butea superba*, *Dracaena conferta*, *Leucaena leucocephala*, against lung cancer cells (CHAGO). The plant ethanolic crude extracts were incubated with the tested cells prior to MTT assay for 3 days. The results revealed that the cell survival rates were decreased significantly following the treatment with 9 plant ethanolic crude extracts that exhibited the IC\textsubscript{50} within 10-1,000 μg/ML, including *S. multiflorum*, *F. fragrans*, *P. emblica*, *T. crispa*, *M. azedarach*, *A. luzonensis*, *S. asper*, *B. superba*, *D. conferta* which *D. conferta* is the most potent antiproliferation against the tested lung cancer cells (IC\textsubscript{50} = 160.09). Thus this study confirms the potential of certain Thai rejuvenating herbal plants in antiproliferation against lung cancer cells.

Key Words: lung cancer, rejuvenating herbs, *Suregada multiflorum*, cytotoxic assay

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INTRODUCTION

The use of natural products as anticancer agents has a long history including traditional medicines and several phytochemical-derived drugs currently used in chemotherapy (Diwanay et al., 2004). Over 50% of the drugs in clinical trials for anticancer activity were isolated from natural sources (Costa-Lotufo et al., 2005). In Asian countries, herbal formulations from a mixture of plants are often used by traditional medical practitioners for the treatment of cancer. Ayurveda, the traditional Indian medicine has been consumed for various tumor prevention or suppression (Abu-Dahab and Afifi, 2007). Phytotherapy is considered as an alternative to reduce the adverse effect on the use of synthetic drug.

In the recent study of Thai medicinal plants, Dioscorea membranacea contained dioscorealide B and dioscoreanone, which were potent antiproliferation against the breast adenocarcinoma MCF-7 (Tewtrakul and Itharat, 2006). Mucuna colletti was potent antiproliferation against MCF-7 (Cherdshewasart et al., 2004). The study on the essential oil from the Thai medicinal plants, Psidium guajava L. leaf and Ocimum basilicum L. showed anti-proliferative activity against human mouth epidermal carcinoma KB cells and murine leukemia P388 cells (Manosroi et al., 2006).

Polyphenol and sterol group from tea exhibited anti-proliferation against lung cancer cell (Naghma and Hasan, 2007). Flavonoids from Brazilian propolis inhibited the growth of lung cancer cell (Chao-Rui et al., 2007). The stem extracts of Euphorbia antiquorum (Kunlapana R., 2001), the bark extracts of Croton oblongifoli (Boonjira B., 2006), the root extracts of Kaempferia parviflora (Deachodomphan S., 2001) and the flower extracts of Melodorum fruticosum (Chaichantipyuth et al., 2001) have potential antiproliferation against lung cancer cells (CHAGO). This is definitely very low numbers of tested plants. We, therefore make a study in the 10 Thai rejuvenating plants against the proliferation of CHAGO cells.

MATERIALS AND METHODS

1. Plant materials

The 10 Thai rejuvenating herbal plants were collected from certain sources and the parts of plant used were identified by the domestic traditional medicinal expert and the medicinal expert at the largest herbal plant materials in Bangkok. The plant Thai names were adapted to plant scientific name with the aid of the reference book (วงศ์สถิตย์ ฉั่วกุล และคณะ, 2543) (Table 1). The plant materials were sliced into pieces and dried in hot air oven at 70°C. The dried materials were ground into powder and submitted to extraction with 95% ethanol for 7 days in the dark place. The supernatants were filtered through No.1 filter paper and subsequently evaporated in the rotary
evaporator until completely dried. The crude extracts were collected and stored in a light-protect bottle at 4°C.

2. Preparation of plant crude extracts

The plant crude extracts were dissolved in 100% dimethyl sulfoxide; DMSO (Merck, Darmstadt, Germany) to establish the 100 mg/mL stock solution which were diluted to the serial concentration of 0.1, 1, 10, 100 and 1,000 µg/mL for screening activity test.

3. Cell Culture

The lung cancer cells (CHAGO) were grown as monolayers in RPMI 1640 medium supplemented with 10% heat-inactivated Fetal bovine serum; FBS (Sigma, Taukirchen, Germany), 1.5 mM L-glutamine, 25 mM HEPES buffer, 1 mL of 10,000 units/mL penicillin and 10,000 µg/mL streptomycin (HyClone, Logan, UT, USA) for antibiotic reagent and 2 g NaHCO₃ were added in each liter of RPMI 1640 medium. The culture cells were maintained at 37°C with 95% humidity atmosphere in 5% CO₂ incubator (Thermo Forma, Marietta, Ohio, USA).

4. Cell cytotoxicity assay

Lung cancer cell cytotoxicity assay was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT assay) (Sigma, Saint Louis, MI, USA). The cells (5×10⁴ cells/well) were seeded in 96-well culture plate (Nunc, Roskilde, Denmark), cultured overnight and then treated with the plant crude extracts at the concentrations of 0.1, 1, 10, 100 and 1000 µg/ml for 72 hr. Genistein (10⁻² - 10⁻¹¹ M) is used as a positive control in comparison with the negative control DMSO. After treatment, the medium was removed and the cells were submitted to MTT assay and analyzed at the absorbance of 540 nm using a microplate reader (Tecan, Durham, NC, USA). The results were shown as a plot between the percentage of cell viability (Y-axis) and the concentrations of the sample (X-axis) and calculated the concentration of 50% cytotoxicity (IC₅₀) based on the obtained graph.

Calculation of the percentage of cell viability

The percentage of cell viability = \( \frac{\text{Absorbance of treated cells}}{\text{Absorbance of viable cells}} \times 100 \)

The IC₅₀ value could be calculated from the derived growth curve. It was defined as the 50% reduction of the absorbance or 50% of the percentage of cell viability compared with cells that were treated by DMSO as a negative control in the MTT assay.
5. Statistical analysis
The results were shown as mean ± standard error mean (S.E.M.) of five replicated experiments \((n = 5)\). Statistical analysis was performed using a one-way ANOVA for the analysis of the test results and Duncan analysis of variance at the significance levels of \(p < 0.05\) were considered significantly. (SPSS® version 14.0)

RESULTS AND DISCUSSION

Table 1 The Scientific name, plant family, part of plant used, source, yield of extract and IC\(_{50}\) of the 10 Thai rejuvenating herbal plants used to prepare crude extract and incubated with CHAGO cells.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Plant family</th>
<th>Part of plant used</th>
<th>Source</th>
<th>Yield of extract (%)</th>
<th>IC(_{50}) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suregada multiflorum</td>
<td>Euphorbiaceae</td>
<td>Stem and leaves</td>
<td>Khon Kaen</td>
<td>4.18</td>
<td>160.09±0.02</td>
</tr>
<tr>
<td>Fagraea fragrans</td>
<td>Potaliaceae</td>
<td>Whole stem</td>
<td>Khon Kaen</td>
<td>11.64</td>
<td>201.45±0.03</td>
</tr>
<tr>
<td>Phyllanthus emblica</td>
<td>Euphorbiaceae</td>
<td>Fruit</td>
<td>Sa Kaeo</td>
<td>9.96</td>
<td>211.01±0.02</td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td>Menispermaceae</td>
<td>Whole stem</td>
<td>Khon Kaen</td>
<td>10.56</td>
<td>223.51±0.02</td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>Meliaceae</td>
<td>Whole stem</td>
<td>Khon Kaen</td>
<td>18.84</td>
<td>224.94±0.03</td>
</tr>
<tr>
<td>Anaxagorea luzonensis</td>
<td>Annonaceae</td>
<td>Whole stem</td>
<td>Chiang Mai</td>
<td>4.72</td>
<td>297.67±0.03</td>
</tr>
<tr>
<td>Streblus asper</td>
<td>Moraceae</td>
<td>Seed</td>
<td>Bangkok</td>
<td>5.34</td>
<td>482.32±0.02</td>
</tr>
<tr>
<td>Butea superba</td>
<td>Papilionaceae</td>
<td>Tuberous root</td>
<td>Chiang Mai</td>
<td>15.14</td>
<td>778.75±0.02</td>
</tr>
<tr>
<td>Dracaena conferta</td>
<td>Agavaceae</td>
<td>Whole stem</td>
<td>Chiang Mai</td>
<td>4.52</td>
<td>796.51±0.04</td>
</tr>
<tr>
<td>Leucaena leucocephala</td>
<td>Mimosaceae</td>
<td>Root</td>
<td>Bangkok</td>
<td>4.9</td>
<td>&gt;1,000</td>
</tr>
</tbody>
</table>
From the cytotoxicity test it is found that 9 of 10 plant samples exhibited IC\textsubscript{50} within the range of 100 - 1000 µgML\textsuperscript{-1} which are classified as the potential harmful agents (Balantyne et al., 1999). They are including *Suregada multiflorum* (Figure 1), *Fagraea fragrans* (Figure 2), *Phyllanthus emblica* (Figure 3), *Tinospora crispa* (Figure 4), *Melia azedarach* (Figure 5), *Anaxagorea luzonensis* (Figure 6), *Streblus asper* (Figure 7), *Butea superba* (Figure 8), *Dracaena conferta* (Figure 9).

\[
y = -8.32\ln(x) + 92.23
\]

Figure 1 The cytotoxic effect of *S. multiflorum* crude extract against the growth of CHAGO

\[
y = -7.79\ln(x) + 91.33
\]

Figure 2 The cytotoxic effect of *F. fragrans* crude extract against the growth of CHAGO

\[
y = -8.44\ln(x) + 95.17
\]

Figure 3 The cytotoxic effect of *P. emblica* crude extract against the growth of CHAGO

\[
y = -7.18\ln(x) + 88.84
\]

Figure 4 The cytotoxic effect of *T. crispa* crude extract against the growth of CHAGO

\[
y = -10.1\ln(x) + 104.7
\]

Figure 5 The cytotoxic effect of *M. azedarach* crude extract against the growth of CHAGO

\[
y = -12.5\ln(x) + 121.2
\]

Figure 6 The cytotoxic effect of *A. luzonensis* crude extract against the growth of CHAGO
Phytochemicals with anti-cancer properties are increasing potential alternative for cancer treatment. 26-hydroxyfriedelane-1,3-dione isolated from the stems of *Salacia verrucosa* (Celastraceae) exhibited significant antiproliferation to CHAGO cells with an IC$_{50}$ value of 17.74 $\mu$M (Somwong *et al.*, 2011).

*S. multiflorum* is the strongest candidate within the category of the potentially harmful crude extract against the proliferation of CHAGO cells (IC$_{50}$ = 160.09). *S. multiflorum* also exhibited other bioactivity. The plant extract possessed potent nitric oxide (NO) inhibitory effect with an IC$_{50}$ value of 8.6 mg/ml. The isolated plant compounds were including helioscopinolide A which exhibited the highest activity against NO release with an IC$_{50}$ value of 9.1 $\mu$M, helioscopinolide C and suremulol D with IC$_{50}$ values of 24.5 and 29.3 $\mu$M, respectively (Tewtrakul *et al.*, 2011). Some plant isolated compounds possessed anti-allergic activities in RBL-2H3 cells model with IC$_{50}$ values ranging from 22.5 to 42.2 $\mu$M (Cheenpracha *et al.*, 2006). However, the anti-cancer activity of the plant extract had not yet evaluated. Thus this plant is an interesting sample for the further study with the approach of active chemical identification and retest with the same cancer cell line.
CONCLUSION

*S. multiflorum* is the best candidate within the category of the potentially harmful crude extract against the proliferation of CHAGO cells. There should be a further study in this plant to isolate and do bioassays of the plant pure compounds, especially the anti-cancer properties.

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