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The Anti-Inflammatory Potential of Fermented Red Ginger Extract (Zingiber officinale var. Rubrum) Against In Vitro Protein Denaturation

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ABSTRACT

Red ginger (*Zingiber officinale* var. Rubrum) is known for many phytochemical components and various pharmacological activities, such as anti-inflammation. However, red ginger also has a high cellulose level. Special treatment is needed to degrade the cellulose so that phytochemicals can be maximally extracted. *Trichoderma harzianum*, a cellulase enzyme-producing fungus, can degrade cellulose and increase the efficiency of phytochemical extraction. This study aimed to evaluate the in vitro anti-inflammatory potential of red ginger extract fermented with *Trichoderma harzianum*. This research is an experimental method involving the fermentation of red ginger powder before extraction, followed by an in vitro protein denaturation inhibition assay using a UV-Vis spectrophotometer. Bovine Serum Albumin (BSA) was used as a negative control, diclofenac sodium as a positive control, and the fermented red ginger extract (FRGE) was tested at 25, 50, 75, 100, and 125 ppm. Phytochemical screening of FRGE confirmed the presence of alkaloids, flavonoids, saponins, phenols, and triterpenoids. The extract yield was 14.29%, with the highest inhibition of protein denaturation at 62.62% observed at 125 ppm. The IC50 value is 93.61 ppm. Based on these research results, FRGE had strong antiinflammatory potency *in vitro*.

Keywords: Red ginger, Fermentation, Anti-inflammatory, Protein denaturation, Trichoderma harzianum

ABSTRAK

Jahe merah (*Zingiber officinale* var. Rubrum) dikenal sebagai tanaman yang memiliki beragam komponen fitokimia dengan berbagai aktivitas farmakologi, salah satunya sebagai antiinflamasi. Jahe merah juga memiliki kadar selulosa yang cukup tinggi sehingga diperlukan perlakuan khusus untuk mendegradasi selulosa tersebut hingga didapatkan komponen fitokimia yang lebih maksimal saat proses ekstraksi. *Trichoderma harzianum* adalah kapang yang memiliki enzim selulase yang mampu mendegradasi selulosa tersebut sehingga luas permukaan kontak pelarut saat ekstraksi menjadi lebih maksimal. Penelitian ini bertujuan untuk mengetahui potensi antiinflamasi secara *in vitro* ekstrak jahe merah yang difermentasi dengan *Trichoderma harzianum*. Penelitian ini menggunakan metode eksperimental dengan melakukan fermentasi pada serbuk jahe merah sebelum diekstraksi dan diuji antiinflamasi dengan metode penghambatan denaturasi protein secara *in vitro* menggunakan spektrofotometer UV Vis. Penelitian ini menggunakan Bovine Serum Albumin (BSA) sebagai kontrol negatif, natrium diklofenak sebagai kontrol positif dan Ekstrak Fermentasi Jahe Merah (EFJM) dengan konsentrasi 25, 50, 75,100, dan 125 ppm. EFJM yang didapatkan memiliki alkaloid, flavonoid, saponin, fenol, dan triterpenoid berdasarkan hasil skrining fitokimianya. Penelitian ini menghasilkan rendemen EFJM sebanyak 14,29% dengan %inhibisi denaturasi protein tertinggi sebesar 62,62% pada konsentrasi EFJM 125 ppm dengan nilai IC50 93,61 ppm. Berdasarkan hasil penelitian ini, dapat disimpulkan bahwa EFJM memiliki potensi anti-inflamasi yang kuat secara *in vitro*.

Kata kunci: Jahe merah, Fermentasi, Antiinflamasi, Denaturasi protein, Trichoderma harzianum

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INTRODUCTION

Inflammation is often the cause of various diseases, such as atherosclerosis. Atherosclerosis is caused by chronic inflammation in the artery walls due to lipid buildup, marking the beginning of coronary heart disease (CHD) [1]. This disease is categorised as a non-communicable disease (NCD). NCDs are the leading cause of death worldwide, accounting for 72%, particularly in low- and middle-income countries [2]. Inflammation is essentially an immunological reaction that is protective, resulting from a stimulus that can be acute or chronic [3]. The inflammatory process begins with the formation of pro-inflammatory mediators resulting from the metabolism of arachidonic acid. Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used drug classes by the public as anti-inflammatory medications. However, this class of drugs has side effects, such as gastric ulcers and blood clots, making it necessary to promote the use of traditional medicines from natural sources that have fewer side effects [4].

One of the plants known for its anti-inflammatory effects is red ginger. Red ginger (*Zingiber officinale* var. rubrum) contains various bioactive compounds, such as gingerols and shogaols, which studies have shown to possess significant anti-inflammatory and antioxidant properties [5]. This makes it a promising candidate for natural remedies. However, red ginger contains a considerably high amount of cellulose, ranging from 60–80% [6]. Special treatment is needed to maximise the extraction yield from red ginger because cellulose is rigid and can reduce solvent contact, such as fermentation with Trichoderma harzianum. Trichoderma harzianum is a fungus recognised for its ability to produce a variety of hydrolytic enzymes, most notably cellulases. These enzymes break down complex cellulose molecules into simpler sugars, making the plant material more accessible for extracting other valuable compounds [7]. If the extraction yield increases by fermentation, then the anti-inflammatory effects of fermented red ginger are expected to also increase. This study aimed to evaluate the in vitro anti-inflammatory potential of red ginger extract fermented with Trichoderma harzianum.

MATERIALS AND METHODS

This research design employs an experimental method. The design used is the posttest-only control design group, which involves conducting an *in vitro* anti-inflammatory activity test of fermented red ginger extract using the protein denaturation inhibition method.

a. Red Ginger Preparation

Fresh red ginger rhizomes were collected directly from Rendang Subdistrict in Karangasem Regency, Bali. Following collection, the red ginger rhizomes were meticulously sorted and thoroughly washed. The subsequent stage involved slicing the rhizomes to enhance their surface area. Thereafter, they were air-dried for a duration of 6 to 7 days and subsequently underwent dry sorting to eliminate any remaining impurities [8]. In the next phase, simplisia haksel was processed using a blender and then subjected to sieving through a 40 mesh sieve. This particular mesh size was selected due to the high fibre content of red ginger, which renders it challenging to grind finely [9].

b. Fermentation with *T. harzianum*

Trichoderma harzianum was preserved on potato dextrose agar (PDA) slants. It was inoculated on potato dextrose broth (PDB). 100 grams of red ginger powder were combined with 100 ml of liquid inoculum of the *Trichoderma harzianum* fungus and distributed within the fermenter vessel. The fermentation process will be conducted for six days, as the optimal content of red ginger oil is achieved within this period. Throughout the fermentation, measures will be implemented to sustain humidity levels between 40-45% every 24-hour basis. Should humidity levels drop below this range, the liquid fungal medium will be applied, while excessive humidity will necessitate the circulation of air until humidity levels revert to the 40% to 45% range [7].

c. Extraction of Fermented Red Ginger

100 grams of fermented red ginger powder (*Zingiber officinale* var. rubrum) were macerated by incorporating a 1:6 ratio of 96% ethanol solvent, which entailed the addition of 600 mL of 96% ethanol to immerse the powdered simplicia fully [10]. The sample was agitated for 30 minutes and subsequently allowed to soak for 24 hours. After this period, it was filtered and re-macerated three additional times. The resulting filtrate was concentrated using a rotary vacuum evaporator until a viscous extract of red ginger was obtained [11].

d. Phytochemical screening

The active compounds contained in the fermented red ginger extract were qualitatively identified through secondary metabolite group testing, specifically examining for alkaloids [12], flavonoids [4], phenolics [12] terpenoids/steroids [13], and saponins [12].

e. *In vitro* anti-inflammatory activity test of fermented red ginger extract

The anti-inflammatory testing method utilised was the protein denaturation method using a UV-Vis spectrophotometer. This facilitated the measurement of absorbance, which indicates the capacity of the fermented red ginger extract to inhibit protein denaturation.

Sodium Diclofenac was used as the positive control, and Bovine Serum Albumin (BSA) was the negative control. Both the fermented red ginger extract and Sodium Diclofenac (as the positive control) were prepared as stock solutions with a concentration of 1000 ppm, and then diluted to 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm. The test was conducted by measuring the absorbance at a wavelength of 660 nm [14].

The data acquired from the UV-Vis spectrophotometer, specifically the absorbance readings, will be collected and analysed. The percentage inhibition of protein denaturation will be calculated for each fermented red ginger extract concentration, the positive control (Sodium Diclofenac), and compared with the negative control (BSA). The percentage of protein denaturation inhibition can be calculated using the following formula:

$$\frac{abs\ negative\ control - abs\ sample}{abs\ negative\ control} \times 100\%$$

Furthermore, the IC50 value, which refers to the concentration necessary to inhibit 50% of protein denaturation, for the red ginger fermentation extract is anticipated to be calculated and compared with that of the positive control.

Table 1. IC50 Value Categories [15]

IC50 Value (ppm)	Category
<50	Potent
50-100	Strong
101-250	Moderate
251-500	Weak
>500	Inactive

RESULTS AND DISCUSSIONS

This research has been carried out, generating data such as the results of phytochemical screening and *in vitro* anti-inflammatory tests, as presented in Tables 2 and 3.

Table 2. Phytochemical Screening Results

Test Parameter	Results
Alkaloids	Positive
Flavonoids	Positive
Steroids/terpenoids	Positive terpenoid
Phenols	Positive
Saponins	Positive

Source: Primary, 2025

Table 2 indicates that the results of the phytochemical screening of the red ginger fermented extract are consistent with previous research. Red ginger extract is known to contain alkaloids such as *cyclohexamine* [16]. Flavonoids in red ginger have been identified, such as *7,4-dihydroxyflavone* [17]. Terpenoids, such as *zingiberene* [18]. Phenols like *gingerol*s, *shogaol*s, and saponin [11].

Table 3. Percentage of Protein Denaturation Inhibition

Group	Concentration (ppm)	%inhibition
Negative control (BSA)	-	0
Positive control (diclofenac sodium)	25 ppm	8,74 %
	50 ppm	23,79 %
	75 ppm	27,18 %
	100 ppm	37,38 %
	125 ppm	40,29 %
Fermented red ginger extract (FRGE)	25 ppm	10,68 %
	50 ppm	33,01 %
	75 ppm	48,06 %
	100 ppm	50,49 %
	125 ppm	62,62 %

Source: Primary, 2025

Table 3 presents the protein denaturation inhibition percentages for each research group. Inhibition abilities above 20% are potentially anti-inflammatory *in vitro*, have anti-inflammatory activity, and can guide drug development [11].

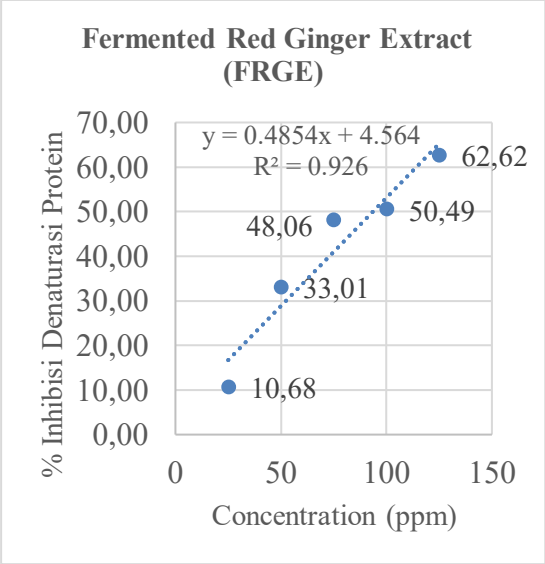

Figure 1. Linear regression curve FRGE

According to Figure 1, the fermented red ginger extract yielded a linear regression equation represented by y = 0.4854x + 4.564, and an IC50 value of 93.61 ppm, classifying it within the strong anti-inflammatory category *in vitro*. 50 ppm is the minimum concentration of FRGE at which the percentage of protein denaturation inhibition exceeds 20%. The highest protein denaturation inhibition is 62,62% with 125 ppm FRGE.

This result surpasses previous research that reported a maximum inhibition level of 39.66% at a concentration of 600 ppm of ginger methanol extract [19]. Furthermore, another study indicated that red ginger ethanol extract exhibited the highest protein denaturation inhibition of 28.28% at its peak concentration of 100 ppm [20].

The regression coefficient indicates a positive influence of increasing FRGE concentration on the percentage of protein denaturation inhibition. The results are much better compared to the linear regression results of unfermented red ginger ethanol extract from previous research [20].

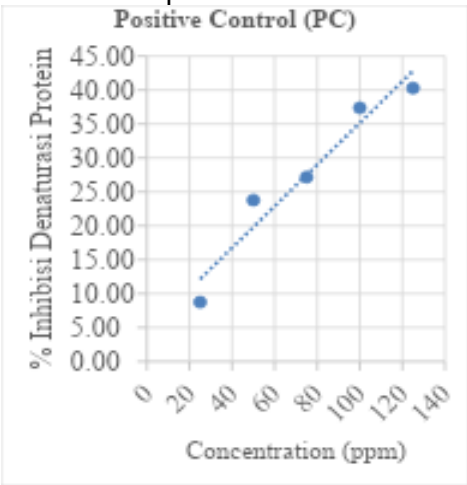

Figure 2. Linear regression curve PC

According to Figure 2, diclofenac sodium as a positive control yielded a linear regression equation represented by y = 0.3068x + 4.469 and an IC50 value of 148.41 ppm, which classifies it within the moderate anti-inflammatory category *in vitro* in this research.

CONCLUSIONS

Based on this study's findings, Fermented Red Ginger Extract (FRGE) demonstrates strong anti-inflammatory activity. FRGE at a concentration of 125 ppm exhibited the highest percentage of protein denaturation inhibition, at 62.62%, with an IC50 value of 93.61 ppm.

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