



Ethnopharmacological communication

Anti-allergic activity of some selected plants in the Zingiberaceae family

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Abstract

Ethanollic and water extracts, together with volatile oils from the rhizomes of six selected Zingiberaceous plants, including *Curcuma mangga*, *Kaempferia galanga*, *Kaempferia parviflora*, *Zingiber cassumunar*, *Zingiber officinale* and *Zingiber zerumbet* were investigated for their anti-allergic activities using a RBL-2H3 cell line. The ethanollic (EtOH) extract of *Kaempferia parviflora* 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 10.9 μ g/ml, followed by *Zingiber cassumunar* (EtOH, IC_{50} = 12.9 μ g/ml) and *Curcuma mangga* (water, IC_{50} = 36.1 μ g/ml). The volatile oils of these six plants were apparently inactive (IC_{50} > 100 μ g/ml). The crude extracts were also tested on β -hexosaminidase activity to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. As a result, the plant extracts were inactive against the enzyme activity of β -hexosaminidase. These findings support the use in Thai traditional medicine of these selected Zingiberaceous plants, especially *Kaempferia parviflora* and *Zingiber cassumunar*, for treatment of allergy and allergic-related diseases.

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1. Introduction

An allergy is one of the immune dysfunctions, which is a serious health problem worldwide. Substances that cause allergy are called allergens, such as pollen, food, dust mites, cosmetics, mold spores and animal hairs. Second exposure to the allergen leads to the crosslinking of the bound IgE, triggering the release of pharmacological active mediators, vasoactive amines, from mast cells and basophils. These mediators cause smooth muscle contraction, increased vascular permeability and vasodilation (Goldsby et al., 2002).

When granules in mast cells or basophils degranulate, an enzyme β -hexosaminidase is usually released along with histamine, this enzyme is thus used as a biomarker for antigen-induced degranulation in rat basophilic leukemia (RBL-2H3) cell line (Cheong et al., 1998).

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) and *Alpinia galanga* (Yoshikawa et al., 2004). 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. *Kaempferia galanga* has been used in treatment of urticaria and allergy, *Kaempferia parviflora* for treatment of allergy and gastrointestinal disorders as well as an aphrodisiac (Pengcharoen, 2002), *Zingiber cassumunar* for treatment of inflammation and skin disease, *Zingiber officinale* as an anti-asthmatic agent and *Zingiber zerumbet* as antitumor and anti-inflammatory agent (Wutthithamavet, 1997).

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

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2. Materials and methods

2.1. Plant materials

The rhizomes of some Zingiberaceous plants including *Curcuma mangga* Val. & Zijp., *Kaempferia galanga* Linn., *Kaempferia parviflora* Wall ex Baker, *Zingiber cassumunar* Roxb., *Zingiber officinale* Roscoe and *Zingiber zerumbet* (L.) Sm. were collected in June 2005 in Songkhla province, Thailand, except for *Kaempferia parviflora* rhizomes which were bought from a Thai traditional drug store in Songkhla province, Thailand. The voucher specimens are SKP 2060313, SKP 2061107, SKP 2061116, SKP 2062603, SKP 2062615 and SKP 2062616, respectively. The plant materials were identified by Assoc. Prof. Dr. Sanan Subhadhirasakul and the voucher specimens are kept at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, 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. The yields of ethanolic extracts of *Curcuma mangga*, *Kaempferia galanga*, *Kaempferia parviflora*, *Zingiber cassumunar*, *Zingiber officinale* and *Zingiber zerumbet* were found to be 15.6%, 17.3%, 16.9%, 21.9%, 11.4% and 8.7% (w/w), respectively; whereas the water extracts were 15.1%, 15.5%, 10.0%, 6.5%, 10.4% and 24.6% (w/w), respectively. Stock solutions (10 mg/ml) of the extracts were prepared in DMSO and stored at 4 °C until use.

2.3. Preparation of the volatile oils

One hundred grams of each powdered plant was added to 500 ml of distilled water and the volatile oil collected by water distillation for 3 h. The yields of each volatile oil were 0.5%, 2.2%, 0.2%, 2.5%, 0.7% and 3.0% (v/w) for *Curcuma mangga*, *Kaempferia galanga*, *Kaempferia parviflora*, *Zingiber cassumunar*, *Zingiber officinale* and *Zingiber zerumbet*, respectively. The oils were kept in air tight containers at 4 °C before use.

2.4. Anti-allergic activity assay

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

Inhibitory effects on the release of β -hexosaminidase from RBL-2H3 cells (purchased from ATCC) 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 minimum essential medium eagle (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 transferred into a 96-well plate and incubated with 50 μ l 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 μ l of stop solution (0.1 M 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:

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

control (C): DNP-BSA (+), test sample (–); test (T): DNP-BSA (+), test sample (+); blank (B): DNP-BSA (–), test sample (+); normal (N): DNP-BSA (–), test sample (–).

2.4.2. β -Hexosaminidase inhibitory activity

The following assay was carried out in order to clarify that the anti-allergic effects of samples were due to the inhibition of β -hexosaminidase release, and not from the inhibition of β -hexosaminidase activity.

The cell suspension (5×10^7 cells) in 6 ml of PBS (phosphate buffer saline) was sonicated. The solution was then centrifuged; and the supernatant was diluted with Siraganian buffer and adjusted to equal the enzyme activity of the degranulation tested above. The enzyme solution (45 μ l) and test sample solution (5 μ l) were transferred into a 96-well microplate and incubated with 50 μ l of the substrate solution at 37 °C for 1 h. The reaction was stopped by adding 200 μ l of the stop solution and the absorbance was measured using a microplate reader at 405 nm.

2.5. Statistical analysis

The results were expressed as mean $\pm$ S.E.M. of four determinations at each concentration for 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 ethanolic extract of *Kaempferia parviflora*, locally known in Thai as Kra-Chai-Dam, was the most active against allergic reaction with an IC₅₀ value of 10.9 μ g/ml, followed by *Zingiber cassumunar* (EtOH, IC₅₀ = 12.9 μ g/ml) and *Curcuma mangga* (water, IC₅₀ = 36.1 μ g/ml), whereas other plants possessed moderate

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

Plant extract	% inhibition at various concentrations (µg/ml)					IC ₅₀ (µg/ml)	β-Hexosaminidase inhibitory activity at 100 µg/ml
	0	3	10	30	100		
<i>Curcuma mangga</i> Valetton and Zijp (EtOH)	0.0 ± 5.6	–	21.1 ± 9.2	37.1 ± 8.4**	79.1 ± 4.1**	36.7	–0.5
<i>Curcuma mangga</i> (Water)	0.0 ± 10.5	–	18.3 ± 5.7	36.3 ± 5.7*	82.6 ± 6.1**	36.1	0.1
<i>Curcuma mangga</i> (Vol. oil)	0.0 ± 8.7	–	–	–	33.9 ± 5.7	>100	–
<i>Kaempferia galanga</i> Linn. (EtOH)	0.0 ± 10.6	–	14.3 ± 8.1	32.5 ± 9.1	54.5 ± 5.3**	78.6	3.9
<i>Kaempferia galanga</i> (Water)	0.0 ± 10.9	–	11.9 ± 8.6	24.4 ± 6.3	75.0 ± 8.0**	49.5	2.5
<i>Kaempferia galanga</i> (Vol. oil)	0.0 ± 8.5	–	0.3 ± 2.1	20.3 ± 4.2	57.8 ± 5.7**	80.5	–
<i>Kaempferia parviflora</i> Wall. ex Baker (EtOH)	0.0 ± 8.6	6.5 ± 4.4	57.6 ± 6.6**	83.2 ± 2.6**	101.3 ± 0.9**	10.9	–0.2
<i>Kaempferia parviflora</i> (Water)	0.0 ± 10.9	–	–16.7 ± 15.8	39.9 ± 11.3*	74.4 ± 3.4**	48.4	8.8
<i>Kaempferia parviflora</i> (Vol. oil)	0.0 ± 8.7	–	–	–	–7.7 ± 9.7	>100	–
<i>Zingiber cassumunar</i> Roxb. (EtOH)	0.0 ± 9.5	14.6 ± 3.7	39.1 ± 5.1*	73.2 ± 2.0**	103.5 ± 0.9**	12.9	0.4
<i>Zingiber cassumunar</i> (Water)	0.0 ± 11.1	–	–7.7 ± 5.9	34.1 ± 9.2*	81.8 ± 7.9**	44.4	4.6
<i>Zingiber cassumunar</i> (Vol. oil)	0.0 ± 5.4	–	–	–	27.6 ± 3.0*	>100	–
<i>Zingiber officinale</i> Roscoe (EtOH)	0.0 ± 10.8	–	–5.1 ± 6.2	36.9 ± 7.2	86.9 ± 1.7**	40.3	–6.1
<i>Zingiber officinale</i> (Water)	0.0 ± 10.2	–	–16.1 ± 5.9	18.6 ± 7.0	69.3 ± 9.2**	62.6	0.6
<i>Zingiber officinale</i> (Vol. oil)	0.0 ± 8.7	–	–	–	–97.0 ± 7.9	>100	–
<i>Zingiber zerumbet</i> (L.) Sm. (EtOH)	0.0 ± 10.6	–	8.4 ± 4.5	24.2 ± 3.8*	53.7 ± 3.2**	91.0	1.5
<i>Zingiber zerumbet</i> (Water)	0.0 ± 9.4	–	10.9 ± 2.8	30.5 ± 5.6	59.1 ± 6.2**	68.2	2.5
<i>Zingiber zerumbet</i> (Vol. oil)	0.0 ± 8.7	–	–	–	–27.3 ± 6.7	>100	–
Ketotifen fumarate	0.0 ± 6.9	–	12.8 ± 0.5	38.3 ± 3.2**	68.2 ± 1.5**	47.5 µM = (20.2 µg/ml)	–

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

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

and mild activities. The volatile oils of these six plants were apparently inactive ($IC_{50} > 100 \mu\text{g/ml}$). The anti-allergic effects of *Kaempferia parviflora* and *Zingiber cassumunar* were two-fold higher than that of Ketotifen fumarate ($IC_{50} = 20.2 \mu\text{g/ml}$), the positive control. It is suggested that these plant extracts may contain some certain compounds that have IC_{50} values considerably lower than that of ketotifen fumarate. These substances are likely to be useful as lead compounds for the development of a novel class of anti-allergic agents. The crude extracts of these Zingiberaceous plants were also tested on β-hexosaminidase activity to clarify whether their effects were due to the inhibition of enzyme activity or of degranulation. As a result, the plant extracts were inactive against the enzyme activity of β-hexosaminidase (Table 1).

Kaempferia parviflora has recently been reported to possess antibacterial and antiparasmodial activities (Yenjai et al., 2004), as well as anti-peptic ulcer effect (Rujjanawate et al., 2005). It was reported that the rhizome of *Kaempferia parviflora* contained some bioactive flavonoids such as 5,7,4'-trimethoxyflavone and 5,7,3',4'-tetramethoxyflavone and these compounds possessed antiparasmodial effects (Yenjai et al., 2004). Moreover, the wine preparation of this plant is increasingly used in Thailand as a tonic and as an aphrodisiac. From the present study, some flavonoid derivatives containing in the rhizomes of *Kaempferia parviflora* might be responsible for anti-allergic effect of this plant. *Zingiber cassumunar*, locally known in Thai as Plai, has been used extensively as an anti-inflammatory agent. The extract of this plant was demonstrated to have anti-inflammatory activity in animal model induced rat paw edema (Panthong et al., 1997). However, the biological activity of *Kaempferia parviflora* and *Zingiber cassumunar* as anti-allergen have not been studied so far.

In conclusion, the present finding supports the use of *Kaempferia parviflora* and *Zingiber cassumunar* for treatment of allergy and allergic-related diseases as claimed by Thai traditional doctors. The isolation of anti-allergic sub-

stances from the rhizomes of *Kaempferia parviflora* is now in progress.

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