Anti-HIV-1 integrase activity of compounds from *Cassia garrettiana* heartwood

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Abstract

The ethanol extract of heartwood from Cassia garrettiana had strong anti-HIV-1 integrase (IN) activity with an IC₅₀ value of 3.0 µg/mL. Therefore, its fractions and compounds were investigated for their anti-HIV-1 IN effect using the multiplate integration assay (MIA). From bioassay-guided isolation, the ethyl acetate fraction was then separated to give five compounds which are chrysophanol (1), piceatannol (2), aloe-emodin (3), emodin (4) and cassigarol E (5). Of the tested samples, piceatannol (2) showed the highest activity against HIV-1 IN with an IC₅₀ of 17.9 µM, followed by cassigarol E (5, IC₅₀ = 72.9 µM), respectively. From the present study, this is the first report on anti-HIV-1 IN activity of Cassia garrettiana.

Key words: HIV-1 integrase, Cassia garrettiana, Caesalpiniaceae
1. Introduction

An acquired immunodeficiency syndrome (AIDS) has been rapidly spreading in many countries and is worldwide public health problem. Three enzymes that are essential for the HIV-1 life cycle are HIV-1 protease (PR), reverse transcriptase (RT) and integrase (IN). HIV-1 IN has become an appealing target for AIDS treatment since there are only two HIV-1 IN inhibitors named raltegravir and elvitegravir that are now available in the market. HIV-1 IN functions as a dimer and the integration process is composed of two steps: 3′ processing and 3′ joining (strand transfer) which finally integrates viral DNA into host chromosome (Katz & Skalka, 1994; Lucia, 2007). *Cassia garrettiana* Craib, locally known in Thai as Samae-sarn, is one of the plants in the Caesalpiniaceae family. In Thai traditional medicine, the heartwood of this plant has been used as emmenagogue and as blood tonic for women (Tewtrakul et al, 2007). Moreover, *C. garrettiana* heartwood has been used to treat Herpes zoster, leukemia, constipation and nematodes (Boonyapraphatsara & Chokchaicharoenporn, 1998). *C. garrettiana* has been reported to show many biological activities such as antifungal (Inamori et al., 1984), antitumor and antimetastatic effects (Yoshiyuki et al., 2008). Thus, searching for HIV-1 IN inhibitors from natural sources is becoming an interesting target for AIDS treatment.

2. Materials and methods

2.1 Plant material

*Cassia garrettiana* heartwoods were collected in 2010 at the Suan Ya Thai Thongnoppakhun herbal garden in Chonburi province and were identified by Thai traditional doctor, Mr. Sraupsin Thongnoppakhun and the voucher specimen number is SKP 072021901.
The sample was kept at the Herbarium of Department of Pharmacognosy and Pharmaceutical 
Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

2.2 Isolation of compounds from Cassia garrettiana extract

The dried power of Cassia garrettiana heartwood (2.1 kg) was extracted three times 
with ethanol (34 L) at room temperature. The EtOH extract (124.4 g) was successively 
partitioned to obtain hexane (8.5 g), chloroform (0.7 g), ethyl acetate (47.2 g) and water 
fractions (68.0 g) respectively. The fractions were dried under reduced pressure and then re-
dissolved in 50% DMSO for bioassay. These fractions were prepared in the concentration of 
3-100 μg/mL. The ethyl acetate fraction (10.0 g) which showed good separation on thin layer 
chromatography (TLC) and exhibited marked anti-HIV-1 IN activity (IC50 = 23.2 μg/mL), 
was further separated by silica gel column chromatography using 10% CHCl3 in methanol to 
afford 15 fractions (F1-F15). Fraction F2 (40.0 mg) was purified by column chromatography 
on sephadex LH-20 using 100% methanol to give chrysophanol (1) (orange solid, 11.3 mg, 
0.042% w/w). Fraction F3 (2.0 g) was purified by column chromatography on silica gel 
using 10 % methanol in chloroform to give subfraction (F3/1a–F3/7a). Subfraction F3/2a 
(120.0 mg) was purified by column chromatography on silica gel using 10% methanol in 
chloroform to give piceatannol (2) (white solid, 50.3 mg, 0.190% w/w). Fraction F3/5a 
(600.0 mg) was purified by column chromatography on silica gel using 20% methanol in 
chloroform to give cassigarol E (5) (pale yellow solid, 207.5 mg, 0.786% w/w). Fraction 
F3/7a (60.0 mg) was purified by sephadex LH-20 using 100% methanol to give aloe-emodin 
(3) (yellow solid, 2.0 mg, 0.007% w/w) and emodin (4) (yellow solid, 2.3 mg, 0.009% w/w), 
respectively.
The structures of compounds 1-5 were elucidated using spectroscopic techniques and compared with reported spectral data (García-Sosa et al., 2006; Li et al., 2005, a; Kametani et al., 2007; Yao et al., 2006; Li et al., 2005, b).

2.3 Anti-HIV-1 IN assay

The inhibitory effect on HIV-1 IN activity was evaluated according to a modified method from that previously reported (Tewtrakul et al, 2001). Briefly, a mixture (45 µL), composed of 12 µL of IN buffer [containing 150 mM 3-(N-morpholino) propane sulfonic acid, pH 7.2 (MOPS), 75 mM MnCl\(_2\), 5 mM dithiothritol (DTT), 25% glycerol and 500 µg/mL bovine serum albumin], 1 µL of 5 pmol/mL digoxigenin-labelled target DNA and 32 µL of sterilized water, were added into each well of a 96-well plate. Subsequently, 6 µL of sample solution and 9 µL of 1/5 dilution of integrase enzyme was added to each well and incubated at 37°C for 80 min. The wells were then washed with PBS 4 times, and 100 µL of 500 mU/mL alkaline phosphatase (AP) labelled anti-digoxigenin antibody was then added to all wells and incubated at 37°C for 1 h. The plate was washed again with washing buffer containing 0.05% Tween 20 in PBS 4 times and with PBS 4 times. Then, AP buffer (150 µL) containing 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM MgCl\(_2\) and 10 mM p-nitrophenyl phosphate was added to each well and incubated at 37°C for 1 h. Finally, the plate was measured with a microplate reader at a wavelength of 405 nm. A control consisted of a reaction mixture, 50% DMSO and an integrase enzyme, while a blank was buffer-E containing 20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1 mM ethylenediaminetetraacetate disodium salt (EDTA. 2Na), 0.1% Nonidet-P 40 (NP-40), 20% glycerol, 1 mM DTT and 4 M urea without the integrase enzyme. Suramin, a polyanionic HIV-1 IN inhibitor was used as a positive control. The % inhibition against HIV-1 IN was calculated as follows:
% Inhibition against HIV-1 IN = [(OD control - OD sample)/ OD control] x 100

Where OD = absorbance detected from each well

2.4 Statistical analysis

The values are expressed as mean ± S.E.M of four determinations. The IC₅₀ values were calculated using the Microsoft excel programme.

3. Results and discussion

Since the EtOH extract of *Cassia garrettiana* possessed potent anti-HIV-1 IN effect (IC₅₀ = 3.0 µg/mL), the hexane, chloroform, ethyl acetate and water fractions of *C. garrettiana* heartwood were then tested for anti-HIV-1 IN activity. Among the tested samples, the water fraction exhibited the most potent inhibitory activity with an IC₅₀ value of 16.9 µg/mL, followed by the ethyl acetate (IC₅₀ = 23.2 µg/mL), chloroform (IC₅₀ = 73.1 µg/mL), and hexane fractions (IC₅₀ > 100 µg/mL), respectively (Table 1). From bioassay-guided fractionation, five compounds were isolated from the ethyl acetate fraction of *C. garrettiana* and they were carried out for testing on anti-HIV-1 IN activity. The % inhibition and IC₅₀ values are shown in Table 2. The result indicated that compound 2 (piceatannol) showed the highest activity against HIV-1 IN with an IC₅₀ value of 17.9 µM, followed by compound 5 (cassigarol E) with an IC₅₀ value of 72.9 µM. Whereas, compounds 3, 4 and 1 were found to be 44.8, 40.1 and 6.1 % inhibition at concentration of 100 µM, respectively. Piceatannol has been reported to show anti-cancer (Potter et al., 2002), anti-viral (Clouser et al., 2012), anti-oxidant (Ovesná et al., 2006) and anti-inflammatory effects (Ashikawa et al., 2002). For cassigarol E, it has been reported to show anti-oxidant (Xiang et al., 2005) and
anti-allergic activities (Morikawa et al., 2010). Thai medicinal plants known as “Hua-Khao-Yen” were also reported to possess anti-HIV-1 IN activity. It was indicated that the EtOH extract of *Smilax corbularia* exhibited potent anti-HIV-1 IN effect with an IC$_{50}$ value of 1.9 µg/mL (Tewtrakul et al., 2006). It has been revealed that hydroxylated aromatic or catechol moiety is crucial for the potency against HIV-1 IN (Lameira et al., 2006). The structure activity relationships (SARs) are proposed that the aromatic moiety is claimed to interact with the divalent cation in a cation $\pi$ type interaction (Nicklaus et al., 1997). There is also a possibility of a typical charge-charge interaction between the metal ions and ionic or partial charges of the ligands. The catechol moiety is expected to produce IN inhibitors by chelating a divalent metal such as Mg$^{2+}$ or Mn$^{2+}$ on the IN active site (Fan et al., 2011). Thus piceatannol (2) and cassigarol E (5), whose structures bearing the catechol moiety may have the mechanism against HIV-1 IN through this interaction. Cassigarol E (5, IC$_{50}$ = 72.9 µM) is a dimer of piceatannol (2, IC$_{50}$ = 17.9 µM). However, it was found to exhibit lower anti-HIV-1 IN activity than the piceatannol. This result suggested that the stearic effect of compound 5 may have an influence to interfere the binding of this compound with the active site of the IN enzyme.

4. Conclusions

From the present study, three anthraquinones (1, 3 and 4) and two stilbene derivatives (2, 5) were isolated from the heartwood of *Cassia garrettiana*. It is concluded that piceatannol (2) and cassigarol E (5) are responsible for anti-HIV-1 IN activity of *C. garrettiana*. Based on anti-HIV-1 IN activity of *C. garrettiana* and its compounds, it might be suggested that this plant could be useful for treatment of AIDS.
Acknowledgments

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References


**Figure 1.** Structures of compounds 1-5 isolated from *Cassia garrettiana* heartwood
### Table 1. IC₅₀ values of the extract and fractions of *Cassia garrettiana* against HIV-1 IN activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH extract</td>
<td>3.0</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>73.1</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>23.2</td>
</tr>
<tr>
<td>Water fraction</td>
<td>16.9</td>
</tr>
<tr>
<td>Suramin (positive control)</td>
<td>10.0</td>
</tr>
</tbody>
</table>

### Table 2. % Inhibitiona and IC₅₀ values of isolated compounds from ethyl acetate fraction of *C. garrettiana* against HIV-1 IN activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>% Inhibition at various concentrations (µM)</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>Chrysophanol (1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piceatannol (2)</td>
<td>44.3±3.6</td>
<td>53.1±3.3</td>
</tr>
<tr>
<td>Aloe-emodin (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Emodin (4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cassigarol E (5)</td>
<td>13.7±3.8</td>
<td>25.4±4.7</td>
</tr>
<tr>
<td>Suramin</td>
<td>60.4±1.7</td>
<td>82.4±1.3</td>
</tr>
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*Each value represents mean ± S.E.M. of four determinations.*