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Short Communication

Anti - HIV-1 integrase activity of Thai Medicinal Plants

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Abstract

For the purpose of discovering anti-HIV-1 agents from natural sources, the aqueous and EtOH extracts of eight Thai plants including *Clerodendron indicum* (whole plant), *Tiliacora triandra* (stem), *Capparis micracantha* (wood), *Harrissonia perforata* (wood), *Ficus glomerata* (wood), *Diospyros decandra* (wood), *Dracaena loureiri* (heartwood), and *Tinospora crispa* (stem) were screened for their inhibitory activities against HIV-1 integrase (IN) using the multiplate integration assay (MIA). Of the EtOH extracts, *Ficus glomerata* (wood) was the most potent with an IC_{50} value of 7.8 µg/ml; whereas the water extract of *Harrisonia perforata* (wood) was the most potent aqueous extract with an IC_{50} value of 2.3 µg/ml. The isolation of active principles against HIV-1 IN from *Ficus glomerata* is now actively pursued.

Keywords: Anti-HIV-1 integrase activity, Thai plants, *Ficus glomerata, Harrisonia perforata*

1. Introduction

The acquired immunodeficiency syndrome (AIDS) has been rapidly spreading in many countries and is worldwide a public health problem. AIDS is caused by the human immunodeficiency virus type 1 or HIV-1. 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 only one HIV-1 IN inhibitor, raltegravir, is 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 and Skalka, 1994; Lucia, 2007). Nowadays, there are several drugs available used clinically as HIV-1 RT and HIV-1 PR inhibitors; however, they have some side effects such as nausea, headache and fever (Richman *et al.*, 1987). Thus, searching for HIV-1 IN inhibitors from natural sources is become an interesting target for AIDS treatment.

Eight Thai plants used for treatment of blood-realted diseases in Thai traditional medicine were investigated for their HIV-1 IN inhibitory activity. They are *Diospyros decandra* Lour., *Dracaena loureiri* Gagnep., *Clerodendron indicum* Kuntze, *Tiliacora triandra* Diels., *Harrisonia perforata* Merr., *Capparis micracantha* DC., *Ficus glomerata* Roxb, and *Tinospora crispa* Miers ex Hook. F & Thoms. Since the anti-HIV-IN activity of these plants have not been studied so far, we were interested in this topic, so that these plants might be developed as natural anti-HIV-IN agents in the future.

2. Materials and Methods

2.1 Preparation of plant extracts

Twenty grams of each dried plant were extracted two times with water and ethanol separately (150 ml each) under reflux for 3 hrs. The solvents were removed under reduced pressure to give the respective dry extracts (Table 1) and dissolved in 50% dimethyl sulfoxide (DMSO) for bioassay. Sample solutions of these extracts were prepared in the concentration range 3-100 µg/ml.

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2. 2 Multiplate integration assay (MIA)

2.2.1 Enzyme

The HIV-1 IN protein was kindly provided by Dr. Robert Craigie, National Institute of Health, Bethesda, Maryland, USA. This enzyme was expressed in *Escherichia coli* and purified according to the method described in Goldgur *et al.* (1999), and stored at -80°C before use.

2.2.2 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 -25°C before use. The sequence of biotinylated LTR donor DNA and its unlabelled complement were 5'-biotin-ACCCTTTTAGTCAGTGTGG AAAATCTCTAGCAGT-3' (LTR-D1) and 3'-GAAAATCA GTCACACCTTTTAGAGATCGTCA-5' (LTR-D2), respectively. Those of the target substrate DNA (digoxigenin-labelled target DNA, TS-1) and its 3'-labelled complement were 5'-TGACCAAGGGCTAATTCACT-digoxigenin and digoxigenin-ACTGGTTCCCGATTAAGTGA-5' (TS-2), respectively.

2.2.3 Annealing of the substrate DNA

The anti-HIV-1 IN assay was carried out following the procedure described by Tewtrakul *et al.* (2001). Two separate solutions, the first containing LTR-D1 and LTR-D2, and the second containing TS-1 and TS-2 were made to concentrations of 2 pmol/µl and 5 pmol/µl, respectively, by dilution with a buffer solution [containing 10 mM Tris-HCl (pH 8.0), 1 mM EDTA and 100 mM KCl]. The LTR- and TS solutions were heated at 85°C for 15 min in an incubator. After heating, each solution was gradually cooled down to room temperature. Both solutions were then stored at -20°C until use.

2.2.4 Pretreatment of the multiplate (microplate)

A 96 well plate was coated overnight with 50 µl of streptavidin solution containing 40 µg/ml streptavidin, 90 mM Na_2CO_3 and 10 mM KCl. After discarding the streptavidin coating solution, each of the coated plates was washed twice with sterilized water (270 µl) and twice with PBS solution (270 µl). The blocking buffer (270 µl) containing 1% skim milk in PBS was then added into each well, and the plate was kept at room temperature for 30 min. After discarding the blocking buffer, each well was washed three times with PBS solution (270 µl). A biotinylated-LTR donor DNA (50 µl) solution containing 10 mM Tris-HCl (pH 8.0), 1 mM NaCl, and 40 fmol/µl of LTR donor DNA was added into each well and kept at room temperature for 60 min. After discarding the LTR donor solution, the microplate was washed three times with PBS solution (270 µl) and then each well filled with 270 µl of PBS solution. Just before the integration reaction, the PBS solution of each well was discarded and each well again rinsed three times with 270 µl of distilled water.

2.2.5 Integration reaction

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/µl digoxigenin-labelled target DNA and 32 µl of sterilized water were added into each well of the 96-well plate. Subsequently, 6 µl of plant extract sample solution and 9 µl of 1/5 dilution of the integrase enzyme was added to the plates and incubated at 37°C for 80 min. After washing the wells three times with 270 ml PBS, 100 µl of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody were added and incubated at 37°C for 1 h. The plates were washed again three times with 270 ml washing buffer containing 0.05% Tween 20 in PBS and there after another three times with 270 ml PBS. Then, an 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 absorbance in each well was measured with a microplate reader under a wavelength of 405 nm. A control composed of a reaction mixture, 50% DMSO and integrase enzyme, while a blank was buffer-E containing 20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1mM ethylenediaminetetraacetate disodium salt (EDTA, 2Na) 0.1% Nonidet-P 40 (NP-40), 20% glycerol, 1mM DTT, and 4 M urea without the integrase enzyme (Tewtrakul *et al.*, 2001). Suramin, an HIV-1 IN inhibitor, was used as a positive control.

2.6 Statistics

For statistical analysis, the values are expressed as mean ± SEM of four determinations. The IC_{50} values were calculated using the Microsoft Excel programme.

3. Results and Discussion

The aqueous and EtOH extracts of eight Thai plants including *Clerodendron indicum* (whole plant), *Tiliacora triandra* (stem), *Capparis micracantha* (wood), *Harrissonia perforata* (wood), *Ficus glomerata* (wood), *Diospyros decandra* (wood), *Dracaena loureiri* (heartwood), and *Tinospora crispa* (stem) were screened for their inhibitory activities against HIV-1 integrase using the multiplate integration assay. From these plant extracts, *Dracaena loureiri* (heartwood, EtOH) possessed high %yield with 39.9 %w/w, followed by *Tinospora crispa* (stem, water, 12.6 %w/w), whereas those of other plants were 1.2-6.8 %w/w. Of the EtOH extracts, *Ficus glomerata* (wood) showed the highest activity against HIV-1 IN with an IC_{50} value of 7.8 µg/ml; whereas the water extract of *Harrisonia perforata* (wood) was the most potent for aqueous extracts (IC_{50} = 2.3 µg/ml). It was found that the aqueous extract of *Harissonia perforata* exhibited anti-HIV-1 IN activity higher than that of suramin, a positive control (IC_{50} = 3.4 µg/ml). Other plant extracts possessed moderate to weak activity with IC_{50} values ranging from 22.1 to over 100 µg/ml (Table 1 and Figure 1).

From the previous studies, it was reported that *Dracaena loureiri* exhibited antinociceptive and anti-pyretic activities in rats (Reanmongkol *et al.*, 2003). The extract of *Dracaena loureiri* and *Myristica fragrans* significantly inhibited proliferation of leukemia cell line (Chiratawom *et al.*, 2005). The extracts of *Tiliacora triandra* and *Harrisonia perforata* inhibited *Plasmodium falciparum* (Saiin and Markmee, 2003; Julie *et al.*, 2007). A water extract of *Tinospora crispa* decreased blood glucose and increased insulin levels in diabetic rats (Noor and Ashscoff, 1989) as well as decreased fever in male white rats (Kongsaktrakoon *et al.*, 1994), had a bitter tonic effect (Temsiririrkkul *et al.*, 1986) and possessed antioxidant activity (Cavin *et al.*, 1998). The extract of *Ficus glomerata* was found to exhibit a gastroprotective effect in rats (Rao *et al.*, 2008). Regarding constituents of *Ficus glomerata*, it has been reported to contain lupeol, β-sitosterol, β-sitosterol-β-D-glucoside, friedelin, tiglic acid ester of taraxasterol, gluanol acetate and racemosic acid (Suresh *et al.*, 1979; Baslas *et al.*, 1985; Li *et al.*, 2004). Since the EtOH extract of *Ficus glomerata* exhibited marked HIV-1 IN inhibitory activity, the isolation of active principles responsible for HIV-1 IN inhibitory effects from this plant is now in progress.

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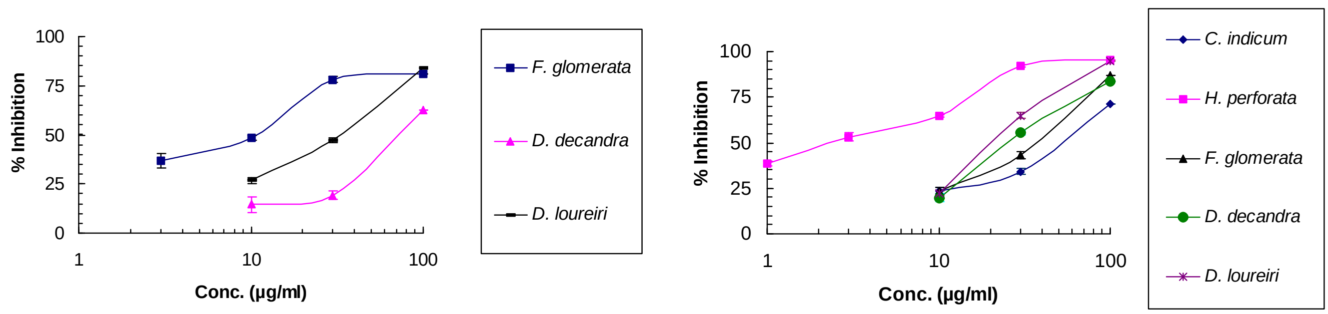
Table 1. IC_{50} values of aqueous- and ethanolic extracts of eight Thai plants against HIV-1 IN activity.

Botanical name	Family	Part used	Yield (% w/w)	Extract	IC_{50} (µg/ml)
Clerodendron indicum	Verbenaceae	whole plant	1.3	Ethanol	>100
			4.4	Water	43.5
Tiliacora triandra	Menispermaceae	stem	2.0	Ethanol	>100
			3.5	Water	>100
Capparis micracantha	Capparidaceae	wood	2.8	Ethanol	>100
			5.4	Water	>100
Harrisonia perforata	Simaroubaceae	wood	1.6	Ethanol	>100
			6.8	Water	2.3
Ficus glomerata	Moraceae	wood	1.2	Ethanol	7.8
			4.4	Water	29.5
Diospyros decandra	Ebenaceae	wood	3.0	Ethanol	69.9
			5.4	Water	27.8
Dracaena loureiri	Agavaceae	heart wood	39.9	Ethanol	28.0
			3.6	Water	22.1
Tinospora crispa	Menispermaceae	stem	6.41	Ethanol	>100
			2.6	Water	>100
Suramin(Positive control)	-	-	-	-	3.4



(A) (B)

Figure 1. Dose-response curves of EtOH (A) and aqueous extracts (B) of Thai plants against HIV-1 IN.

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