ภาคผนวก 2 : ผลงานวิจัยตีพิมพ์ เรื่อง **HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt.**

SHORT COMMUNICATION

HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt.

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Abstract

Tewtrakul, S., Subhadhirasakul, S., Puripattanavong, J., and Panphadung, T.
HIV-1 protease inhibitory substances from the rhizomes of *Boesenbergia pandurata* Holtt.
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Four flavonoids (pinostrobin, pinocembrin, cardamonin and alpinetin) isolated from the ethanol extract of *Boesenbergia pandurata* Holtt. (yellow rhizome) were tested for their activities against HIV-1 protease (HIV-PR). The result showed that cardamonin exhibited an appreciable anti-HIV-1 PR activity with an IC_{50} value of 31 μg/ml.

Key words : HIV-1 protease, inhibitory substance, *Boesenbergia pandurata*

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บทคัดย่อ

สุภิญญา ติ๋วตระกูล สนั่น ศุภธีรสกุล จินดาพร ภูริพัฒนาวงษ์ และ ทัศนีย์ ปานผดุง
สารต้านเอนไซม์ HIV-1 protease จากเหง้ากระชาย (*Boesenbergia pandurata* Holtt.)
ว. สงขลานครินทร์ วทท. 2546 25(4) : 503-508

ได้นำ Flavonoids 4 ชนิด ได้แก่ pinostrobin, pinocembrin, cardamonin และ alpinetin ซึ่งแยกได้จากสารสกัดด้วยเอทานอลของเหง้ากระชายเหลือง (*Boesenbergia pandurata* Holtt.) มาศึกษาฤทธิ์ต้านเอนไซม์ HIV-1 protease (HIV-1 PR) พบว่า cardamonin มีค่าการยับยั้งการทำงานของ HIV-1 PR อย่างน่าพอใจ ด้วยค่า IC_{50} เท่ากับ 31 µg/ml

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Boesenbergia pandurata Holtt., yellow variety (so called Kra-chai in Thai) is a perennial herb belonging to the Zingiberaceae family. The rhizome has been reported to contain essential oil (Ultee, 1957), boesenbergin, cardamonin, pinostrobin (Jaipetch *et al.*, 1982), 5, 7-dimethoxyflavone, 1,8-cineole, panduratin (Pancharoen *et al.*, 1987). In the primary health care project of Thailand, the rhizome of this plant is used for the treatment of dyspepsia. As regards its biological activities, *B. pandurata* exhibited antibacterial (Ungsurungsie *et al.*, 1982), antifungal (Achararit *et al.*, 1983), anti-inflammatory, analgesic, antipyretic (Pathong *et al.*, 1989), antispasmodic (Apisaksiriyakul and Ananthasarn, 1984; Thamaree *et al.*, 1985), antitumor (Murakami *et al.*, 1993) and insecticidal activities (Areekul *et al.*, 1987).

An acquired immunodeficiency syndrome (AIDS) has evolved rapidly into an epidemic and world-wide health crisis. Many researches have been carried out to discover compounds as anti-HIV-1 agents and enzyme inhibitors of the HIV-1. However, the effective agents for treatment of this disease are still in demand since HIV-1 is resistant to some synthetic anti-HIV-1 PR inhibitors (Borman *et al.*, 1996). HIV-1 PR hydrolyzes viral polyproteins into functional enzymes and structural proteins that are essential for viral assembly (Kohl *et al.*, 1988). Therefore, HIV-1 PR is considered to be an important target for development of anti-HIV-1 drugs. The HIV-1 PR functions as a dimer which cleaves the amino acid sequence of Phe-Pro, Pro-Tyr or Leu-Phe in polyprotein (Orosalan, 1989). In a previous study, the extract of *Boesenburgia pandurata* was screened for anti-HIV-1 PR activity (Tewtrakul *et al.*, 2003). Herein, we report the isolation and the activity against HIV-1 PR of the isolated compounds from this plant.

Materials and Methods

The fresh rhizomes of *B. pandurata* Holtt. were bought from Hat Yai Market, Hat Yai, Thailand. The voucher specimen was identified and kept at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand (accession number : SKP 2060216).

Extraction and isolation

The fresh rhizomes (3 kg.) of *B. pandurata* were homogenized in 95% ethanol (2 L.) and extracted by percolation for 3 days. After filtration, the residue was repeated twice by the same procedure. The solvent was evaporated from the combined extract, affording the crude extract and a pure compound, pinostrobin (compound **1**, 3.4 g). The crude extract (15 g) was fractionated on a column of silica gel with *n*-hexane, dichloromethane, ethyl acetate and methanol as the mobile phase. Each fraction was evaporated to dryness under reduced pressure to give residues of 2.9, 1.8, 1.7 and 1.8 g of *n*-hexane, dichloromethane, ethyl acetate and methanol eluates, respectively. The hexane fraction was chromatographed over silica

gel and eluted with ethyl acetate-hexane (15 : 85) to afford compounds **2** (pinocembrin, 0.22 g) and **3** (cardamonin, 0.21 g). The dichloromethane fraction was chromatographed on silica gel and eluted with methanol-ethyl acetate (10 : 90) to give compound **4** (alpinetin, 0.12 g). The identification of compounds **1-4** was performed by comparing the ^{1}H- and ^{13}C-NMR spectra with those in the literature (Burke and Nair, 1986; Tanaka *et al.*, 1985 and Itokawa *et al.*, 1981).

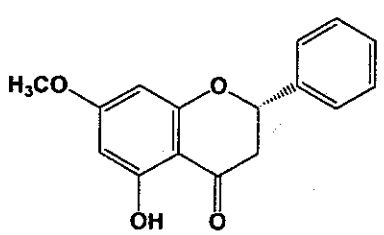
Assay of HIV-1 protease activity

This assay followed the method described in the previous paper (Tewtrakul *et al.*, 2003).

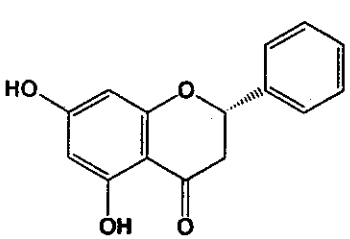
Results and Discussion

From an ethanol extract of *B. pandurata*, three flavanones (**1, 2** and **4**) and one chalcone (**3**) were isolated (Figure 1). The results showed that compound **3** (cardamonin) was the most potent against HIV-1 PR with an IC_{50} of 31 µg/ml, whereas flavanones exhibited mild inhibitory activities (Table 1). However, some flavanones have shown many biological activities such as antiherpetic activity by inhibition of plaque information of HSV-1 and HSV-2 (Lee *et al.*, 1999), heptatoprotective activity (Lin *et al.*, 1996) and anticancer activity (Min *et al.*, 1996). Acetyl pepstatin, which was a positive control, possessed 98.47% inhibition in the same condition (IC_{50} = 0.32 µg/ml). The HIV-1 PR inhibitory effects of some flavonoids such as gardenin A, myricetin and morin have previously been investigated (Brinkworth *et al.*, 1992); however the activity of chalcone compounds has not been reported so far. Both natural and synthetic chalcones are known to exhibit anti-inflammatory (Tuchinda *et al.*, 2002), anticancer (Saydam *et al.*, 2003), anti-tuberculosis (Lin *et al.*, 2002) and immunostimulatory activities (Barfod *et al.*, 2002).

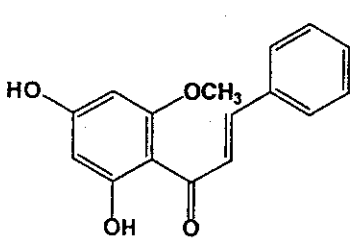
Regarding the chemical constituents of *B. pandurata*, there are reports of flavonoids (Hirunsalee *et al.*, 1987), chalcones (Trakoontiyakorn *et al.*, 2001)), flavonols (Jaipetch *et al.*, 1983), flavones (Jaipetch *et al.*, 1982) and essential oil (Ultee, 1957 and Pandji *et al.*, 1993). Kra-chai (*B.*



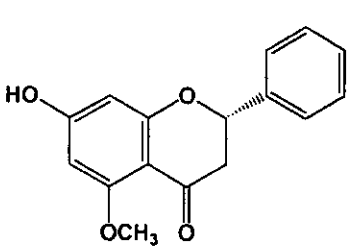
Pinostrobin (1)



Pinocembrin (2)



Cardamonin (3)



Alpinetin (4)

Figure 1 Chemical constituents isolated from the rhizomes of *B. pandurata* Holtt.

Table 1. HIV-1 protease inhibitory activities of substances isolated from the rhizomes of *B. pandurata* at a concentration of 100 μg/ml and their IC_{50} values.

Compound	Inhibition (%)	IC_{50} (μg/ml)
Pinostrobin (1)	25.52±0.56	>100
Pinocembrin (2)	25.48±0.44	>100
Cardamonin (3)	**75.11±1.44**	**31.0**
Alpinetin (4)	23.76±3.65	>100
Acetyl pepstatin (positive control)	**98.47±0.27**	**0.32**

The results are mean ± S.D (n = 3)

pandurata, yellow variety) is one of the plants in the primary health care project of Thailand for the treatment of dyspepsia and its rhizomes are used in cooking. Therefore, this plant may have a high potency to be used as self medication by AIDS patients since it possesses appreciable *in vitro* anti-HIV-1 PR activity. Its safety is also supported by a previous report on the low toxicity and lack of mortality in rats after 7 days of treatment (Pathong *et al.*, 1989). Moreover, this plant also displayed antibacterial (Ungsurungsie *et al.*, 1982) anti-inflammatory (Pathong *et al.*, 1989) and antitumor activities (Murakami *et al.*, 1993). These biological activities are also supporting evidence for using this plant in the treatment of some opportunistic infections in AIDS patients.

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