Abstract

Extracted compounds from *Caesalpinia sappan* L. were examined for the inhibitory activity against NO, PGE_2 and TNF-α productions and on associated transcription levels using RAW264.7 cells. They were also tested for their effects on wound healing using fibroblast L929 cells. Among the compounds tested, brazilin (**8**) was the most effective against LPS-induced NO production in RAW264.7 cells with an IC_{50} value of 10.3 μM, followed by sappanchalcone (**2**, 31.0 μM). Brazilin (**8**) also inhibited PGE_2 and TNF-α production with IC_{50} values of 12.6 and 87.2 μM, respectively. The anti-inflammatory mechanism of brazilin involved down regulation of the mRNA expressions of the iNOS, COX-2 and TNF-α genes in a dose-dependent manner. An ethanol (EtOH) extract of *Caesalpinia sappan* significantly increased fibroblast proliferation, fibroblast migration and collagen production, whereas brazilin (**8**) only stimulated fibroblast migration. In addition, the EtOH extract showed no acute toxicity in mice and it was therefore safe to make use of its potent anti-inflammatory and wound healing activities. Brazilin was mainly responsible for its anti-inflammatory effect through its ability to inhibit the production of NO, PGE_2 and TNF-α. This study supports the traditional use of *Caesalpinia sappan* for treatment of inflammatory-related diseases.

Caesalpinia sappan L. (Caesalpiniaceae) has been traditionally used as blood tonic, expectorant and astringent by boiling with water. Searching for HIV-1 integrase (HIV-1 IN) inhibitors from this plant is a promising approach. The EtOH extract of *Caesalpinia sappan* and its isolated compounds were tested for their anti-HIV-1 IN effect using the multiplate integration assay (MIA) and the active compounds were determined for their mechanisms by molecular docking technique. Extraction from the heartwoods and roots of *Caesalpinia sappan* led to the isolation of nine compounds. Among the compounds tested, sappanchalcone (**2**) displayed the strongest effect against HIV-1 IN with an IC_{50} value of 2.3 μM, followed by protosappanin A (**9**, IC_{50} = 12.6 μM). Structure-activity relationships (SARs) of compounds from *Caesalpinia sappan* were found, in which the vicinal hydroxyl moiety were essential for anti-HIV-1 IN effect of compounds **2** and **9** by binding with the amino acid residues Gln148 and Thr66 in the core domain of the HIV- 1 IN enzyme, respectively.

The leaves from *Aglaia andamanica* were determined for their anti-allergic and anti-inflammatory effects using RBL-2H3 and RAW264.7 cells, respectively. Among the isolated compounds, 24-epi-piscidinol A (**5**) exhibited the highest anti-allergic activity against β-hexosaminidase release with an IC_{50} value of 9.4 μg/mL, followed by pachypodol (**2**, IC_{50} = 13.2 μg/mL) and (-)-yangambin (**3**, IC_{50} = 15.1 μg/mL); whereas other compounds possessed moderate to mild effects (IC_{50} = 24.2->85.9 μg/mL). For anti-inflammatory activity, 24-epi-piscidinol A (**5**) possessed potent activity with an IC_{50} value of 11.4 μg/mL, followed by pachypodol (**2**, IC_{50} = 11.9 μg/mL), (-)-yangambin (**3**, IC_{50} = 16.7 μg/mL) and pyramidaglain B (**9**, IC_{50} = 17.8 μg/mL), respectively; whereas other compounds exhibited moderate to mild effects (IC_{50} = 24.1->46.1 μg/mL). These active compounds could be developed as anti-allergic and anti-inflammatory agents in the futures and this is the first report of *A. andamanica* for anti-allergic and anti-inflammatory activities.

The leaves and compounds (3-100 μg/mL) from *Aglaia andamanica* were determined for the anti-HIV-1 IN effect using the multiplate integration assay (MIA) by detection the absorbance of the final product, *p*-nitrophenol, at 405 nm. The molecular docking with the HIV-1 IN of the active compound *N*-methyl-trans-4-hydroxy-L-proline (**10**) was also studied. The Swiss albino mice were used for an acute toxicity test. Among the isolated compounds, **10** showed marked anti-HIV-1 IN effect with an IC_{50} value of 11.8 μg/mL, whereas other compounds were inactive (IC_{50} > 100 μg/mL). The molecular docking of compound **10** with an HIV-1 IN enzyme was also studied. The result revealed that this compound formed the hydrogen bonding with the Thr66, Asn155 and Lys159 of the HIV-1 IN binding site. The acute toxicity of the *Aglaia andamanica* extract was not observed at the dose 2,000 mg/kg mice. This is the first report of *Aglaia andamanica* for anti-HIV-1 IN activity.

Keywords: NO production; iNOS; COX-2; RAW264.7 cells; fibroblast L929 cells; anti-HIV-1 IN activity, molecular docking; *Caesalpinia sappan*; *Aglaia andamanica*

Executive summary

Anti-inflammatory and anti-HIV-1 integrase activities of *Caesalpinia sappan* heartwood and *Aglaia andamanica* leaves

Introduction

Nitric oxide (NO) is one of the inflammatory mediators that causes inflammation in several organs. This free radical has been implicated in pathological and physiological processes including vasodilation, non-specific host defense and acute or chronic inflammation. NO has a role in host defense mechanisms by damaging pathogenic DNA and as a regulatory molecule for homeostatic activities (Kou and Schroder, 1995). However, excessive production of this free radical is pathogenic to the host tissue itself, because NO can bind with other superoxide radicals and acts as a reactive radical that directly damages the function of normal cells (Moncada *et al*., 1991).

Wound healing processes comprise a complex series of events in which repair to the damaged tissue partially or completely depends on the severity of the wounding. This process can be characterized by three overlapping phases; an inflammatory phase (consisting of hemostasis and inflammation), a proliferative