



# Bioactivity-Guided Isolation and Stability Analysis of the Active Anti-Allergic Compound from *Kaempferia galanga* L. Extract and Its Anti-Inflammatory Activity

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## ABSTRACT

*Kaempferia galanga* L. rhizomes (KG) have been used in Thai traditional medicine for treating the common cold and fevers. A previous study found ethyl-*p*-methoxycinnamate (EPMC) to be a main compound of KG. There have been many studies on the bioactivities of EPMC, but none on its anti-allergic activity and its stability. In this study, bioassay-guided fractionation was used to isolate an anti-allergic compound from KG. EPMC (an active compound) and KG extracts were investigated for their anti-allergic activities using immunoglobulin E (IgE)-sensitized b-hexosaminidase in rat basophilic leukemia-2H3 (RBL-2H3) cells and anti-inflammatory activities using lipopolysaccharide (LPS)-induced nitric oxide (NO). In addition, the stability of active marker content was determined by HPLC. The crude ethanolic extract of KG showed strongly anti-allergic activity ( $IC_{50} = 19.85 \pm 2.14 \mu\text{g/ml}$ ). The crude extract was then separated by bioassay-guided fractionation. The two purified compounds were then identified as ethyl-*p*-methoxycinnamate (EPMC) and ethyl cinnamate (EC). There was no significant difference in the anti-allergic activity between EPMC, KG, and

chlorpheniramine (CPM) ( $IC_{50}=18.87 \pm 0.36$ ,  $19.85 \pm 2.14$ , and  $16.10 \pm 0.16$ , respectively). KG and EPMC showed less anti-inflammatory activity compared to prednisolone. The para-methoxy group ( $OCH_3$ ) of EPMC is the most important functional group contributing to the anti-allergic and anti-inflammatory activities. EPMC has strong anti-allergic activity and can be used as a marker for the anti-allergic activity of KG for drug quality control.

**Keywords:** Anti-allergic activity; Ethyl *p*-methoxycinnamate; Ethyl cinnamate; *Kaempferia galanga* L.; Structure-activity relationships

## 1. Introduction

*Kaempferia galanga* L. (KG) has long been utilized in dietary medicine in Asia, including China, India, Bangladesh, and Japan. In Thai traditional medicine, a considerable amount of KG (50% w/w) is present in the polyherbal medicine Prasaprophyai (PSPY), which is used to treat fever and allergic rhinitis [1]. Early studies revealed that ethanolic extract of KG exerted an anti-allergic effect by inhibiting  $\beta$ -hexosaminidase ( $IC_{50}=14.91 \pm 0.86 \mu\text{g/ml}$ ) and possessed anti-inflammatory effects by inhibiting nitric oxide (NO) ( $IC_{50}=30.30 \pm 1.23 \mu\text{g/ml}$ ) [2]. A previous study reported that ethyl *p*-methoxycinnamate (EPMC) is a main component in KG. EPMC has many proven pharmacological activities including anti-inflammatory [3-5], insect repellent, insecticidal [6], anti-cancer [7, 8], antimicrobial [9], anti-tuberculosis [10], and anti-sedative activities [11]. However, to date there have been no reports on the anti-allergic activities of active compounds of KG. Although EPMC is a good candidate for a PSPY marker [12], the stability and anti-allergic activity of this compound has not been established.

In this study, we performed bioassay-guided fractionation of KG's anti-allergic compound and investigated the anti-allergic and anti-inflammatory effects of KG and its isolated compound. The stability of the isolated compound was also tested using HPLC.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

The analytical grade solvents ethyl acetate (EtOAc), methanol (MeOH), chloroform ( $CHCl_3$ ), and hexane were purchased from RCI Labscan (Bangkok, Thailand). Chromatographic columns (4.5×54 cm, glass) were purchased from Becthai (Bangkok, Thailand). Filter funnels (1,000 ml, 130×115 mm) were purchased from ROBU. Silica gel 60 (grade numbers 0.07734 and 0.09385, mesh 70-230 and 230-400) and TLC silica gel 60 F254 were purchased from Merck (Darmstadt, Germany).

### 2.2 Cell culture

The RBL-2H3 rat basophilic leukemia cell line (ATCC CRL-2256, VA, USA) was purchased from American Type Culture Collection. Trypan blue, trypsin-EDTA, and fetal bovine serum (FBS) were purchased from Gibco (OK, USA). Cells were cultured in MEM supplemented with 15% FBS, penicillin (100  $\mu\text{g/ml}$ ), 0.1% sodium bicarbonate, and 2 mM glutamine and streptomycin (100  $\mu\text{g/ml}$ ). Anti-dinitrophenylated bovine albumin (DNP-BSA), 4-nitrophenyl *N*-acetyl- $\beta$ -D-glucosaminide (PNAG), anti-DNP IgE (monoclonal anti-DNP), and chlorpheniramine (CPM) were purchased from Sigma (MO, USA). Piperazine-*N,N'*-bis (2-ethanesulfonic acid) (PIPES) was purchased from Amresco (OH, USA). Sodium chloride and sodium hydroxide (analytical grade) were purchased from Univar (Ajax Finechem, Australia). Calcium chloride dehydrate, magnesium chloride

hexahydrate, potassium chloride, and sodium carbonate were purchased from Merck (Darmstadt, Germany). Sodium bicarbonate was purchased from BDH (Poole, U.K.).

RAW 264.7, murine macrophage cells (ATCC® TIB-71™) were cultured in RPMI-1640 medium supplemented with 2 mM glutamine, 0.1% sodium bicarbonate, streptomycin (100 µg/ml), penicillin (100 µg/ml), and 10% FBS. Lipopolysaccharide (LPS, from *Salmonella enteritidis*), 3-(4,5-dimethyl-2-thiazolyl)-2,5 diphenyl 2H-tetrazolium bromide (MTT), and L-nitroarginine (L-NA) were purchased from Sigma (MO, USA). Phosphate-buffer saline (PBS), penicillin-streptomycin, and fetal bovine serum (FBS) were purchased from Biochrome. Dimethyl sulfoxide (DMSO) was purchased from Fluka (Munich, Germany) and 96-well sterile microplates were purchased from Costar. Prednisolone was purchased from Sigma (MO, USA).

### 2.2.1 Plant materials and preparation of extract

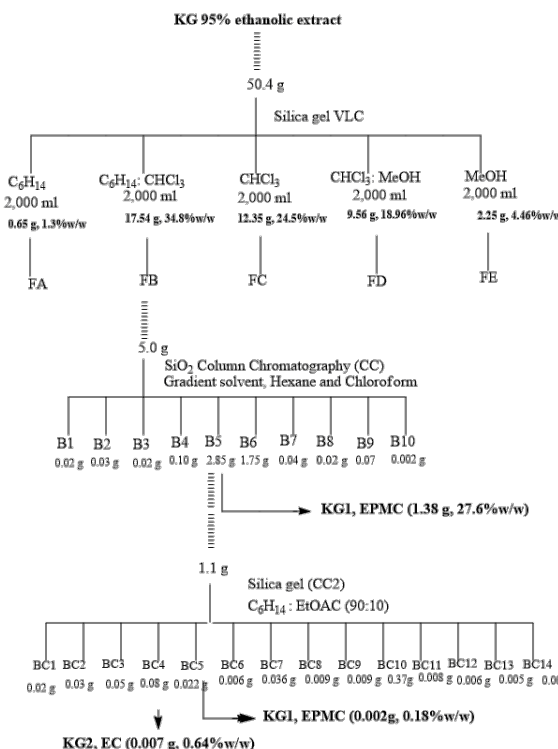
Fresh rhizomes of KG were collected from Sukhothai province, Thailand. The KG specimen (voucher number SKP206110701) was deposited at the Herbarium of Southern Center of Thai Medicinal Plants at the Faculty of Pharmaceutical Science, Prince of Songkla University, Songkhla, Thailand. KG rhizomes were cleaned and dried at 50 °C for 24 h. The dried rhizomes were powdered using an electric grinder (40 mesh particle size). The powdered KG was macerated with 95% ethanol (L/S ratio=1:2) and evaporated under reduced pressure at 40 °C to obtain the dry ethanolic extracts. The extract was dried to constant weight in a vacuum desiccator. This process produced a sticky brown extract. The extract was then kept at -20 °C until further use.

### 2.2.2 Isolation of compounds from the ethanolic extract of *Kaempferia galanga* L. rhizomes

A schematic flow chart showing the isolation of compounds in KG rhizomes is presented in fig. 1. The process of isolation was performed as follows.

### 2.2.3 Vacuum Liquid Chromatography

The vacuum liquid chromatography (VLC) column was packed with silica gel 60 (230–400 mesh). The crude KG extract (50.40 g) was adsorbed on silica gel and applied to the top of the column. The extract was then eluted using the following solvent systems (in order of increasing polarity, denoted as FA, FB, FC, FD, and FE, respectively): hexane (2,000mL), hexane:chloroform (1:1, 2000mL), chloroform (2,000mL), chloroform:methanol (1:1, 2,000mL), and methanol (2,000 mL). The solvent from each fraction was then removed by rotary evaporator at 40°C, drying the extract until constant weight. Each fraction was tested for its anti-allergic activity. The



**Fig. 1.** Flow chart for separating two compounds from the ethanolic extract of KG.

fraction with the best anti-allergic activity was chosen for compound isolation.

### 2.2.4 Column chromatography

With regards to the bioassay-guided fractionation, the FB fraction showed the highest anti-allergic activity and was chosen to reperform chromatograph by gravity column chromatography (CC) (4.50 cm diameter, 54 cm length) using silica gel G (70-230 mesh). The selected fraction (5.0 g) was loaded onto the column, eluting with hexane: chloroform (1:1). Fifteen ml fractions were collected during elution with each solvent. Each collected fraction was examined by TLC. Fractions with similar TLC profiles were pooled. The B5 fraction showed two intense spots.

The B5 fraction was suspended in hexane with gentle shaking; crystals and a brown-colored supernatant formed. The crystals were separated from the supernatant by repeated decanting with hexane until the supernatant became transparent (completely washed out). KG1 compound (white crystals, 1.38 g, 27.6% w/w) appeared in this.

The supernatant from fraction B5 was subjected to a second gravity column chromatography (CC2). A single spot appeared in fractions BC4 and BC5. They were designated compounds KG1 (0.002 g, 0.18% w/w) and KG2 (0.007 g, 0.64 % w/w), respectively.

### 2.2.5 Identification of the isolated compounds

The structures of the isolated compounds were elucidated by <sup>1</sup>H-NMR (500 MHz) and <sup>13</sup>C-NMR (125 MHz) using a Varian Unity Inova 500 NMR spectrometer (Bruker Company, USA). The ESI mass spectrums of the compounds were obtained from a high-resolution LCMS (Bruker).

### 2.2.6 Anti-allergic activity

The KG extract, VLC fractions, and pure compounds were evaluated for anti-allergic activity via inhibition of  $\beta$ -

hexosaminidase release assay from rat basophilic leukemia cells (RBL-2H3) using a method by Tewtrakul and Itharat with slight modification [13]. Briefly, the RBL-2H3 cells were seeded in 24-well plates ( $5 \times 10^5$  cells/mL) and incubated to adhere at 37 °C in 5% CO<sub>2</sub> for 1.5 h. RBL-2H3 cells were sensitized with anti-DNP IgE (anti-dinitrophenyl-immunoglobulin E) (0.45  $\mu$ g/mL), and incubated at 37 °C in 5% CO<sub>2</sub> for 24 h. The cells were washed with 400  $\mu$ L of Siraganian buffer (buffer A) [119mM NaCl, 5mM KCl, 5.6mM glucose, 0.4mM MgCl<sub>2</sub>, 1mM CaCl<sub>2</sub>, 25 mM piperazine-*N,N'*-bis (2-ethanesulfonic acid) (PIPES), 0.1% bovine serum albumin (BSA), and 40mM NaOH, pH 7.2]. An aliquot (160  $\mu$ L) of buffer A was added. Incubation was continued for an additional 10 min at 37 °C. The test sample solution (20  $\mu$ L) was added to each well and incubated for 10min, followed by the addition of 20  $\mu$ L of antigen (DNP-BSA, final concentration 10  $\mu$ g/mL) at 37 °C for 20 min to stimulate cell degranulation. The supernatants were transferred into a 96-well plate at 50  $\mu$ L/well and incubated with 50  $\mu$ L of substrate PNAG (1mM *p*-nitrophenyl-*N*-acetyl- $\beta$ -D-glucosaminide) in 0.1M citrate buffer (pH 4.5) at 37 °C for 2 h. The reaction was stopped by adding 200  $\mu$ L of stop solution (0.1M Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. CPM was used as positive control. Percent inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = [1 - (T - B - N)] \times 100.$$

$$(C - B - N)$$

Control (C): DNP-BSA (+), Test sample (-);

Test (T): DNP-BSA (+), Test sample (+);

Blank (B): DNP-BSA (-), Test sample (+);

Normal (N): DNP-BSA (-), Test sample (-).

### 2.2.7 Effects on LPS-induced NO release using RAW 264.7 cells

The effect of extracts on NO production by murine macrophage-like RAW 264.7 cells was determined using a method by Tewtrakul and Itharat with slight modification [14]. Briefly, the cells were seeded in 96-well plates with  $1 \times 10^5$  cells/100  $\mu$ l/well and allowed to adhere for 1 h. After that, the medium was replaced with fresh medium containing 10 ng/ml of LPS, together with test samples at various concentrations (100  $\mu$ l/well), and incubated for 24 h. NO production was determined by measuring the accumulation of nitrite in the culture supernatant using Griess reagent. Cytotoxicity was also determined using the 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric method. Briefly, after 24 h incubation with test samples, MTT solution (10  $\mu$ l, 5 mg/ml in PBS) was added to the wells. After 2 h incubation, the medium was removed and 100  $\mu$ l of isopropanol containing 0.04 M HCl was added to dissolve the dry formazan in the cells. The optical density of the formazan solution was measured with a microplate reader at 570 nm. The test compounds or extract were considered to be cytotoxic if the optical density of the sample-treated group had a level of viable cells less than 70% of that in the control (vehicle-treated) group. Prednisolone was used as a positive control. The stock solution of each test sample was dissolved in DMSO and the solution was added to the medium RPMI-1640 (final DMSO concentration < 0.2%). Percent inhibition was calculated using the following equation and  $IC_{50}$  values were determined graphically:

$$\text{Inhibition (\%)} = \frac{[(\text{OD control} - \text{OD sample}) / \text{OD control}] \times 100}{100}$$

### 2.2.8 Stability test of *Kaempferia galanga* L. Rhizomes extract

The stability of the KG ethanolic extract was investigated under accelerated conditions. Chemical and biological changes were determined at different storage times (Days 0, 15, 30, 60, 90, 120, 150, and 180).

The ethanolic extracts were kept under heat-accelerated conditions (40 °C, EPMC content was correlated with anti-allergic activity ( $p=0.008$ ). The coefficient value line was -0.782, indicating a strong correlation between anti-allergic activity and EPMC content. Therefore, the highest EPMC content showed the strongest anti-allergic activity (Table 1).

**Table 1.** The percent yield of each fraction of KG extract, anti-allergic activity, EPMC content, and the correlation coefficient between anti-allergic activity and EPMC content.

Fraction	Weight (g)	%w/w	Anti-allergic activity ( $IC_{50}$ , $\mu$ g/ml)	EPMC content (mg/g)	The correlation coefficient <sup>s</sup>
A	0.65	1.30	$42.33 \pm 5.74$	14.98	-0.782**
B	17.54	34.80	$13.61 \pm 1.44$	750.95	
C	12.35	24.50	$15.76 \pm 2.34$	572.53	
D	9.56	18.96	$17.02 \pm 5.88$	16.74	
E	2.25	4.46	$29.97 \pm 3.25$	15.65	

Note: <sup>s</sup> Spearman's rank correlation coefficient.

\*\*Correlation is significant at the 0.01 level (2-tailed).

EPMC showed the highest content in the hexane:chloroform fraction (FB). Thus, FB was chosen for repeat chromatography by column chromatography (Table 1), 75% RH for 6 months) [15]. The chemical changes were determined by EPMC content using HPLC. The mobile phase gradient was a mixture of methanol (A) and purified water (B), programmed as follows: 0 - 23 min, 60% A, 40% B; 23-30 min, 90% A, 10% B; 30-35 min, 60% A, 40% B. Samples (10  $\mu$ l) were injected into the HPLC system with a flow rate of 1 ml/min. The diode array detector was set at 227 nm. The operating temperature was maintained at room temperature (25°C) [12]. The biological activity was determined by anti-allergic assay.

### 2.2.9 Data analysis

Each sample was analyzed in triplicate in all assays. The  $IC_{50}$  values are expressed as mean  $\pm$  SEM. Values were calculated using Prism software. The  $IC_{50}$  values were compared between EPMC, EC, KG, and

standard control using one-way ANOVA (Dunnett t-test). The correlation coefficient between anti-allergic activity and EPMC content was calculated using Spearman's rank correlation coefficient.

### 3. Results and Discussion

#### 3.1 Isolation of compounds from *Kaempferia galanga* L. rhizomes.

An aliquot of 50.4 g of KG extract was separated by VLC using serial solvent systems as follows: hexane, hexane:chloroform, chloroform, chloroform:methanol, and methanol. Then, each fraction was tested for anti-allergic activity against antigen-induced  $\beta$ -hexosaminidase release as a marker of degranulation in RBL-2H3 cells. The results showed that the  $IC_{50}$  of anti-allergic activity of VLC fraction of KG ranged from 13.61-42.33  $\mu\text{g/ml}$ . The FB fraction (hexane:chloroform) showed the highest percent yield (34.80% w/w) and had the strongest anti-allergic activity ( $IC_{50} = 13.61 \pm 1.44 \mu\text{g/ml}$ ). While the FA fraction (hexane) showed a low % w/w and weak anti-allergic activity.

EPMC content was correlated with anti-allergic activity ( $p=0.008$ ). The coefficient value line was -0.782, indicating a strong correlation between anti-allergic activity and EPMC content. Therefore, the highest EPMC content showed the strongest anti-allergic activity (Table 1). EPMC showed the highest content in the FB fraction. Thus, FB was chosen for repeat chromatograph by column chromatography (Table 1).

The fraction B of VLC was purified using a silica gel chromatogram. Then, two pure compounds (KG1 and KG2) were isolated.

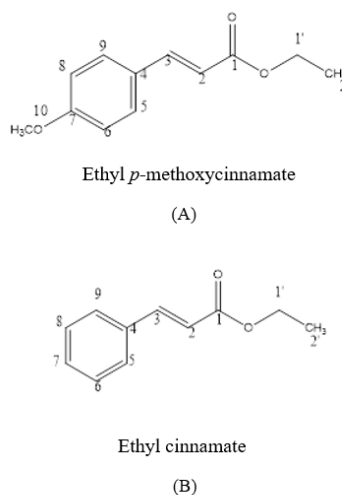
#### 3.2 Identification of the isolated compounds

KG 1 (1.58g, 3.16% w/w) appeared as colorless crystal. The structure of the compound was determined by its physicochemical and spectral data ( $^1\text{H-NMR}$

and  $^{13}\text{C-NMR}$ ), which agreed with the structure reported in the literature of ethyl *p*-methoxycinnamate [16]. The structure was also then confirmed by mass spectroscopy. KG1 was identified as ethyl *p*-methoxycinnamate (EPMC) (Fig. 2A).

KG 2 (0.07 g, 0.14% w/w) appeared as a pale-yellow liquid. The structure of the compound was determined by its physicochemical and spectral data ( $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ ), which agreed with the structure reported in the literature of ethyl cinnamate [17]. The structure was also then confirmed by mass spectroscopy. Thus, KG2 was identified as ethyl cinnamate (EC) (Fig. 2B).

EPMC and EC are the main constituent compounds in the essential oil of KG [18].



**Fig. 2.** Two compound structures purified from KG extract.

Two isolated cinnamate derivatives were found, having similar structures to one another. The functional group of EPMC is the methoxy group in the *para*-position (C-10). When this functional group is absent, the molecular structure is that of EC (Table 2).

**Table 2.** Comparison of <sup>1</sup>H- and <sup>13</sup>C-NMR data between ethyl-*p*-methoxycinnamate: KG1 (recorded in CDCl<sub>3</sub>) and ethyl cinnamate: KG2 (recorded in CDCl<sub>3</sub>).

Position	Ethyl <i>p</i> -methoxycinnamate		Ethyl cinnamate	
	$\delta_c$ (100 MHz)	$\delta_H$ (J in Hz, 500MHz)	$\delta_c$ (100 MHz)	$\delta_H$ (J in Hz, 600MHz)
1	167.4	-	166.9	-
2	115.8	6.30 (1H, d, 15.94)	118.5	6.44 (1H, d, 16.02)
3	144.2	7.64 (1H, d, 15.96)	144.5	7.69 (1H, d, 16.03)
4	127.2	-	134.6	-
5,9	129.7	7.48 (2H, d, 8.73)	128.0	7.53 (2H, m)
6,8	114.3	6.91 (2H, d, 8.75)	128.9	7.38 (2H, m)
7	161.3	-	130.2	7.38 (1H, m)
10	55.3	3.83 (3H, s)	-	-
1'	60.3	4.25 (2H, q)	60.4	4.27 (2H, dd, 7.13)

2'	14.3	1.32 (3H, t, 7.12)	14.3	1.33 (3H, t, 7.13)
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### 3.2 Anti-allergic activity and Anti-inflammatory activity

The anti-allergic and anti-inflammatory activities of the pure compounds isolated from KG were investigated. The anti-allergic activity of EC was weak, a significant difference from CPM. The anti-allergic activity of KG and EPMC were not significantly different from CPM (Positive control). With regard to anti-inflammatory activity, prednisolone was used as a positive control for anti-inflammatory activity ( $IC_{50} = 0.18 \pm 0.02$  g/ml). The mean  $IC_{50}$  value of KG, EPMC, and EC were significantly different from prednisolone at the 0.05 level. EPMC, EC, and KG showed weak anti-inflammatory activity as shown in Table 3.

**Table 3.** The percent inhibition and  $IC_{50}$  value of KG extract and its pure compound on  $\beta$ -hexosaminidase release and nitric oxide production.

Crude extracts and pure compound	%inhibition at various concentrations of extract (mean ± SEM, µg/ml)					IC <sub>50</sub> ±SEM (µg/ml)
	0.1	1	10	50	100	
Anti-allergic activity						
KG	-	9.04±4.72	31.96±1.74	76.21±1.23	90.77±1.58	19.85 ± 2.14
EPMC	-	11.87±2.92	33.84±0.89	74.05±4.91	89.20±0.90	18.87 ± 0.36 (91.48 µM)
EC	-	6.26±3.21	17.48±5.15	46.31±3.13	64.77±3.84	56.12 ± 3.52 * (318.46 µM)
CPM	-	6.49±4.47	35.72±1.50	69.88±5.89	81.03±4.48	16.10 ± 0.16 (58.58 µM)
Anti-inflammatory activity <sup>a</sup>						
KG		-7.16±4.20	1.81±3.11	60.54±1.79	91.78±1.95	42.08 ± 1.83 *
EPMC		-14.97±10.90	1.61±4.01	51.45±0.50	74.52±5.49	47.24 ± 1.94 * (229.20µM)
EC		-7.32±6.61	-2.68±4.50	43.16±1.91	82.00±3.69	57.06 ± 2.29 * (324.06µM)
Prednisolone	9.35±1.39	46.54±2.98	66.01±1.99	72.67±1.77		0.18 ± 0.02 (0.49µM)

**Note:** (-) mean not tested; a cytotoxic effect was observed; \* significant difference (compared with positive control), the mean difference is significant at the .05 level. One-way ANOVA, Dunnett t-tests treat CPM or Prednisolone as a control and compare all other groups against it.

For the structure-activity relationship (SAR), we suggested that the para-methoxy group (OCH<sub>3</sub>) of EPMC as an important functional group contributing to the anti-allergic and anti-inflammatory activities. The

anti-allergic and anti-inflammatory activities were reduced when the functional group was absent, as in the structure of EC. Thus, EPMC has the potential to be an anti-allergic chemical marker of KG.

### 3.2 Stability test

KG was kept under accelerated conditions (40°C, 75% RH) for 180 days, and regularly monitored for quality control, as shown in Table 4. The percentage of remaining EPMC content was higher than 97% for the 180 days when compared with day 0. EPMC content was in the range of 482.60-532.09 mg/g, indicating overall stability for the chemical content of EPMC.

In Thai traditional medicine, PSPY, a medication used to treat patients with allergic rhinitis, has KG as a significant constituent (50%) of its formula. A clinical study revealed a reduction in the severity of nasal symptoms after taking PSPY for 6 weeks. During this time, the liver and kidneys did not suffer from toxicity [19]. There is no quality control marker, however. EPMC could be a marker for PSPY's anti-allergic efficacy.

**Table 4.** Stability of EPMC contents of KG extract in accelerated condition (40 °C, 75% RH).

Sample	EPMC contents	
	mg/g	% Remain
Day 0	495.39 ± 5.57	100
Day 15	521.81 ± 19.08	105
Day 30	502.66 ± 18.91	101
Day 60	482.60 ± 12.93	97
Day 90	545.40 ± 22.43	110
Day 120	532.09 ± 25.68	107
Day 150	535.22 ± 15.70	108
Day 180	531.07 ± 25.01	107

### 4. Conclusion

The bioassay-guided fractionation of KG led to the isolation of EPMC as the active compound of anti-allergic activity; further research should support the ethnobotanical usage of KG as an herbal remedy for allergic rhinitis and use EPMC as a marker compound for KG quality control. Additionally, pure compounds for anti-allergic agents should be isolated from other fractions besides FB.

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### References

- [1] Mukkasombut N, Chanvimalueng W, Itharat A, Budsaba K, Ooraikul B. Clinical Trial Phase I of Prasaprophyai Extract Capsules, J Med Assoc Thai 2020;103(Suppl.4): 94-101.
- [2] Itharat, A. and Makchuchit, S. Anti-allergic and anti-inflammatory activities of Thai traditional medicine preparation called Prasaprophyai. Full report, 2009: 62-66.
- [3] Umar, M.I., M.Z. Asmawi, A.Sadikun, A. M. Majid, F.S. Al-Suede, L.E. Hassan, R. Altaf and M. B. Ahamed. Ethyl- *p*-methoxycinnamate isolated from *Kaempferia galanga* inhibits inflammation by suppressing interleukin-1, tumor necrosis factor- $\alpha$ , and angiogenesis by blocking endothelial functions. Clinics (Sao Paulo) 2014; 69(2): 134-44.
- [4] FazhuangYao, Yuying Huang, Yihai Wang, Xiangjiu He. Anti-inflammatory diarylheptanoids and phenolics from the rhizomes of kencur (*Kaempferia galanga* L.). Industrial Crops and Products 2018; 125:454-61.
- [5] M.R. Sulaiman , Z.A. Zakaria, I.A. Daud, F N. Ng, Y.C. Ng, M.T. Hidayat. Antinociceptive and anti-inflammatory



- activities of the aqueous extract of *Kaempferia galanga* leaves in animal models. J Nat Med 2008; 62(2):221-7.
- [6] Kim, N.J., S.G. Byun, J.E. Cho, K. Chung and Y.J. Ahn. Larvicidal activity of *Kaempferia galanga* rhizome phenylpropanoids towards three mosquito species. Pest Manag Sci 2008; 64(8): 857-62.
- [7] Liu, B., F. Liu, C. Chen and H. Gao. Supercritical carbon dioxide extraction of ethyl *p*-methoxycinnamate from *Kaempferia galanga* L. rhizome and its apoptotic induction in human HepG2 cells. Nat Prod Res 2010; 24(20): 1927-32.
- [8] Melanny Ika Sulistyowaty, Nugroho A.E, Putra G.S and a. B.T. Ekowati J. Syntheses, Molecular Docking Study and Anticancer Activity Examination of *p*-Methoxycinnamoyl Hydrazides. Int J Pharm Clin Res 2016 ; 8(6): 623-7.
- [9] Lin Song, Xiaofei Wua, Jingwen Xie, Hui Zhang, Huanbin Yang, Qingpei Zeng, Xihong Yang and Wan cui Xie. *Kaempferia galanga* Linn. Extract - A potential antibacterial agent for preservation of poultry products. LWT 2021; 147:111553.
- [10] Lakshmanan, D., J. Werngren, L. Jose, K.P. Suja, M.S. Nair, R.L. Varma, S. Mundayoor, S. Hoffner and R. A. Kumar. Ethyl *p*-methoxycinnamate isolated from a traditional anti- tuberculosis medicinal herb inhibits drug resistant strains of Mycobacterium tuberculosis in vitro. Fitoterapia 2011; 82(5): 757-61.
- [11] Mohammad Shawkat Ali, Pritesh Ranjan Dash & Mahmuda Nasrin. Study of sedative activity of different extracts of *Kaempferia galanga* in Swiss albino mice. BMC Complementary and Alternative Medicine 2015; 15:158
- [12] Mukkasombut, N., Pipatrattanaseree, W. and Itharat, A. Validation of HPLC Method for the Determination of Anti-allergic Compounds in Ethanolic Extract of Prasaprophyai remedy, a Thai Traditional Medicine. Thammasat Medical Journal 2020; 20 (1), 74-83.
- [13] Tewtrakul, S. and A. Itharat. Anti-allergic substances from the rhizomes of *Dioscorea membranacea*. Bioorg Med Chem 2006; 14(24): 8707-11.
- [14] Tewtrakul, S and Itharat, A. Nitric oxide inhibitory substances from the rhizomes of *Dioscorea membranacea*, J.Ethnopharmacol 2007; 109 (3): 412-6.
- [15] ICH Harmonised Tripartite Guideline. Evaluation for stability data Q1E. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use.2003
- [16] Hasali, N.H.M, Omar, M.N, Zuberdi, A.M, and AlFarra, H. Y. Biotransformation of ethyl *p*-methoxycinnamate from *Kaempferia galanga* L. using *Aspergillus niger*. Int. J. Biosci 2013; 3(7): 148-55.
- [17] Li, Y. C, Ji, H, Li, X. H, Zhang, H. X. and Li, H. T. Isolation of nematocidal constituents from essential oil of *Kaempferia galanga* L. rhizome and their activity against *Heterodera avenae* Wollenweber. Trop J Pharm Res 2017; 16 (1): 59-65.
- [18] Nupur Srivastavaa, Ranjanaa, Shilpi Singhb, Amit Chand Guptab, Karuna Shankera, Dnyaneshwar U. Bawankuleb and Suaib Luqman. Aromatic ginger (*Kaempferia galanga* L.) extracts with ameliorative and protective potential as a functional food, beyond its flavor and nutritional benefits. Toxicology Reports 2019; 6 : 521-8.
- [19] Pimsen, N., Chanvimalueng, W., Mukkasombut N. , Yamprasert, R. and

Itharat, A. Preliminary Study of Effectiveness and Safety of Prasaprophyai Extract in Patients with Moderate to Severe Allergy Rhinitis. Thai Journal of Pharmacy Practice 2021; 13 (2): 508-17.