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

Supinya Tewtrakul¹, Sanan Subhadhirasakul², Jindaporn Puripattanavong³ and Tassanee Panphadung⁴

Abstract

Tewtrakul, S., Subhadhirasakul, S., Puripattanavong, J., and Panphadung, T.
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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 IC₅₀ value of 31 µg/ml.

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

¹Ph.D.(Pharmaceutical Sciences), Asst. Prof., ²Ph.D.(Pharmaceutical Sciences), Assoc. Prof., ³Ph.D.(Pharmaceutical Sciences), Asst. Prof., ⁴Master Student in Pharmaceutical Sciences, 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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**Abstract**

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), anti-tumor (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 Boesenbergia 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 (Trakootiyakorn 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. pandurata* Holtt.)

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

<table>
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<tr>
<th>Compound</th>
<th>Inhibition (%)</th>
<th>IC_{50} (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td>Pinostrobin (1)</td>
<td>25.52±0.56</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Pinocembrin (2)</td>
<td>25.48±0.44</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Cardamonin (3)</td>
<td>75.11±1.44</td>
<td>31.0</td>
</tr>
<tr>
<td>Alpinein (4)</td>
<td>23.76±3.65</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Acetyl pepstatin (positive control)</td>
<td>98.47±0.27</td>
<td>0.32</td>
</tr>
</tbody>
</table>

The results are mean ± S.D (n = 3)

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