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# Accelerated Stability Study on Anti-Allergic, Anti-inflammatory Activities and Phytochemical Contents of the Ethanolic Extract of Zingiber officinale Roscoe

Rodsarin Yamprasert[1], Waipoj Chanvimalueng[2], Napaporn Pattanacharoenchai[3]
Weerachai Pipatrattanaseree[4], Nichamon Mukkasombut[5], Arunporn Itharat[1,5,*]

[1]*Department of Applied Thai Traditional Medicine, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[2]*Department of Otolaryngology, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*
[3]*Faculty of Dentistry, Thammasat University, Pathum Thani 12120, Thailand*
[4]*Regional Medical Science Centre Songkhla, Department of Medical Science, Ministry of Public Health, Songkhla 90100, Thailand*
[5]*Center of Excellence on Applied Thai Traditional Medicine Research, Faculty of Medicine, Thammasat University, Pathum Thani 12120, Thailand*

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## ABSTRACT

Allergic rhinitis (AR) is a global health problem and herbs are one of the alternative treatments for AR. Ginger is widely used as a spice food. Many reports have revealed that ginger extract has potential anti-allergic and anti-inflammatory activities. However, there is no report on the stability of the anti-allergic and anti-inflammatory activities of ginger extract after storage. This study aimed to investigate the stability of ginger extract on the accelerated condition by determining anti-allergic and anti-inflammatory activities and measuring 6-gingerol and 6-shogaol. The ethanolic extract of ginger was kept under the accelerated condition for six months. The extracts at various storage times were investigated for anti-allergic and anti-inflammatory activities by measuring the inhibition of β-hexosaminidase release from RBL-2H3 cells and nitric oxide production in RAW 264.7 cells, respectively. Levels of 6-gingerol and 6-shogaol were determined by HPLC. The results showed that under the accelerated storage condition, the anti-allergic activity of ethanolic ginger extract was stable until day 90; however, after day 90 the $IC_{50}$ values were still less than 20 µg/ml. There was no significant difference in anti-inflammatory activity of all stored ginger extracts

***Corresponding author:** iarunporn@yahoo.com
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comparing to day 0. At the end of the stability storage, the level of 6-gingerol, one of major components, was reduced, but 6-shogaol level was increased. However, the anti-allergic and anti-inflammatory activities were maintained at acceptable levels. Therefore, ginger extract can be stored within 2 years at room temperature without the loss of both activities. This study provided the stability data for development of ginger extract to be a drug for AR treatment.

**Keywords:** Ginger; Anti-allergic activity; Anti-inflammatory activity; HPLC; Stability study

## 1. Introduction

Allergic rhinitis (AR) is a worldwide health problem that causes major illness and significant impairment in quality of life [1]. AR is caused by IgE-mediated inflammation of the nasal mucosa initiated by an allergic immune response after the exposure to allergens. Exposure to the allergen leads to mast cell or basophil degranulation, causing the release of histamine, serotonin and other biologically active mediators [2]. Various drugs for treatment of allergic diseases have been developed based on mediators involved in Type I hypersensitivity reactions and found after the degranulation of sensitized mast cells. Nevertheless, chemical synthetic drugs for allergy have the risk of adverse reactions and high development costs. Thus, the search for safer food-derived ingredients with anti-allergic and anti-inflammation effects is important.

Drug regulatory agencies like the World Health Organization [3], European Medicines Agency [4], and the International Conference on Harmonization (ICH) [5] define the guidelines for maintaining quality, safety, and efficacy of herbal products through stability studies. The stability study provides quality information over the time of the stored plant extract under the influence of storage condition including environmental factors, such as temperature, humidity, light, oxygen, and moisture [6]. The purpose of stability testing is to determine the storage time under the storage condition of the extract. The extract should be stable when kept in an airtight container protected from light and stored at room temperature for at least two years [5].

*Zingiber officinale Roscoe* (Ginger) has been widely used as a spice for a long time and throughout the world. In Thai traditional medicine, ginger has been used as a component of herbal remedies for maintaining the balance of elements in the body and prescribed for treatment of common cold, constipation, sleeplessness, , relieving flatulence, etc. [7]. 6-gingerol, 6-shogaol, 8-gingerol and 10-gingerol have been identified as the major pungent compounds in ginger [8-10]. In a previous study, the ethanolic extract of ginger and pure compounds such as 6-gingerol and 6-shogaol showed good anti-allergic activity by inhibiting allergic reactions in rat basophilic leukemia (RBL-2H3) cells with $IC_{50}$ values of 12.93 ± 1.28μg/ml, 18.30 ± 3.38 μg /ml (62.16 μM) and 0.28 ± 0.11 μg /ml (1.01 μM), respectively [11]. In an *in vivo* study, 50 μM of 6-gingerol inhibited the expression of Th2 cytokines (IL-4, IL-10 and IL-13) and Th1 cytokine (IFN-$\gamma$) in ovalbumin (OVA) -sensitized spleen cells. Moreover, 6-shogaol reduced the passive cutaneous anaphylaxis reaction compared to the control group, and the significant reduction of mast cells and histamine can be detected in the rat peritoneum [12]. Moreover, ginger is well known for its ability to inhibit expression of several pro-inflammatory cytokines and inflammatory mediators synthesized from various types of cells, including nitric oxide, IL-1, TNF-α

and IL-8 [13,14], and affect Th1-derived responses [15].

Ginger extract exhibits potential anti-allergic and anti-inflammatory activities for the development as a drug for allergic treatment. However, there is no report on the stability of the anti-allergic activity and anti-inflammatory activities for quality control of crude extract. Therefore, the purpose of this study was to determine the biological activities and the change of 6-gingerol and 6-shogaol levels of the ginger extract after an accelerated stability test procedure. Inhibition of β-hexosaminidase release from RBL-2H3 cells can be used as a standard test for anti-allergic activity. Inhibition of nitric oxide release from LPS-stimulated RAW 264.7 cells can be used as a standard test for anti-inflamatotory activity.

## 2. Materials and Methods

### 2.1 Chemicals and reagents

Rat basophilic leukemia cell line (RBL-2H3: ATCC® CRL-2256™), and murine macrophage leukemia cell line (RAW 264.7: ATCC® TIB-71™) were purchased from ATCC. Anti-DNP IgE (Monoclonal Anti-DNP), anti-dinitrophenylated bovine serum albumin (DNP-BSA), p-nitrophenyl-N-acetyl-b-D-glucosaminide (PNAG), albumin bovine fraction V power, D- (+)-glucose, chlorpheniramine, lipopolysaccharide (LPS), sulfanil-amide, N-(1-naphthyl) ethylenediamine dihydrochloride, phosphoric acid, thiazolyl blue tetrazolium bromide (MTT) and prednisolone were purchased from Sigma–Aldrich Inc. (MO, USA). Calcium chloride dehydrate, citric acid monohydrate, magnesium chloride $6H_2O$, potassium chloride, sodium carbonate and sodium bicarbonate were purchased from Merck (Darmstadt, Germany). Fetal bovine serum (FBS), minimum essential medium (MEM), penicillin-streptomycin (P/S), trypan blue, RPMI 1640 medium and trypsin-EDTA were purchased from Gibco BRL Life Technologies (NY, USA). Phosphate buffer saline (PBS) and piperazine-N, N'-bis (2-ethanesulfonic acid) (PIPES) were purchased from Amresco (OH, USA). Sodium chloride and sodium hydroxide were purchased from Univar (NSW, Australia). Commercial grade ethanol was purchased from Sasol Chemical Pacific LTD (Shenton, Singapore). Water was purified using a Milli-Q water purification system from Millipore (MA, USA). Analytical grade reagents (e.g. dimethyl sulfoxide, hydrochloric acid, isopropanol) were purchased from Labscan Limited (Bangkok, Thailand). 6-Gingerol and 6-Shogaol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Acetronitrile and purified water (HPLC grade) from Labscan (Bangkok, Thailand).

### 2.2 Plant materials

Fresh rhizomes of ginger were collected in May 2015 from Ratchaburi province Thailand. The specimen voucher (BKF 192198) was deposited at Bangkok Forest Herbarium, Herbarium Department of National Parks, Wildlife and Plant Conservation Thailand.

### 2.3 Preparation of crude extract

The dried ginger rhizomes were cleaned and dried with hot air oven at 50 °C. Then, the dried rhizomes were mechanically powdered, and extracted by maceration with 95% ethanol for 3 days followed by filtration. This process was repeated two times with the residue. After that, the extract was concentrated under reduced pressure by a rotary evaporator and percentage of yield was calculated.

### 2.4 Stability study of ginger extract under the accelerated condition

Accelerated stability of the ethanolic extract of ginger was investigated according

to ICH guidelines [5]. The ginger extract was packed into capped opaque glass vials and stored under controlled temperature (40 ± 2 °C) and relative humidity (75 ± 5%) for 6 months. The samples were withdrawn at periods of 0, 15, 30, 60, 90, 120, 150 and 180 days and analyzed for anti-allergic activity, anti-inflammatory activity and levels of 6-gingerol and 6-shogaol.

### 2.5 Inhibitory effect of ginger extract on the release of β-hexosaminidase from RBL-2H3 cell lines

After contact with an allergen, histamine is usually released from granules in mast cells or basophils. Direct measurement of histamine is complicated more than the detection of β-hexosaminidase, an enzyme released along with histamine in the degranulation processes of mast cells. Therefore, the indirect method for testing anti-allergic activity is determined by analyzing the inhibitory effect on the release of β-hexosaminidase [16]. In this study, we investigated the anti-allergic activity of ginger extract by measuring its inhibitory effects on the release of β-hexosaminidase in IgE-sensitised and DNP-BSA stimulated rat basophilic leukemia RBL-2H3 cells.

The inhibitory effect of the extract on β–hexosaminidase release from RBL-2H3 cells was evaluated by the following modified method [17]. The RBL-2H3 cells were dispensed in 24-well plates at a concentration of $2 \times 10^5$ cells/well and allowed to adhere for 2 hr at 37 °C in 5% $CO_2$. The cells were then sensitized with anti-dinitrophenyl-immunoglobulin E (anti-DNP IgE) (0.45 mg/ml), and incubated at 37 °C in 5% $CO_2$ for 24 hr. The cells were washed twice with 400 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 PIPES, 0.1% 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 were added to each well and incubated for 10 min, and then 20 µl of antigen (DNP-BSA, final concentration 10 g/ml) were added and incubated 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 PNAG substrate (1 mM p-nitrophenyl-N-acetyl-d-glucosaminide) in 0.1 M citrate buffer (pH 4.5) at 37 °C for 1.5-2 hr. The reaction was stopped by adding 200 µl of stop solution (0.1 M $Na_2CO_3$ /$NaHCO_3$, pH 10.0). The absorbance was measured with a microplate reader at 405 nm. The inhibition (%) of β-hexosaminidase release was calculated by the following equation.

$$\text{inhibition } (\%) = \left[1 - \frac{(T - B - N)}{(C - B - N)}\right] x100$$

Control (C): DNP-BSA (+), test sample (−)
Test (T): DNP-BSA (+), test sample (+)
Blank (B): DNP-BSA (−), test sample (+)
Normal (N): DNP-BSA (−), test sample (−).

$IC_{50}$ values were calculated using a Prism software.

### 2.6 Inhibitory effects of ginger extract on the release of NO production from RAW264.7 cell lines

Nitric oxide is a free radical molecule produced from inflammation response. Nitric oxide is associated with homeostatic and pro-inflammatory roles in nasal mucosa, sinuses and airway [18]. Several studies reported the association between IgE mediated type I hypersensitivity and inflammatory reaction. Previous research reported that iNOS expression was elevated in the nasal epithelial cells of allergic rhinitis patients, especially in the nasal submucosal glands. Therefore, the continuous mucosal inflammation generally occurring in allergic

rhinitis patients could increase iNOS activity [19-21]. Consequently, inhibitoryeffect on NO production of crude ginger extract was investigated in this study.

The inhibition of NO production from RAW 264.7 cells was evaluated using the following modified method [22]. The cells were seeded in 96-well plates at a density of $1 \times 10^5$ cells/ 100 µl/ well and allowed to adhere for 24 hr at 37 °C in 5% $CO_2$. After that, the media was replaced with fresh media (100 µl /well) containing 10 ng/ml of LPS. Samples (100 µl) at various concentrations were applied into each well and then the plate were incubated at 24 hr. NO production from LPS activation was determined by the Griess reagent. Aliquots (100 µl) of supernatant were transferred into other 96-well plates and mixed with the same volume of Griess reagent (1% sulfanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% phosphoric acid). The optical density (OD) was measured by a microplate reader at 570 nm. The inhibition (%) of NO production was calculated by the following equation, and $IC_{50}$ values were calculated using a Prism software:

$$\text{inhibition}\,(\%) = \left[ \frac{OD_{control} - OD_{sample}}{OD_{control}} \right] x100,$$

where $OD_{control}$ = mean of control media (+LPS) - mean of control media (-LPS)
$OD_{sample}$ = mean of control sample (+LPS) - mean of control sample (-LPS)

### 2.7 Measurement of 6-gingerol and 6-shogaol levels in ginger extract

Determination of markers for ethanolic extract of ginger was carried out using the high performance liquid chromatographic (HPLC) method according to Pattanacharoenchai [23]. Both 6-gingerol and 6-shogaol responded for anti-allergic and anti-inflammatory activities [8]. The Chromatographic system is composed of a C18 reverse-phase column (250 x 4.60 mm 5 micron; Phenomenex, Inc., USA) protected by a Security Guard Cartridge (C18, 4 x 3.0 mm; Phenomenex, Inc., USA). Briefly, gradient mobile phase consists of water (A): acetonitrile (B): 0-25 min, 60:40; 25-40 min, 50:50; 40-45 min, 5:95; 45-45.10 min, 0:100; 45.10-50 min, 60:40. The flowrate was 1 ml/min and the peak response was detected with a diode array detector at absorbance of 227 nm. The operating temperature was maintained at room temperature.

Standard solutions of 6-gingerol and 6-shogaol were prepared in methanol and diluted serially for constructing calibration curves at the concentrations of 1, 5, 10, 25, 50, 80 and 100 µg/ml. Sample solutions were prepared by dissolving the extract in methanol and sonicating for 10 min to produce the extract solution at concentration of 10 mg/ml. All solutions were filtered through a 0.45 µm membrane filter prior to injection to HPLC for determining chemical content.

### 2.8 Statistical analysis

Data were expressed as the mean ± standard error of mean (SEM) of triplicate experiments. Statistical analysis was performed using a standard statistical software. Mean difference between samples at various time points compared with day 0 were analyzed by one-way analysis of variance (ANOVA) and followed by Dunnett's test.

## 3. Results and Discussions

### 3.1 Inhibitory effect of ginger extract on β-hexosaminidase release from RBL-2H3 cell line

The results found that ginger extract exhibited strong anti-allergic activity with $IC_{50}$ value of 12.73 ± 0.46 µg/ml, while positive control (chlorpheniramine: CPM)

showed less activity with $IC_{50}$ value of24.08 ± 2.44 µg/ml. The results of this study are consistent with the previous study that reported IC50 value of ginger extract at 12.93 mg/ml [11]. After storage in the accelerated condition, the anti-allergic activity of crude extract was slightly different with IC50 values ranging from 13.66 -18.63 µg/ml. The anti-allergic activity of crude extract at days 0, 15, 30, 60 and 90 was similar to day 0 (Table 1 and Fig. 1). The anti-allergic activity of crude extract was significantly decreased from day 120 – 180 with IC50 values of 18.63 ± 1.30, 17.64 ± 1.52 and 16.78 ± 1.02 µg/ml (p-value < 0.05), respectively. However, all stability samples exhibited anti-allergic activity more potent than positive control (chlorpheniramine) with IC50 values less than 20 µg/ml. The present study is the first report on accelerated stability study of ethanolic extract of ginger.

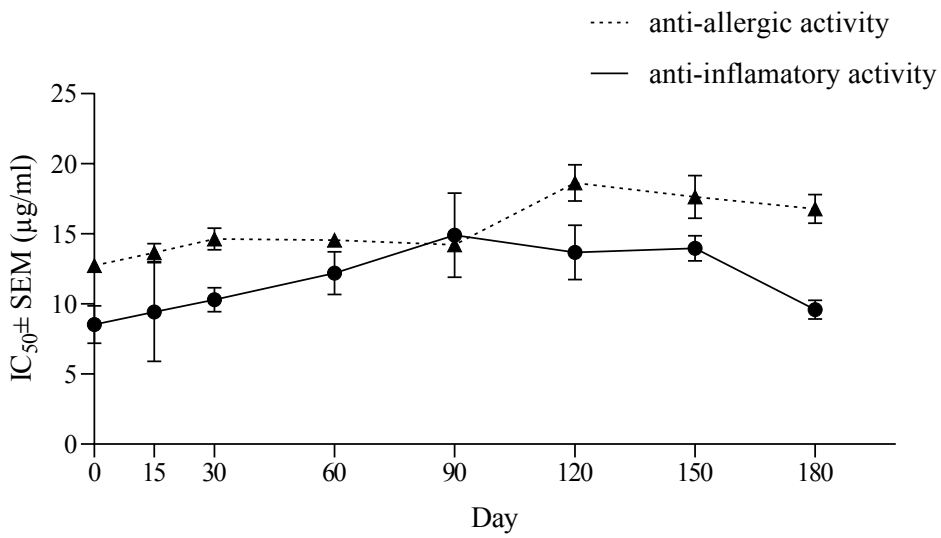


**Fig. 1.** Effect of ginger extracts at various storage times on anti-allergic and anti-inflammatory activity. Data were analyzed by one-way ANOVA and Dunnett's multiple comparison tests. Results are presented as $IC_{50}$ ± SEM (µg/ml) values (n = 3).
*Significant differences ($p < 0.05$) compared with Day 0.

**Table 1.** The inhibition (%) at various concentrations and $IC_{50}$ values of ginger extract on the inhibition of β-hexosaminidase release from RBL-2H3 cells (mean ± SEM; n = 3)

| Crude extracts | Inhibition (%) of b-hexosaminidase release from RBL-2H3 cells at various concentrations (µg/ml) | | | | $IC_{50}$± SEM (µg/ml) | p-value |
|---|---|---|---|---|---|---|
| | 1 | 10 | 50 | 100 | | |
| Day 0 | 18.51 ± 1.34 | 44.24 ± 1.09 | 55.14 ± 4.83 | 81.15 ± 0.72 | 12.73 ± 0.46 | control |
| Day 15 | 30.59 ± 0.53 | 45.49 ± 0.71 | 60.78 ± 1.23 | 89.39 ± 1.53 | 13.66 ± 0.64 | 0.919 |
| Day 30 | 25.44 ± 3.09 | 43.12 ± 0.06 | 68.41 ± 1.22 | 89.41 ± 1.02 | 14.64 ± 0.77 | 0.377 |
| Day 60 | 16.98 ± 2.47 | 41.18 ± 0.08 | 61.12 ± 5.84 | 82.85 ± 5.49 | 14.55 ± 0.26 | 0.422 |
| Day 90 | 19.12 ± 4.42 | 41.70 ± 1.65 | 72.19 ± 1.45 | 91.87 ± 3.20 | 14.22 ± 0.44 | 0.616 |
| Day 120 | 15.46 ± 0.07 | 36.03 ± 1.00 | 69.22 ± 1.90 | 94.86 ± 0.55 | 18.63 ± 1.30 | 0.000* |
| Day 150 | 17.88 ± 1.49 | 36.32 ± 2.46 | 68.00 ± 2.29 | 87.56 ± 4.51 | 17.64 ± 1.52 | 0.002* |
| Day 180 | 17.18 ± 0.43 | 37.22 ± 1.04 | 74.31 ± 2.44 | 90.72 ± 1.65 | 16.78 ± 1.02 | 0.010* |
| Chlorpheniramine | 12.48 ± 2.47 | 29.33 ± 3.76 | 70.81 ± 1.90 | 91.97 ± 1.24 | 24.08 ± 2.44 | - |

* Significant differences ($p < 0.05$) compared with Day 0.

The responsive markers of the anti-allergic activity are 6-gingerol and 6-shogaol. A previous study showed that 6-shogaol exhibited potent anti-allergic activity with an $IC_{50}$ value of 0.28 ± 0.11 μg/ml (1.01 μM), while 6-gingerol also exhibited the activity with $IC_{50}$ of 18.30 ± 3.38 μg/ml (62.16 μM) [11]. Both compounds showed potent anti-allergic activity compared to an antihistamine, chlorpheniramine. In addition, the pure compounds also exhibited the more potent activity than the crude extract, confirming the important of the pure compounds as markers of the ginger extract.

### 3.2 Inhibitory effects of ginger extract on the release of NO production from RAW264.7 cell line.

The ethanolic extract of ginger exhibited strong activity on the inhibition of NO production from RAW 264.7 cells with $IC_{50}$ value of 8.54 ± 1.34 μg/ml. However, the positive control (prednisolone) which is a potent inhibitor showed the highest potency. The inhibitory effect of the crude ginger extract was stable under the accelerated condition showing with $IC_{50}$ values ranging from 8.54 – 14.90 μg/ml (Table 2 and Figure 1). The inhibitory effect of the stability samples was not significantly different when compared with day 0 (*p*-value > 0.05).

Similar to the anti-allergic activity, 6-gingerol and 6-shogaol are also the responsive markers for anti-inflammatory by detecting the inhibition of NO production in RAW 264.7 cells. 6-Gingerol inhibited the NO production with $IC_{50}$ value of 72.25 ± 7.75 μg/ml (245.42 μM), while 6-shogaol inhibited with $IC_{50}$ value of 0.92 ± 0.31 μg/ml (3.33 μM) [11].

**Table 2.** The inhibition (%) at various concentrations and IC50 values of ginger extract from stability test of the inhibitory effect on LPS-induced NO production from RAW264.7 cells (mean ± SEM; n = 3).

| Crude extracts | Inhibition of NO production (%) from RAW264.7 cells at various concentration (μg/ml) | | | | | $IC_{50}$± SEM (μg/ml) | p-value |
|---|---|---|---|---|---|---|---|
| | 001 | 0.1 | 1 | 10 | 20 | | |
| Day 0 | - | -11.40± 1.51 | -1.33± 4.34 | 51.32± 2.64 | 81.14± 3.14 | 8.54± 1.34 | control |
| Day 15 | - | -12.72± 3.52 | - 4.79± 4.90 | 51.95± 5.56 | 80. 74± 2.69 | 9.42± 3.52 | 0.998 |
| Day 30 | - | -7.09± 5.18 | - 0.06± 4.84 | 49.52± 3.19 | 82.46± 3.65 | 10.31± 0.86 | 0.937 |
| Day 60 | - | -9.27± 11.04 | -1.19± 11.04 | 43.32± 4.24 | 74.63± 3.27 | 12.20± 1.52 | 0.719 |
| Day 90 | - | -14.31± 10.64 | -9.18± 8.44 | 35.09± 9.49 | 63.89± 7.58 | 14.90± 3.00 | 0.051 |
| Day 120 | - | -28.56± 8.49 | - 18.97± 8.92 | 36.88± 6.01 | 66.92± 4.76 | 13.67± 1.94 | 0.144 |
| Day 150 | - | -25.70± 5.50 | -17.17± 10.87 | 36.94± 4.10 | 68.73± 2.97 | 13.96± 0.90 | 0.114 |
| Day 180 | - | -10.42± 7.02 | -5.25± 7.99 | 54.57± 4.53 | 82.58± 3.62 | 9.59± 0.66 | 0.995 |
| prednisolone | 6.77 ± 2.61 | 54.92± 3.77 | 73.19± 1.35 | 77.47± 1.67 | - | 0.09± 0.01 | - |

* Significant differences ($p < 0.05$) compared with Day 0

## 3. Levels of 6-gingerol and 6-shogaol

Stability of 6-gingerol and 6-shogaol in the ethanolic ginger extract under accelerated condition was determined by HPLC method.

The chromatogram of 6-gingerol and 6-shogaol is shown in Fig. 2. The results show that 6-gingerol level was significantly reduced with high decreasing rate when compared with day 0. The 6-gingerol levels of day 0 and day 180 were 71.13 ± 0.80 mg/g (100% remaining) and 27.38 ± 1.66 mg/g (38.49% remaining). In contrast, 6-shogaol levels significantly increased

from day 15 and up to 2.45 times by day 180. The levels of 6-shogaol in the crude ginger extract at days 0, 15, 30, 60, 90, 120, 150 and 180 were 19.65 ± 0.35, 32.12 ± 0.25, 36.34 ± 0.73, 39.84 ± 0.79, 44.98 ± 0.89, 32.93 ± 3.36, 33.86 ± 3.30 and 48.21 ± 1.03 mg/g, respectively. The data is shown in Table 3 and Fig. 3.

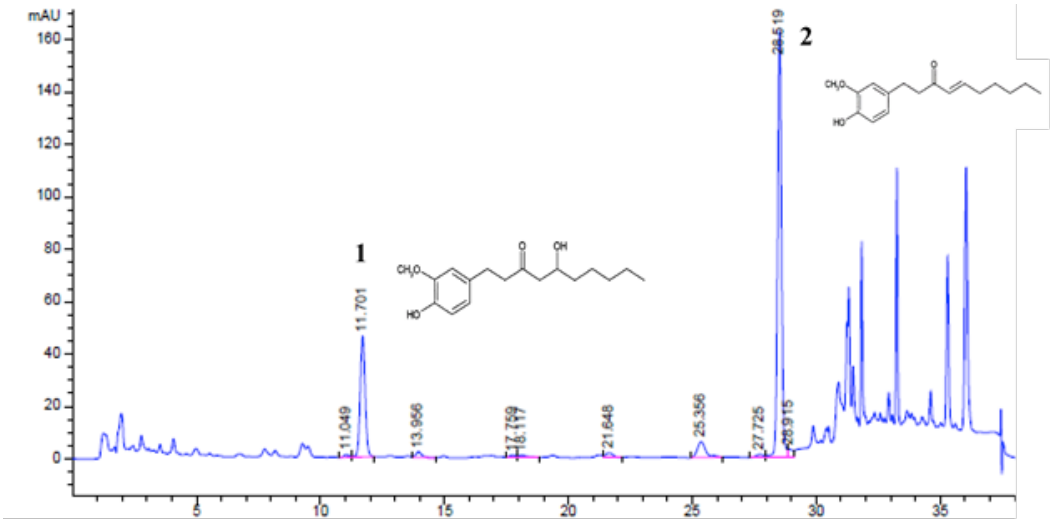


**Fig. 2.** HPLC chromatogram of ginger extract preparation: (1) 6-gingerol, (2) 6-shogaol.

**Table 3.** Levels of 6-gingerol and 6-shogaol of the ethanolic extract after storage under the accelerated condition (mean ± SEM; n = 3).

| Sample | 6-gingerol (mg/g) | % remaining | p-value | 6-shogaol (mg/g) | % remaining | p-value |
|---|---|---|---|---|---|---|
| Day 0 | 71.13 ± 0.80 | 100.00 | control | 19.65 ± 0.35 | 100.00 | control |
| Day 15 | 67.53 ± 0.69 | 94.94 | 0.594 | 32.12 ± 0.25 | 163.51 | 0.002* |
| Day 30 | 65.45 ± 1.13 | 92.02 | 0.184 | 36.34 ± 0.73 | 184.98 | < 0.001* |
| Day 60 | 53.79 ± 1.17 | 75.63 | < 0.001* | 39.84 ± 0.79 | 202.79 | < 0.001* |
| Day 90 | 46.15 ± 2.16 | 64.88 | < 0.001* | 44.98 ± 0.89 | 228.94 | < 0.001* |
| Day 120 | 24.98 ± 2.31 | 35.11 | < 0.001* | 32.93 ± 3.36 | 167.62 | 0.001* |
| Day 150 | 18.91 ± 2.61 | 26.59 | < 0.001* | 33.86 ± 3.30 | 172.33 | < 0.001* |
| Day 180 | 27.38 ±1.66 | 38.49 | < 0.001* | 48.21 ± 1.03 | 245.41 | < 0.001* |

* Significant differences ($p < 0.05$) compared with Day 0.

Regarding stability of 6-gingerol and 6-shogaol, which are the responsive components in ginger extract, the 6-gingerol level was significantly and greatly reduced, while the 6-shogaol level was significantly increased. This result relates to the previous report that gingerols are thermally labile compounds due to the presence of the β-hydroxy keto group in the structure, and they undergo hydrolysis to form the corresponding shogaols [24]. However, 6-gingerol and 6-shogaol were reduced in days 120 and 150 probably due to the change of 6-gingerol to other derivative compounds. Finally, at day 180, levels of both compounds were increased. The results relate to the anti-allergic and anti-inflammatory activities (Fig. 1). The potency of both activities of ginger

extracts at day 180 were slightly increased associating with the increasing of both responsive compounds. We suggest that it may due to the loss of volatile substances and moisture of the stored ginger extract that concentrated the levels of both compounds and other active non-volatile substances. However, the exact mechanism should be investigated further.

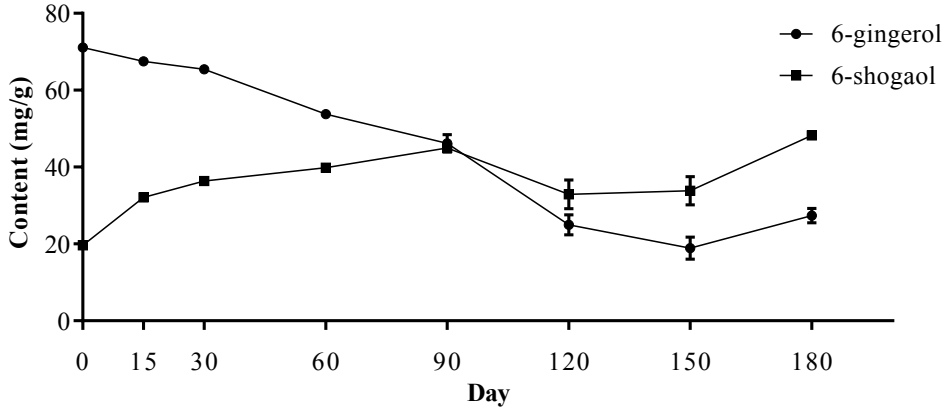


**Fig. 3.** 6-gingerol and 6-shogaol levels of the ethanolic extract after storage under the accelerated condition

A recent study found that ginger can be used for prevention of IgE-mediated allergic disease, especially allergic rhinitis. 6-gingerol is a potential immunosuppressive agent with a mechanism of markedly decreasing infiltration of mast cells in nasal mucosa, and potently suppressing the expression of IL-4, one of the key cytokines secreted by Th2 cells [25].

## 4. Conclusion

The purpose of this study was to investigate the stability of the ethanolic extract of ginger on the accelerated condition at 40 ± 2 °C and 75 ± 5% RH for 6 months. The 95% ethanolic extract of ginger showed stable anti - inflammatory activity. Although the extract showed significantly less potency at day 120 – 180 for anti-allergic activity when compared with day 0, the anti-allergic activity at day 120 – 180 was still higher than chlorpheniramine. Therefore, it could be concluded that both activities of ginger extract are stable under the accelerated condition. This is the first report of the stability of anti-allergic activity and anti-inflammatory activity of ginger extract. Furthermore, 6-gingerol and 6-shogaol levels vary over time but the activities of ginger extract remains stable. According to the guideline, therefore, the crude ginger extract can be stored for at least 2 years without the loss of activity in an airtight container protected from light at room temperature. We propose that ginger extract can be developed to be a drug or healthcare product for treatment or prevention of allergic rhinitis patients.

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