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Original Article

Anti-allergic activity of compounds from *Boesenbergia thorelii*

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Abstract

Boesenbergia thorelii extract of rhizomes and its compounds were investigated for anti-allergic activities using antigen-induced β-hexosaminidase release in RBL-2H3 cells. From bioassay-guided fractionation, the chloroform fraction was separated to afford four compounds: asaronaldehyde (1), β-sitosterol-D-glucoside (2), protocatechuic acid methyl ester (3) and 2-hydroxy-1-(3, 4-dimethoxyphenyl) ethanols (4). Their structures were elucidated on the basis of UV, IR and NMR spectroscopic data. Of these, compound 1 (asaronaldehyde) was the most active with an IC_{50} value of 24.3 μM, followed by 2 (IC_{50} = 63.3 μM) and 4 (IC_{50} = 72.6 μM); whereas compound 3 was inactive (IC_{50} > 100 μM). Anti-allergic effect of 1 (IC_{50} = 24.3 μM) was higher than that of ketotifen fumarate (IC_{50} = 41.1 μM), the positive control. This study may support the use of *B. thorelii* for treatment of allergy-related diseases. Moreover, this is the first report on biological study and chemical constituents of *B. thorelii*.

Keywords: RBL-2H3 cells, anti-allergic activity, *Boesenbergia thorelii*

1. Introduction

The immunological definition of atopy is an immediate hypersensitivity reaction to environmental antigens, mediated by IgE. Such reactions tend to run in families and these families are said to have inherited the atopy trait. Although the term allergy was originally defined as altered reactivity to exogenous antigens, it is now often used synonymously with atopy. Usually, allergies are very rapid reactions mediated by IgE. However, some allergic reactions continue for a long time (for example, when the environmental antigen cannot be easily avoided) and they develop into a late phase reaction characterized by T cell infiltrates, which is called the late phase response (Nairn and Helbert, 2002).

Rat basophilic leukemia (RBL-2H3) cells display properties of mucosal-type mast cells. The RBL-2H3 cells contain several hundred thousand IgE receptors on the membrane surface, and after sensitization with mouse monoclonal IgE, the cells respond to antigen and release histamine. Therefore, RBL-2H3 cells are used as a model cell line for histamine release (Nakatani *et al*., 2002). β-hexosaminidase is the enzyme that is stored in the secretory granules of mast cells and basophiles, and is released along with histamine when mast cells and basophils are activated. Thus, this enzyme is used as the marker of mast cell or basophils degranulation (Cheong *et al*., 1998).

Nowadays, there are many drugs available as anti-histamine and anti-allergic agents. However, many have undesirable side effects and induce adverse reactions, such as drowsiness, headache, gastrointestinal tract disturbance, fatigue and dry mouth. Since these modern medicines have some limitation in use, traditional herbs are being more closely examined for their abilities to treat allergies.

Boesenbergia thorelii,(Ganep) Loes, locally known in Thai as Kra-Chai-Pa, belongs to the Zingiberaceae family and is widely cultivated in Thailand, Malaysia and Laos.

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Sometimes, it is called in Thai as Waan-Petch-Krub or Kra-Chai-Khao. This plant is a perennial ground herb that grows to 30 cm height and has brown rhizomes. The leaf is simple with lanceolate shape. The inflorescence is terminal which comes from separate shoots arising from the rhizomes. Calyx is tubular whereas the corolla tube is exserted from calyx. There are no reports of its pharmacological activities and bioactive constituents. However, there was a report on the evaluation of genetic variation and evolutionary relationships of *Boesenbergia* in Thailand using multilocus DNA fingerprints generated by AFLP analysis (Techaprasan *et al*., 2008).

Since the ethanol (EtOH) extract of *B. thorelii* showed good anti-allergic effect (Madaka and Tewtrakul, 2011), the present study aimed to investigate the acute toxicity of the EtOH extract in mice and the anti-allergic activity of compounds from *B. thorelii* using RBL-2H3 cells.

2. Materials and Methods

2.1 Plant materials

Boesenbergia thorelii (Kra-Chai-Pa) was bought from the JATUJAK market in Bangkok, Thailand. The voucher specimen is Songklanakarind Pharmacy (SKP) 206022001. The plant material was identified by Dr Jarun Maknoi and kept in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

2.2 Preparation of the plant extract and isolation

Four kilograms (dried weight) of *B. thorelii* was ground and macerated with ethanol four times (24 L×4) at room temperature. The EtOH extract (231.25 g) was then concentrated and partitioned between 90% MeOH and hexane, the MeOH removed, water added and the extract partitioned with chloroform. After that, the water layer was partitioned with ethyl acetate (EtOAc). Each partition was evaporated to dryness *in vacuo* to give residues of hexane (93.65 g), chloroform (107.31 g), EtOAc (1.28 g) and water fractions (15.4 g), respectively. The chloroform fraction (30.0 g), which possessed marked anti-allergic activity on the release of β-hexosaminidase, was chromatographed on silica gel (800 g) using hexane/EtOAc (100:0 to EtOAc 100%) and EtOAc/methanol (100:0 to methanol 20%), to afford fifteen fractions (F1-F15).

Fraction F5 (2.76 g) was subjected to column chromatography on 120 g of silica gel eluted with hexane/EtOAc (70: 30 to EtOAc 100%) which finally afforded asaronaldehyde (1) (white needles, 20 mg). Fraction F12 (1.75 g) was subjected to column chromatography on 100 g of silica gel eluted with $CHCl_3$/EtOAc (100: 0 to EtOAc 100%) and EtOAc/methanol (100:0 to methanol 20%) to give twelve subfractions (F1a-F12a). Subfraction F12a gave β-sitosterol-D-glucoside (2) (white powder, 15 mg). Fractions F6-F7 (1.28 g) were purified by column chromatography on 100 g of silica gel using hexane/EtOAc (50:50 to EtOAc 100%) to give eight subfractions (F1b-F8b). Further column chromatography of the subfraction F6b (35 mg) was performed on 50 g of silica gel using hexane/EtOAc (70: 30 to EtOAc100%) to obtain protocatechuic acid methyl ester (3) (pale yellow solid, 10 mg). Subfraction F7b was purified by preparative TLC with EtOAc 100% (eluted 2 times) to afford 2-hydroxy-1-(3, 4-dimethoxyphenyl) ethanols (4) (white solid, 16 mg). These four compounds were isolated for the first time from *B. thorelii*. The structures of compounds 1-4 were elucidated using spectroscopic techniques and compared with reported spectral data (Patra and Mitra, 1981; Kadowaki *et al*., 2003; Chin *et al*., 2008; Takenaka *et al*., 2000).

2.3 Animals

Male and female Swiss albino mice of weight ranging from 30-40 g were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand. They were fed with standard rodent diet and water *ad libitum*. Animal study protocol was approved by The Animal Ethics Committee, Prince of Songkla University (MOE0521.11/303).

2.4 Acute toxicity test of *Boesenbergia thorelii* extract in mice

The 50% lethal dose (LD_{50}) of the ethanol extract of *B. thorelii* rhizome was estimated by the up-and down method in mice (Bruce, 1985). The animals were fasted for 6 h prior to dosing. Doses were adjusted by a constant multiplicative factor; viz. 1.5, for this experiment. The dose for each successive animal was adjusted up or down depending on the previous outcome. The extract was homogenized in Tween-80 (1%) and dissolved in distilled water and orally administered in a single dose by gavage using a stomach tube to both groups of male and female mice. Animal behaviors were observed individually at least once during the first 30 minutes after administration, periodically during the first 8 hours and daily thereafter, for a total of 7 days. Signs of toxicity, including tremor, convulsion, diarrhea, hyperactivity, sedation, grooming, loss of righting reflex, increased or decreased respiration, coma and death, were observed.

2.5 Anti-allergic activity assay

2.5.1 Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells

Inhibitory effects on the release of β-hexosaminidase from RBL-2H3 cells were evaluated by the following modified method (Matsuda *et al*., 2004). Briefly, RBL-2H3 cells were dispensed in 24-well plates at a concentration of 2×10^5 cells/well using Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 U/ml), and anti-dinitrophenyl immunoglo-

bulin E (anti-DNP IgE) (0.45 µg/ml), then incubated overnight at 37°C in 5% CO_2 for sensitization of the cells. The cells were washed twice with 500 µl of Siraganian buffer (119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM piperazine-*N*-*N*'-*bis* (2-ethanesulfonic acid) (PIPES), 0.1% bovine serum albumin (BSA) and 40 mM NaOH, pH 7.2) and then incubated in 160 µl of Siraganian buffer for an additional 10 min at 37°C. After that, 20 µl of test sample solution was added to each well and incubated for 10 min, followed by addition of 20 µl of antigen (DNP-BSA, final concentration 10 µg/ml) at 37°C for 20 min to stimulate the cells to degranulate. The supernatant was transferred into a 96-well plate and incubated with 50 µl of substrate (1 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200 µl of stop solution (0.1M Na_2CO_3/ $NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The test sample was dissolved in dimethylsulfoxide (DMSO), and the solution was added to Siraganian buffer (final DMSO concentration was 0.1%). The inhibition (%) of the release of β-hexosaminidase by the test samples was calculated by the following equation, and IC_{50} values were determined graphically:

$$\%\text{Inhibition} = \left[1 - \frac{T - B - N}{C - N}\right] \times 100$$

Control (C): DNP-BSA (+) and test sample (-); test (T): DNP-BSA(+) and test sample (+); blank (B):DNP-BSA(-) and test sample (+); normal (N): DNP-BSA(-) and test sample (-).

2.6 Statistical analysis

The results were expressed as mean ± S.E.M. of four determinations at each concentration for each sample. The IC_{50} values were calculated using the Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett's test.

3. Results and Discussion

In the acute toxicity test, the signs of toxicity, including loss of righting reflex, sleep and death, were observed. The LD_{50} values for oral administration of the ethanol extract of *B. thorelii* in male and female mice were found to be 471 mg/kg and 636 mg/kg, respectively. The obtained LD_{50} values will be useful for optimizing the suitable dose by oral administration.

Since the EtOH extract of *B. thorelii* showed appreciable anti-allergic effect (IC_{50} = 23 µg/ml) (Madaka and Tewtrakul, 2011), the hexane, chloroform, ethyl acetate and water fractions from *B.thorelii* rhizomes were then investigated for their anti-allergic activities. As shown in Table 1, the chloroform fraction of *B. thorelii* extract exhibited the most potent anti-allergic effect against antigen-induced β-hexosaminidase release as a marker of degranulation in RBL-2H3 cells with an IC_{50} value of 22.9 µg/ml, followed by the water- and hexane fractions with IC_{50} values of 49.6 and 59.1 µg/ml, respectively; whereas the ethyl acetate fraction was apparently inactive (IC_{50}>100 µg/ml). From bioassay-guided fractionation, compounds 1-4 were isolated from the chloroform fraction of *B. thorelii*, and their structures are shown in Figure 1. These compounds were investigated for their anti-allergic activities using RBL-2H3 cells. As shown in Table 2, the result indicated that compound 1 (asaronaldehyde), a monoterpene derivative, possessed the highest activity with an IC_{50} value of 24.3 µM, followed by β-sitosterol-D-glucoside (2, IC_{50} = 63.3 µM) and 2-hydroxy-1-(3, 4-dimethoxyphenyl) ethanols (4 ,IC_{50} = 72.6 µM), respectively, whereas proto-

Table 1. Anti-allergic activity[a] of fractions from *B. thorelii* rhizomes

Sample	IC_{50} (µg/ml)
EtOH extract	23.0
Hexane fraction	59.1
Chloroform fraction	22.9
EtOAc fraction	>100
Water fraction	49.6
Ketotifen fumarate (positive control)	27.4

[a]Each value represents mean ± S.E.M. of four determinications.

Table 2. Anti-allergic activity[a] of compounds from *B. thorelii* rhizomes

Compound	% Inhibition at various concentrations (µM)				IC_{50} (µM)
	0	10	30	100	
Asaronaldehyde (1)	0.0±8.1	31.8±4.0**	58.3±2.2**	73.0±3.2**	24.3
β-Sitosterol-D-glucoside (2)	0.0±3.6	-10.7±9.0	39.6±1.7**	59.5±8.0**	63.3
Protocatechuic acid methyl ester (3)	0.0±1.4	6.5±1.9	35.0±1.2**	48.7±5.3*	>100
2-Hydroxy-1-(3, 4-dimethoxyphenyl) ethanols (4)	0.0±1.3	3.9±6.7	33.6±4.2**	55.5±1.0**	72.6
Ketotifen fumarate(Positive control)	0.0±4.0	12.0±2.1*	37.3±3.5**	77.0±2.1**	41.1

Statistical significance, *$p < 0.05$; **$p < 0.01$

[a]Each value represents mean ± S.E.M. of four determinications.

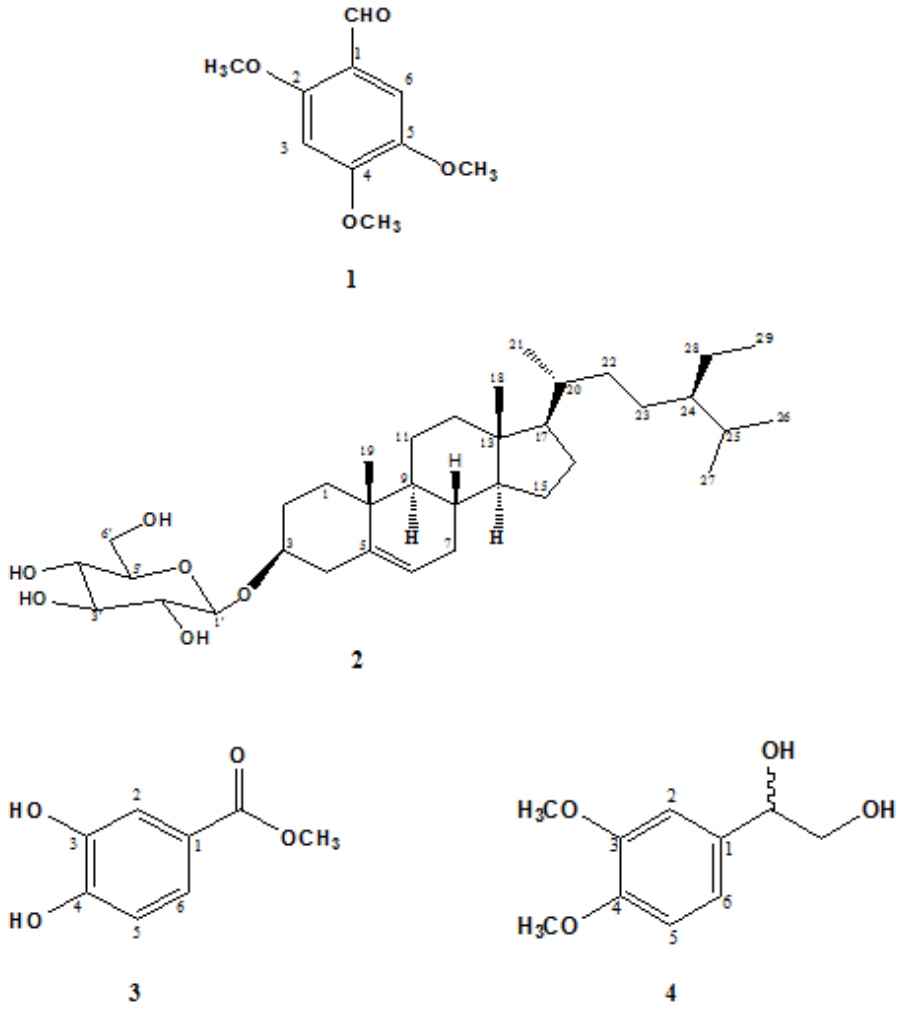

Figure 1. Structures of isolated compounds 1-4 isolated from *B. thorelii*

catechuic acid methyl ester (3) was inactive (IC_{50} > 100 µM). It was shown that the anti-allergic effect of 1 (24.3 µM) was two-fold higher than that of ketotifen fumarate (IC_{50}= 41.1 µM), the positive control.

It has been reported that some monoterpene derivatives such as 1′S-1′-acetoxychavicol acetate and 1′S-1′-acetoxyeugenol acetate possessed potent inhibitory activity against allergic reaction with IC_{50} values of 15 and 19 µM, respectively. Moreover, these two compounds also inhibited the ear passive cutaneous anaphylaxis reactions in mice and the antigen-IgE-mediated TNF-α and IL-4 production that participate in the late phase of type-I allergic reaction (Matsuda *et al*., 2003).

Regarding biological activities of the isolated compounds, the fungicidal activity of asaronaldehyde (1) applied at various concentrations against six phytopathogenic fungi, were determined *in vivo* (Lee, 2007). β-Sitosterol-D-glucoside (2) has been reported having antibacterial activity (Bayor *et al*., 2009), uv-radiation protection, antioxidant, moisture holding (Fan, 2010), antimicrobial (Chung *et al*., 2005) and gastroprotactive activities (Navarrete *et al*, 2002). Protocatechuic acid methyl ester (3) has been reported having antioxidant activity against DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical (Azizuddin and Choudhary, 2010).

4. Conclusion

From the present study, it could be concluded that asaronaldehyde (1) isolated from *B. thorelii* is mainly responsible for anti-allergic effect, followed by β-sitosterol-D-glucoside (2) and 2-hydroxy-1-(3, 4-dimethoxyphenyl) ethanols (4). Moreover, this is the first report on biological study and chemical constituents of *B. thorelii*. The results suggest that *B. thorelii* and its compounds exert anti-allergic activity and may support the use of *B. thorelii* for treatment of allergy and allergy-related diseases.

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