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Short communications

Anti-HIV-1 integrase activity of Thai medicinal plants in longevity preparations

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Abstract

For the purpose of discovering anti-HIV-1 agents from Thai plant preparations with a history of use in Thai traditional medicine as agents assisting longevity, the aqueous and EtOH extracts from 24 Thai plants were screened for their inhibitory activities against HIV-1 integrase (IN) using the multiplate integration assay (MIA). 11 extracts (23%), 5 ethanol and 6 water, from 8 plants *Albizia procera*, *Areca catechu*, *Bauhinia strychnifolia, Betula alnoides, Blumea balsamifera, Caesalpinia sappan, Cassia garrettiana* and *Stephania venosa*, produced extracts with IC_{50} values of < 20 µg/ml. 27 extracts (56%) had no detectable activity with IC_{50} values >100 µg/ml, while 10 extracts (21%) had IC_{50} values of between 20-97.2 µg/ml. Both the EtOH and water extracts of *Cassia garrettiana* (heartwood) and *Bauhinia strychnifolia* (vine), were the most potent with IC_{50} values of 3.0 and 5.2 µg/ml, and 6.4 µg/ml and 11.2 µg/ml, respectively. The isolation of active principles against HIV-1 IN from *Cassia garrettiana* and *Bauhinia strychnifolia* is now being further investigated.

Keywords: anti-HIV-1 integrase activity, longevity preparation, Thai plants

1. Introduction

The acquired immunodeficiency syndrome (AIDS) has been rapidly spreading in many countries and is a world-wide public health problem. AIDS develops from a retrovirus infection called human immunodeficiency virus or “HIV”. 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 named *raltegravir* (Merck & Co., Inc., U.S.A) 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) that finally integrates the viral DNA into the host chromosome (Katz and Skalka, 1994; Lucia, 2007). Currently available therapeutics against HIV infection includes inhibitors of the reverse transcriptase, protease, integrase, and fusion inhibitors. Despite the beneficial effects of these drugs in improving the quality of life of HIV/AIDS patients, the development of virus resistance is a continuing problem (Pomerantz and Horn, 2003). As a result, the search for better anti-HIV-1 IN agents continues, and much attention has been focused on natural sources, particularly plant species (Bessong *et al*., 2005). Thai medicine longevity preparations have been recommended by the Thai national health physicians for improving the quality of life and health. In the present study, 24 Thai plants described as longevity preparations were investigated for their HIV-1 IN inhibitory activity. Extracts were obtained from *Caesalpinia sappan* L., *Bauhinia strychnifolia* Craib, *Cassia garrettiana* Craib, *Cassia timoriensis* DC, *Cryptolepis buchanani* Roem & Schult, *Betula alnoides* Buch.-Ham, *Anamirta cocculus* L., *Derris scandens* Benth, *Piper chaba* Hunt, *Spilanthes acmella* L., *Albizia procera* Benth, *Fagraea fragrans* Roxb, *Ficus foveolata* Wall, *Piper nigrum* L., *Diospyros rhodocalyx* Kurz, *Morinda elliptica* Ridl, *Artocarpus heterophyllus*, *Areca catechu* L., *Stephania pierrei* Diels, *Stephania venosa* Blume, *Zingiber ottensii* Valeton, *Piper ribesioides* Wall, *Cyperus rotundus* L. and *Blumea balsamifera* L. Since the anti-HIV-IN activity of these plants has not been previously

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studied, it will be of interest to establish if any of these Thai plant preparations could assist against HIV-1 infections and in particular if they might inhibit HIV-1 IN activity. Any such effects could lead to the development of new natural anti-HIV-IN agents.

2. Materials and Methods

2.1 Plant materials

The twenty four Thai plants were collected from the Suan Ya Thai Thongnoppakhun herbal garden in 2010 and were identified by Thai traditional doctor, Mr. S. Thongnoppakhun.

2.2 Preparation of plant extracts

Twenty grams of each dried plant were extracted twice with water and ethanol separately (150 ml each) under reflux for 1 hr. The extracts were dried under reduced pressure and then re-dissolved in 50% DMSO for bioassay. Sample solutions of these extracts were prepared in the concentration range of 3-100 mg/ml.

2.3 Multiplate integration assay

The anti-HIV-1 IN assay was carried out following the procedure in a previous report (Tewtrakul *et al.*, 2001).

2.4 Statistics

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

3. Results and Discussion

The aqueous and EtOH extracts of Thai plants including *Caesalpinia sappan, Bauhinia strychnifolia, Cassia garrettiana, Cassia timoriensis, Cryptolepis buchanani, Betula alnoides, Anamirta cocculus*, *Derris scandens*, *Piper chaba*, *Spilanthes acmella*, *Albizia procera*, *Fagraea fragrans*, *Ficus foveolata*, *Piper nigrum*, *Diospyros rhodocalyx*, *Morinda elliptica*, *Artocarpus heterophyllus*, *Areca catechu*, *Stephania pierrei*, *Stephania venosa*, *Zingiber ottensii*, *Piper ribesioides*, *Cyperus rotundus* and *Blumea balsamifera* were screened for their inhibitory activities against HIV-1 IN using the MIA assay (Table 1). Both, the EtOH and water extracts of *Cassia garrettiana* (heartwood), were the most potent with IC_{50} values of 3.0 and 5.2 µg/ml respectively; whereas the EtOH extract of *Areca catechu* (fruit) was found to be 3.2 µg/ml, followed by *Bauhinia strychnifolia* (vine, IC_{50} = 6.4 µg/ml), *Stephania venosa* (tuber, IC_{50} = 9.3 µg/ml), and *Albizia procera* (bark, IC_{50} = 19.5 µg/ml). For the water extract, the most potent activity was from *Albizia procera* bark with an IC_{50} value of 5.9 µg/ml, followed by *Blumea balsamifera* (leaf, 7.8 µg/ml), *Betula alnoides* (wood, 10.2 µg/ml), *Bauhinia strychnifolia* (vine, 11.2 µg/ml), and *Areca catechu* (fruit, 15.7 µg/ml). Other plant extracts possessed moderate to weak activity with IC_{50} values that ranged from 20.1 to more than 100 µg/ml (Table 1 and Figure 1).

As preparations from most of these plants have been claimed to affect health, it was of interest to check if those with good anti HIV-1-IN activity had any common characteristics. It seemed that most have been used previously as antiinflamatory agents, anticancer agents, and perhaps anti oxidative agents, characteristics that might have some relevance to AIDS patients.

Previously, *Caesalpinia sappan* extracts exhibited antioxidant (Badami *et al.*, 2003; Yingming *et al.*, 2004; Wetwitayaklunga *et al.*, 2005), hepatoprotective (Moon *et al.*,

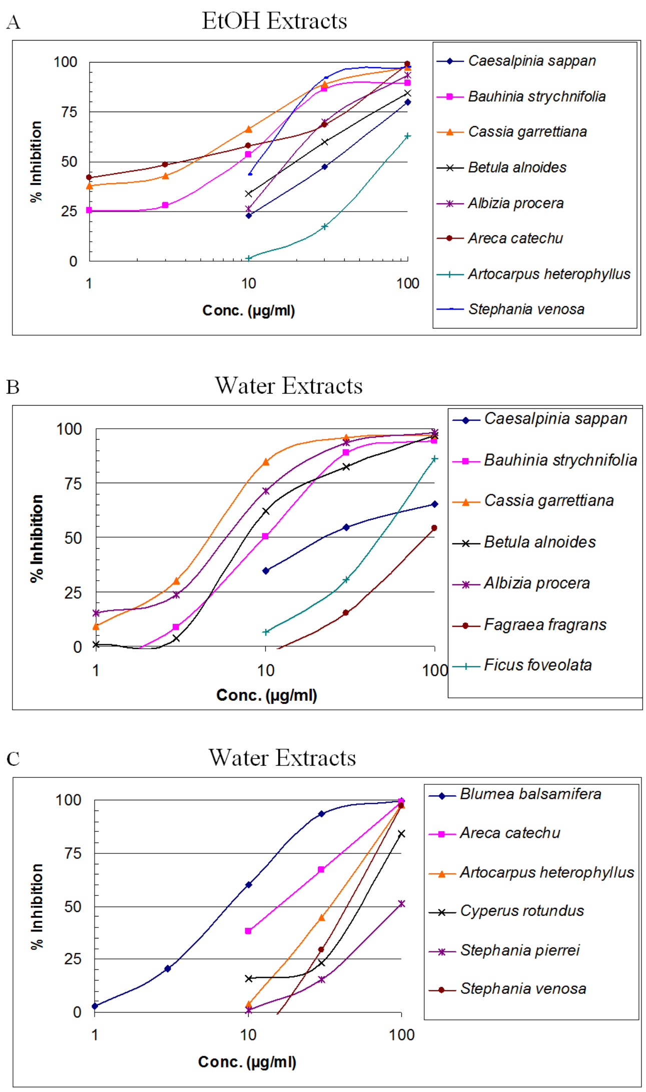

Figure 1. Dose-response curves of the EtOH (A) with IC_{50} range of 3.0-80.2 µg/ml and water extracts with IC_{50} range of 5.2-97.2 µg/ml on anti-HIV-1 IN activity (B, C) from Thai longevity plants.

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

Botanical name	Family	Part used	Yield(% w/w)	Extract	IC_{50}(μg/ml)
Cryptolepis buchanani Roem &Schult	Asclepiadaceae	vine	6.0	Ethanol	>100
			8.0	Water	>100
Betula alnoides Buch.-Ham	Betulaceae	wood	3.6	Ethanol	20.1
			6.4	Water	10.2
Caesalpinia sappan L.	Caesalpiniaceae	heartwood	17.0	Ethanol	31.0
			11.2	Water	27.7
Cassia garrettiana Craib	Caesalpiniaceae	heartwood	7.3	Ethanol	3.0
			11.6	Water	5.2
Cassia timoriensis DC	Caesalpiniaceae	wood	4.2	Ethanol	>100
			6.21	Water	>100
Blumea balsamifera L.	Compositae	leaf	12.6	Ethanol	>100
			18.3	Water	7.8
Spilanthes acmella L.	Compositae	flower	13.8	Ethanol	>100
			44.5	Water	>100
Cyperus rotundus L.	Cyperaceae	rhizome	1.9	Ethanol	>100
			10.5	Water	58.0
Diospyros rhodocalyx Kurz	Ebenaceae	bark	16.2	Ethanol	>100
			7.3	Water	>100
Bauhinia strychnifolia Craib	Fabaceae	vine	19.6	Ethanol	6.4
			13.1	Water	11.2
Fagraea fragrans Roxb	Gentianaceae	leaf	20.6	Ethanol	>100
			26.0	Water	94.3
Anamirta cocculus L.	Menispermaceae	wood	4.8	Ethanol	>100
			6.8	Water	>100
Stephania pierrei Diels	Menispermaceae	tuber	17.1	Ethanol	>100
			24.0	Water	97.2
Stephania venosa Blume	Menispermaceae	tuber	5.6	Ethanol	9.3
			3.8	Water	41.2
Albizia procera Benth	Mimosaceae	bark	20.1	Ethanol	19.5
			25.1	Water	5.9
Artocarpus heterophyllus Lam	Moraceae	heartwood	3.4	Ethanol	80.2
			2.9	Water	32.1
Ficus foveolata Wall	Moraceae	vine	6.7	Ethanol	>100
			16.1	Water	40.1
Areca catechu L.	Palmaceae	fruit	9.0	Ethanol	3.2
			14.2	Water	15.7
Derris scandens Benth	Papilionaceae	vine	5.3	Ethanol	>100
			11.2	Water	>100
Piper chaba Hunt	Piperaceae	fruit	10.1	Ethanol	>100
			6.2	Water	>100
Piper nigrum L.	Piperaceae	fruit	9.9	Ethanol	>100
			20.3	Water	>100
Piper ribesioides Wall	Piperaceae	vine	5.3	Ethanol	>100
			11.1	Water	>100
Morinda elliptica Ridl	Rubiaceae	bark	0.5	Ethanol	>100
			8.0	Water	>100
Zingiber ottensii Valeton	Zingiberaceae	rhizome	8.2	Ethanol	>100
			18.3	Water	>100
Suramin(Positive control)	-		-	-	3.4

1992), hypoglycemic (Kim *et al*., 1998; Moon *et al*., 1988), anti-inflammatory and antibacterial activities (Nirajan-Reddy *et al*., 2004). Extracts from *Cassia garrettiana* had antifungal (Inamori *et al*., 1984), antitumor and antimetastatic effects (Yoshiyuki *et al*., 2008). From *Blumea balsamifera* there were reports of anti-angiogenic (Ng *et al*., 2010), anti-inflammatory (Chen *et al*., 2010), anti-hepatocellular carcinoma (Norikura *et al*., 2008), and anti-radical scavenging activities (Fazilatun *et al*., 2005). Extracts of *Betula alnoides* had exhibited anti-inflammatory activity (Sur *et al*., 2002). *Artocarpus heterophyllus* had seed-derived lectin of versatile application in immunobiological research (Kabirs, 1998). *Areca catechu* extracts exhibited antifungal (Matan *et al*., 2011), antiradical (Li and Lin, 2010), analgesic, anti-inflammatory and anti-oxidant properties (Bhandare *et al*., 2010). Extracts from *Stephania venosa* had an anticancer effect (Nantapap *et al*., 2010) and from *Albizia procera* had anti-inflammatory (Yadava and Tripathi, 2000) and anticancer activities (Ignacimuthu *et al*., 2008).

The *Derris scandens* extracts had anti-radical scavenging effect (Sridhar *et al*., 2007), increase lymphocyte proliferation (Srivanthana ans Chavalittumrong, 2001). The *Stephania venosa* extract showed anticancer effect (Nantapap *et al*., 2010). Since the EtOH extracts of *Caesalpinia sappan* and *Bauhinia strychnifolia* exhibited marked HIV-1 IN inhibitory activity, the isolation of active principles responsible for HIV-1 IN inhibitory effect from these plants is now in progress.

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