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Study of Gamma Ray Irradiation Effect on Red Ginger (*Zingiber officinal roscoe*) 70% Ethanol Extract Level Markers and Its Anti-Inflammatory Activity

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Abstract

Background: In Indonesia, the use of herbal plants in overcoming several health problems shows a reasonably high rate. Red ginger is one of the herbs widely consumed and empirically has the property of relieving or reducing inflammation. However, as is well known, the microbiological Contamination of herbs is relatively high in general. Special treatment is required to maintain the quality of herbal plants to ensure that microbial Contamination is within safe limits. This study aims to determine the effect of gamma irradiation on the number of microbial Contamination and the bioactive content of 6,8,10-gingerol; 6-shogaol in 70% ethanol extract of red ginger and its activity as an anti-inflammatory. **Methods:** Samples of 70% ethanol extract of red ginger were irradiated with doses of 0, 5, 7.5, 10, and 15 KGy. Microbiological Contamination is determined by Total Plate Number and Yeast Mold Number. The content of compounds 6,8,10-gingerol and 6-shogaol was observed by the high-performance liquid method, and their anti-inflammatory activity was followed by protein denaturation inhibition (BSA). **Results:** Gamma irradiation at doses of 0, 5, 7.5, 10, and 15 KGy reduced microbial contamination as the exposure dose increased and did not affect the levels of bioactive 6,8,10-gingerol; 6-shogaol and its anti-inflammatory activity. The content of bioactive compounds influences the anti-inflammatory activity of the 70 % ethanol extract of red ginger. **Conclusion:** Gamma irradiation is effective for contaminant decontamination, improves the quality of red ginger, and does not affect its bioactive levels and anti-inflammatory activity (*in vivo*).

Keywords: 6,8,10-gingerol, 6-shogaol; Anti-inflammatory ; Irradiation gamma rays; *Zingiber officinale roscoe*; Bovine serum albumin

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Introduction

The use of plants as herbal medicine or traditional medicine is every day in Indonesia. Red ginger is one of the plants often used in conventional medicine. Ginger (Zingiber officinale) is one of the herbs trusted and used empirically in various treatments. Ginger comes from the Zingiberaceae family, which has pharmacological activities including anti-arthritis, anti-inflammatory, anti-diabetic, antibacterial, anti-fungal, and anti-cancer (Ghasemian et al., 2016; Mbaveng & Kuete, 2017).

The active substances in ginger responsible for anti-inflammatory activity through specific inhibition of COX-2 are shogaol and gingerol compounds (Badoni et al., 2015). Gingerols and shogaols are flavonoid compounds abundant in ginger and have antioxidant,

anti-inflammatory, and antitumorigenic activities (Dugasani et al., 2010; Fitzpatrick et al., 2017).

Inflammation or inflammation is a protective response of the body to outside disturbances (Karunakaran & Sadanandan, 2019). Inflammation is characterized by pain, swelling, redness, heat, and loss of function due to the dilation of blood vessels, which causes an increase in blood supply and an increase in the space between cells that causes the movement of leukocytes, proteins, and fluids into the swollen area (Iwalewa et al., 2007; Weisburger, 2002). Inflammation can develop as a cause of diseases such as cancer, heart, and blood vessel disease, diabetes, obesity, osteoporosis, autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease, asthma, and other diseases related to the central nervous system such as Alzheimer's disease (Badoni et al., 2015; Feuerstein et al., 2007). Therefore, inflammatory therapy is needed to prevent the development of these various diseases (Feuerstein et al., 2007).

Treatment or therapy of inflammation can use non-steroidal anti-inflammatory drugs, anti-inflammatory steroids, and immunosuppressants (Ghasemian & Owlia, 2016; Zetoune et al., 2014). However, the use of these synthetic drugs has side effects such as gastrointestinal irritation, kidney damage, diarrhea, headaches, depression, and pancreatitis, and this therapy is sometimes aggressive and ineffective in some cases (Santika et al., 2015). Therefore, using natural or herbal ingredients is an alternative to inflammation therapy to minimize side effects caused by synthetic drugs.

Herbal plants, especially rhizomes such as ginger, tend to be contaminated by insects and microorganisms during production and storage. This Contamination causes the storage time (expiration) to be shorter. Conventional decontamination with chemicals or heating can be chosen to deal with microorganism contamination. However, this method can change the sensory profile due to the loss or degradation of volatile aroma components (a decrease in the quality of the herb). Another way that can overcome the problem of the conventional method is by using gamma-ray irradiation (Chatterjee et al., 2015)

The gamma irradiation technique can preserve food and herbs and extend their shelf life. The use of gamma irradiation has several advantages, including having high penetrating power to the material, not increasing the temperature of the material being processed, the material can be irradiated after being packaged, does not leave residue, and is environmentally friendly (Katrin et al., 2014). From various studies, the amount of irradiation dose affects the activity of irradiated plants. Gamma irradiation with a dose of ≥ 10 kGy on the crown of the gods' meat Simplicia could inhibit the growth and kill all bacteria and yeasts present. Still, there was a significant decrease in the cytotoxic activity of the ethanol extract (Katrin et al., 2014). Almeida (2012) reported that the antioxidant activity of the rhizome of temu putih decreased at a dose of 20 kGy. Gamma irradiation at ten kGy on Sambiloto aqueous extract did not interfere with its activity as an anti-inflammatory (Mamatha et al., 2010). However, from further literature searches, no journals were found that examined the effect of variations in gamma irradiation doses on bioactive levels (6,8,10-gingerol, 6-shogaol) and anti-inflammatory activity in 70% ethanol extract of Red Ginger (*Zingiber officinale var. rubrum*).

In this study, gamma-ray irradiation was carried out on 70% ethanol extract of Red Ginger (*Zingiber officinale var. rubrum*) and then tested for bioactive levels using the HPLC method and anti-inflammatory activity using the protein denaturation method (Ezzat et al., 2018; Williams, 2009). Tests were carried out on irradiated extracts with variations in exposure doses of 5, 7.5, 10, and 15 kGy (Abdeldaiem & Helal, 2009) to determine the effect of gamma irradiation on bioactive levels and anti-inflammatory activity in 70% ethanol extract of Red Ginger.

Methods

Samples of 70% ethanol extract of red ginger rhizome were irradiated with various doses of 0, 5, 7.5, 10 and 15 kGy. Microbiological contaminants were determined as total

plate number (ALT) and yeast mold number (AKK). Levels of bioactive 6,8,10-gingerol and 6-shogaol, anti-inflammatory activity (protein inhibition) were also investigated by various in vitro colorimetric methods.

Sample

The materials used in this study included red ginger rhizome Simplicia (*Zingiber officinale var. rubrum*) from material supplier PT Phytochemindo Reksa, 70% ethanol, Bovine serum albumin (Sigma Aldrich), diclofenac sodium (Kimia Farma), aquadest, Tris base (Merck), acetic acid (Merck), Acetonitrile (Merck), glacial acetic acid (Merck).

Instrument

In this study, the HPLC Gradient Waters e2695 instrument was used with the PDA 2998 detector. SymmetriShield C-18 column, 3.5µm (4.6 X 150 mm) for analysis of bioactive compounds content and Shimadzu UV-Vis 1900i for measuring the absorbance of protein denaturation were also used in this study.

Data collection

Data presented for ALT, AKK, bioactive levels in ginger, and anti-inflammatory activity were obtained from triplicate measurements, and results are expressed as mean ± standard deviation.

Procedure

Preparation of 70% Ethanol Extract of Red Ginger Rhizome

1 kg of powdered rhizome Simplicia was extracted by maceration method for 24 hours using 5L 70% ethanol. Then the extract was concentrated using a rotary evaporator at 500C to produce a thicker consistency. Extract yield was calculated by comparing the initial weight of the simplicial and the final weight of the resulting extract. The 70% ethanol extract of red ginger rhizome was put into a dark bottle to be irradiated with various doses of 5; 7.5; 10; and 15 Kgy, as well as 70% ethanol extract of red ginger rhizome without irradiation as control blank.

Microbiological Analysis

Total Plate Count

The irradiated 70% ethanol extract of Red Ginger was tested for its quality by analyzing total bacterial Contamination using the pour plate method. Samples were diluted at 10-1 to 10-3 dilutions. 1 ml of each dilution was taken with a pipette and poured into a sterile petri dish, then given 15-20 ml of Tryptic Soy Agar medium at 37-40°C and then homogenized. The petri dish containing the sample was incubated at 30-35°C for ± 72 hours. The growing bacterial colonies were observed and counted (Pharmacopeia, 2019).

Total Yeast Mold

Calculate the number of yeast molds using the pour plate method. Red Ginger 70% ethanol extract samples that had been irradiated were diluted at 10-1 to 10-2 dilutions. 1 ml of each dilution was taken with a pipette and poured into a sterile petri dish, then given 15-20 ml of Sabouraud Dextrose Agar medium at 37-40°C and then homogenized. The petri dish containing the sample was incubated at 20-25°C for ± 5 days. The yeast colonies that grew were observed and counted (Pharmacopeia, 2019).

Determination of Bioactive Content of Red Ginger 70% Ethanol Extract by High-Performance Liquid Chromatography

Stir the sample until homogeneous, then ultrasonic for 5 minutes. Weigh the sample as much as 200 mg in a 50 mL measuring flask. Added 30 mL of methanol, ultrasonic for 15 minutes, and let stand. Added methanol to 50 mL (limit mark). Filter the solution with 0.2 µm filter paper. 20 µl of the solution was tested for levels carried out by the internal

method with the HPLC instrument at a wavelength of 230 nm. The sample area was calculated against the common room with each standard solution concentration (6,8,10-gingerol, 6-shogaol) 10 ppm.

In vitro Anti-inflammatory Activity Test (Protein Denaturation Inhibition)

Anti-inflammatory activity of 70% ethanol extract of Red Ginger rhizome, evaluated by protein denaturation method as described by (Alhakmani et al., 2013; Padmanabhan & Jangle, 2012; Tatti et al., 2012; Whittaker & Vogler, 2008; Bailey-shaw et al., 2017) with slight modifications.

First, a solution of Tris Buffer Saline was made as a primary solvent. A total of 24 g of Tris and 88 g of NaCl were weighed and dissolved in distilled water, then the pH was adjusted with glacial acetic acid to 6.5, and the volume of the solution was up to 1 L. Then a 0.2% BSA (bovine serum albumin) solution was made by weighing 1.0 g BSA and diluting it with TBS to a volume of 50 mL. Preparation of a test solution from each sample of 70% ethanol extract of the red ginger rhizome exposed to gamma irradiation (doses of 0, 5, 7.5, 10, and 15 kGy) with a concentration of 100 ppm. In the test sample group. A sample comparison of 50 µL of 70% ethanol extract of red ginger 100 ppm per irradiation dose was made and added with 0.2% BSA to a solution volume of 5 mL. The negative control was prepared by adding 50 µL of distilled water and dissolving it with 0.2% BSA to a volume of 5 mL. The positive control used a 50 ppm diclofenac sodium solution of 50 µL and added 0.2% BSA to a solution volume of 5 mL.

Each blank solution, positive control, negative control, and test solution was diluted 1:10 by adding TBS as a solvent. All samples were heat induced by incubation in a water bath at 70oC for 5 minutes, then allowed to stand at room temperature for 20 minutes and vortexed for 3 minutes to prevent protein aggregation in the sample solution. Anti-inflammatory activity was measured by calculating the absorbance ratio of the test solution and the negative control absorbance ratio at a wavelength of 200-400nm, and the optimum absorbance was obtained at a wavelength of 278 nm with a UV-Vis spectrophotometer for BSA protein (Arya & Malik, 2015; Sudha, Srinivasan, et al., 2016). The following formula can calculate the percentage of protein denaturation inhibition: (Williams, 2009)

$$\% \text{ inhibition} = \left[\frac{Absorbansi\ sampel - Absorbansi\ kontrol}{Absorbansi\ kontrol}\right]$$

From the test results and calculations above, compounds that inhibit denaturation of more than 20% in the range of concentrations have anti-inflammatory properties and can be helpful for drug development. (Williams et al., 2002).

Data analysis

Data were analyzed using one-way ANOVA following Bonferroni and Tukey's posttest to assess significant differences ($p<0.05$) between samples. The correlation between doses of gamma irradiation, ALT, AKK, bioactive levels in ginger, and anti-inflammatory activity was analyzed using Pearson's correlation.

Result

Extraction Result of Red Ginger Rhizome with 70% Ethanol

From Simplicia, ground red ginger rhizome, as much as 1Kg, was extracted with 5L 70% ethanol and then concentrated with a rotary evaporator at 500C, obtained ± 200 grams of extraction results, a yield percentage of 20%.

Microbiological Analysis

Microbiological analysis Table 1 shows the effect of gamma irradiation on total plate count (ALT) and yeast mold rate (AKK). In this study, gamma irradiation reduced the levels of bacteria, yeast, and mold in the samples. ALT contamination was higher than AKK in all

models examined. The total microbial count decreased with increasing irradiation dose. No bacteria were detected at an amount of 15 kGy, whereas at a dose of 10 kGy, it was sufficient to destroy all molds and yeasts in the sample.

Table 1. Effect of 60Co Gamma Irradiation on Microbiological Contamination of 70% Ethanol Extract of Red Ginger Rhizome

Irradiation Dosage (kGy)	Microbiological Contamination (cfu/g)	
	ALT	AKK
0	16 ± 1,73	19 ± 1,00
5	28 ± 3,00	16,67 ± 0,58
7,5	15,67 ± 2,08	12 ± 0,00
10	29,33 ± 2,52	0 ± 0,00
15	0 ± 0,00	0 ± 0,00

Results of Bioactive Levels of 70% Ethanol Extract of Red Ginger HPLC Method

Table 2 shows the effect of gamma irradiation on the levels of bioactive 6,8,10-gingerol and 6-shogaol. Gamma irradiation up to 15 kGy did not change the compound's macro marker (bioactive) content. The comparison of the bioactive levels of the control and gamma-irradiated samples showed that the gamma-irradiated examples did not show a significant difference ($p>0.05$).

Table 2. Effect of ^{60}Co Gamma Irradiation on Bioactive Levels of 70% Ethanol Extract of Red Ginger Rhizome

Irradiation Dosage (kGy)	Bioactive Levels (%)			
	6-gingerol	8-gingerol	10-gingerol	6-shogaol
0	2,97 ± 0,66	0,25 ± 0,01	0,88 ± 0,03	0,47 ± 0,01
5	3 ± 0,01	0,25 ± 0,01	0,9 ± 0,01	0,49 ± 0,01
7,5	2,90 ± 0,01	0,24 ± 0,01	0,87 ± 0,01	0,47 ± 0,01
10	2,92 ± 0,03	0,25 ± 0,01	0,92 ± 0,01	0,48 ± 0,01
15	2,94 ± 0,06	0,25 ± 0,01	0,86 ± 0,01	0,48 ± 0,01

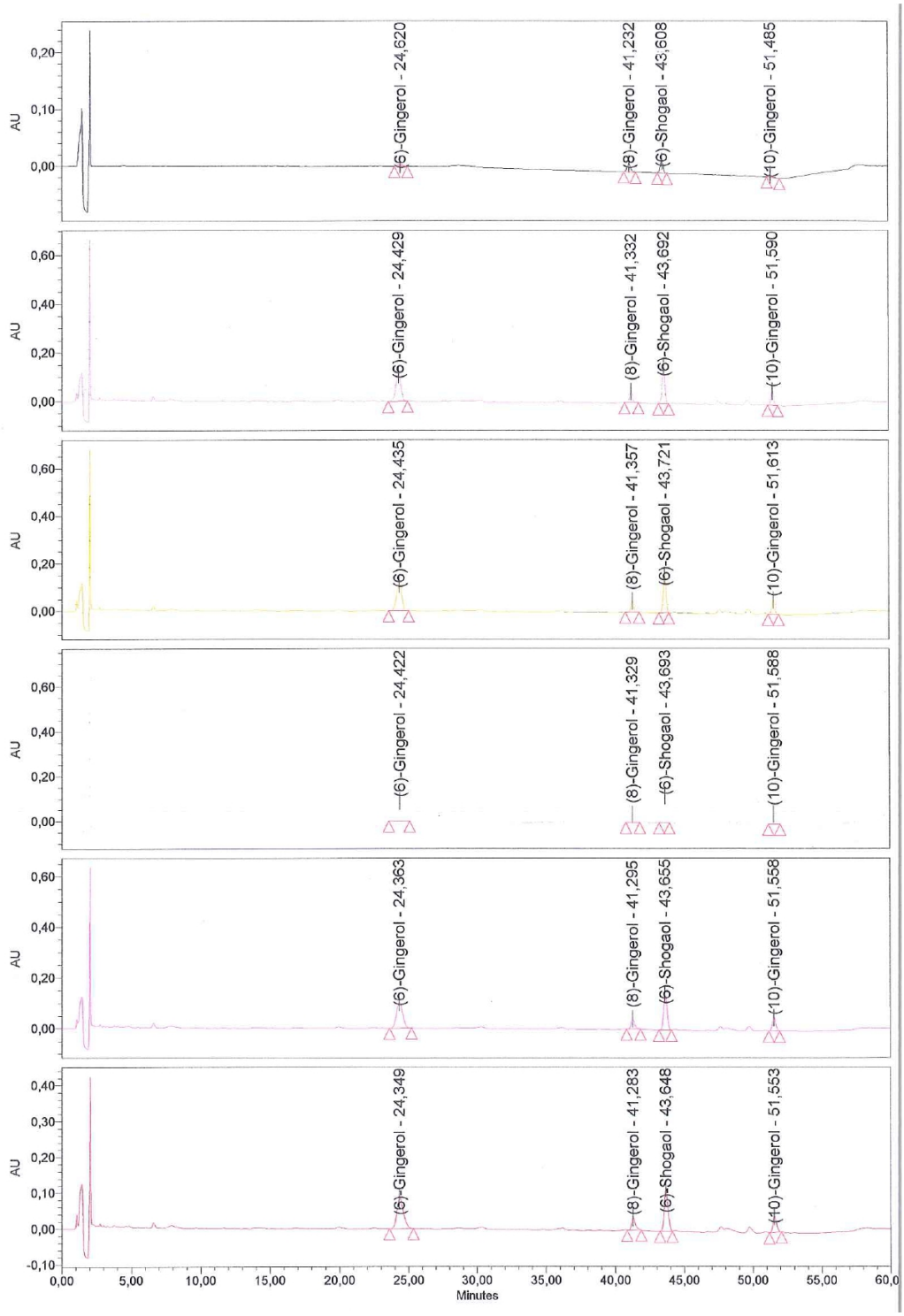

Figure 1. Chromatogram of 70% ethanol extract of Red Ginger irradiated doses of 0, 5, 7.5, 10, 15 Kgy.

Anti-inflammatory activity in vitro (Protein Denaturation Inhibition) with UV-Vis Spectrophotometer

Bioactive compounds 6,8,10-gingerol, 6-shogaol They were proven to inhibit heat-induced denaturation of bovine serum albumin (BSA) protein. It can be seen by comparing the absorbance of the sample compound with the negative control group. Diclofenac sodium at a five µg/ml concentration showed an inhibitory effect on BSA protein denaturation of 66.21%. The impact of sample denaturation inhibition can be seen in Table. 3.

Table 3. %Heat-induced sample protein denaturation inhibition

Irradiation Dose (kGy)	BSA Absorbance	Inhibition of Protein Denaturation
0	0,709 ± 0,003	123,65%
5	0,742 ± 0,004	133,84%
7,5	0,722 ± 0,002	127,54%
10	0,743 ± 0,002	134,37%
15	0,738 ± 0,002	132,58%
negative control (Blank)	0,317 ± 0,005	0,000
positive control (Na diclofenac)	0,527 ± 0,005	66,21%

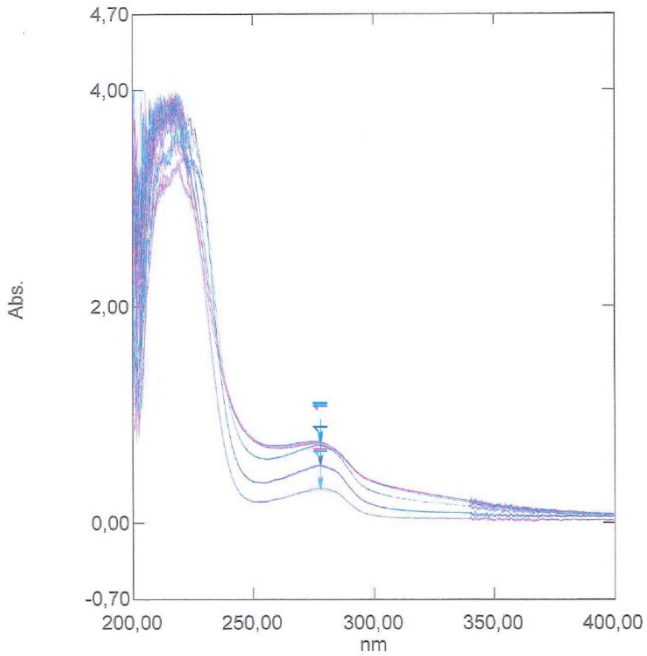

Figure 2. Overlay spectrum of BSA protein denaturation inhibition with λ max. 278nm

Discussion

Gamma irradiation is an effective method for reducing the number of microbial Contamination, maintaining food quality and safety, and increasing product shelf life. In this study, increasing the irradiation dose was equivalent to increasing the reduced microbial count ($p<0.05$). Irradiation can damage microbial cells by damaging DNA mechanisms, inducing the death of organisms, and causing organisms to be unable to reproduce (Mustapha et al., 2014; Sudprasert et al., 2006). However, several studies reported the effect or effects of irradiation on the active substance content. (biomarkers) of herbal plants. Several studies are reporting gamma irradiation's effect on phenolic compounds content. Of the seven plants studied, there was a general decrease in the levels of total phenolic compounds and flavonoid compounds (Naveed et al., 2018). In another study, gamma irradiation on fruit juices could increase total phenols, flavonoids, and anthocyanin levels (Kalaiselvan et al., 2018). The effect of gamma irradiation was also reported to affect the increase in total phenol levels in Curcuma alismatifolia (*Zingiberaceae*) plants (Taheri et al., 2014).

The effect of gamma irradiation on the bioactive content of ground red ginger Simplicia was reported in a study (Andrews et al., 1995), with the result that, in general, there was a

decrease in the levels of volatile compounds in irradiated red ginger ground Simplicia. In 1997 Variyar et al. researched the fresh red ginger rhizome and found an increase in all the bioactive in the tested red ginger. The decrease in bioactive levels only occurred in the ranks of linalool+α-terpineol and β-sesquiphellandrene+β-bisabolene compounds, which are contained in the oleoresin of red ginger plants (Variyar et al., 1997). The content of oleoresin and gingerol in ginger rhizomes that were irradiated with gamma. From the results of this study, during the storage period (9 months), there was a significant decrease in 6-gingerol levels in the radiated red ginger group, while in the control group, the reduction of 6-gingerol levels was described by a more sloping curve. (Onyenekwe, 2000). During the results obtained from this study, gamma irradiation did not affect the content of the phenolic compounds 6,8,10-gingerol and 6-shogaol in the 70% ethanol extract of red ginger. From the results of correlation testing of increasing gamma irradiation doses with levels of phenolic compounds, variations in irradiation doses had no significant effect on phenol levels (6,8,10-gingerol and 6-shogaol) ($p>0.05$).

Phenol compounds have various biological activities, one of which is anti-inflammatory activity (Zhang et al., 2022). The anti-inflammatory activity of phenolic compounds contained in ginger is related to phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and nuclear of activated B cells (NF-κB) (Mao et al., 2019; Sang et al., 2009; Zhang et al., 2022). Anti-inflammatory activity in this study showed a significant correlation ($p<0.05$) to the content of phenolic compounds in the tested samples. BSA protein added with 70% ethanol extract of red ginger with various doses of gamma irradiation showed the potential to inhibit protein denaturation compared to the control group without ginger extract. These results are by several other studies which concluded that the content of phenolic compounds has potential anti-inflammatory activity (Ambriz-Pérez et al., 2016; Bouhlali et al., 2020; Shahidi & Yeo, 2018)

Conclusions

The results of this study indicate that a dose of 10 kGy gamma irradiation effectively improves quality by ensuring the microbiological safety of Zingiber officinale Roscoe. Gamma irradiation did not affect the phenolic content of 70% ethanol extract of red ginger rhizome. And the biological activity of red ginger containing phenolic compounds is considered potential as an anti-inflammatory.

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Declaration statement

The authors reported no potential conflict of interest

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