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Original Article

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 IC_{50} value of 3.0 µg/mL. Therefore, its fractions and compounds were investigated for their anti-HIV-1 IN effect using the multi-plate 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 IC_{50} of 17.9 µM, followed by cassigarol E (5, IC_{50} = 72.9 µM), respectively. This is the first report on anti-HIV-1 IN activity of *Cassia garrettiana*.

Keywords: HIV-1 integrase, *Cassia garrettiana*, Caesalpiniaceae

1. Introduction

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 a 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.

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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 (IC_{50} = 23.2 µg/mL), was further separated by silica gel column chromatography using 10% $CHCl_3$ 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.*, 2005a; Kametani *et al.*, 2007; Yao *et al.*, 2006; Li *et al.*, 2005b).

2.3 Anti-HIV-1 IN assay

The inhibitory effect on HIV-1 IN activity was evaluated according to a modification of the method 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 sterilzed water, was 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:

$$\% \text{ Inhibition against HIV-1 IN} = [(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100$$

Where OD = absorbance detected from each well

2.4 Statistical analysis

The values are expressed as mean ± S.E.M of four determinations. The IC_{50} 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 (IC_{50} = 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 IC_{50} value of 16.9 µg/mL, followed by the ethyl acetate (IC_{50} = 23.2 µg/mL), chloroform (IC_{50} = 73.1 µg/mL), and hexane fractions (IC_{50} > 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 each tested for anti-HIV-1 IN activity. The % inhibition and IC_{50} values are shown in Table 2. The result indicated that compound 2 (piceatannol) showed the highest activity against HIV-1 IN with an IC_{50} value of 17.9 µM, followed by compound 5 (cassigarol E) with an IC_{50} value of 72.9 µM, whereas, compounds 3, 4 and 1 were found to produce 44.8, 40.1 and 6.1 % inhibition at concentration of 100 µM, respectively. Piceatannol has been reported to show anti-cancer

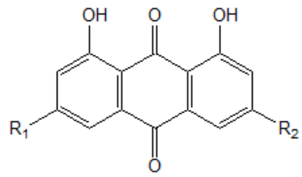
Table 1. IC_{50} values of the extract and fractions of *Cassia garrettiana* against HIV-1 IN activity

Sample	IC_{50} (µg/mL)
EtOH extract	3.0
Hexane fraction	>100
Chloroform fraction	73.1
EtOAc fraction	23.2
Water fraction	16.9
Suramin (positive control)	10.0

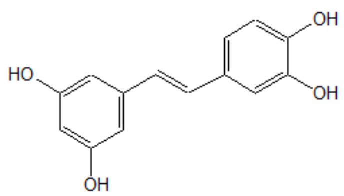
Table 2. % Inhibition[a] and IC_{50} values of isolated compounds from ethyl acetate fraction of *C. garrettiana* against HIV-1 IN activity

Compound	% Inhibition at various concentrations (mM)			
	10	30	100	IC_{50} (μM)
Chrysophanol (1)	-	-	6.1±3.9	>100
Piceatannol (2)	44.3±3.6	53.1±3.3	85.6±2.8	17.9
Aloe-emodin (3)	-	-	44.8±0.8	>100
Emodin (4)	-	-	40.1±2.7	>100
Cassigarol E (5)	13.7±3.8	25.4±4.7	65.6±4.6	72.9
Suramin(positive control)	60.4±1.7	82.4±1.3	83.1±0.7	7.0

[a]Each value represents mean ± S.E.M. of four determinations.



1: $R_1 = H$; $R_2 = CH_3$
3: $R_1 = H$; $R_2 = CH_2OH$
4: $R_1 = CH_3$; $R_2 = OH$



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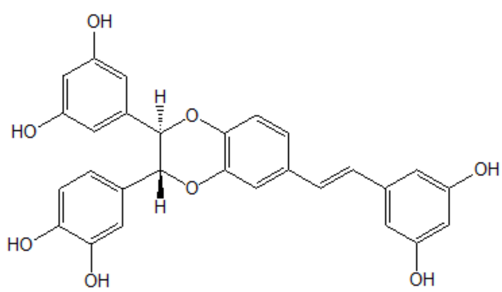

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Figure 1. Structures of compounds 1-5 isolated from *Cassia garrettiana* heartwood

(Potter *et al.*, 2002), anti-viral (Clouser *et al.*, 2012), anti-oxidant (Ovesná *et al.*, 2006) and anti-inflammatory effects (Ashikawa *et al.*, 2002). Cassigarol E 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 reported 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 proposed structure- activity relationships (SARs) involved the aromatic moiety interacting with the divalent cation in a cation π type interaction (Nicklaus *et al.*, 1997). There is also the 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 a 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 piceatannol. This result suggests that the stearic effect of compound 5 may interfere with the binding of this compound with the active site of the IN enzyme.

4. Conclusions

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 is suggested that this plant might be useful for treatment of AIDS.

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