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Original Article

Anti-HIV-1 integrase and anti-allergic activities of *Bauhinia strychnifolia*

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Abstract

A stem ethanol extract of *Bauhinia strychnifolia* and its compounds were investigated for their anti-HIV-1 integrase (IN) and anti-allergic activities. From bioassay-guided isolation, five compounds including quercetin (**1**), 3,5,7,3',5'-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside (**2**), 3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside (**3**) and a mixture of β-sitosterol (**4**) and stigmasterol (**5**) were isolated. Of the tested samples, compound **1** (quercetin) showed the highest activity against HIV-1 IN with an IC_{50} value of 15.2 μM, followed by **3** (3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside), **4+5** (mixture of β-sitosterol and stigmasterol) and **2** (3,5,7,3',5'-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside) with % inhibition of 28.2, 26.2 and 6.7 at 100 μM, respectively. With regard to anti-allergic activity, quercetin (**1**) possessed the highest anti-allergic activity with an IC_{50} of 8.1 μM, followed by **3** (3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside) and **4+5** (mixture of β-sitosterol and stigmasterol) with IC_{50} values of 52.1 and 77.5 μM, respectively. Compound **2** (3,5,7,3',5'-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside) was inactive. The present study is the first report of chemical constituents and biological activities of *Bauhinia strychnifolia*.

Keywords: anti-HIV-1 integrase, anti-allergy, *Bauhinia strychnifolia*, Fabaceae

1. Introduction

AIDS is derived from infection by a retrovirus called human immunodeficiency virus (HIV). The estimated number of people living with HIV in the world is 39.4 million. The HIV virus infects the host cell, then destroys several parts of the hosts immune system and this facilitates infections by other microbial pathogens (bacteria, virus, fungi, or protozoa). These opportunistic pathogens can cause disease and death in AIDS patients especially from tuberculosis and pneumonia. HIV-1 integrase (IN) is a dimer and its function comprises two steps: 3′ processing and 3′ joining (strand transfer), which finally integrates the viral DNA into the host chromosome (Katz and Skalka, 1994**;** Lucia, 2007). HIV-1 IN is becoming an important target for the development of novel anti HIV drugs for several reasons. First, it is an essential enzyme in the retroviral life cycle. Second, integration of the proviral DNA into transcriptional active sites of the host DNA represents a point of no return. Moreover, a mutation in any of its conserved residues (D64, D116, and E152) reduces the virus ability to be replicated (Deng *et al.*, 2006) and there are only two HIV-1 IN inhibitors named raltegravir and elvitegravir, that are available in the market.

Allergies usually occur in AIDS patients because they have high levels of allergic antibody (IgE), especially as the CD_4^+ T-cell levels drop. Allergic reactions are induced upon the binding of an allergen to IgE, which is tethered to the high-affinity IgE receptor (FcεRI) on the surface of mast cells. Following this aggregation of cell-surface receptors a cascade of intracellular events are induced, including an increase of intracellular Ca^{2+} levels, and the release of preformed inflammatory mediators from secretory granules such as histamine and β-hexosaminidase (Galli *et al.*, 2008). When an AIDS person is exposed to normally harmless

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environmental substances, (mediators) such as animal dander, house dust mites, foods, pollen, insects, and chemical agents, these events can easily occur (Vo *et al.*, 2012). These mediators are the originators of various pathophysiologic events such as acute allergic reactions including airway constriction, mucous production, and recruitment of other inflammatory cells (Galli *et al.*, 2008). When granules in mast cells or basophils degranulate, an enzyme β-hexosaminidase is usually released along with histamine; this enzyme is thus used as a biomarker for antigen-induced degranulation in a rat basophilic leukemia (RBL-2H3) cell line (Cheong *et al.*, 1998). *Bauhinia strychnifolia* Craib (Fabaceae) is known in Thai as Yanang Dang or Kha yan (Larsen and Larsen, 1984). In Thai traditional medicines, the leave, stem and root have been used to relieve fever and alcohol intoxication. The stem and leaves have also been used as anticancer, anti-allergic agents and treatment of leaves or stems with boiling water yields a tonic (Wutthithammavet, 1997). *B. strychnifolia* has been reported to possess strong cytotoxic effects against human cancer cell lines (Kaewpaiboon *et al.*, 2012). Other *Bauhinia* species, such as *B. variegata* exhibited trypsin and HIV-1 reverse transcriptase (RT) inhibitory effects (Fang *et al.*, 2010). *B. purpurea* had anti-trypsin-chymotrypsin effect and anti-cancer potential against hepatocellular carcinoma (Fang *et al.*, 2012).

Since the stem ethanol extract of *B. strychnifolia* showed good anti-HIV-1 IN and anti-allergic activities and there has been no report of the phytochemical and biological studies of this plant, this study was aimed to investigate anti-HIV-1 IN and anti-allergic activities of compounds from *B. strychnifolia.*

2. Materials and Methods

2.1 Chemicals and instruments

For anti-HIV-1 IN assay, the recombinant HIV-1 IN was expressed in *Escherichia coli*, purified according to the method described in a previous report (Jenkins *et al.*, 1996). The HIV-1 IN enzyme was stored at -80°C until used. Other chemicals were from Sigma.

For anti-allergic assay, Eagle's Minimum Essential Medium (MEM) and anti-DNP-IgE (Monoclonal anti-DNP) were purchased from Sigma; fetal calf serum (FCS) was from Gibco; dinitrophenylated bovine serum albumin was from Sigma. Other chemicals were from Sigma. 24-well and 96-well plates were from Nunc. Microplate reader (Power wave X model) was from BIO-TEK.

2.2 Plant material

Bauhinia strychnifolia stems 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, by comparison with the Flora of Thailand Vol. 4 (Larsen and Larsen, 1984). 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. The *B. strychnifolia* stems were cleaned and cut into small pieces. After that, they were dried at 50°C for 48 h in a hot air oven and then reduced to powder using a grinder.

2.3 Preparation of the plant extract and isolation

A dried powder of *Bauhinia strychnifolia* stems (4.5 kg) was extracted for 7 days with ethanol (14 x 2 L) at room temperature. The solvent was removed under reduced pressure to give the EtOH extract with 993.56 g and was kept at 4°C. The EtOH extract (993.56 g) was successively partitioned to obtain hexane (5.23 g), chloroform (153.92 g), ethyl acetate (58.64 g), water (458.99 g) and a precipitate from the ethyl acetate : water fractions (49.0 g), respectively. Using bioassay-guided fractionation, the fractions were tested for their inhibition on HIV-1 IN and allergy.

The water fraction (40.0 g) was chromatographed on Dianion HP-20 using water, water /methanol and methanol (100 ml, each) to afford 6 fractions (F1-F6). Fraction F6 (6.0 g) was separated by silica gel column chromatography using 10% methanol in ethyl acetate (50 ml, each) to give six subfractions (F6/1a–F6/6a). Subfraction F6/2a (3.47 g) was purified by column chromatography on silica gel using 10% methanol in ethyl acetate (25 ml, each) to give subfractions F6/1b-F6/3b (1.79 g). Subfraction F6/2b was purified by column chromatography on sephadex LH-20 using 100% methanol (10 ml, each) to give quercetin (**1**) (yellow solid, 3.0 mg, 0.0075% w/w). Subfraction F6/3b was purified by column chromatography on silica gel using 20% methanol in chloroform (10 ml, each) to obtain 3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside (**3**) (white solid, 5.3 mg, 0.01325% w/w).

The ethyl acetate fraction (2.0 g) was separated by chromatography on silica gel using 20% methanol in chloroform (50 ml, each) to afford 5 fractions (F1-F5). Fraction F2 (100.2 mg) was purified by column chromatography on sephadex LH-20 using 100% methanol (10 ml, each) to give 3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside (**3**) (white solid, 36 mg, 0.09% w/w). Fraction F5 (158.2 mg) was recrystallized to obtain 3,5,7,3',5'-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside (**2**) (white solid, 65 mg, 0.1625% w/w). The hexane fraction (5.23 g) was separated by chromatography on silica gel using 30% chloroform in hexane (30 ml, each) to afford 9 fractions (F1-F9). Fraction F5 (3.0 g) was recrystallized to obtain a mixture of β-sitosterol (**4**) and stigmasterol (**5**) (white solid, 50 mg, 0.956% w/w).

The structures of compounds **1-5** were elucidated using spectroscopic techniques (EIMS, IR, UV, ^{1}H NMR and ^{13}C NMR) and compared with previously reported spectral data (Guvenalp and Demimezer, 2005; Konishi *et al.*, 2003; Lu and Foo, 1999; Daengrot, 2006).

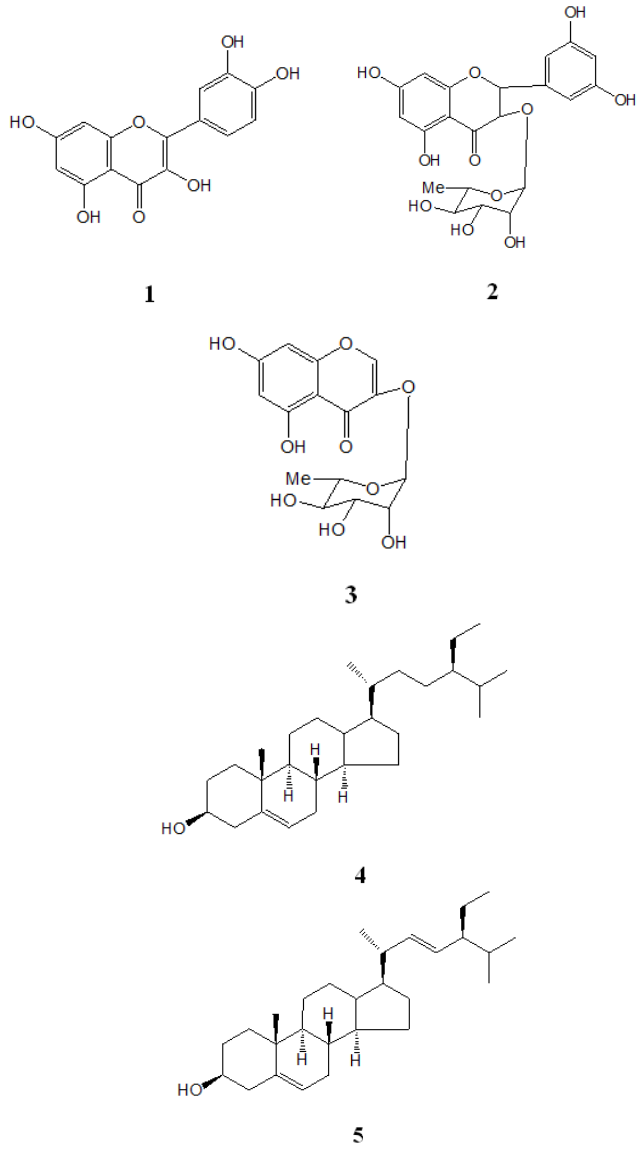

Figure 1. Structures of compounds **1-5** isolated from *Bauhinia strychnifolia* stem

2.4 Inhibitory effect on HIV-1 IN activity

The inhibitory effect on HIV-1 IN activity was evaluated according to a modification of a previously reported method (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 were 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 the 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.

2.5 Inhibitory effect on allergic reaction

The inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells, (obtained from ATCC) were evaluated by the modified method (Matsuda *et al.*, 2004). Briefly, RBL-2H3 cells were dispensed in 24-well plates at a concentration of 2×10^5 cells/well using Eagle's Minimum Essential Medium (MEM) containing 10% fetal calf serum (FCS), penicillin (100 units/mL), streptomycin (100 unit/mL) and anti-dinitrophenyl-immunoglobulin E (anti-DNP IgE) (0.45 µg/mL) and then incubated overnight at 37°C in 5% CO_2 for sensitization of the cells. The cells were washed twice with 500 µL of siraganian buffer [119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES), 0.1% bovine serum albumin (BSA) and 40 mM NaOH, pH 7.2] and then incubated in 160 µL of siraganian buffer for an additional 10 min at 37°C. After that, 20 µL of test sample solution was added to each well and incubated for 10 min, followed by addition of 20 µL of antigen (DNP-BSA, final concentration 10 µg/mL) at 37°C for 20 min to stimulate the cells to degranulate. The supernatant was transferred into a 96-well plate and incubated with 50 µL of substrate (1mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200 µL of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to siraganian buffer (final DMSO concentration was 0.1%). Ketotifen fumarate (anti-histamine drug) was used as a positive control. The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the following equation, and IC_{50} values were determined graphically:

% Inhibition = [1- (T-B-N)/(C-N)] × 100
Control (C): DNP-BSA (+), Test sample (-); Test (T) : DNP-BSA (+), Test sample (+); Blank (B) : DNP-BSA (-), Test sample (+); Normal (N) : DNP-BSA (-), Test sample (-)

2.6 Statistical analysis

The values are expressed as a mean ± S.E.M of four determinations. The IC_{50} values were calculated using the Microsoft Excel program.

3. Results and Discussion

The EtOH extract from *B. strychnifolia* stem was partitioned into hexane, chloroform, ethyl acetate and water fractions and each was tested for its anti-HIV-1 IN activity. Among them, the water fraction exhibited the most potent inhibitory activity with an IC_{50} value of 0.03 μg/mL, followed by the precipitated ethyl acetate:water fraction (IC_{50} = 7.4 μg/mL), chloroform fraction (IC_{50} = 21.1 μg/mL), ethyl acetate fraction (IC_{50} = 50.3 μg/mL) and hexane fraction (IC_{50} > 100 μg/mL), respectively (Table 1). For anti-allergy activity, the ethyl acetate fraction exhibited the most potent inhibitory activity with an IC_{50} value of 25.4 μg/mL, followed by the chloroform fraction (IC_{50} = 34.3 μg/mL), water fraction (IC_{50} = 35.1 μg/mL), hexane fraction (IC_{50} = 83.7 μg/mL) and the precipitated ethyl acetate:water fraction (IC_{50} > 100 μg/mL), respectively (Table 1). Using bioassay-guided fractionation, five compounds were isolated from the extract of *B. strychnifolia* and were tested for their inhibition of HIV-1 IN (Table 2) and allergy (Table 3).

Regarding anti-HIV-1 IN activity, compound **1** (quercetin) exhibited the highest activity against HIV-1 IN with an

Table 1. IC_{50} values[a] of the extract and fractions of *Bauhinia strychnifolia* against HIV-1 IN and allergy

Sample	IC_{50} (μg/mL)	
	Anti-HIV-1 IN	Anti-allergy
EtOH extract	6.4±0.4	47.1±1.1
Hexane fraction	>100	83.7±1.4
Chloroform fraction	21.1±1.3	34.3±1.8
EtOAc fraction	50.3±1.7	25.4±1.2
Water fraction	0.03±0.01	35.1±0.8
Precipitate ethyl acetate :Water	7.4±0.7	>100

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

Table 2. IC_{50} values[a] of isolated compounds from *Bauhinia strychnifolia* against HIV-1 IN activity

Compound	% Inhibition at various concentrations (μM)				
	3	10	30	100	IC_{50} (μM)
Quercetin **(1)**	-	47.0±2.0	66.2±1.3	88.5±0.2	15.2
3,5,7,3′,5′-Pentahydroxy-flavanonol-3-*O*-α-L-rhamnopyranoside **(2)**	-	-	-	6.7±3.5	>100
3,5,7-Trihydroxy-chromone-3-*O*-α-L-rhamnopyranoside **(3)**	-	-	-	28.2±3.2	>100
Mixture of β-sitosterol and stigmasterol **(4+5)**	-	-	-	26.2±3.6	>100
Suramin (Positive control)	29.0±2.9	60.4±1.7	82.4±1.3	83.1±0.7	7.0

[a]Each value represents a mean ± S.E.M. of four determinations.
(-) = not determined.

Table 3. IC_{50} values[a] of isolated compounds from *Bauhinia strychnifolia* against allergic reaction

Compound	% Inhibition at various concentrations (μM)				
	3	10	30	100	IC_{50} (μM)
Quercetin **(1)**	21.6±8.0	69.2±2.4	84.0±2.9	97.0±3.5	8.1
3,5,7,3′,5′-Pentahydroxy-flavanonol-3-*O*-α-L-rhamnopyranoside **(2)**	-	-	-	35.5±4.6	>100
3,5,7-Trihydroxy-chromone-3-*O*-α-L-rhamnopyranoside **(3)**	-	-13.4±3.3	25.5±5.8	77.0±7.0	52.1
Mixture of β-sitosterol and stigmasterol **(4+5)**	-	-7.2±6.2	25.2±1.8	56.3±1.6	77.5
Ketotifen fumarate (Positive control)	-	11.9±2.1	37.2±3.5	76.9±2.0	41.1

[a]Each value represents a mean ± S.E.M. of four determinations.
(-) = not determined.

IC_{50} of 15.2 μM, followed by **3** (3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside), **4+5** (mixture of β-sitosterol and stigmasterol) and **2** (3,5,7,3′,5′-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside) with 28.2, 26.2 and 6.7% inhibition at concentration of 100 μM, respectively. Moreover, compounds **1-5** are isolated for the first time from *B. strychnifolia*. It has been reported that synthesized pyridinone derivative exhibited potent anti-HIV-1 IN activity with an IC_{50} value of 70 nM (Seo *et al.*, 2011).

For anti-allergic effect, quercetin (**1**) again possessed the highest activity with an IC_{50} 8.1 μM, followed by **3** (3,5,7-trihydroxychromone-3-α-L-rhamnopyranoside, IC_{50} = 52.1 μM) and **4 + 5** (mixture of β-sitosterol and stigmasterol, IC_{50} = 77.5 μM), respectively. Whereas compound **2** (3,5,7,3′,5′-pentahydroxyflavanonol-3-*O*-α-L-rhamnopyranoside) was inactive (IC_{50} > 100 μM). The anti-allergic activity of quercetin (**1**, IC_{50} = 8.1 μM) was higher than that of ketotifen fumarate (IC_{50} = 41.1 μM), a positive control. Quercetin (**1**) has been reported to have antiviral (Ohnishi & Banna**,** 1993; Yu *et al.*, 2007), anti-inflammatory (Hämäläinen *et al.*, 2007) and anti-hypertensive activities (Perez-Vizcaino *et al.*, 2009), to prevent atherosclerotic plaque formation and to have anti-thrombotic, antihypertensive and antiarrhythmic effects (Formica & Regelson, 1995) as well as anti-allergic activity (Chirumbolo, 2011).

For anti-HIV-1 IN activity, it has been reported that hydroxylated aromatic or catechol moiety is required for the potency against HIV-1 IN (Lameira *et al.*, 2006). Structure-activity relationships (SAR) are proposed in which the aromatic moiety is found to interact with the divalent cation of the IN enzyme in a cation π 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. It has been shown that both types of interaction can co-exist in a binding site of IN enzyme and the optimal integration of salicylic acid and catechol is expected to produce HIV-1 IN inhibitors by chelating a divalent metal such as Mg^{2+} or Mn^{2+} on the IN active site (Fan *et al.*, 2011). Thus, quercetin (**1**), which is a kind of flavonoid bearing a catechol moiety may exert its action against HIV-1 IN through this interaction.

Regarding anti-allergic activity, it was found that 5-hydroxy-3,7,3′,4′-tetramethoxyflavone possessed potent anti-allergic activity against antigen-induced β-hexosaminidase release in RBL-2H3 cells. The mechanism on inhibition of cell degranulation by this compound mainly involves the inhibition of Ca^{2+} influx to the cells (Tewtrakul *et al.*, 2008). Therefore, quercetin, whose structure is similar to that of the methoxyflavone, might have its anti-allergic mechanism through this inhibition.

The secondary metabolites of plant species in the genus *Bauhinia* are mainly flavonoids. Moreover, stilbenes, steroids, terpenoids, phenolic acid and other groups such as cyanogenetic glycosides and quinones have also been found. The pharmacological activities reported in this genus include hypoglycemic, antioxidant and anticancer activities (Kaewamatawong, 2008).

4. Conclusion

The present study is the first report of chemical constituents and biological activities of *B. strychnifolia*, and quercetin is mainly responsible for both anti-HIV-1 IN and anti-allergic effects of this plant. Based on anti-HIV-1 IN and anti-allergic activities of *B. strychnifolia* and its bioactive compounds, it is suggested that this plant could be useful in the treatment of AIDS and allergy-related diseases.

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