ORIGINAL ARTICLE

Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients

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Abstract

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Anti-HIV-1 integrase activity of medicinal plants used as self medication by AIDS patients

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The extracts of selected medicinal plants used as self medication by AIDS patients were investigated for their inhibitory activities against HIV-1 integrase (HIV-1 IN) using the multiplate integration assay (MIA). Of these, the water extract of *Eclipta prostrata* (whole plant) exhibited the most potent inhibitory activity with an IC_{50} value of 4.8 μg/ml, followed by the methanol extract of *Eclipta prostrata* (whole plant, IC_{50} = 21.1 μg/ml), the water extract of *Barleria lupulina* (stem, IC_{50} = 26.4 μg/ml), the chloroform extract of *Barleria lupulina* (stem, IC_{50} = 33.0 μg/ml), the methanol extract of *Barleria lupulina* (stem, IC_{50} = 38.2 μg/ml) and the chloroform extract of *Piper betle* (leaf, IC_{50} = 39.3 μg/ml), respectively.

Key words : HIV-1 integrase, self medication, AIDS patients

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บทคัดย่อ

สุภิญญา ติ๋วตระกูล สนั่น ศุภธีรสกุล และ โสภา คำมี
ฤทธิ์ต้านเอนไซม์ HIV-1 integrase ของสมุนไพรที่ผู้ป่วยเอดส์ใช้รักษาตนเอง
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การศึกษาฤทธิ์ต้านเอนไซม์ HIV-1 integrase ของสารสกัด 36 ชนิด ที่ได้จากการสกัดด้วยคลอโรฟอร์ม เมทานอล และน้ำ ของสมุนไพรที่ผู้ป่วยเอดส์ใช้รักษาตนเอง พบว่า สารสกัดด้วยน้ำของต้นกะเม็งมีฤทธิ์ดีที่สุด (IC_{50} = 4.8 μg/ml) ตามด้วยสารสกัดด้วยเมทานอลของต้นกะเม็ง (IC_{50} = 21.1 μg/ml) สารสกัดด้วยน้ำของลำต้นเสลดพังพอนตัวผู้ (IC_{50} = 26.4 μg/ml) สารสกัดด้วยคลอโรฟอร์มของลำต้นเสลดพังพอนตัวผู้ (IC_{50} = 33.0 μg/ml) สารสกัดด้วยเมทานอลของลำต้นเสลดพังพอนตัวผู้ (IC_{50} = 38.2 μg/ml) และสารสกัดด้วยคลอโรฟอร์มของใบดีปลี (IC_{50} = 39.3 μg/ml) ตามลำดับ

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

The effectiveness of combination therapy for HIV infection with drugs targeting protease and reverse transcriptase has recently been acknowledged. However, issues of patient compliance, drug toxicity, the emergence of multidrug-resistant phenotypes, and the presence of persistent reservoir of virus replication have launched the need to develop alternative therapeutic approaches utilizing other drug targets in the viral replication cycle. One of the essential enzymes in HIV-1 life cycle is the integrase. HIV-1 integrase (HIV-1 IN) integrates transcribed double strand DNA into the host chromosome (Kat and Skalka, 1994). Fusion of viral DNA with host chromosome subsequently triggers immunodeficiency that make patients susceptible to fatal opportunistic infections. Eleven Thai medicinal plants were studied for their inhibitory activities against HIV-1 IN. Most of them have been used in the primary health care project in Thailand and by AIDS patients. They are *Zingiber zerumbet* (rhizome), *Boesenbergia pandurata* (rhizome), *Piper chaba* (fruit), *Eclipta prostrata* (whole plant), *Barleria lupulina* (leaf, stem), *Acanthus ilicifolius* (leaf and stem), *Alpinia galanga* (rhizome), *Piper betle* (leaf), *Spilanthus acmella* (whole plant), and *Coccinia glandis* (leaf). Previously, we reported HIV-1 protease (HIV-1 PR) inhibitory effects and antifungal activities against opportunistic fungal pathogens of these Thai plants (Tewtrakul *et al.*, 2003; Phongpaichit *et al.*, 2005). The aim of the present study was therefore to investigate HIV-1 IN inhibitory effects of these Thai medicinal plants used as self medication for AIDS treatment.

Materials and Methods

Plant materials and preparation of extracts

The plants were collected at the botanical garden of Prince of Songkla University and some areas in Songkhla province, Thailand, and were identified by Assoc. Prof. Dr. Sanan Subhadhirasakul. The voucher specimens are deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Ten grams of each dried plant were extracted successively by maceration for 1 week (3 times) with 200 ml of chloroform and methanol. After that, the marc left from methanol extraction was extracted with boiling water 200 ml for 3 hrs (3 times). The solvents were removed under reduced pressure to give chloroform-, methanol- and water extracts, respectively. The extracts were dissolved in 50% DMSO for bioassay.

Enzymes and chemicals

HIV-1 IN protein was expressed in *Escherichia coli*, purified according to the method described in the previous paper (Jenkins *et al.*, 1996) and stored in -80ºC before use.

Assay for HIV-1 IN inhibitory activity

Oligonucleotide substrates

Oligonucleotides of long terminal repeat donor DNA (LTR-D) and target substrate (TS) DNA were purchased from QIAGEN Operon, USA and stored at -25ºC before use. The sequence of biotinylated LTR donor DNA and its unlabelled complement were 5'-biotin-ACCCTTTTAGTCA GTGTGGAAAATCTCTAGCAGT-3' (LTR-D1) and 3'-GAAAATCAGTCACACCTTTTAGAGA TCGTCA-5' (LTR-D2), respectively; while those of the target substrate DNA (digoxigenin-labelled target DNA, TS-1) and its 3'-labelled complement were 5'-TGACCAAGGGCTAATTCACT-digoxigenin and digoxigenin-ACTGGTTCCCGATTAA GTGA-5' (TS-2), respectively.

Multiplate integration assay (MIA)

Annealing of the substrate DNA

Firstly, LTR-D1 and LTR-D2, TS-1 and TS-2 were mixed separately and then the former solution was diluted to a concentration of 2 pmol/ml, while the later one was made to 5 pmol/ml using a buffer solution containing 10 mM Tris-HCl (pH 8.0), 1mM EDTA and 100 mM KCl. Both solutions were then heated at 85ºC for 15 min in an incubator. After heating, each solution was gradually cooled to room temperature and stored at -20ºC until use.

Pretreatment of the multiplate

A 96-well plate was coated with 50 µl of a streptavidin solution containing 40 µg/ml streptavidin, 90 mM Na_2CO_3 and 10 mM KCl. After discarding streptavidin coating solution, the coated plate was washed with 300 µl of sterile water twice and phosphate buffer saline (PBS, 300 µl) twice. The blocking buffer (300 µl) containing 1% skim milk in PBS was added into each well and the plate was kept at room temperature for 30 min. After discarding the blocking buffer, each well was washed with PBS solution (300 µl) four times and then the PBS solution was completely removed. A biotinylated LTR donor DNA (50 µl) solution containing 10 mM Tris-HCl (pH 8.0), 1mM NaCl and 40 fmol/ml of LTR donor DNA was added into each well and the plate was shaken well, centrifuged and kept at room temperature for 60 min. After discarding the LTR donor solution, the microplate was washed with PBS solution four times and then each well was filled with 300 µl of PBS solution. Just before the integration reaction, the PBS solution of each well was discarded and rinsed with 300 µl of distilled water four times, and then the distilled water was removed completely.

Integration reaction

The integration reaction was evaluated according to the method previously described (Tewtrakul *et al*., 2001). 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 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 the plate and incubated at 37ºC for 80 min. After wells were washed with PBS 4 times, 100 µl of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody were added and incubated at 37ºC for 1 hr. 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 37ºC for 1 hr. Finally, the plate was measured with a microplate reader at a wavelength of 405 nm. A control composed of a reaction mixture, 50% DMSO and an integrase enzyme, and a blank composed of 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 were employed. Suramin, a polyanionic HIV-1 IN inhibitor was used as a positive control.

% Inhibition against HIV-1 IN
= [(OD control - OD sample)/ OD control] x 100
* OD = absorbance detected from each well

Statistics

For statistical analysis, the results of anti-HIV-1 IN were expressed as mean ± S.D of four determinations. The IC_{50} values were calculated using the Microsoft Excel program.

Results and Discussion

Chloroform-, MeOH- and water extracts of Thai medicinal plants widely used in the primary health care project of Thailand, were prepared and tested for their HIV-1 IN inhibitory activities. As shown in Table 1, Table 2 and Figure 1, the results indicated that the water- and MeOH extracts of *Eclipta prostrata* (whole plant) strongly inhibited HIV-1 IN activity with IC_{50} values of 4.8 and 21.1 µg/ml, respectively, followed by the water extract of *Barleria lupulina* (IC_{50} = 26.4 µg/ml), the $CHCl_3$ extract of *B. lupulina* (IC_{50} = 33.0 µg/ml), the MeOH extract of *B. lupulina* (IC_{50} = 38.2 µg/ml), the $CHCl_3$ extract of *P. betel* (IC_{50} = 39.3 µg/ml) and the water extract of *Coccinia glandis* (IC_{50} = 44.6 µg/ml). This is the first report indicating that *E. prostrata* possesses active compounds having anti-HIV-1 IN activity. Previously, we reported HIV-1 PR inhibitory activities of these Thai plants (Tewtrakul *et al*., 2003). The result indicated that *Boesenbergia pandurata* extract exhibited the most potent activity against HIV-1 PR, whereas *Eclipta prostrata* possessed only weak activity. However, *E. prostrata* significantly inhibited HIV-1 IN activity in the present study. The result may imply that this plant shows selective inhibition of HIV-1 IN but not of HIV-1 PR. Regarding the chemical constituents of *E. prostrata*, it has been reported to contain coumestans (Wagner *et al*., 1986; Yahara *et al*., 1997), triterpeniod glycosides (Singh and Bhargava, 1992), thiophene derivatives (Yahara *et al*., 1997), triterpenoid saponins (Zhao *et al*., 2001), flavonoids (Sheih and Tsai, 1985) and wedelolactone (Samiulla *et al*., 2003). Previous reports indicated that *E. prostrata* possessed various biological activities including anti-inflammatory (Kobori *et al*., 2004), immunomodulatory effect on T-lymphocytes (Liu *et al*., 2001), antimicrobial activity (Wiart *et al*., 2004) and hepatoprotective activities (Han *et al*., 1998). Therefore, the present result on anti-HIV-1 IN activity provides support for the beneficial effects of using this plant in AIDS treatment. The isolation of active principles against HIV-1 IN from *E. prostrata* is now in progress.

Table 1. HIV-1 integrase inhibitory activities of some Thai medicinal plants used as self medication for AIDS treatment at concentration of 100 µg/ml.

Botanical name	Family	Part used	% Inhibition		
			$CHCl_3$ extract	MeOH extract	Water extract
1. *Zingiber zerumbet* L.	Zingiberaceae	Rhizome	53.68±3.60	38.32±6.91	15.00±4.55
2. *Boesenbergia pandurata* Holtt.	Zingiberaceae	Rhizome	51.42±3.40	28.71±6.53	13.02±7.43
3. *Alpinia galanga* L.	Zingiberaceae	Rhizome	-5.14±5.90	55.77±4.17	56.00±5.66
4. *Piper chaba* Hunt..	Piperaceae	Fruit	28.05±2.49	23.37±2.61	-3.76±3.62
5. *Piper betle* L.	Piperaceae	Leaf	76.32±0.68	49.86±4.07	36.43±4.07
6. *Eclipta prostrata* L.	Compositae	Whole- plant	25.76±2.31	91.58±0.36	101.06±0.20
7. *Spilanthus acmella* L.	Compositae	Whole-plant	40.53±2.28	2.90±1.61	19.25±5.33
8. *Barleria lupulina* Lindl.	Acanthaceae	Leaf	29.17±3.49	61.62±0.78	60.66±3.48
9. *Barleria lupulina* Lindl.	Acanthaceae	Stem	72.58±0.88	83.69±0.56	83.93±0.59
10. *Acanthus ilicifolius* L.	Acanthaceae	Leaf and stem	37.99±5.46	45.65±2.85	40.63±1.93
11. *Murraya paniculata* L.	Rutaceae	Leaf	29.10±1.02	12.91±1.58	10.16±4.83
12. *Coccinia glandis* L.	Cucurbitaceae	Leaf	9.64±1.41	14.66±3.09	75.62±0.39

The results are expressed as mean ± S.D (n = 4).

Table 2. The IC_{50} against HIV-1 IN of active plant extracts used as self medication by AIDS patients

Botanical name	Family	Part used	% Inhibition at various concentrations				IC_{50} (µg/ml)
			3 µg/ml	10 µg/ml	30 µg/ml	100 µg/ml	
1. *Piper betle* L. ($CHCl_3$)	Piperaceae	Leaf	-	10.25±5.28	43.07±3.37	76.32±0.68	39.3
2. *Eclipta prostrata* L. (MeOH)	Compositae	Whole-plant	-	21.65±4.80	71.24±2.22	91.58±0.36	21.1
3. *Eclipta prostrata* L. (Water)	Compositae	Whole-plant	37.50±2.54	67.59±1.09	85.68±0.43	101.06±0.20	4.8
4. *Barleria lupulina* Lindl. (Water)	Acanthaceae	Stem	-	24.06±2.23	54.57±1.06	83.93±0.59	26.4
5. *Barleria lupulina* Lindl. (MeOH)	Acanthaceae	Stem	-	0.20±2.22	43.58±4.56	83.69±0.56	38.2
6. *Barleria lupulina* Lindl. ($CHCl_3$)	Acanthaceae	Stem	-	24.84±4.81	48.75±2.21	72.58±0.88	33.0
7. *Coccinia glandis* L. (Water)	Cucurbitaceae	Leaf		6.43±7.95	35.32±5.15	75.62±0.39	44.6
Suramin, a positive control			43.62±1.62	88.45±2.43	98.42±0.64	99.89±0.45	3.2

The results are expressed as mean ± S.D (n = 4)

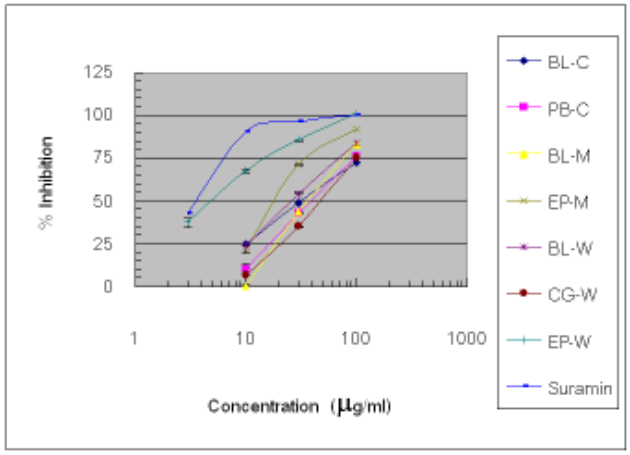

Figure 1. Dose concentration dependence against HIV-1 IN activity of some plants used as self medicaction by AIDS patients. BL (*Barleria lupulina*), PB (*Piper betle*), EP (*Eclipta prostrata*), CG (*Coccinia glandis*), C (Chloroform extract), M (Methanol extract) and W (Water extract).

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