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ภาคผนวก 1 : ผลงานวิจัยตีพิมพ์ เรื่อง HIV-protease inhibitory effects of medicinal plants used as self medication by AIDS patients.

SHORT COMMUNICATION

HIV-1 protease inhibitory effects of medicinal plants used as self medication by AIDS patients

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Abstract

Tewtrakul, S., Subhadhirasakul, S., and Kummee, S.
HIV-1 protease inhibitory effects of medicinal plants used as self medication by AIDS patients
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Thirty-six chloroform-, methanol-, and water- extracts of some plants used as self mediciation by AIDS patients were investigated for their HIV-1 protease (HIV-1 PR) inhibitory activities. Of these extracts, *Boesenbergia pandurata* (rhizome, chloroform extract) showed the most potent inhibitory activity against HIV-1 PR, followed by *Boesenbergia pandurata* (rhizome, MeOH extract) and *Alpinia galanga* (rhizome, MeOH extract) with the inhibitions of 64.92, 51.92 and 48.70%, respectively, at concentration of 100 µg/ml.

Key words : HIV-1 protease, inhibitory effect, self medication, AIDS patients

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บทคัดย่อ

สุภิญญา ติ๋วตระกูล สนั่น ศุภธีรสกุล และ โสภา คำมี
ฤทธิ์ต้านเอนไซม์ HIV-1 protease ของสมุนไพรที่ผู้ป่วยเอดส์ใช้รักษาตนเอง
ว. สงขลานครินทร์ วทท. 2546 25(2) : 239-243

การศึกษาฤทธิ์ต้านเอนไซม์ HIV-1 protease ของสารสกัด 36 ชนิด ที่ได้จากการสกัดด้วยคลอโรฟอร์ม เมทานอล และน้ำ ของสมุนไพรที่ผู้ป่วยเอดส์ใช้รักษาตนเอง พบว่าสารสกัดด้วยคลอโรฟอร์มของเหง้ากระชายมีฤทธิ์ดีที่สุด ตามด้วยสารสกัดด้วยเมทานอลของเหง้ากระชาย และสารสกัดด้วยเมทานอลของเหง้าข่า โดยมีเปอร์เซนต์การยับยั้งการทำงานของ HIV-1 protease เท่ากับ 64.92, 51.92 และ 48.70% ตามลำดับ ที่ความเข้มข้นของสารสกัด 100 µg/ml

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Acquired immunodeficiency syndrome (AIDS) has evolved rapidly into an epidemic and world-wide health crisis. Extensive researches have been carried out to discover some active compounds as anti-HIV-1 agents and HIV enzyme inhibitors. However, effective agents for treatment of this disease are still in demand. Up to now, only a few drugs have been licensed for clinical use in AIDS therapy. Three HIV-1 enzymes are essential for the life cycle of the virus, HIV-1 protease (PR) processes viral proteins into functional enzymes and structural proteins, HIV-1 reverse transcriptase (RT) transcribes viral RNA to viral DNA, whereas the HIV-1 integrase (IN) integrates transcribed double strand DNA into the host chromosome (Katz and Skalka, 1994). HIV-1 PR is considered to be an important target for development of anti-HIV-1 drugs, since it plays a key role in the process of maturation and infectivity of the virus (Kohl *et al.*, 1988). This protease functions as a dimer of 11 kDa each, which contains a conserved catalytic site of Asp-Thr-Gly, and the amino acid site sequences that can be catalyzed by PR are Phe-Pro, Pro-Tyr and Leu-Phe in polyprotein (Orosalan, 1989).

The screening of medicinal plants as HIV-1 PR inhibitors has been a promising approach. Until now, there are many available antiviral drugs as HIV-1 PR inhibitors such as saquinavir (SQV), nelfinavir (NFV) and amprenavir (APV). 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). Twelve Thai medicinal plants were studied for their activities against HIV-1 PR; most of them have been used in the primary health care project of Thailand but the HIV-1 PR inhibitory activities of these plants have not been reported. They were *Zingiber zerumbet* (rhizome), *Boesenburgia pandurata* (rhizome), *Piper chaba* (fruit), *Eclipta prostrata* (whole plant), *Barleria lupulina* (leaf, stem), *Acanthus ilicifolius* (leaf and stem), *Alpinia galanga* (rhizome), *Piper betel* (leaf), *Spilanthes acmella* (whole plant), and *Coccinia grandis* (leaf). Therefore, the aim of the study was to investigate HIV-1 PR 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. The voucher specimens are deposited at the Herbarium of Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Ten grams of 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 then 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.

Apparatus

Vortex Genie 2 for mixing sample solutions was purchased from Scientific Industries, USA. Incubator for sample incubation was purchased from Memmert company, Germany. Block incubator, BT3 for stop reaction was purchased from Grant Instruments (Cambridge) Ltd., Cambridge, United Kingdom. Centrifuge 2500 was purchased from General Enterprises Marketing L.P., Bangkok, Thailand. HPLC instrument composed of SCL-10A (system controller), LC-10AD (Liquid chromatograph), DGU-14A (Degasser), SPD-10A (UV-Vis detector) and SIL-10AD (Autoinjector) for detection of substrate and products was purchased from Shimadzu Corporation, Kyoto, Japan.

Enzymes and chemicals

HIV-1 PR recombinant, substrate peptides and acetyl pepstatin, were purchased from Sigma Chemical Co., St. Louis, USA.

Assay of HIV-1 protease activity

This assay followed the method as previously described (Min *et al.*, 1999). Recombinant HIV-1 PR solution was diluted with a buffer composed of [50 mM of sodium acetate (pH 5.0), 1 mM ethylenediamine disodium (EDTA.2Na) and 2 mM 2-mercaptoethanol (2-ME)] and glycerol in the ratio of 75 : 25. The substrate peptide, His-Lys-Ala-Arg-Val-Leu-(pNO2-Phe)-Glu-Ala-Nle-Ser-NH2, was diluted with a buffer solution of 50 mM sodium acetate (pH 5.0). To a reaction mixture containing 2 µl of 50 mM buffer solution (pH 5.0) and 2 µl of substrate solution (2 mg/ml), 2 µl of plant extract and 4 µl of HIV-1 PR solution (0.025 mg/ml) were added. The reaction mixture 10 µl was incubated at 37°C for 1 hr. A control reaction was performed under the same condition, without the plant extract solution. The reaction was stopped by heating the reaction mixture at 90°C for 1 min. Then, 20 µl of sterililized water was added and an aliquot of 10 µl was analyzed by HPLC using RP-18 column. Ten microlitres of the reaction mixture was injected to a RP-18 column (4.6 x 150 mm I.D., Supelco 516 C-18-DB 5 µm, USA) and gradiently eluted with acetonitrile (15-40%) and 0.1% trifluoroacetic acid (TFA) in water, at a flow rate of 1.0 ml/min. The elution profile was monitored at 280 nm. The retention times of the substrate and *p*-NO_2-Phe-bearing hydrolysate were recorded at 10.329 and 8.976 min, respectively (Figure 1). The inhibitory activity on HIV-1 PR was calculated as follows : % inhibition = $(A_{control} - A_{sample}) \times 100/A_{control}$; where A is the relative peak

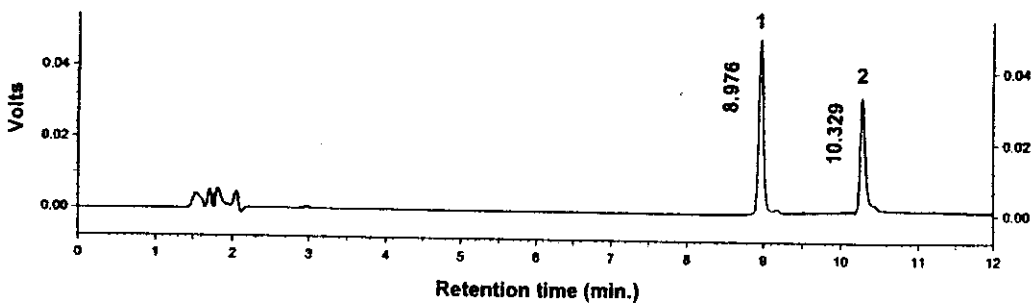

Figure 1. HPLC profile of a reaction mixture of HIV-1 PR and substrate incubated for 1 hr at 37°C. The substrate and its hydrolysate were detected at 280 nm and their retention times were 10.329 and 8.976 min, respectively. Peak 1 was the product hydrolysate (*p*-NO_2-Phe-Glu-Ala-Nle-Ser-NH_2), whereas peak 2 was the substrate (His-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-Nle-Ser-NH_2).

area of the product hydrolysate. Acetyl pepstatin was used as a positive control with the IC_{50} of 0.32 µg/ml.

Results and Discussion

Thirty-six extracts of chloroform-, MeOH- and water- extracts of Thai medicinal plants widely used in the primary health care project of Thailand, were tested for their HIV-1 PR inhibitory activities. The results showed that the chloroform- and MeOH extracts of *Boesenbergia pandurata* (rhizome) inhibited the HIV-1 PR activity by 64.92 and 51.92% inhibition, respectively, at a concentration of 100 µg/ml as shown in Table 1. Other plants that exhibited moderate activity (40-50 % inhibition at 100 µg/ml) were the MeOH extract of the rhizome of *Alpinia galanga* (48.70%) and the water extract of the whole plant of *Eclipta prostrata* (42.53%). Acetyl pepstatin, which was the positive control, exhibited strong activity, causing 98.47% inhibition at concentration of 100 µg/ml (IC_{50} = 0.32 µg/ml). Regarding the chemical constituents, the rhizome of *Boesenbergia pandurata* was reported to contain flavonoids (Herunsalee *et al.*, 1987; Trakoontiyakorn *et al.*, 2001)), flavonols (Jaipetch *et al.*, 1983), flavones (Jaipetch *et al.*, 1982) and essential oil (Pandji *et al.*, 1993). It has been reported that *Boesenbergia pandurata* exhibited antitumor (Murakami *et al.*, 1993), anti-inflammatory (Pathong *et al.*, 1989) and smooth muscle relaxant activities (Apisaksiriyakul and Ananthasarn, 1984). However, no anti-HIV-1 PR activity has been reported for this plant. Since the chloroform- and MeOH extracts of *Boesenbergia pandurata* exhibited appreciable activity against HIV-1 PR, the isolation of active principles against HIV-1 PR from this plant is now in progress.

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Table 1. HIV-1 protease inhibitory activities of some Thai 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	9.64±1.64	9.93±2.29	25.30±0.54
2. *Boesenbergia pandurata* Holtt.	Zingiberaceae	rhizome	**64.92±4.75**	**51.92±0.22**	8.21±1.75
3. *Alpinia galanga* L.	Zingiberaceae	rhizome	6.11±0.75	**48.70±1.21**	16.30±0.68
4. *Piper chaba* Hunt.	Piperaceae	fruit	9.25±0.02	28.52±0.41	-1.66±0.76
5. *Piper betel* L.	Piperaceae	leaf	15.28±0.77	16.65±0.69	25.19±0.92
6. *Eclipta prostrata* L.	Compositae	Whole plant	3.62±0.08	10.82±0.86	**42.53±2.30**
7. *Spilanthes acmella* L.	Compositae	Whole plant	12.79±1.28	19.69±0.71	15.91±0.74
8. *Barleria lupulina* Lindl.	Acanthaceae	leaf	11.28±1.19	24.93±0.63	17.48±2.63
9. *Barleria lupulina* Lindl.	Acanthaceae	stem	27.50±0.15	9.71±0.97	26.70±0.95
10. *Acanthus ilicifolius* L.	Acanthaceae	leaf, stem	15.36±0.91	9.27±0.59	19.03±0.79
11. *Murraya paniculata* L.	Rutaceae	leaf	21.83±0.14	0.62±1.08	14.95±0.71
12. *Coccinia grandis* L.	Cucurbitaceae	leaf	13.00±2.07	6.95±0.37	22.23±1.58
Acetyl pepstatin (positive control)			**98.47±0.27**		

The results are mean ±S.D (n=3)

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