



Short Communication

Anti-allergic activity of some selected plants in the genus *Boesenbergia* and *Kaempferia*

Fameera Madaka and Supinya Tewtrakul*

*Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences,
Prince of Songkla University, Hat Yai, Songkhla, 90112 Thailand.*

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Abstract

Ethanol and water extracts from the rhizomes of eight selected Zingiberaceous plants, including *Boesenbergia thorelii*, *Boesenbergia* sp 1, *Boesenbergia* sp 2, *Boesenbergia* sp 3, *Kaempferia angustifolia*, *Kaempferia marginata*, *Kaempferia rotunda* and *Kaempferia* sp were tested for their anti-allergic activities using the rat basophilic leukemia strain 2H3 (RBL-2H3) cell line. Both the ethanol (EtOH) and water extracts of *Boesenbergia thorelii* exhibited the most potent anti-allergic effects against antigen-induced β -hexosaminidase release as a marker of degranulation in RBL-2H3 cells, with IC₅₀ values of 23.0 and 26.3 μ g/ml, respectively. Other extracts also had some activity from *Boesenbergia* sp 2 (EtOH, IC₅₀ = 38.3 μ g/ml), *Kaempferia marginata* (water, IC₅₀ = 38.4 μ g/ml), *Boesenbergia* sp 3 (EtOH, IC₅₀ = 49.4 μ g/ml) and *Kaempferia angustifolia* (water, IC₅₀ = 55.6 μ g/ml), whereas other *Boesenbergia* and *Kaempferia* spp had little or no activity. These findings support the use of *Boesenbergia thorelii* for the treatment of allergies and allergy-related diseases.

Keywords: RBL-2H3 cells, anti-allergic activity, Zingiberaceae

1. Introduction

The Zingiberaceae is among the plant families which are widely distributed throughout the tropics particularly in Southeast Asia. Zingiberaceae is one of the largest plant families from the order Zingiberales, with approximately 50 genera and over 1,000 species (Holttum, 1950). Various species of Zingiberaceae are used as spices, medicines, flavouring agents and as the source of certain dyes (Burkill, 1966). Several studies have shown that members of the Zingiberaceae family contain a wide variety of biologically active phytochemicals including some with antioxidative, anti-inflammatory, anticancer and anti-tumour properties (Ling et al., 2009).

Type I allergy is induced by certain types of antigens such as from foods, dust mites, medicines, cosmetics, mold

spores, and pollen. This class of antigen induces the production of antigen-specific IgE antibodies that bind to receptors on mast cells or basophils (Matsuda et al., 2004). It has been shown that the development of this allergy is a biphasic reaction, comprising an early and a late phase. The early phase reaction is a type I allergy and occurs within minutes when the sensor cells are activated to produce the mediators such as histamine and serotonin that are released from the cell. These mediators induce vasodilation, mucous secretion and bronchoconstriction (Matsuda et al., 2004). β -hexosaminidase is located in the secretory granules of mast cells where histamines are stored, and is released along with histamine when mast cells are immunologically activated. For this reason, β -hexosaminidase is considered to be a degranulation marker, and has been widely used for the biochemical studies of allergy. It is also used for screening for anti-allergic agents (Cheong et al., 1998). The late phase reaction occurs within 4-6 h after the early phase reaction in type I allergy. The mediators such as the cytokines (TNF- α , IL-4, etc) from the cells are involved in the late phase. These mediators increase

* Corresponding author.

Email address: supinyat@yahoo.com

endothelial cell adhesion and the recruitment of inflammatory cells to the affected site (Matsuda *et al.*, 2004).

There have been many reports on the anti-allergic effects of some Thai plants in the Zingiberaceae family, such as *Curcuma longa* (Yano *et al.*, 2000), *Curcuma zedoaria* (Matsuda *et al.*, 2004), *Alpinia galanga* (Yoshikawa *et al.*, 2004), and *Kaempferia parviflora* (Tewtrakul *et al.*, 2007). The rhizome of *Curcuma longa* has long been used in Thai traditional medicine for treatment of itching and other skin diseases, whereas *Curcuma zedoaria* has been used as a substitute for *Curcuma longa* and has recently been reported to show anti-allergic activity. *Alpinia galanga* has been used to treat stomachache and as a carminative, antiflatulant, anti-fungal and anti-itching agent. *Kaempferia parviflora* has been used for treating allergies, gastrointestinal disorders, fungal infections and impotence, *Zingiber cassumunar* for treatment of inflammation and skin diseases (Jeenapongsa *et al.*, 2003), *Zingiber officinale* as an antiasthmatic agent (Mascolo *et al.*, 1989) and *Zingiber zerumbet* as an antiflatulant and anti-inflammatory agent (Wuththithamavet, 2006).

Since several Zingiberaceous plants have been used in the traditional treatment of allergies and allergy-related diseases, this study aimed to investigate in more detail the anti-allergic activities of these selected plants in order to acquire scientific support for their uses by Thai traditional doctors.

2. Materials and Methods

2.1 Plant materials

The rhizomes of some Zingiberaceae plants including *Boesenbergia thorelii* (Kra Chai Pa), *Boesenbergia* sp 1 (Ron Thong), *Boesenbergia* sp 2 (Kai Dang), *Boesenbergia* sp 3 (Kai Dam), *Kaempferia angustifolia* (Thao Nhang Haeng), *Kaempferia marginata* (Kra Jae Jhun), *Kaempferia rotunda* (Thip-pa-ya-Nate) and *Kaempferia* sp (Prauh Pa) were all bought from the JATUJAK market in Bangkok province, Thailand. The voucher specimens are Songklanakarin Pharmacy (SKP) 206022001, SKP 2060200-101, SKP 2060200-201, SKP 2060200-301, SKP 206110101, SKP 206111301, SKP 206111801 and SKP 2061100-101, respectively. These plant materials were identified by Dr. Jarun Maknoi and were kept in the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

2.2 Preparation of the plant extracts

Ten grams of each dried plant was powdered and extracted successively by reflux for 3 h with 200 ml of ethanol (EtOH) and water, separately. The solvents were removed under reduced pressure to give ethanolic and water extracts, respectively. Stock solutions (10 mg/ml) of the extracts were prepared in dimethylsulfoxide (DMSO) and stored at 4°C until used.

2.3 Anti-allergic activity assay

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

The 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 immunoglobulin E (anti- DNP IgE) (0.45 μ g/ml), then incubated overnight at 37°C in 5% CO₂ for sensitization of the cells. The cells were washed twice with 500 μ l of Siraganian buffer (119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM MgCl₂, 1 mM CaCl₂, 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 μ l of Siraganian buffer for an additional 10 min at 37°C. After that, 20 μ l of test sample solution was added to each well and incubated for 10 min, followed by addition of 20 μ l of antigen (DNP-BSA, final concentration is 10 μ g/ml) at 37°C for 20 min to stimulate the cells to degranulate. The supernatant was then transferred into a 96-well plate and incubated with 50 μ l of substrate (1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosamide) in 0.1 M citrate buffer (pH 4.5) at 37°C for 1 h. The reaction was stopped by adding 200 μ l of stop solution (0.1M Na₂CO₃ / NaHCO₃, 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 IC₅₀ 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 (-)].

2.4 Statistical analysis

The results were expressed as the mean \pm S.E.M. (N = 4) at each concentration of each sample. The IC₅₀ 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

As shown in Table 1, the EtOH extract of *Boesenbergia thorelii*, locally known in Thai as Kra Chai Pa, was the

Table 1. Anti-allergic activity^a of some selected plants in the Zingiberaceae family

Plant species	% Inhibition at various concentrations (μg/ml)					IC ₅₀ (μg/ml)
	0	3	10	30	100	
<i>Boesenbergia thorelii</i> (Ganep) Loes. (Water)	0.0±2.1	-	17.0±8.9	54.0±6.6**	96.1±3.0**	26.3
<i>B. thorelii</i> (EtOH)	0.0±9.8	-	23.5±3.5	64.4±1.2**	86.6±1.0**	23.0
<i>Boesenbergia</i> sp1 (Water)	0.0±3.9	-	-	-	48.9±0.8**	>100
<i>Boesenbergia</i> sp1 (EtOH)	0.0±3.9	-	-	-	-21.6±2.2	>100
<i>Boesenbergia</i> sp2 (Water)	0.0±2.1	-	-	-	47.2±2.1**	>100
<i>Boesenbergia</i> sp2 (EtOH)	0.0±5.6	-	35.1±4.5**	47.5±7.8**	79.4±2.5**	38.3
<i>Boesenbergia</i> sp3 (Water)	0.0±3.9	-	-	-	23.1±0.7	>100
<i>Boesenbergia</i> sp3 (EtOH)	0.0±8.7	-	24.9±8.6*	32.7±9.7*	86.6±1.1**	49.4
<i>Kaempferia marginata</i> Carey. (Water)	0.0±2.1	-	27.4±6.7*	36.7±5.5**	109.4±7.0**	38.4
<i>K. marginata</i> (EtOH)	0.0±3.9	-	-	-	-51.3±0.9**	>100
<i>Kaempferia</i> sp. (Water)	0.0±3.9	-	-	-	48.7±5.1**	>100
<i>Kaempferia</i> sp. (EtOH)	0.0±3.9	-	-	-	-36.9±4.7**	>100
<i>Kaempferia angustifolia</i> Roscoe. (Water)	0.0±3.9	-	-10.1±2.9	37.7±4.8**	65.4±7.4**	55.6
<i>K. angustifolia</i> (EtOH)	0.0±4.3	-	-	-	10.1±4.2	>100
<i>Kaempferia rotunda</i> L. (Water)	0.0±8.7	-	-	-	39.8±5.0**	>100
<i>K. rotunda</i> L. (EtOH)	0.0±12.5	-	4.5±12.9	18.5±5.9*	73.1±0.2**	70.12
Ketotifen fumarate(positive control)	-	-	-	-	-	0.2

“-” means “not done”; Statistical significance, **p* < 0.05; ***p* < 0.01.

^a Each value represents the mean ± S.E.M. of four determinations.

most active inhibitor of the allergic reaction with an IC₅₀ value of 23.0 μg/ml, followed by *Boesenbergia thorelii* (water, IC₅₀ = 26.3 μg/ml), *Boesenbergia* sp 2 (EtOH, IC₅₀ = 38.3 μg/ml), *Kaempferia marginata* (water, IC₅₀ = 38.4 μg/ml) *Boesenbergia* sp 3 (EtOH, IC₅₀ = 49.4 μg/ml) and *Kaempferia angustifolia* (water, IC₅₀ = 55.6 μg/ml), whereas other *Boesenbergia* and *Kaempferia* spp had much lower activities (IC₅₀ = 70.1-> 100 mg/ml). The result showed that the IC₅₀ values of EtOH and water extracts of *Boesenbergia thorelii* (IC₅₀ = 23.0 and 26.3 μg/ml, respectively) were comparable to that of the positive control, ketotifen fumarate (IC₅₀ = 20.2 μg/ml).

Boesenbergia thorelii, *Boesenbergia* sp 2 and *Boesenbergia* sp 3 have not been previously reported for their 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 amplified fragment length polymorphism (AFLP) analysis (Techaprasan *et al.*, 2008). *Kaempferia marginata* is locally known in Thai as Kra Jae Jun and its roots have been used for treating allergies, fever, and swollen legs. A preliminary biological assay on *K. marginata* indicated that a dichloromethane extract of whole plants exhibited activity against the malarial parasite *Plasmodium falciparum*. Chemical constituents that have been reported for the *K. marginata* include six pimarane-type diterpenes, namely, (1*R*,2*S*,5*S*,7*S*,9*R*,-10*S*,13*R*)-1,2,7-trihydroxypimara-8(14),15-diene, (1*R*,2*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,2,11-trihydroxypimara-8(14),15-diene,

(1*S*,5*S*,7*R*,9*R*,10*S*,11*R*,13*R*)-1,7,11-trihydroxypimara-8(14),15-diene, (1*S*,5*S*,9*S*,10*S*,11*R*,13*R*)-1,11-dihydroxypimara-8(14),15-diene, (5*S*,6*R*,9*S*,-10*S*,13*R*)-6-hydroxypimara-8(14),15-diene-1-one and (1*R*,2*S*,5*S*,7*S*,9*R*,10*S*,

(13*R*)-1,2-dihydroxypimara-8(14),15-diene-7-one. These substances have been evaluated using a panel of bioassays including antimalarial, antituberculous and antifungal activity (Thongnest *et al.*, 2005). *Kaempferia angustifolia*, which is locally known in Thai as Thao Nhang Haeng, has been used to treat abdominal illness, including dysentery and diarrhea. It was reported that the rhizomes of *K. angustifolia* contain cyclohexane diepoxides (e.g. crotapoxide, zeylanol) and other oxygenated cyclohexane derivatives (Woerdenbag *et al.*, 2004).

In conclusion, the present study supports the use of *Boesenbergia thorelii* for treatment of allergy and allergic-related diseases. The isolation of anti-allergic substances from the rhizomes of *Boesenbergia thorelii* is now in progress.

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