SHORT COMMUNICATION

HIV-1 protease inhibitory effects of some selected plants in Caesalpiniaceae and Papilionaceae families

Supinya Tewtrakul[1], Sanan Subhadhirasakul[2] and Pranee Rattanasuwan[3]

Abstract

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HIV-1 protease inhibitory effects of some selected plants in Caesalpiniaceae and Papilionaceae families
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Fifty-two ethanol and water extracts of the plants in Caesalpiniaceae and Papilionaceae families were screened for their HIV-1 protease (HIV-1 PR) inhibitory activities using high performance liquid chromatography (HPLC) technique. Among the tested extracts, *Cassia garrettiana* (wood, water extract) showed the most potent inhibitory activity against HIV-1 PR, followed by *Cassia garrettiana* (wood, EtOH extract) and *Caesalpinia sappan* (wood, EtOH extract) with IC_{50} of 18, 32 and 75 µg/ml, respectively. The isolation of active substances against HIV-1 PR of these two plants will be further investigated.

Key words : HIV-1 protease, inhibitory effect, Caesalpiniaceae, Papilionaceae

[1]Ph.D.(Pharmaceutical Sciences), Asst. Prof., [2]Ph.D.(Pharmaceutical Sciences), Assoc. Prof., [3]Scientist, Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, 90112, Thailand
Corresponding e-mail: supinyat@yahoo.com
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บทคัดย่อ

สุภิญญา ติ๋วตระกูล สนั่น ศุภธีรสกุล และปราณี รัตนสุวรรณ
ฤทธิ์ต้านเอนไซม์ HIV-1 protease ของพืชบางชนิดในตระกูล Caesalpiniaceae และ Papilionaceae
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การทดสอบฤทธิ์ต้านเอนไซม์ HIV-1 protease ของสารสกัดด้วยเอทานอลและน้ำจำนวน 52 ชนิด ของพืชบางชนิดในตระกูล Caesalpiniaceae และ Papilionaceae โดยใช้เทคนิค high performance liquid chromatography (HPLC) พบว่า สารสกัดด้วยน้ำจากแก่นแสมสาร (*Cassia garrettiana*) มีฤทธิ์ต้านเอนไซม์ HIV-1 protease ดีที่สุด รองลงมาได้แก่ สารสกัดด้วยเอทานอลจากแก่นแสมสาร และสารสกัดด้วยเอทานอลจากแก่นฝาง (*Caesalpinia sappan*) โดยมีค่า IC_{50} เท่ากับ 18, 32 และ 75 μg/ml ตามลำดับ ซึ่งการแยกสารสำคัญที่มีฤทธิ์ต้านเอนไซม์ HIV-1 PR จากพืชทั้งสองต้นนี้จะได้ทำการศึกษาต่อไป

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

The human immunodeficiency virus type-1 (HIV-1), a member of retrovirus family, has been a causative agent in an acquired immunodeficiency syndrome (AIDS) which is a world-wide health problem. Therefore, drugs that can effectively prevent this disease are still in demand. A few drugs have been licensed for clinical use in AIDS therapy; they are inhibitors of viral replication such as HIV-1 protease (HIV-1 PR) and HIV-1 reverse transcriptase (HIV-1 RT). HIV-1 PR is considered to be an important target for development of anti-HIV-1 drugs, since this enzyme plays an important role in the process of infectivity and maturation of fully infectious virions of HIV-1 (Kohl *et al*., 1988). This protease belongs to the aspartyl protease class and functions as a dimer of 99 amino acids each, which possesses a residue of Asp^{25}-Thr^{26}-Gly^{27} as the catalytic triad. A nucleophillic attack of the water molecule at the active site of PR results in proteolysis of the substrate peptide. The amino acid site sequences catalyzed by this enzyme are Phe-Pro, Pro-Tyr and Leu-Phe in polyprotein (Orosalan, 1989).

Up to now, many antiviral drugs as peptidyl HIV-1 PR inhibitors are available. However, they have limited clinical benefit due to the rapid development of HIV-1 resistance and side effects (Borman *et al*., 1996; Stclair *et al*.,1991). Thus, searching for HIV-1 PR inhibitors from natural sources has become a promising approach. Recently, it has been reported that *Cassia fistula* (Caesalpiniaceae) and *Abrus precatorius* (Papilionaceae) showed potent activity against HIV-1 PR (Ma *et al*., 1998). This study was therefore aimed to investigate HIV-1 PR inhibitory effects of some Thai medicinal plants in Caesalpiniaceae and Papilionaceae families. These two families contain many plants that are well-known and some of them are used in cooking.

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. The voucher specimens are deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. Five grams of dried plant were separately extracted with 100 ml of water and ethanol (two times) under reflux for 3 hrs. After that, the solvents were removed under reduced pressure to give dry extracts which were dissolved in 50% DMSO for bioassay. The plant extracts showing activities more than 50% inhibition at 100 μg/ml were further serial by 10-

fold diluted to 10 and 1 µg/ml and screened for their IC_{50} values.

Assay of HIV-1 protease activity

This assay followed the method described by Min *et al*. (Min *et al*., 1999). Acetyl pepstatin was used as a positive control. The HPLC profile of a reaction mixture of HIV-1 protease and substrate was shown in Figure 1.

Results and Discussion

Fifty-two EtOH and water extracts of Thai medicinal plants in Caesalpiniaceae and Papilionaceae families have been tested for their HIV-1 PR inhibitory activities. The results showed that three extracts were found to considerably inhibit the HIV-1 PR activity (>50%) at a concentration of 100 µg/ml as shown in Table 1 and their IC_{50} values were displayed in Table 2. They were the EtOH extracts of wood of *Caesalpinia sappan* (57.70%, IC_{50} = 75.0 µg/ml), the wood of *Cassia garrettiana* (70.66%, IC_{50} = 32.0 µg/ml) and the water extract of the wood of *Cassia garrettiana* (87.21 %, IC_{50} = 18.0 µg/ml). Other plants that exhibited moderate activity (40-50% inhibition at 100 µg/ml) were the EtOH extracts of the pod of *Cassia grandis* (44.83%), the pod of *Cassia tora* (44.13%), the leaf of *Derris trifolia* (43.73%) and the water extract of the pod of *Cassia grandis* (43.89%). Acetyl pepstatin, which was a positive control, exhibited the strong activity by 98.47% inhibition at concentration of 100 µg/ml (IC_{50} = 0.32 µg/ml). In 1998, Ma *et al.*, reported that the seeds of *Abrus precatorius* exhibited potent HIV-1 PR inhibitory activity (Ma *et al.*, 1998), which was the contrary with our results. This may be because the collecting place, collecting time and stage of growth are different from each other, that could make the variation in constituents and number of compounds'

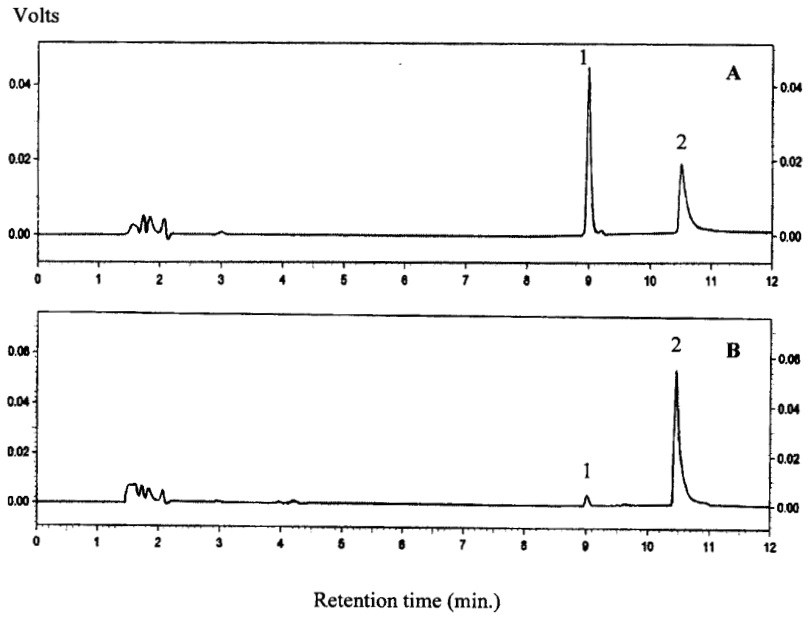

Figure 1. HPLC profile of a reaction mixture of HIV-1 PR and substrate without plant extract (control) incubated for 1 hr. at 37°C (A) and an HPLC profile of reaction mixture with the water extract of *C. garrettiana* at 100 µg/ml (B). The substrate and its hydrolysate were detected at 280 nm and their retention times were 10.447 and 8.995 min, respectively. Peak 1 was the product hydrolysate (*p*NO_2-Phe-Glu-Ala-Nle-Ser-NH_2), whereas peak 2 was the substrate (His-Lys-Ala-Arg-Val-Leu-(*p*NO_2-Phe)-Glu-Ala-Nle-Ser-NH_2).

Table 1. HIV-1 protease inhibitory activities of extracts of some plants in the Caesalpiniaceae and Papilionaceae families at concentrations of 100 µg/ml.

Botanical name	Family	Part used	% Inhibition	
			EtOH extract	Water extract
1. *Caesalpinia sappan* L.	Caesalpiniaceae	wood	**57.70±0.14**	13.42±0.10
2. *Cassis alata* L.	Caesalpiniaceae	leaf	5.27±0.12	0.14±0.11
3. *Cassis alata* L.	Caesalpiniaceae	pod	8.06±1.98	16.51±1.23
4. *Cassis alata* L	Caesalpiniaceae	seed	37.17±0.04	25.86±1.65
5. *Cassis tora* L.	Caesalpiniaceae	leaf	7.06±0.21	0.32±0.06
6. *Cassis tora* L.	Caesalpiniaceae	Pod	15.78±1.58	16.04±1.04
7. *Cassis tora* L.	Caesalpiniaceae	seed	44.13±0.34	0.43±0.27
8. *Cassia grandis* L.	Caesalpiniaceae	pod	44.83±0.47	43.89±0.49
9. *Cassia siamea* L.	Caesalpiniaceae	leaf	6.96±0.10	19.02±0.16
10. *Cassia siamea* L.	Caesalpiniaceae	pod	19.83±0.48	5.87±0.90
11. *Cassia siamea* L.	Caesalpiniaceae	seed	25.50±0.46	1.92±0.23
12. *Cassia siamea* L.	Caesalpiniaceae	flower	25.97±0.12	0.93±0.21
13. *Cassia garrettiana* Craib	Caesalpiniaceae	wood	**70.66±0.29**	**87.21±0.12**
14. *Abrus fructiculosus* Wall ex. Wight & Arn.	Papilionaceae	leaf	7.07±0.08	0.42±0.14
15. *Abrus fructiculosus* Wall ex. Wight & Arn.	Papilionaceae	seed	31.50±0.44	0.48±0.12
16. *Abrus precatorius* L.	Papilionaceae	leaf	0.90±0.44	0.15±0.20
17. *Abrus precatorius* L.	Papilionaceae	seed	21.14±3.20	5.29±1.20
18. *Glycine max* (L.) Merr.	Papilionaceae	seed	0.28±0.78	0.49±0.13
19. *Clitorea ternatea* L.	Papilionaceae	leaf	2.57±0.08	0.05±0.02
20. *Clitorea ternatea* L.	Papilionaceae	flower	0.62±0.05	5.40±0.15
21. *Crotalaria laburnifolia* L.	Papilionaceae	leaf	7.35±0.88	0.51±0.24
22. *Crotalaria laburnifolia* L.	Papilionaceae	pod	18.82±0.95	0.90±0.73
23. *Crotalaria laburnifolia* L.	Papilionaceae	seed	26.86±0.51	41.39±0.69
24. *Derris trifolia* L.	Papilionaceae	leaf	43.73±0.99	0.06±0.08
25. *Sesbania grandiflora* Pers	Papilionaceae	leaf	-0.04±0.09	8.85±0.10
26. *Sesbania grandiflora* Pers	Papilionaceae	flower	17.55±0.12	9.77±0.09
Acetyl pepstatin (positive control)			**98.47±0.27**	

The results are mean ± S.D (n = 3)

production, which finally affected the activity against HIV-1 PR.

As regards the chemical constituents, *Cassia garrettiana* was reported to contain stilbenosides named cassigarols C-G (Baba *et al*., 1991 and Baba *et al*., 1994), flavonoids, triterpenes (Hata *et al*., 1979) and some quinoids (Hata *et al*., 1978). It has been known that *Cassia garrettiana* exhibited antihistamine (Mokkhasmit *et al*., 1971), antitumor activity (Kimura *et al*., 2000), gastric secretory stimulation (Murakami *et al*., 1992), arachidonate-5-lipoxygenase inhibition and uterine stimulant activity (Loewsoponkul, 1982). However, anti-HIV-1 PR activity has not been reported so far. Concerning the chemical compounds of *Caesalpinia sappan*, flavonoids, anthraquinones and triterpenes are isolated (Namikashi *et al*., 1987). The flavonoids isolated from this plant also possess anti-complementary, antihypercholesteremia (Fuke *et al*., 1985), antibacterial, anti-inflammatory (Hong *et al*., 2002), vasorelaxing (Xie *et al*., 2000) and antioxidant activities (Hong *et al*., 2001). Since

Table 2. Inhibitory concentrations at 50% inhibition against HIV-1 PR (IC_{50}) of extracts of some plants in the Caesalpiniaceae and Papilionaceae families

Botanical name	Family	Part used	IC_{50} (μg/ml)	
			EtOH extract	Water extract
1. *Caesalpinia sappan* L.	Caesalpiniaceae	wood	75.0	>100
2. *Cassia alata* L.	Caesalpiniaceae	leaf	>100	>100
3. *Cassia alata* L.	Caesalpiniaceae	pod	>100	>100
4. *Cassia alata* L.	Caesalpiniaceae	seed	>100	>100
5. *Cassis tora* L.	Caesalpiniaceae	leaf	>100	>100
6. *Cassis tora* L.	Caesalpiniaceae	Pod	>100	>100
7. *Cassis tora* L.	Caesalpiniaceae	seed	>100	>100
8. *Cassis grandis* L.	Caesalpiniaceae	pod	>100	>100
9. *Cassia siamea* L.	Caesalpiniaceae	leaf	>100	>100
10. *Cassia siamea* L.	Caesalpiniaceae	pod	>100	>100
11. *Cassia siamea* L.	Caesalpiniaceae	seed	>100	>100
12. *Cassia siamea* L.	Caesalpiniaceae	flower	>100	>100
13. *Cassia garrettiana* Craib	Caesalpiniaceae	wood	32.0	18.0
14. *Abrus fructiculosus* Wall ex. Wight & Arn.	Papilionaceae	leaf	>100	>100
15. *Abrus fructiculosus* Wall ex. Wight & Arn.	Papilionaceae	seed	>100	>100
16. *Abrus precatorius* L.	Papilionaceae	leaf	>100	>100
17. *Abrus precatorius* L.	Papilionaceae	seed	>100	>100
18. *Glycine max* (L.) Merr.	Papilionaceae	seed	>100	>100
19. *Clitorea ternatea* L.	Papilionaceae	leaf	>100	>100
20. *Clitorea ternatea* L.	Papilionaceae	flower	>100	>100
21. *Crotalaria laburnifolia* L.	Papilionaceae	leaf	>100	>100
22. *Crotalaria laburnifolia* L.	Papilionaceae	pod	>100	>100
23. *Crotalaria laburnifolia* L.	Papilionaceae	seed	>100	>100
24. *Derris trifolia* L.	Papilionaceae	leaf	>100	>100
25. *Sesbania grandiflora* Pers	Papilionaceae	leaf	>100	>100
26. *Sesbania grandiflora* Pers	Papilionaceae	flower	>100	>100
Acetyl pepstatin (positive control)			**0.32**	

the extracts of *Cassia garrettiana* and *Caesalpinia sappan* were active against HIV-1 PR, the isolation of active principles from these two plants will be further investigated.

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