HIV-1 protease inhibitory substances from \textit{Cassia garrettiana}

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Abstract

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HIV-1 protease inhibitory substances from \textit{Cassia garrettiana}

\textit{Cassia garrettiana} Craib, a Thai medicinal plant locally known as Samae-sarn, was investigated for its active constituents against HIV-1 protease (HIV-1 PR). Bioassay-guided fractionation of the heart wood of this plant led to the isolation of a stilbene derivative (1, piceatannol) and an anthraquinone derivative (2, chrysophanol). Piceatannol exhibited appreciable inhibitory effect against HIV-1 PR with an IC₅₀ value of 25.4 \textmu g/ml, whereas that of chrysophanol was 73.5 \textmu g/ml. In addition, other two stilbenoids together with three anthraquinone derivatives were also investigated for their anti-HIV-1 PR activities. The result indicated that resveratrol possessed anti-HIV-1 PR activity with an IC₅₀ value of 85.0 \textmu g/ml, whereas other stilbenoid (oxyresveratrol) and anthraquinone derivatives (emodin, aloe-emodin, rhein) were inactive (IC₅₀ > 100 \textmu g/ml).

Key words : HIV-1 protease, inhibitory substances, \textit{Cassia garrettiana}
HIV-1 protease inhibitory substances from C. garrettiana

Cassia garrettiana Craib, locally known in Thai as Samae-sarn, is one of the plants in the Caesalpiniaceae family. In Thai traditional medicine, the heart wood of this plant has been used to cure feminine disease and as blood tonic for women. C. garrettiana has been reported to show many biological activities such as anticancer (Kimura et al., 2000), antifungal (Inamori et al., 1984), acid secretion inhibitor (Murakami et al., 1992), anti-allergy (Inamori et al., 1991) and anti-hypertension activities (Inamori et al., 1984).

Previously, we reported anti-HIV-1 PR activities of Thai medicinal plants in the Caesalpiniaceae family (Tewtrakul et al., 2003). It was found that the ethanolic extract of C. garrettiana showed appreciable activity against HIV-1 PR (IC$_{50}$ = 32 µg/ml). The present study is therefore aimed to investigate HIV-1 PR inhibitory activity of compounds isolated from C. garrettiana, as well as their related compounds.

Materials and Methods

Plant materials and preparation of an extract

The heart wood of C. garrettiana was bought from the Thai traditional drug store in Hat Yai, Songkhla, Thailand. The specimen was identified by Assoc. Prof. Dr. Sanan Subhadhirasakul, Dept. of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences. The voucher specimen (No. SKP 2060307) is kept at the Southern Center of Thai Medicinal Plants, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

The dried heart wood of C. garrettiana (3 kg) was crushed and extracted with ethanol (EtOH) 6 L x 6. The crude EtOH extract (214 g) was obtained and fractionated further into four fractions of n-hexane (10.7 g), chloroform (4.2 g), ethyl acetate (113.9 g) and water fractions (25.6 g), respectively. These fractions were then tested against HIV-1 PR activity and the isolation of active principles from the chloroform fraction was carried out using the preparative HPLC (Apollo C18, 5 µ, 250 mm x 22 mm).

The chloroform fraction (2 g) was further isolated over silica gel column using chloroform and methanol in the ratio of 9:1, 8:2, 7:3, 6:4 and 1:1, respectively and three subfractions of fr.A (0.10 g), fr. B (1.3 g) and fr. C (0.61 g) were obtained. The inhibitions at 100 µg/ml of these three subfractions against HIV-1 PR were found to be 6.7%, 90.1% and 93.3%, respectively. Fr. B was then purified using preparative HPLC with a mobile phase of 100% MeOH at flow rate 3 ml/min, to give compounds 1 (piceatannol, 35 mg) and 2...
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(Chrysophanol, 25 mg), whereas that from fr. C was compound 1 (50 mg). The condition of the preparative HPLC for fr. C was acetonitrile and 0.2% TFA (in water) in the ratio of 45:55 at flow rate of 3 ml/min.

**Enzymes and chemicals**

Recombinant HIV-1 PR, substrate peptides and acetyl pepstatin, were purchased from Sigma Chemical Co., St. Louis, USA. Resveratrol, emodin, aloe-emodin and rhein were obtained from Sigma chemical Co., Ltd., whereas oxyresveratrol was isolated from the extract of *Artocarpus lakoocha* heart wood.

**Assay of HIV-1 protease inhibitory activity**

This assay was modified from the previously reported method (Tewtrakul *et al.*, 2003). Briefly, the recombinant HIV-1 PR solution was diluted with a buffer. Two microliters of plant extract and 4 µl of HIV-1 PR solution were added to a solution containing 2 µl of buffer solution and 2 µl of substrate solution, and the reaction mixture 10 µl was incubated at 37°C for 1 hr. A control reaction was performed under the same condition but without the plant extract. The reaction was stopped by heating the reaction mixture at 90°C for 1 min. Subsequently, 20 µl of sterile water was added and an aliquot of 10 µl was analyzed by HPLC using RP-18 column. The inhibitory activity on HIV-1 PR was calculated as follows: % inhibition = (A<sub>control</sub> - A<sub>sample</sub>) x 100/ A<sub>control</sub>; whereas A is a relative peak area of the product hydrolysate. Acetyl pepstatin was used as a positive control.

**Statistics**

The results of anti-HIV-1 PR activity were expressed as mean ± S.D of three determinations. The IC<sub>50</sub> values were calculated using the Microsoft Excel program.

**Results and discussion**

The n-hexane, chloroform, ethyl-acetate and water fractions of *C. garrettiana* were tested against HIV-1 PR activity. The result indicated that chloroform fraction possessed the most potent inhibitory activity with an inhibition of 95.2% at 100 µg/ml, followed by water fraction (94.6%), ethyl acetate fraction (56.0%) and hexane fraction (0.4%), respectively (Table 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>% inhibition at 100 µg/ml</th>
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</thead>
<tbody>
<tr>
<td>EtOH extract</td>
<td>72.0±1.2</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>-0.4±0.3</td>
</tr>
<tr>
<td>Chloroform</td>
<td>95.2±0.8</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>56.0±1.5</td>
</tr>
<tr>
<td>Water</td>
<td>94.6±2.0</td>
</tr>
<tr>
<td>Acetyl pepstatin (positive control)</td>
<td>98.4±0.2</td>
</tr>
</tbody>
</table>

Compounds 1 and 2, together with their related compounds (3-7) were tested against HIV-1 PR. The result (Table 2) showed that compound 1 (piceatannol) possessed marked activity with an IC<sub>50</sub> value of 25.4 µg/ml, followed by 2 (chrysophanol, IC<sub>50</sub> = 73.5 µg/ml) and 4 (resveratrol, IC<sub>50</sub> = 85.0 µg/ml), whereas others (3, 5, 6, and 7) were inactive (IC<sub>50</sub> > 100 µg/ml). Acetyl pepstatin was used as a positive control, its IC<sub>50</sub> value was found to be 2.2 µg/ml. The structures of compounds 1-7 are shown in Figure 1.

**Table 2. HIV-1 PR inhibitory activities of compounds 1-7 isolated from *C. garrettiana***

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</th>
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</thead>
<tbody>
<tr>
<td>Piceatannol (1)</td>
<td>25.4</td>
</tr>
<tr>
<td>Chrysophanol (2)</td>
<td>73.5</td>
</tr>
<tr>
<td>Oxyresveratrol (3)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Resveratrol (4)</td>
<td>85.0</td>
</tr>
<tr>
<td>Emodin (5)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Aloe-emodin (6)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Rhein (7)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Acetyl pepstatin (positive control)</td>
<td>2.2</td>
</tr>
</tbody>
</table>
Regarding of biological activities, piceatan-nol, the main component in the chloroform fraction of \textit{C. garrettiana}, was reported to show anti-allergic activity in RBL-2H3 cell line (Inamori, et al., 1991), anti-inflammatory activity (Matsuda et al., 2000) and coronary vasodilatory activity (Inamori, et al., 1984). Chrysophanol and its derivatives, emodin, aloe-emodin and rhein, were found to be laxative agents by induction chloride uptake into the intestinal lumen (Dewick et al., 2000) and exhibited anticancer activity (Fenig et al., 2004; Cha et al., 2005).

As a result, it may be concluded that piceatan-nol and chrysophanol containing in the heart wood of \textit{C. garrettiana} are responsible for anti-HIV-1 PR activity. In addition, this is the first time to report inhibitory effect against HIV-1 PR of \textit{C. garrettiana} heartwood.

**Acknowledgments**

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**References**


