篇章 2 : ผลงานวิจัยดีพิมพ์เรื่อง HIV-1 protease inhibitory substances from the rhizomes of Boesenbergia pandurata Holtt.
HIV-1 protease inhibitory substances from the rhizomes of *Boesenhergia pandurata* Holtt.

Supinya Tewtrakul\(^1\), Sanan Subhadhirasakul\(^2\), Jindaporn Puripattanavong\(^3\) and Tassanee Panphadung\(^4\)

---

**Abstract**

Tewtrakul, S., Subhadhirasakul, S., Puripattanavong, J., and Panphadung, T.

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


Four flavonoids (pinostrobin, pinocembrin, cardamonin and alpinetin) isolated from the ethanol extract of *Boesenhergia 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, *Boesenhergia pandurata*

\(^1\)Ph.D.(Pharmaceutical Sciences), Asst. Prof., \(^2\)Ph.D.(Pharmaceutical Sciences), Assoc. Prof., \(^3\)Ph.D.(Pharmaceutical Sciences), Asst. Prof., \(^4\)Master 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

Received, 29 January 2003  Accepted, 22 April 2003
**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 \(\mu\)g/ml, whereas flavanones exhibited mild inhibitory activities (Table 1). However, some flavanones have shown many biological activities such as antitherpetic activity by inhibition of plaque formation of HSV-1 and HSV-2 (Lee et al., 1999), hepatoprotective 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 \(\mu\)g/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 (Hirunsa-lee 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.

![Chemical constituents isolated from the rhizomes of B. pandurata Holtt.](image-url)
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<sub>50</sub> values.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Inhibition (%)</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; (μg/ml)</th>
</tr>
</thead>
<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>Alpinetin (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)

*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.

Acknowledgments

The authors thank the Thai Government Budget for awarding the grant. Thanks are also due to the Central Laboratory of Faculty of Pharmaceutical Sciences for providing an HPLC instrument, the Department of Clinical Pharmacy for providing a block incubator and the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, for providing laboratory facilities. They also thank Dr. Chatchai Watanapiromsakul, Department of Pharmacognosy and Pharmaceutical Botany, for valuable suggestions.

References

Achararit, C., Panyayong, W., and Ruchatakomut, E. 1983. Inhibitory action of some Thai herbs (medicinal plants) to fungi. Special project for the degree of B.Sc. (Pharm.), Faculty of Pharmacy, Mahidol University.


