Biological activities of Antidesma thwaitesianum Muell

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Abstract:
Antidesma thwaitesianum Muell is a member of Euphorbiaceae, some parts of it were used to treatment several diseases worldwide but there is no scientific report. The objective of this research is to test biological activity of all parts of this plant extract such as cytotoxic activity against lung cancer cell by SRB assay, antioxidant activity by DPPH assay and antimicrobial activity by disc diffusion method. The results found that the ethanolic extract showed high antioxidant activity because they showed antioxidant activity more than BHT (and wood exhibited the highest antioxidant activity. Antidesma thwaitesianum wood extract also exhibited the highest cytotoxic against lung cancer cells. Its leaves extract showed the highest antimicrobial against Staphylococcus aureus and Candida albican. In the conclusion, wood of this plant appeared to be the best part used for cancer treatment because it showed both antioxidant and cytotoxic activities and the leaves appeared to be the best part for antimicrobial.

Keywords: Antidesma thwaitesianum Muell. cytotoxic and antioxidant activities assay.

Introduction
Antidesma thwaitesianum Muell is Thai name Maoluang. Its fruits were use as soft drink and vine in Thaland. Indian and Indonesian like to eat leaves of this plant for food. In Cambodia using its leaves were used as antipyretic, headache and nausea, stem bark used for wound healing in animal and antidiarrhea. The previous report found that root of this plant showed anti HIV-1 integrase. Surprisingly there is no research to investigate cytotoxic activity against cancer cell by comparison all parts of this plant extract. Thus, the objectives of this research were to study on biological activities such as cytotoxic activity against cancer cell, antioxidant and antimicrobial activities. The comparison activities of each part of this plant was also investigated.

Methodology
1. Plant Materials
Antidesma thwaitesianum Muell. were collected from Amphor wang-juan, Chonbure Province, Thailand.
2. Preparation of Plant Extracts
The each part of Antidesma thwaitesianum Muell. were washed, sliced thinly, dried in an oven at 45°C and made it be powdered. Dried plant material (500 g) were macerated by 1.5 L of 95% ethanol, for 3 days, filtered and dried by using an evaporator. The percentage of yielded were evaluated and showed in table 1.

In vitro assay for Antioxidant activity
DPPH radical scavenging assay, pipetted sample solution in each concentration 100 μl in 96-well plate, added DPPH solution 100 μl in each sample and mixed.(Final concentration of sample 100, 50, 10, 1, 0.5 μg/ml). The absorbance (A) was measured at 520
nm. Calculated by formula \( \% \text{inhibition} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \) and \( EC_{50} \) value calculated by linear regression analysis by prism program.

**In vitro Assay for Cytotoxic Activity**

The cytotoxicity assay was carried out using sulphorhodamine B (SRB) assay\(^4\). The target cell lines were human lung cancer cells (COR-L23). The 50 \( \mu \)g/ml of the extracts were tested first against all cell lines by SRB assay and the active plants extracts were diluted and tested for calculating \( IC_{50} \).

The monolayered culture of each cell line in a 96-well microtiter plate was treated with each plant extracts for 6 replicates. The plates were incubated for an exposure time of 72 hours, then the medium was removed and washed. The plates were incubated for a recovery period of 6 days. The survival percentage was measured colorimetrically using SRB assay and \( IC_{50} \) values was calculated by means of Prism program.

**Antimicrobial assay**\(^5,6\)

In the preliminary studies, all extracts were evaluated for antibacterial activity by disc diffusion method. All extracts were tested against two types of gram positive bacteria (Staphylococcus aureus, Bacillus subtilis), one type of gram negative bacteria (Escherichia coli) and one type of fungi (Candida albicans). The active plant extracts were diluted to determine the minimum inhibitory concentration (MIC).

**Results:**

**Table 1** Percentage of yield, cytotoxic activity against lung cancer cells and antioxidant activity of the ethanolic extract of four parts of Antidesma thwaitesianum Muell. (n=3)

<table>
<thead>
<tr>
<th>Part of plant</th>
<th>% yield</th>
<th>Cytotoxic against CORL23</th>
<th>Antioxidant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC(_{50}) (( \mu )g/ml) ±SEM</td>
<td>EC(_{50}) (( \mu )g/ml) ±SEM</td>
</tr>
<tr>
<td>leaves</td>
<td>8.40</td>
<td>34.675±2.5</td>
<td>3.88±0.1</td>
</tr>
<tr>
<td>Stem bark</td>
<td>8.61</td>
<td>19.320±0.12</td>
<td>3.59±0.5</td>
</tr>
<tr>
<td>Wood</td>
<td>7.82</td>
<td>15.411±1.2</td>
<td>2.42±0.12</td>
</tr>
<tr>
<td>Root</td>
<td>4.52</td>
<td>22.253±1.9</td>
<td>9.53±0.9</td>
</tr>
<tr>
<td>BHT</td>
<td></td>
<td>12.12±1.5</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2** Inhibition zone (mm) ±SD of all part of Antidesma thwaitesianum Muell.extract against four microbial (n=3)

<table>
<thead>
<tr>
<th>Parts of plant (mg/ml)</th>
<th>E.coli</th>
<th>S. aureus</th>
<th>B.subtilis</th>
<th>C.albican</th>
</tr>
</thead>
<tbody>
<tr>
<td>leaves</td>
<td>-</td>
<td>17.00± 0.57</td>
<td>-</td>
<td>15.00 ± 0.57</td>
</tr>
<tr>
<td>Stem bark</td>
<td>-</td>
<td>10.33 ±0.88</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Wood</td>
<td>-</td>
<td>11.33 ±0.66</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>-</td>
<td>10.00 ±1.15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Ampicilllin (0.003mg/ml)</td>
<td>16±0.0</td>
<td>30.0±0.1</td>
<td>25.1±0.3</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT= no test

The results found that the ethanolic extract of all part of this plant extract showed high antioxidant activity and more than BHT and wood exhibited the highest antioxidant activity.
(EC$_{50}$=2.42 µg/ml). Antidesma thwaitesianum wood extract also exhibited the highest cytotoxic against lung cancer cells (IC$_{50}$=15.4µg/ml). Its leaves extract showed the highest antimicrobial against Staphylococcus aureus and Candida albican. In the conclusion, wood of this plant was evaluated as the best part for cancer treatment because it showed both antioxidant and cytotoxic activities and leaves is the best for antimicrobial. This result related with using for wound healing (Narod, 2004). Surprisingly, Its root have ever been report that it showed good activity for anti-HIV integrase but it has ever been studied that another part of this plant showed anti HIV. Thus, these studied can conclude that each part should be studied to compare each activity for the highest useful for each activity

Conclusion
In summary from this research can conclude that wood is the best part for using anticancer and antioxidant but leaves is the best part for antimicrobial especially Staphylococcus aureus

References:
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