



Extraction and Identification of Polyphenol Compounds from Red Galangal Rhizome (*Alpinia officinarum*) Fermented by *Lactobacillus plantarum* using GC-MS Method

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Abstract. Using galangal-derived animal feed additives requires proper treatment to enhance its bioactive components. This study aimed to increase the polyphenol content in red galangal flour (*Alpinia officinarum*) through fermentation and extraction. The experiment used a completely randomized design with four treatments. Each treatment was extracted using two solvents: 70% ethanol and distilled water (aquadest) (T1 non-fermented galangal, 70% ethanol solvent, T2 non-fermented galangal, aquadest solvent, T3 fermented galangal, 70% ethanol solvent, and T4 fermented galangal, aquadest solvent). The results showed that extraction of galangal fermented with *Lactobacillus plantarum* and solvent 70% ethanol has a significant effect ($p < 0.01$) on elevating total phenols, flavonoids, and antioxidant activity. Polyphenol content analysis using GC-MS revealed that the primary components in the fermented galangal extract included 1-pentanol, 2,3-dimethyl; silanol, trimethyl; pentanoic acid, 4-oxo-, ethyl ester; 2,3-diethoxy-propionic acid, ethyl ester; 9-octadecanoic acid (Z), methyl ester; and 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethyl ester.

Keywords: Galangal Derived Additive, Bioactive Component, Antioxidant Activity, Flavonoid, GC-MS Analysis.

1 Introduction

Medicinal plants have great potential in the development of innovative products in the fields of health, animal feed, and pharmacy. Red galangal (*Alpinia officinarum*) is one of the rhizomes in Indonesia that comes from the Zingiberaceae family which is used as a spice [1] and also as a traditional medicine for digestion such as for stomach ache and other digestive tract problems, respiratory problems, fever, malaria, muscle and joint pain, diabetes [2]. Red galangal is rich in secondary metabolite compounds such as polyphenols, flavonoids, and essential oils which are known to have antioxidant, antimicrobial, and anticancer activities. Red galangal has 17 bioactive components including nootkatone, yakuchinone, diarylheptanoid, hexahydrocurcumin, galangin, pinocembrin, isorhamnetin, luteolin, rutin, apigenin, quercetin, acacetin, chrysin,

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nocembrin, isorhamnetin, luteolin, rutin, apigenin, quercetin, acacetin, chrysin, tectochrysin, izalpinin, kaempferol, and kaempferide [3]. The main content of *Alpinia officinarum* rhizome is flavonoids, diarylheptanoids, and essential oils, besides it also contains 9 flavanols, galangin-3-methyl ether, galangin, kaempferide, kaempferol, quercetin-3-methyl ether, quercetin, apigenin, isorhamnetin, and rhamnocitrin; two flavanones, pinobanksin and pinocembrin; and epicatechin [4]. However, these bioactive compounds are often bound in complex matrices and thus require special treatment to increase their availability [5].

One effective method to increase the polyphenol content in galangal is through fermentation using *Lactobacillus plantarum* that its not only able to increase the bioavailability of active compounds but also improve the functional properties of the material. The research of the use the fermentation process by *Lactobacillus plantarum* on plant products have been shown to increase bioactive components, which are indicated by increased levels of polyphenols, flavonoids, antioxidant activity and secondary metabolite components [6-11]. *Lactobacillus* sp have glucoside enzyme that able to break down glycoside bonds in bioactive compounds and do not require high temperatures without causing damage to bioactive compounds [11]. *Lactobacillus plantarum* is a type of microbe that is widely used in the herbal fermentation process [12-13]. The fermentation process with *Lactobacillus plantarum* in red galangal is expected to be able to change the chemical structure of polyphenols, increase their biological activity, and produce useful secondary metabolite compounds.

The gas chromatography-mass spectrometry (GC-MS) method is a powerful technique for analyzing volatile and semi-volatile compounds including fermented polyphenols. Gas Chromatography-Mass Spectrometry (GC-MS) is an analytical technique that combines gas chromatography (GC) and mass spectrometry (MS) to separate and identify compounds in complex samples. GC separates components based on their volatility and interaction with the column stationary phase, while MS ionizes and detects compounds based on their mass-to-charge (m/z) ratio [14]. The use of GC-MS in this study was to identify polyphenol compounds in red galangal extract and evaluate the effectiveness of fermentation by identifying and measuring polyphenol compounds before and after fermentation.

2 Materials and Methods

2.1 Red Galangal and *Lactobacillus plantarum* Culture

Red galangal rhizome flour (*Alpinia officinarum*) was obtained from Materia Medika Batu Laboratory (Malang, Indonesia), derived from red galangal harvested in February and dried at 50 °C and packed in plastic packaging. Pure culture of *Lactobacillus plantarum* FNCC 0026 bacteria was obtained from the Center for Food and Nutrition Studies, Gadjah Mada University.

2.2 Fermentation Process

The first stage, Pure culture of *Lactobacillus plantarum* was rejuvenated in Medium deMan Rogosa Sharpe Broth (MRSB) media. A total of 5,22 grams of MRSB was mixed with 100 ml of distilled water in an Erlenmeyer flask, heated on an electric stove and a magnetic stirrer was inserted into it. After mixing, the media was sterilized in an autoclave at a temperature of 121 °C for 15 minutes. In cold conditions, the MRSB media was inserted into an incubator at a temperature of 37 °C for 24 hours to determine the presence of contamination. The uncontaminated media was ready to be used for rejuvenation, by inserting 2 loops of pure culture of *Lactobacillus plantarum* into the broth media in Laminar Air Flow, incubated at a temperature of 37 °C in an incubator for 48 hours until colony growth was visible. The second stage was to dilute the *Lactobacillus plantarum* culture and find the dilution according to the Mc Farland 0.5 standard which would be used for the herbal fermentation process. The third stage, carrying out the fermentation process on red galangal flour by mixing galangal and distilled water in a ratio of 1:7, the mixture is stirred, then *Lactobacillus plantarum* culture was added as much as 10% of the weight of the galangal flour (v/w). Fermentation was carried out at a temperature of 30-40 °C for 6 days. In the final stage, the fermented galangal was dried in a drying oven at a temperature of 50 °C until dry.

2.3 Extraction

The process of extracting fermented and non-fermented galangal flour used the maceration method using 2 types of solvents, namely 70% ethanol and distilled water. Maceration 1 used a ratio of galangal flour: solvent = 1:10 for 24 hours. The extraction was filtered using a filter cloth and continues with maceration 2, using a ratio of galangal flour: solvent = 1:7 for 24 hours. The extraction results in the form of maceration solutions 1 and 2 were mixed and evaporated using a rotary evaporator.

2.4 Total Polyphenol

The determination of total polyphenol content is the Folin-Ciocalteu method with slight modifications [15]. A total of 0.1 mL of sample solution was put into a cuvette, added with 1 mL of Follin-Ciocalteu reagent (1:10 v/v water), left for 5 minutes. The mixture was added with 0.8 mL of Na₂CO₃ (75 g/L water), left for 30 minutes at room temperature. Then the absorbance was measured at a wavelength of 750 nm. The absorbance of the extract solution was measured using a UV-Vis spectrophotometer. The total polyphenol content is expressed in mg GAE (gallic acid equivalent) in 1 gram of extract.

2.5 Total Flavonoids

The analysis method follows Purnamasari et al. [16]. A total of 1 ml of sample was added with 1.5 ml of ethanol then 2.8 ml of distilled water, 0.1 ml of aluminum (III) chloride and 0.1 ml of potassium acetate. Incubate for 30 minutes and measure the absorbance using a UV-Vis spectrophotometer at a maximum wavelength of 439 nm. In

determining the flavonoid content, the regression equation from the standard curve between absorbance and concentration of the standard solution is used. Systematically it can be written: $y = ax + b$.

Description:

y = Absorbance value

a = Intersection of the straight line curve

b = Intersection of the curve with the coordinate

x = Extract concentration (mg/L)

Furthermore, the absorbance value is substituted into the regression equation as (y) so that to determine the flavonoid content in herbal samples, the following equation can be used:

$$\text{Flavonoid content} = CxV/M \quad (1)$$

2.6 Antioxidant Activity

The test method is based on Molyneux [17]. 70% ethanol extract and water extract were weighed as much as 50 mg into a 50 ml measuring flask then dissolved with methanol to the limit mark, this is the stock solution (1000 ppm). The test solution was made in several concentrations, namely 100, 200, 300, 400 and 500 ppm, by pipetting as much as 500, 1000, 1500, 2000 and 2500 μ l of the test solution (1000 ppm) then added 1 ml of 1 mM DPPH solution and pro analis methanol into a 5 ml measuring flask to the limit mark, homogenized and the flask was covered with aluminum foil. Antioxidant Test with 1,1-diphenyl-2-picrylhydrazyl (DPPH). The test solution, blank solution and vitamin C solution (positive control) were then immediately incubated at 37°C for the optimum time. After that, the absorbance was measured by Spectrophotometry at the maximum wavelength that had been determined. The determination was carried out in duplicate. The measurement of the percentage of inhibition against DPPH was calculated using the formula:

$$\text{Resistance (\%)} = (\text{blank absorption} - \text{sample absorption}) / (\text{blank absorption}) \times 100 \quad (2)$$

Determining the IC₅₀ value is obtained by drawing a line between 50% inhibition power and the concentration axis, with the equation $y = ax + b$, where $y = 50$ and x is the concentration of the test solution that is able to inhibit 50% of the free radical solution 1,1-diphenyl-2-picrylhydrazyl (IC₅₀).

2.7 GC-MS

GC-MS was detected using an electron ionization system with an ionization energy of 70 eV. The carrier gas in the form of ultrapure helium was used with a constant flow rate of 1 mL/min. The ion source, mass transfer line, and injector temperatures were set at 230 °C, 250 °C, and 290 °C, respectively. The oven temperature was set between 50 and 150 °C at 3 °C/min and was isothermal for 10 min and then increased to 250 °C at 10 °C/min. Diluted samples (1/100, v/v in ethanol) of 1 μ L were manually injected in a 120 split mode. The mass spectral scan was in the range of 45-450 m/z with a solvent

delay of 2 min. The extract components were identified based on the comparison of the relative GC retention times and mass spectra with components from the NIST MS Search Library Software version 2.0. Cat 10 °C/min [14].

2.8 Statistical Analysis

The research used a completely randomized design, if there was a significant difference it was continued with an honest significant difference test. The significant difference was based on $P < 0.05$.

3 Results and Discussion

Fermentation of red galangal was carried out for 6 days, using a temperature of 30-40 °C and semi-anaerobic. *Lactobacillus plantarum* is one type of homofermentative lactic acid bacteria, has optimal growth at a temperature of 30-37 °C, pH 5-7 [18], and its a gram - positive bacteria. Every day the sample was stirred once a day so that the microbes were evenly mixed in all red galangal flour. After 6 days, the fermented galangal was dried in a drying oven at a temperature of 50 °C until dry. Furthermore, non-fermented and fermented galangal were added with 70% ethanol solvent and distilled water for the maceration process. The soluble extracts from maceration 1 and maceration 2 in each treatment were mixed and filtered again using double fine filter paper and vacuumed to obtain the extraction results. The extraction results of each repetition were evaporated using a rotary evaporator until a thick extract was obtained. The results of the analysis of fermented and non-fermented galangal extracts are presented in Table 1.

Total phenols were calculated using the Folin-Ciocalteu method, with gallic acid as a standard for phenolic compounds. Total phenols and flavonoids of fermented galangal macerated using 70% ethanol increased significantly ($p < 0.01$) (Table 1). Fermentation in herbs involves the role of microbes that mainly have enzymes to break down β -glycoside bonds into aglycones to produce small phenolic components. During the fermentation process, various polyphenol-associated enzymes (PAE) such as tannase, esterase, phenolic acid decarboxylase, and glycosidase are involved so that they will maximally hydrolyze phenolic glycosides to produce sugars and aglycones (free phenolics) which can be proven by increasing phenolic levels [19]. *Lactobacillus plantarum* has 11 genes that are thought to encode β -glucoside/cellobiose enzymes. *Lactobacillus plantarum* hydrolyzes β -glucoside through the PEP-PTS transport system to produce glucose-6-phosphate and glucose. Glucose-6-phosphate can directly enter the glycolysis pathway, so that it can be utilized by cells efficiently in terms of energy [20]. *Lactobacillus plantarum* can break down glycosylated isoflavones into aglycones during the fermentation process, so that the amount of aglycone will increase the total amount of phenol and antioxidant activity. Based on Table 1, there was an increase in phenol and flavonoid levels in fermented galangal compared to non-fermented galangal, while the use of 70% ethanol solvent gave the highest results in both parameters compared to the use of aquades solvent. This correlates with the antioxidant activity value where the T3

treatment resulted in the lowest decrease in antioxidant activity value, namely 299.185 $\mu\text{g/mL}$.

Table 1. Total phenol content, total flavonoids, and antioxidant activity of non-fermented and fermented red galangal.

| Treatment | Total Phenol (mg GAE/100 g) | Total Flavonoid (mg/EQ/100 g) | Antioxidant Activity (IC ₅₀ = $\mu\text{g/mL}$) |
|--|--------------------------------|----------------------------------|--|
| T1 (non-fermented galangal, 70% ethanol solvent) | 1046.56 ^b ±11.22 | 22.31 ^a ±0.4 | 349.3 ^a ±79.98 |
| T2 (non-fermented galangal, aquades solvent) | 659.66 ^a ±27.4 | 15.94 ^a ±0.75 | 590.29 ^b ±51.09 |
| T3 (fermented galangal, 70% ethanol solvent) | 1414.93 ^c ±96.3 | 66.11 ^b ±6.79 | 299.185 ^a ±24.30 |
| T4 (fermented galangal, aquades solvent) | 859.79 ^a ±75.94 | 56.93 ^b ±1.62 | 335.715 ^a ±1.97 |
| BNT (p<0.01) | | | |

The fermentation process increases the pharmacological effectiveness of herbal plants by transforming active ingredients through polysaccharide breakdown, fiber degradation, protein breakdown and then producing secondary metabolites such as lactic acid, amino acids and peptides, as well as phenol and flavonoid compounds [5]. It is hoped that by increasing the concentration of active compounds and producing new metabolites, the pharmacological effects of botanical medicines can be strengthened. *Lactobacillus* has the ability to hydrolyze bound polyphenolic compounds and release them. Enzymes such as glucosidase or decarboxylase produced by *Lactobacillus* can break down glycosidic bonds and release phenolic compounds from plant cell walls, thereby increasing the availability of phenolic biocompounds. The difference in results from the use of 2 solvents is due to the extraction technique used and the selection of extract solvents. This study is in line with several research results on the use of *Lactobacillus plantarum* fermentation to increase phenol and flavonoid components in Jicama fermentation [6], fermentation by *Lactobacillus plantarum* produces β -glucosidase, which hydrolyzes isoflavone-glycosides into glycones and aglycones, which are free phenolics. Similar research, the results of the analysis of the total polyphenol content of basil and peppermint leaves fermented by *Lactobacillus plantarum* were higher than in fresh form [12]. Fermentation using *Lactobacillus plantarum* for 12 hours on fresh galangal juice produced the highest total polyphenol compared to galangal 2 treatments, namely non-fermentation and fermentation treatment using *Lactobacillus casei*, which produced 23 mg GAE/100 g and also produced antioxidant activity higher by 79% compared to non-fermentation (74%) [13]. Fermentation by both strains of *Lactobacillus plantarum* was able to increase the total phenol content and antioxidant activity of avocado leaf extract [21].

During the fermentation process, flavonoid and phenolic compounds will be broken down into free flavonoid and phenolic aglycones that have high antioxidant properties. This process occurs through an enzymatic hydrolysis mechanism where polysaccharide depolymerization occurs including depolymerization of phenolic components that pre-

viously had a high molecular weight changing into components with a smaller molecular weight [22 - 24]. The released polyphenol aglycones are more reactive and have higher antioxidant activity than their glycosides. Antioxidants usually have the ability to neutralize free radicals through mechanisms such as electron or hydrogen donation, and their chemical structures often contain certain functional groups such as phenolics, conjugated aromatics, or resonance-stabilized compounds. The increase in antioxidant activity is thought to be due to the presence of phenolic components that have antioxidant properties such as 9-Octadecanoic acid (Z)-, methyl ester [25], diethyl fumarate, and 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester [11]. These antioxidant properties come from their aromatic rings conjugated with double bonds. These three components are known to be identified in fermented galangal extract (Figure 1).

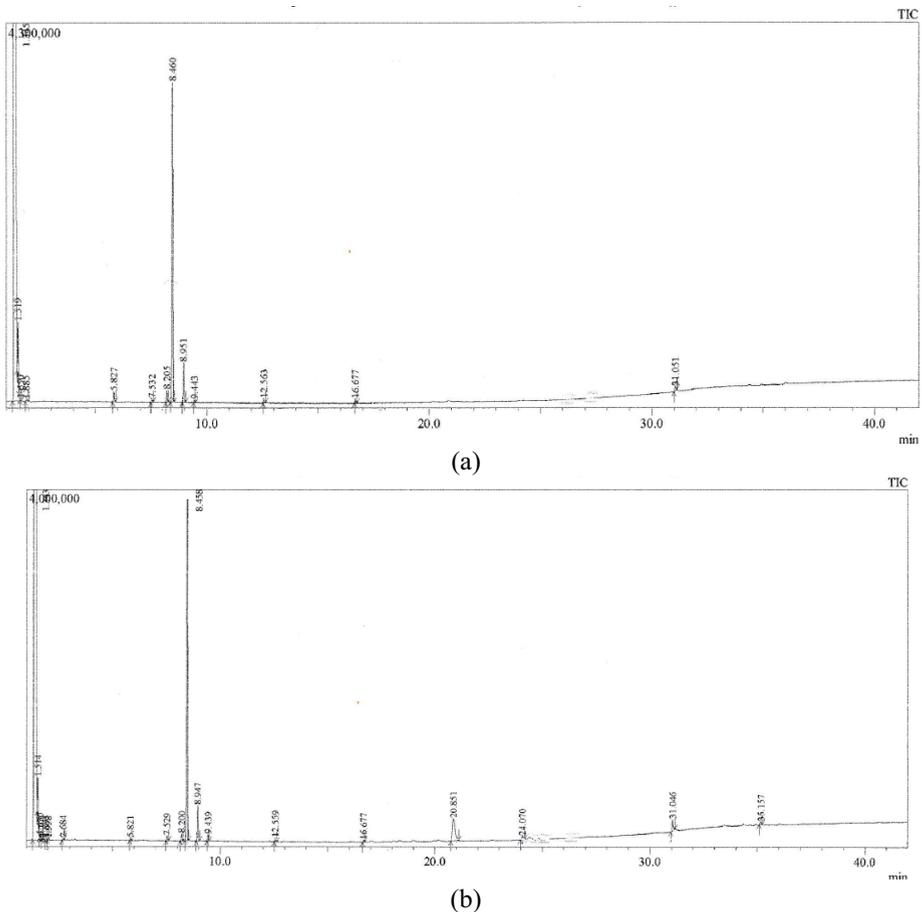


Fig. 1. Results of observations using GC-MS on red galangal extract (a) non-fermented, ethanol 70% solvent (b) fermented *Lactobacillus plantarum* ethanol 70% solvent.

The increase in polyphenol levels is positively correlated with the increase in antioxidant activity. This is because many polyphenol compounds have strong antioxidant activity, so that the greater the amount, the greater its ability as an electron donor and neutralize free radicals. and overall the synergistic interaction between polyphenol compounds can increase antioxidant capacity [21]. The IC₅₀ value is the effective concentration of extract needed to reduce 50% of the total DPPH. Red galangal juice has significantly higher antioxidant activity than red galangal because it contains alkaloids, flavonoids, phenolics, tannins, saponins, and triterpenoids [26]. Table 1 shows the average value of antioxidant activity above 200 µg/mL, and the lowest activity value was produced in the T3 treatment, namely fermented galangal macerated using 70% ethanol solvent. The content of non-fermented and fermented red galangal extracts can be seen in Figure 1.

In both treatments, extraction using ethanol solvent on fermented and non-fermented galangal both produced 1-Pentanol, 2,3-dimethyl with an area of 96.40% (a) and 95.81% (b) (Figure 1). This compound belongs to the alcohol compound group. Alcohol generally acts as a solvent and has antibacterial, antifungal, and antioxidant properties [27]. The high polyphenol content in the T4 treatment was due to the presence of 1-pentanol, 2,3-dimethyl; silanol, trimethyl; pentanoic acid, 4-oxo-, ethyl ester; 2,3-diethoxy-propionic acid ethyl ester; 9-octadecanoic acid (Z), methyl ester; and 2-propenoic acid, 3-(4-methoxyphenyl)-, 2-ethyl ester. Some of these components are known to have antibacterial and antifungal activity, namely propanoic acid, ethyl ester [27], Silanol, trimethyl- [28], Silane, diethylmethyl- [29], 9-Octadecanoic acid (Z)-, methyl ester [30-31], 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-eth [32].

4 Conclusion

Fermentation using *Lactobacillus plantarum* has been shown to significantly increase the total phenol content, total flavonoids, and antioxidant activity. Based on GC-MS analysis, fermented and non-fermented galangal both produce the dominant metabolite components 1-Pentanol, 2,3-dimethyl, but fermentation by *Lactobacillus plantarum* extracted using 70% ethanol solvent produces propanoic acid, ethyl ester, Silanol, trimethyl-, Silane, diethylmethyl-, 9-Octadecanoic acid (Z) -, methyl ester, 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-eth which is thought to cause an increase in polyphenol content higher than non-fermented galangal.

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