



## Antioxidant and Anti-Inflammatory Activity of Red Ginger (*Zingiber officinale* Roscoe var. *Sunti* Val) Essential Oil Distillation Residues

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### Abstract

Natural resources are the main assets for drug development. Red ginger, a gift from nature proven to have pharmacological activities are widely used in traditional medicine. However, the process of its oil refining by traditional method produces heaps of residue unprocessed, causing excessive waste of chemical contents that may have pharmacological activity. Thus, this study aims to create an extraction model to utilize these residues and access its antioxidant and anti-inflammatory activity. 2,2'-azinobis (3-ethylbenzothiazolinone-6-sulfonic acid) (ABTS), 1-diphenyl-2-picrylhydrazyl (DPPH), and lipoxygenase inhibition activity methods were used. Results showed the use of liquid-liquid extraction method with ethyl acetate solvent produced a fraction abundance with 6-gingerol. Antioxidant activity accessed by ABTS and DPPH methods showed the ethyl acetate fraction collected from red ginger residues gave inhibition concentrations (IC<sub>50</sub>) of 27.95 ± 2.69 µg/ml and 32.83 ± 1.61 µg/ml respectively. The strongest lipoxygenase inhibitory activity is also shown by its ethyl acetate fraction (IC<sub>50</sub> 77.23 ± 8.14 µg/ml). These potent antioxidant activities could be caused by its 6-gingerol content (201.38 ± 6.77 ppm/µg extract). In conclusion, the proposed method provides the opportunity for further utilization of red ginger distillation residues. Based on antioxidant capacity and anti-inflammatory properties, ethyl acetate fraction collected from distillation residues has the potential to be developed as an antihyperlipidemic drug, given its 6-gingerol content which prevents lipid peroxidation.

Keywords: red ginger; 2,2'-azinobis (3-ethylbenzothiazolinone-6-sulfonic acid); 1-diphenyl-2-picrylhydrazyl; lipoxygenase

### 1. Introduction

People in their lifetime are constantly exposed to free radicals that affect the body physiologically. In most cases, reactive oxygen species (ROS) and reactive nitrogen species (NOS) are the main free radicals. It was produced as a metabolism residue or obtained from the environment (pollutants). ROS involvement in inflammations, atherogenesis, degenerative disease, metabolic syndrome, and cancer has been proven scientifically [1]. As a counterattack, our body generates antioxidants to neutralize it. These antioxidants actions are generally classified as antioxidant enzymes, chain-breaking antioxidants, and

metal-binding proteins [2]. Antioxidant intake comes from inside the body (endogenous) and nutritional foods.

Red ginger, a variety of ginger, offers a good source of antioxidants because of its gingerols contents based on the elephant ginger research (*Zingiber officinale* Roscoe var. *officinale*) [3]. Ginger varieties are used widely in the world as herbal medicine or spice whether in China, Turkey, India, and Greek [4-6]. In Indonesia, some ethnic groups use red ginger in traditional medication to treat nausea, vomit, impotency, arthritis, flatulence, and cough [7-8]. The red ginger rhizomes could be boiled, burned, and

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shredded then drink immediately. Traditionally volatile oils of red ginger rhizomes used as a medicine were collected with distillation method, whilst in the more modern factory, supercritical fluid extraction (SFE) is the main extraction method. The distillation residue is always discarded as animal feed especially in red ginger cases even though only a small portion of its chemical contents collected, leading to non-optimal utilization. Another method to collect volatile oils is by utilizing ultrasonic waves. This method consumes less energy and time compared to traditional extraction [9].

Researches about red ginger are developing rapidly. Pharmacologically, red ginger ethanolic extract showed an antihyperlipidemic effect when treated to Sprague Dawley rats for 2 weeks. Rats' lipid profile showed a decrease in total cholesterol and triglyceride levels also an increase in HDL levels [10]. When treated to activated macrophages, the active fraction of red ginger chloroform extract effectively inhibited nitric oxide and prostaglandin E2 productions [11]. Red ginger aqueous extracts also inhibit angiotensin-I converting enzyme (ACE) in rats' hearts [12]. Its oleoresins showed a tonic effect, and volatile oils contained in red ginger exhibit antimicrobial activity when treated to *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa* [13-14]. Not only have many pharmacological activities, but red ginger also easily obtained and cheap thus many people utilize it in everyday use as a spice and aromatherapy. Prompted by stated reasons, we proposed a method to maximize its utilization using liquid-liquid extraction to extract a fraction from the residues. To access its possible pharmacological properties, *in vitro* antioxidant and lipoxygenase inhibitory activity methods were used. We also compared its activity towards red ginger rhizome ethanol extract and volatile oils.

## 2. Experimental

### 2.1. Extractions

Fresh and dried red ginger rhizomes were collected from Ciapus village, Bogor regency, West Java. The rhizomes were 9 months old before cultivated and dried. The rhizomes were determined in the *Herbarium Bandungense* and distilled traditionally in the Manoko Experimental Garden. Fresh red ginger rhizomes were used in the distillation process to produce volatile oils and residues. Dried red ginger rhizomes then refluxed with 96% ethanol

(Bratachem®) for three cycles, each cycle last for 2 hours. All extract collected from red ginger rhizomes were concentrated by rotary evaporator. Chromatograph profile was accessed using TLC F254 plate, with ethyl acetate-toluene (1:9) as mobile phase and sprayed with 10% H<sub>2</sub>SO<sub>4</sub>. 6-gingerol (Phytopurify®) was used as a standard.

### 2.2. TLC-Densitometry

Sample and standard solution at various concentrations were sprayed on to TLC F<sub>254</sub> using Linomat 5 then developed with ethyl acetate-toluene (1:9) as mobile phase. The densities were measured at λ 239 nm by TLC scanner 3 (CAMAG) to produce a regression curve of area under the curve (AUC).

### 2.3. 2,2'-azinobis (3-ethylbenzothiazolinone-6-sulfonic acid (ABTS) Assay

20 μL sample or standard solution at various concentration were mixed with 180 μL ABTS radical (absorbance at λ 734 nm of 0.7±0.2). The mixture was then incubated at room temperature for 7 minutes and measured at λ 734 nm [15]. The experiment was done in triplicate. Percentage of inhibition values were calculated according to the formula below:

$$\% \text{ inhibition} = \frac{\{C-S\}}{C} \times 100\% \quad (1)$$

C represents the absorbance of control (ABTS), while S is the absorbance of the sample. The linear regression of concentration versus % inhibition was used to determine IC<sub>50</sub>. Ascorbic acid and α-tocopherol were used as standards. All data were presented as mean ± deviation standard (SD).

### 2.4. 1-diphenyl-2-picrylhydrazyl (DPPH) Assay

50 μL sample or standard solution at various concentrations were mixed with 200 μL DPPH free radicals (0.005% w/v methanolic solution). The mixture was then incubated at room temperature for 30 minutes and measured at λ 517 nm [15]. The experiment was done in triplicate. Percentage of inhibition values were calculated according to formula (1). C represents the absorbance of control (DPPH).

### 2.5. Lipoxygenase (LOX) Inhibition Assay

Measurement of lipoxygenase inhibitory activity refers to Lyckander and Malterud (1992) [16]. Measurement of the increase in absorbance at a wavelength of 234 nm was carried out in the sample that had been added with the enzyme (200 U/mL) in

0.2 M borate buffer pH 9 with linoleic acid as the substrate (134  $\mu\text{M}$ ). DMSO was used as a blank and sample solvent. The inhibitory activity of the enzyme was expressed in terms of increasing absorbance over time. Quercetin was used as a reference standard.

### 2.6. Statistical analysis

All determinations were conducted in triplicate. The reported value for each sample was calculated as the mean and standard deviation of three independent experiments. Statistical analysis was performed by SPSS 16 for Windows using ANOVA and followed by Tukey posthoc. A P value of less than 0.05 was used as the criterion for statistical significance.

### 3. Results and Discussion

The red ginger rhizomes distillation residues extraction process was based on the method stated below (Fig.1). Based on TLC chromatography profiles ethyl acetate fraction collected from distillation residues exhibited high 6-gingerol presence in it. The TLC-densitometry further confirmed this result (Table 1).

6-gingerol was widely known for its antiinflammation and antioxidant activity and shown numerous pharmaceutical properties. Nearly all aspects from its biosynthesis, pharmacokinetics, and safety were studied with full of promising results to be developed as an anti-inflammation drug. These reasons act as the basis to determine possible pharmacological activity from red ginger distillation residues.

Table 1: 6-gingerol concentration on red ginger extracts

Sample	6-gingerol concentration (ppm/ $\mu\text{g}$ extract)
Red ginger rhizomes ethanolic extract	145.96 $\pm$ 1.65
Red ginger rhizomes ethyl acetate fraction	201.38 $\pm$ 6.77

DPPH radical scavenging activity and ABTS methods were used for their sensitivity, stable radicals, and ABTS solubility properties facilitated antioxidant capacity both hydrophilic and lipophilic compounds. The DPPH radical scavenging activity method is based on DPPH radical reduction detected by its discoloration from purple to yellow. The antioxidant capacity is determined by the amounts of hydrogen donor of a substance [17]. Ascorbic acid and  $\alpha$ -

tocopherol were used as standards in this research because both are widely used in the human body.  $\alpha$ -tocopherol, a lipid phase chain-breaking antioxidant, played an important role in lipid peroxidation. While ascorbic acid acts as aqueous phase chain-breaking antioxidants that scavenge radicals located in the aqueous compartment [3]. ABTS radical cation is also very reactive to ascorbic acid [18].

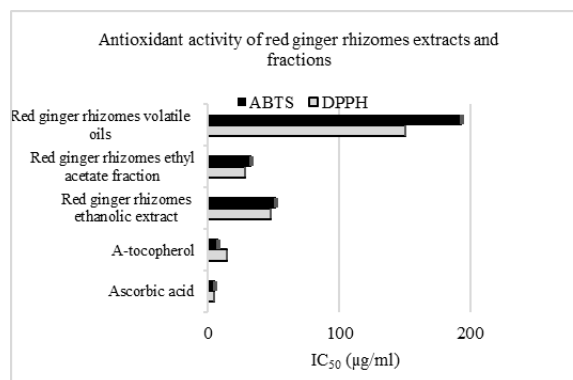


Table 2: Antioxidant activity of red ginger rhizomes extracts and fractions

Sample	IC <sub>50</sub> ( $\mu\text{g/ml}$ )	
	DPPH	ABTS
Ascorbic acid	4.81 $\pm$ 0.13	5.39 $\pm$ 0.55
A-tocopherol	14.13 $\pm$ 4.61	8.36 $\pm$ 0.44
Red ginger rhizomes ethanolic extract	47.77 $\pm$ 1.97	51.74 $\pm$ 1.68
Red ginger rhizomes ethyl acetate fraction	27.95 $\pm$ 2.69	32.83 $\pm$ 1.61
Red ginger rhizomes volatile oils	150.5 $\pm$ 2.39	193.54 $\pm$ 9.45

DPPH radical scavenging activity results showed ethyl acetate fraction collected from distillation residues act as a very strong antioxidant according to Blois classification [19]. Ethyl acetate fraction exhibited potent antioxidant activity compared to red ginger rhizomes ethanolic extract and volatile oils albeit inferior compared to ascorbic acid and  $\alpha$ -tocopherol (Table 2). The antioxidant activity of red ginger rhizomes ethyl acetate fraction was significantly different from its ethanolic extract form, indicating that there was a collection of active compounds with antioxidant properties such as gingerol and shogaol in the fraction. This enables to narrow the possibilities of further purification and separating possible compounds that hinder its pharmacological properties.

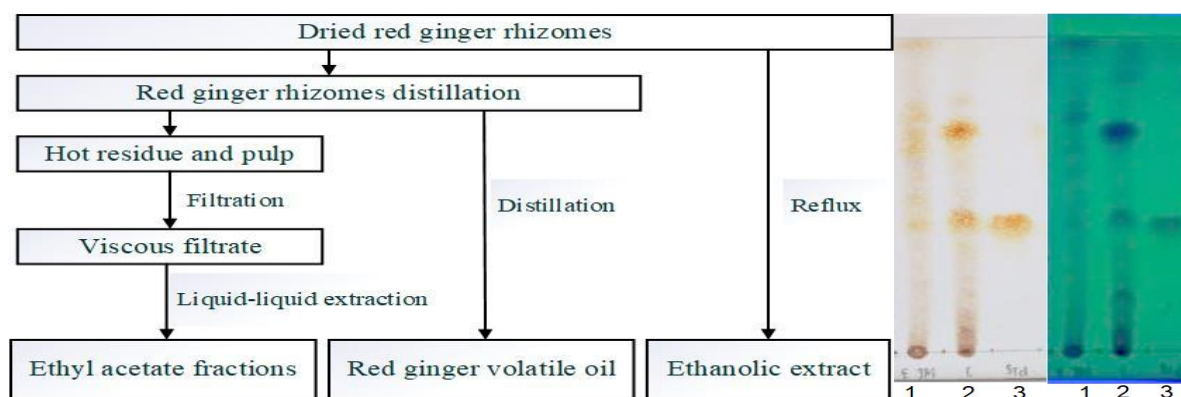


Fig 1. Red ginger rhizome residues extraction using liquid-liquid extraction method with TLC profile comparison of red ginger rhizomes from its ethanolic extract (1), ethyl acetate fractions extracted from residues (2), and 6-gingerol standard (3).

Similar results showed in the ABTS method. Red ginger rhizomes ethyl acetate fraction exhibited potent antioxidant activity that statistically significant compared to its ethanolic extract or volatile oils. These results indicate a positive correlation between the results of both antioxidant methods. These results also in line with previous studies about the correlation between the results of these two methods [20-21].

Lipoxygenase inhibition activity was used to measured possible anti-inflammatory properties. The residual fraction provided the strongest inhibitory activity than ethanol extract and its essential oil although inferior to quercetin. Associated with the chromatography results, potent lipoxygenase inhibition activity from the ethyl acetate fraction of distillation residue may be caused by the 6-gingerol and similar compounds contained in it. 6-gingerol acted as hydrogen atom donors that eliminated peroxide chain reaction as in the lipid peroxidation process [22]. Not only acting as lipoxygenase inhibition activity but judging from the short carbon chain structure from 6-gingerol also contributes to its high antioxidant activity [21].

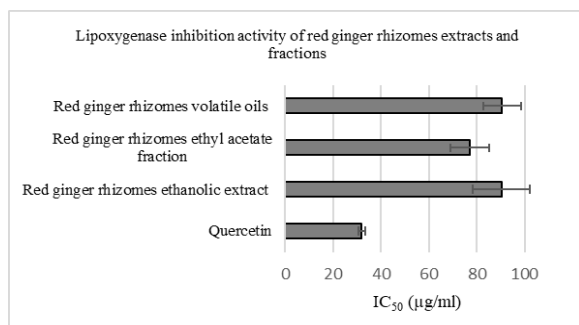


Table 3: Lipoxygenase inhibition activity of red ginger rhizomes extracts and fractions

Sample	IC <sub>50</sub> (µg/ml)
Quercetin	31.94 ± 1.33
Red ginger rhizomes ethanolic extract	90.36 ± 11.9 <sup>a</sup>
Red ginger rhizomes ethyl acetate fraction	77.23 ± 8.14 <sup>ab</sup>
Red ginger rhizomes volatile oils	90.59 ± 7.93 <sup>a</sup>

<sup>a</sup>: statistically significant compared to quercetin (p<0.05)

<sup>b</sup>: statistically significant compared to ethanolic extract and volatile oils (p<0.05)

Lipoxygenase plays an important role in the pathophysiology of several inflammatory diseases such as asthma, psoriasis, and atherosclerosis. Lipoxygenase inhibitory activity is closely related to its antioxidant properties. The mechanisms of lipoxygenase inhibition were through inhibition of the formation of lipid hydroperoxides due to scavenging lipidoxy- or lipidperoxy- radicals, chelation with non-heme bound iron, or reduction of the ferric form.

From the results of the lipoxygenase inhibitory activity test in this study, it can be observed that there were similar results of lipoxygenase inhibition between red ginger ethanolic extract and volatile oils. These results were due to the presence of sesquiterpenes such as ar-curcumene,  $\beta$ -bisabolene, and sesquiphellandrene which are present in red ginger rhizome essential oils that proven to have lipoxygenase inhibitory properties. Zingiberene, a monocyclic sesquiterpenoids was also proven to have potent antioxidant activity [23]. The level of inhibitory activity of red ginger volatile oils will be influenced by its chemical compositions. These results were consistent with previous research on ginger essential oil [24]. Rat paw edema models also showed similar results. Red ginger extract was found to possess potent analgesic and anti-inflammatory effects by inhibiting NO production and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) [25].

#### 4. Conclusions

Proposed liquid-liquid extraction yields an ethyl acetate fraction that contains 6-gingerol. Based on antioxidant capacity and anti-inflammatory properties, ethyl acetate fraction collected from distillation residues has the potential to be developed as an antihyperlipidemic drug, given its 6-gingerol content which prevents lipid peroxidation or other inflammatory diseases. The proposed method also provides the opportunity for further utilization of red ginger distillation residues in food industry. The ethyl acetate fraction obtained from this residue could be developed as a preservative agent in the future considering its high antioxidant activity.

#### 5. Conflicts of interest

There are no conflicts to declare.

#### 6. Acknowledgments

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