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# Extract, fractions, and ethyl-*p*-methoxycinnamate isolate from *Kaempferia galanga* Elicit anti-inflammatory activity by limiting leukotriene B4 (LTB4) production

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ABSTRACT

*Background and aim:* *Kaempferia galanga*, also known as aromatic Ginger (kencur) in Indonesia, has been widely explored and shows potential as an anti-inflammatory agent. However, there has been limited research to show a possible mechanism by which aromatic ginger inhibits lipoxygenase (LOX). Therefore, this study aims to determine the anti-inflammatory activity of aromatic ginger by comparing extract, fractions, and ethyl-*p*-methoxycinnamate (EPMC) isolate, as well as possible LOX inhibition activity, by reducing the production of leukotriene B4 (LTB4).

*Experimental procedure:* Two animal models were used, namely, the carrageenan-induced granuloma air pouch model and the pleurisy model. The test substance was administered 1 h before carrageenan induction, which was performed orally for each animal model. The number of leukocytes and the malondialdehyde (MDA) levels, leukotriene B4 (LTB4) levels, and histology were observed. GC-MS and LC-MS were used for analysis of the chemical compounds in the test samples.

*Results and conclusion:* The results of GC-MS analysis showed that aromatic ginger rhizome extract and fractions were dominated by ethyl-trans-*p*-methoxycinnamate, with the highest level found in the extract. *K. galanga* showed significant anti-inflammatory activity compared to the control ($p < 0.01$) in both the granuloma air pouch and pleurisy models. The results of examining the LTB4 concentration showed comparable activity between *K. galanga* extract, fractions and EMPC isolate, these results were not better than those of zileuton. Overall, this study shows that aromatic ginger extract, fractions and EPMC isolate have anti-inflammatory properties and have the potential to inhibit LOX, thereby reducing LTB4 levels.



## 1. Introduction

Kencur or aromatic ginger (*Kaempferia galanga* L.) is a medicinal plant that has been used by Indonesian people for generations[1] and is included in the Zingiberaceae family. The Zingiberaceae family is well known for its anti-inflammatory activities. Plants in this family have shown potential as anti-inflammatory agents in preclinical and clinical research studies. For example, *Zingiber officinale* reduced inflammatory markers in osteoarthritis and rheumatoid arthritis patients.[2,3] Other studies showed that the essential oils of *Alpinia oxyphylla* Miq. *Kaempferia galanga* L., *Amomum kravanh* Pierre ex Gagnep, and *Alpinia galanga* (Linn.) Willd Yizhiren noticeably inhibit the expression of cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1 (IL-1) in adjuvant arthritic rats.[4]

*K. galanga* contains phytochemicals including esters, terpenoids, flavonoids, diarylheptanoids, phenolic acids, phenolic glycoside, ethyl *p*-methoxycinnamate, ethyl cinnamate, kaempferol, kaempferide, kaempsulfonic acids, kaemgalangol A, xylose, cystargamide

*Abbreviations:* LOX, lipoxygenase; EPMC, ethyl-*p*-methoxycinnamate; MDA, malondialdehyde; LTB4, leukotriene B4; COX, cyclooxygenase; TNF-α, tumor necrosis factor-α; IL, interleukin; WF, water fraction; EAF, ethyl acetate fraction; HF, *n*-hexane fraction; E, ethanol extract; TEP, tetraethoxypropane; TBA, thiobarbituric acid; AA, arachidonic acid; PG, prostaglandin; FLAP, 5-lipoxygenase-activating protein; LTA4H, LTA4 hydrolase; COPD, chronic obstructive pulmonary disease; IBD, inflammatory bowel disease; ARDS, acute respiratory distress syndrome.

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B and 3-caren-5-one.[5] Several studies have been conducted on the anti-inflammatory activity of aromatic ginger. The aqueous extract inhibited edema in rats by 75% at a dose of 300 mg/kg BW,[6] the alcoholic extract inhibited granulation at a dose of 1200 mg/kg BW,[7] and the petroleum ether, chloroform, methanol, and water extracts also inhibited edema at a dose of 2 g/kg BW.[8] Studies also present the anti-inflammatory mechanism of *K. galanga* through inhibition of the cyclooxygenase (COX) enzyme.[9,10] These data show the potential function of aromatic ginger as an anti-inflammatory agent; however, studies on LOX are very limited.

LOX is an enzyme that plays a role in the metabolic process of arachidonic acid (AA), which produces leukotriene (LT), mediating inflammation.[11] A previous study showed that the mediators produced by 5-LOX, 12-LOX, and 15-LOX are associated with allergic reactions and the pathogenesis of atherosclerosis.[12] Although many studies on anti-inflammatory activity in aromatic ginger rhizomes have been carried out, only a few have focused on aromatic ginger as a lipoxygenase inhibitor. This research aims to determine the anti-inflammatory activity of aromatic ginger rhizomes and their possible mechanism as LOX inhibitors.

## 2. Materials and methods

### 2.1. Plant material

Aromatic ginger powder was obtained from Biopharmaca Conservation & Cultivation Station, Bogor Agricultural University, Bogor, Indonesia.

### 2.2. Animals

This study used 70 Wistar rats (*Rattus novergicus* L.) that were 2–3 months old with a body weight of 175–200 g. The animals were divided for use in two test models (granuloma air pouch and pleurisy models), where each model consisted of 7 groups: water fraction (WF), ethyl acetate fraction (EAF), *n*-hexane fraction (HF), ethanol extract (E), EPMC isolate, zileuton and control (received carrier) groups. The protocol was approved by the Health Research Ethics Commission, Muhammadiyah University, Prof. DR HAMKA (KEPK-UHAMKA), Jakarta, No: 03/20.11/0724 and 02/20.11/0722. All animals were acclimatized in a cage at 23 ± 2 °C for one week.

### 2.3. Preparation of aromatic ginger extract

A total of 3 kg of aromatic ginger rhizome powder was macerated with 70% ethanol solvent. The powder was soaked in 70% ethanol for 24 h. Then, the powder was filtered and concentrated by a rotary evaporator at 40 °C°C at a speed of 50 rpm until a thick extract was obtained.

### 2.4. Preparation of tiered aromatic ginger rhizome fraction

A total of 260 g of 70% ethanol extract was partitioned using a separating funnel and solvents with different polarity levels, namely, *n*-hexane, ethyl acetate, and water. The extract was added to ethanol to form a suspension, sufficiently mixed with distilled water, and poured into a separating funnel. Then, *n*-hexane was added at a ratio of 1:1, shaken and left for 30 min to 1 h until 2 layers were formed (the top and bottom layers were the *n*-hexane fraction and the water phase, respectively). The water phase and *n*-hexane fraction were isolated from the separating funnel. Subsequently, the water phase was poured into the separating funnel, and fresh *n*-hexane was added. This process was repeated 3 times or until the *n*-hexane layer was clear. The same procedure was then used to fractionate ethyl acetate. The *n*-hexane and ethyl acetate fractions were then concentrated with a rotary evaporator at 50 °C using a vacuum at a pressure of 130 mBar. The water fraction was further dried using an oven at 50 °C for 18 h. The viscous fraction obtained in this process was prepared for phytochemical and pharmacological testing.

### 2.5. Analysis of chemical compounds in extract, fractions, and EPMC crystal by GC-MS

Analysis of chemical compounds in extract and in the *n*-hexane fraction, ethyl acetate fraction, and in EPMC crystal was carried out at the Regional Health Laboratory, Health Service of the Provincial Government of DKI Jakarta in Indonesia. The ethanolic and crystalline extract were dissolved in methanol and then derivatized with bis-(trimethylsilyl) acetamide and 20 μL of pyridine reagent. The solution was incubated for 60 min at 80 °C. Five microliters of each sample solution was analyzed by gas chromatography with an AutoSampler (Agilent Technologies 7890), 5975 Mass Selective Detector, and Chemstation data system. Separation was carried out using a capillary column (HP Ultra 2) with a length of 30 m × 0.20 mm and D × 0.11 μm film thickness. Helium carrier gas was used at a constant flow rate of 1.2 mL/min. The injection port temperature was set at 250 °C. The initial oven temperature was set at 80 °C, held for 0 min, increased at 3 °C/min to 150 °C, held for 1 min, increased at 20 °C/min to 280 °C, and held for 26 min. The components were identified based on their compatibility with authentic mass spectra in Wiley's electronic library. The main compound criterion in the GC-MS chromatogram was that the compound had a percent area> 5%.[13] The similarity of the sample mass spectra with the library was determined at a qualifying value of at least 80%.

### 2.6. Analysis of chemical compounds in the water fraction using LC-MS

Analyses of the chemical compounds in the water fraction were carried out at the Regional Health Laboratory, Health Service of the Provincial Government of Jakarta in Indonesia. Separation was carried out with the Water Alliance 2695 HPLC Pump system. Chromatographic separation was achieved with an Acquity BEH C18 column (particle size 1.7 μm, 2.1 I. D × 100 mm, Waters, Milford, MA, USA). The mobile phase was methanol (A) and water (B) with a gradient system: 0 min with 10% A and 90% B; 5 min with 25% A and 75% B; 10 min 5 with 0% A and 50% B; 15 min with 75% A and 25% B; 20 min with 50% A and 50% B; 25 min with 25% A and 75% B; and 30 min with 10% A and 90% B. The flow rate was 0.2 mL/min. The sample was initially at 20 °C, while the column was at 40 °C with a maximum pressure of 300 Bar. Mass detection was performed using TQD with positive electrospray ionization (ESI) in multiple reaction monitoring modes. The scan time was set at 5 s to 50 min with a cone voltage of 25.0 V.

### 2.7. Carrageenan-induced granuloma air pouch inflammation

The rats were acclimatized for one week, and their backs were shaved and anesthetized with ketamine. The procedure followed the existing study with slight modification.[14] The shaved backs were swabbed with 70% ethanol and injected with 20 mL of sterile air using a 23G needle and a syringe with a 0.2 mm filter to form an air pouch. After 3 days, the animals were anesthetized and injected with 10 mL of sterile air into the pouch. On the sixth day, the fasting animals were anesthetized and induced with 2 mL of 2% carrageenan into the pouch using a 20 G syringe. One hour before being induced with carrageenan, the test substance was administered (p.o.). Twenty-four hours later, the rats were euthanized, and then

the exudate and pouch tissues were harvested. The tissue pouch was taken and rinsed with NaCl, partially soaked in 10% neutral buffer formalin for histological observation, and partially placed in phosphate buffer for the MDA test. Meanwhile, the exudate was taken using a syringe and then inserted into a sterile tube to determine the number of leukocytes and the level of MDA. A portion of exudate was separated and centrifuged for 10 min at 1000×*g* and 4 °C, and aliquots were used for the LTB4 assay.

### 2.8. Pleurisy

The pleurisy test was conducted based on existing research.[15] Animals were fasted for 18 h and then were given the test substance orally. After 1 h, the animals were anesthetized with ketamine, and a small incision was made under the right arm between ribs 7 and 8. Then, 0.2 mL of 2% w/v carrageenan in saline was injected into the pleural cavity using a 26G needle, and the incision was sutured. Four hours after carrageenan injection, the animals were euthanized. The pleural cavity was flushed with 2 mL of saline containing heparin (20 IU/mL), and then the sutures were carefully opened. Lungs and exudate from the pleural cavity were retrieved (the treatment for the sample was similar to that in the granuloma air-pouch model).

### 2.9. Determination of MDA levels

Tetraethoxypropane (TEP) was used as a standard. Tissue was prepared by adding phosphate buffer, pH 7.4 at a sample ratio of 1:1 and then washing the tissue in a container filled with ice. Furthermore, the sample was centrifuged at 3000 rpm for 10 min, and then an aliquot was separated for analysis. As much as 0.5 mL of exudate or aliquots from tissue samples were added with 0.5 mL of 20% TCA and 1 mL of 0.67% TBA and then homogenized. The mixture was heated in a water bath for 45 min at 50 °C. Subsequently, the samples were chilled on ice and centrifuged at 3000 rpm for 15 min. The supernatant was collected, and the absorption was determined by UV–vis spectrophotometry at a wavelength of 532 nm.

### 2.10. Histopathological examination

For histopathological examination, pouch skin (from the carrageenan-induced granuloma air pouch model) and lung (from the pleurisy model) were collected. The tissue was soaked in 10% neutral buffer formalin and then serially dehydrated in 100% alcohol, 96% alcohol, and 70% alcohol, respectively. Furthermore, the sample was rinsed with xylol and embedded in paraffin. The tissue block was sliced using a microtome with a thickness of 2.5 μm and placed on a slide. The tissues were stained with hematoxylin-eosin for 5 min and rinsed with running water for 10 min. The histopathological preparations were observed under a microscope at 400 × magnification.

### 2.11. Determination of the total leukocyte number

The exudate was aspirated using a Thoma pipette, and Turk reagent was added at a dilution ratio of 1:20 and homogenized. The sample was dropped into the counting chamber, and the leukocytes were allowed to settle for 2–3 min. Observations were made at 40 × magnification.

### 2.12. Determination of leukotriene B4 (LTB4) levels

LTB4 levels were determined using an ELISA Kit (Elabscience) by following the procedure stated in the brochure.

### 2.13. Data analysis

The data were tested for normality and homogeneity and were analyzed by ANOVA with a significance level of 95% and Tukey's test.

## 3. Results and discussion

### 3.1. Chemical compound analysis by GC-MS

GC-MS analysis was used to analyze the compounds of aromatic ginger rhizome extract, *n*-hexane, ethyl acetate fractions, and crystal (Supplementary file S.1). The peaks in retention time were 25,976 (in the aromatic ginger ethanol extract, A), 25,907 (*n*-hexane fraction), 25,604 (ethyl acetate fraction), and 25,742 (aromatic ginger crystal, B) by dominating the chromatogram pattern (Supplementary file S.2). These compounds have a molecular weight of 206 with distinctive fragmentation patterns at 134, 161, and 206 *m/z* (Supplementary file S.3). Based on these data, it was discovered that aromatic ginger rhizome extract and fractions are dominated by ethyl-trans-*p*-methoxycinnamate. This is an ester compound containing a benzene ring and a methoxy group, which is nonpolar, and contains a carbonyl group that binds to a polar ethyl. LC-MS analysis was used to determine the water fraction compounds (Supplementary file S.4). The components of various chemical compounds were described by chromatograms with 13 peaks, each with different molecular weights. The compound at the peak with a retention time of 19.08 min was the dominant compound in the water fraction. This compound has a molecular weight of 481.53.

### 3.2. Anti-inflammatory activity

The metabolism of arachidonic acid (AA) through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways produces several lipid mediators, including prostaglandins (PGs), leukotrienes (LTs), thromboxane (TX), and lipoxins. LTB4 is one of the LTs produced by the sequential action of 5-lipoxygenase (5-LOX), 5-lipoxygenase-activating protein (FLAP) and LTA4 hydrolase (LTA4H).[16] The anti-inflammatory activity of aromatic ginger has been widely studied, especially with COX inhibition. It was of interest to determine whether aromatic ginger could also inhibit lipoxygenase (LOX). In a previous report, we performed a docking study to explore the interaction between *K. galanga* potential compounds and LOX. We examined 21 compounds (ethyl cinnamate, ethyl *p*-methoxycinamic acid, *p*-methoxycinamic acid, 3-carene-5- one, camphene, *δ*-3- carene, *p*-methoxy styrene, *γ*-pinene, *β*-myrcene, *p*-cymene, 1,8-cineole, iso-myrcene, camphor, *α*-terpineol, *p*-cymene- 8-ol, eucarvone, *δ*-cadinene, kaempferol, quercetin, cyanidin, and delphinidin), and almost all compounds interacted with LOX, where the strongest interaction was with *δ*-3-carene. However, in an in vitro study, this compound showed less interaction potential with LOX than zileuton.[17]

Although the docking results showed the potential interactions between aromatic ginger compounds and LOX, the in vitro test results for a single compound showed unsatisfactory results. It is possible that the LOX inhibitory activity of *K. galanga* preferably occurred in a multicomponent form. Therefore, in this study, we retested the aromatic ginger activity of the extract form compared to its fraction and isolate forms to determine which form of preparation was the most active and anti-inflammatory and to determine its ability to inhibit LOX by measuring the concentration of leukotriene B4 (LTB4). The anti-inflammatory activity was tested using two animal models: the carrageenan-induced granuloma air pouch model and the pleurisy model. Carrageenan triggers the release of measurable inflammatory mediators and contributes to

the induction of COX-2 and LOX-5.[18,19] The water fraction (WF), ethyl acetate fraction (EAF), *n*-hexane fraction (HF), ethanol extract (E) and EPMC isolate of *K. galanga* were all tested in rats at the same dose of 300 mg/kg BW. This dose was chosen based on previous research, where aromatic ginger extract and fractions showed an anti-inflammatory effect in a paw edema rat model.[7,20]

Fig. 1 shows that aromatic ginger significantly reduces the number of leukocytes in both the pleurisy and granuloma air-pouch models. Both models showed a remarkable number of leukocytes in the exudate, especially in the pleurisy model. Aromatic ginger in all forms reduced the number of leukocytes in granuloma air-pouch and pleurisy models. The highest activity was observed in aromatic ginger ethyl acetate fractions, extract and EPMC isolate when compared to the results of zileuton. It is also noticeable that WF and HF decreased the leukocyte number slightly more in the pleurisy model than in the granuloma air-pouch model. This result was confirmed by histological observations (Fig. 2).

Carrageenan causes infiltration of many inflammatory cells, including leukocytes, into inflamed tissue.[21] Fig. 2 shows that groups treated with aromatic ginger (C, D, E, F, G) in both models had fewer leukocytes compared to the control (H). Inflammatory changes toward normal were observed in the zileuton group, followed by *K. galanga* EPMC isolate, extract, and fractions of.

In this study, we further examined the activity of *K. galanga* on LTB4. LTB4 is a lipid mediator involved in neutrophil recruitment.[16] Fig. 3 shows that there was a significant difference in LTB4 production in the control group compared to the other groups. In this study, carrageenan induction caused an increase in leukotriene concentrations that were higher in the pleurisy model than in the air pouch granuloma model, and administration of the test substance did not appear to significantly affect the leukotriene concentration in the air pouch granuloma model. In the pleurisy model, the aromatic ginger treatment results showed potential LOX inhibition activity, as indicated by significantly lower LTB4 concentrations than in the controls ($p < 0.05$). Interestingly, the aromatic ginger-treated group showed lower leukotriene concentrations in the extract and fraction form than in the EPMC isolate. This suggests that aromatic ginger contains other compounds that work synergistically to inhibit LOX. However, the activities of the extract, fractions, and EPMC isolate were not comparable to that of zileuton ($p < 0.05$). In addition to LOX, a study also showed a link between LTB4 and myeloperoxidase (MPO), which showed the ability of MPO to reduce the amount of LTB4 in neutrophils.[22] This indicates that MPO affects the LTB4 concentration, so further research is needed to examine the effect of *K. galanga* on this enzyme.

LOX plays an important role in many pathophysiological conditions, such as asthma, COPD, IBD, arthritis, atherosclerosis, dermatitis and cancer.[23] Clinical studies in patients with acute respiratory distress syndrome (ARDS) showed an improvement in oxygenation, which was suspected to be associated with a decrease in LTB4 after 5 days of ginger supplementation at a dose of 120 mg.[24] This indicates that aromatic ginger can possibly be used to treat these conditions. However, compared to *K. galanga, Z. officinale* (ginger) still showed better potential as an anti-inflammatory agent.

Gonçalves & Romano (2017)[25] reviewed that phenolics (flavonoids and stilbenes), as anti-inflammatory agents, were able to inhibit COX and LOX. Therefore, it is possible that the LOX inhibitory activity of *K. galanga* was caused by its flavonoids, kaempferol and kaempferide[5]. Since a study has shown that aromatic ginger inhibits COX,[8] the ability of aromatic ginger to reduce the concentration of LTB4 in this study indicates the possibility of dual COX/LOX inhibitor activity by *K. galanga*. Dual COX/LOX inhibitors could be greatly advantageous, causing less gastric toxicity, and could be developed for other diseases, such as cancer,[26,27] which needs to be investigated further.

Inflammation is also related to oxidative stress.[28] Therefore, the antioxidant capacity of plants often underlies their anti-inflammatory activity. Oxidative stress causes lipid peroxidation, producing malondialdehyde (MDA).[29] In this study, MDA was examined using exudate, tissue and serum samples from pleurisy and granuloma air pouch models (Table 1).

The results showed that carrageenan significantly increased the MDA concentration in blood, exudate, and tissue ($p < 0.01$) compared to that in normal animals. In both animal models, the MDA concentration was higher in tissue than in exudate and serum. All test sample groups inhibited oxidative stress, with the highest level of inhibition observed in EMPC isolate and extract and ethyl acetate fraction. These results were consistent with the conclusion that the test samples exhibited anti-inflammatory activity, which suggests a possible anti-inflammatory mechanism of aromatic ginger through antioxidants.

## 4. Conclusions

The results showed that *K. galanga* extract, fractions, and EPMC isolate have anti-inflammatory activity, as shown by a significantly reduced number of leukocytes and reduced LTB4 concentrations compared to the control. *K. galanga* extract, ethyl acetate fractions

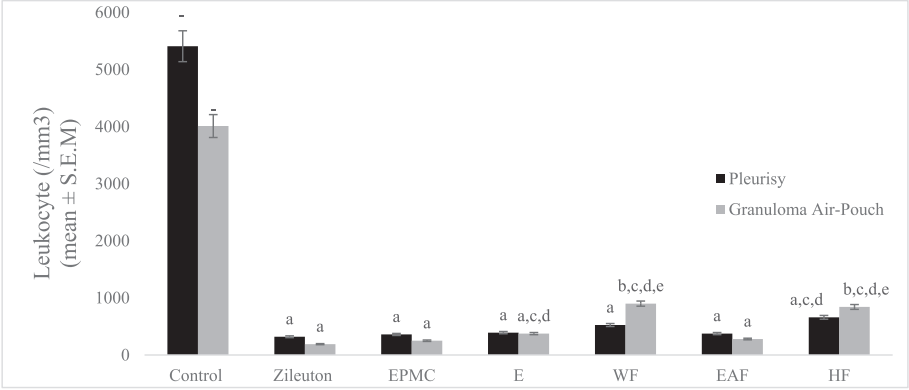


**Fig. 1.** The effect of *K. galanga* on the total leukocyte number in exudate in granuloma air pouch and pleurisy models is shown. [a]Significantly different compared to the control ($p < 0.01$). [b]Significantly different compared to the control ($p < 0.05$). [c]Significantly different compared to zileuton ($p < 0.05$). [d]Significantly different compared to EPMC ($p < 0.05$). [e]Significantly different compared to EAF ($p < 0.05$).

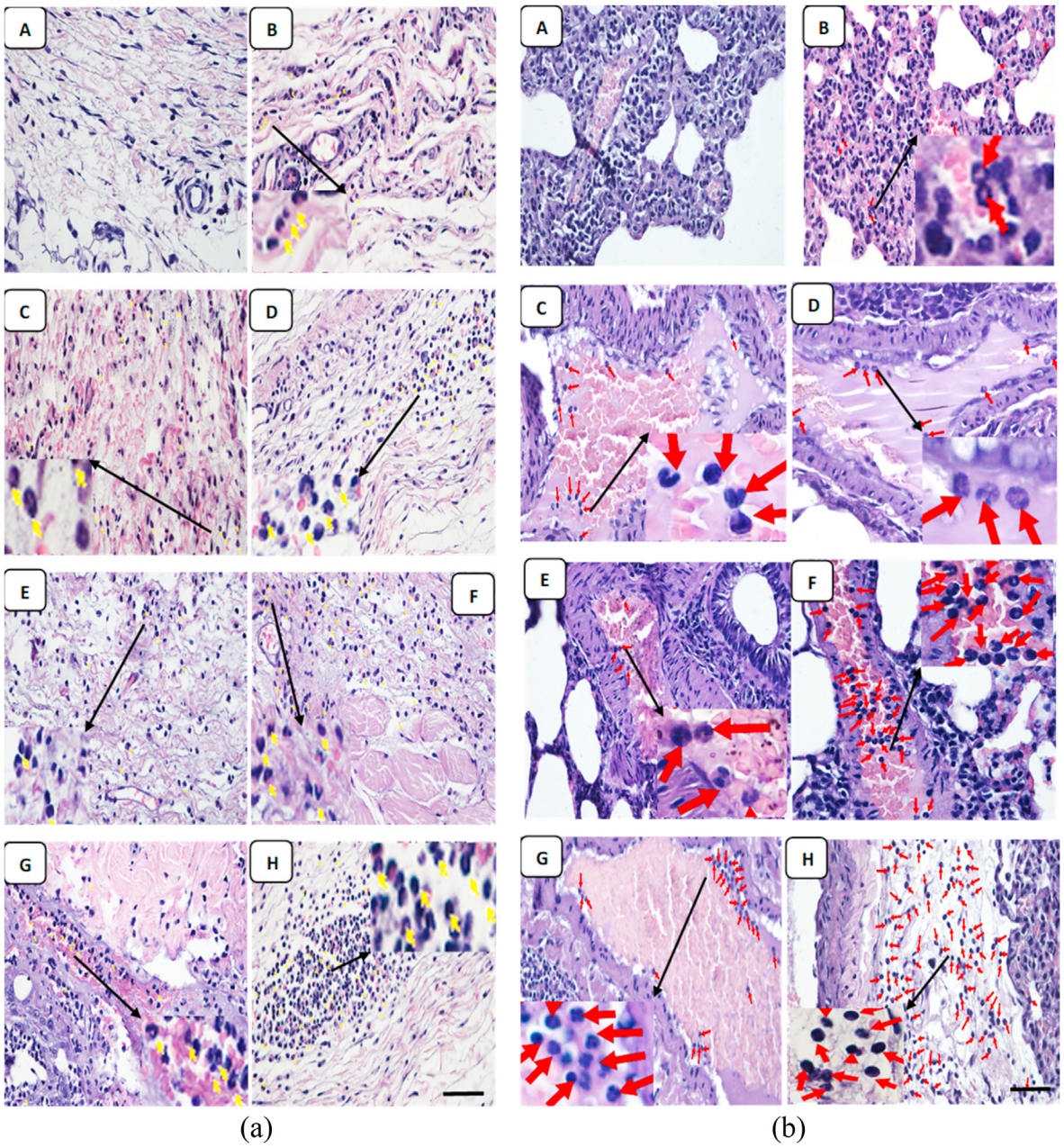


**Fig. 2.** Histopathology experiments were performed to observe tissue. (a) Pouch tissue from the granuloma air pouch model (yellow arrow) and (b) lung tissue from the pleurisy model (red arrow) are shown. The arrow in each picture shows leukocyte infiltration. A = Normal; B = Zileuton; C = EPMC; D = extract; E = EAF; F= HF; G = WF; H = control (hematoxylin and eosin staining; 400 × magnification, scale bar 25 μm).

and EPMC isolate showed comparable results to zileuton in lowering the leukocyte number; however, none of these substances showed comparable results to zileuton in limiting the LTB4 concentration. All in all, this study shows that aromatic ginger extract, fractions and EPMC isolate have anti-inflammatory properties and have the potential to reduce LTB4 levels.

## Author declartion

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved experimental animals has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

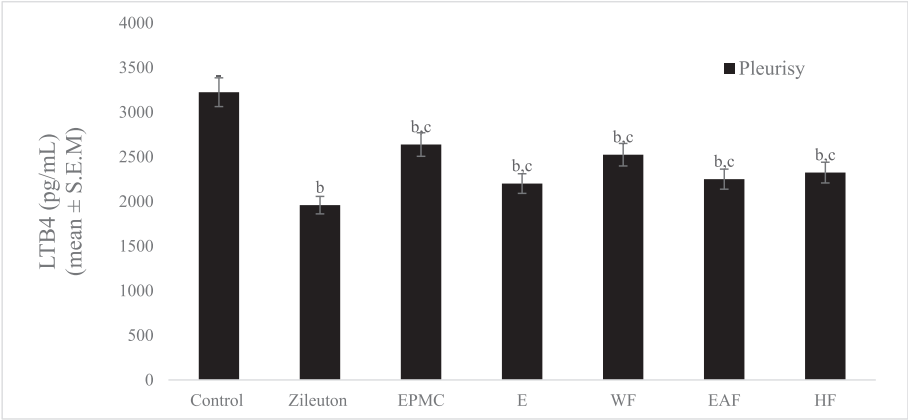


**Fig. 3.** The effect of *K. galanga* on the exudate LTB4 concentration using the pleurisy model is shown. [b]Significantly different compared to the control ($p < 0.05$). [c]Significantly different compared to zileuton ($p < 0.05$).

**Table 1**
Antioxidant activity of *K. galanga*.

| MDA concentration (Mean ± S.E.M) | | | | | |
|---|---|---|---|---|---|
| Groups | Pleurisy | | Granuloma air-pouch | | |
| | Exudate (nmol/mL) | Tissue (nmol/gr) | Exudate (nmol/mL) | Tissue (nmol/gr) | Serum (nmol/mL) |
| Control | $8.63 \pm 0.11^{bc}$ | $32.80 \pm 0.70^{bc}$ | $8.51 \pm 0.2^{bc}$ | $32.30 \pm 0.91^{bc}$ | $6.66 \pm 0.07^{b}$ |
| NC | $5.60 \pm 0.15^{ac}$ | $20.64 \pm 0.70^{ac}$ | $5.49 \pm 0.18^{ac}$ | $20.80 \pm 0.51^{ac}$ | $4.13 \pm 0.17^{ac}$ |
| EPMC | $6.21 \pm 0.09^{abc}$ | $23.49 \pm 0.25^{abc}$ | $6.13 \pm 0.13^{ab}$ | $23.55 \pm 0.16^{abc}$ | $4.68 \pm 0.06^{ab}$ |
| E | $6.77 \pm 0.04^{abc}$ | $25.17 \pm 0.33^{abc}$ | $6.67 \pm 0.06^{abc}$ | $25.47 \pm 0.31^{abc}$ | $5.17 \pm 0.05^{abc}$ |
| WF | $6.91 \pm 0.05^{abc}$ | $25.33 \pm 0.18^{abc}$ | $6.93 \pm 0.10^{abc}$ | $26.30 \pm 0.19^{abc}$ | $5.42 \pm 0.17^{abc}$ |
| EAF | $6.59 \pm 0.08^{abc}$ | $25.07 \pm 0.42^{abc}$ | $6.50 \pm 0.12^{abc}$ | $24.98 \pm 0.30^{abc}$ | $4.48 \pm 0.13^{ab}$ |
| HF | $7.75 \pm 0.08^{abc}$ | $29.87 \pm 0.41^{abc}$ | $7.86 \pm 0.09^{abc}$ | $29.50 \pm 0.16^{abc}$ | $6.02 \pm 0.16^{abc}$ |
| Zileuton | $5.97 \pm 0.06^{ab}$ | $22.98 \pm 0.49^{ab}$ | $5.95 \pm 0.08^{ab}$ | $23.12 \pm 0.37^{ab}$ | $4.05 \pm 0.03^{ab}$ |

[a]Significantly different compared to the control ($p < 0.01$), [b]Significantly different compared to the normal control ($p < 0.01$), [c]Significantly different compared to zileuton ($p < 0.01$).

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from (supandi@uhamka.ac.id).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2021.06.004.

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