The effect of blanching and extraction method on total phenolic content, total flavonoid content and antioxidant activity of Kencur (Kaempferia galanga. L) extract

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Abstract. Kencur (Kaempferia galanga. L) is a rhizome used to make Jamu, a traditional herbal drink commonly consume in Indonesia, that has been studied to be rich in phytochemical sources with biological properties that contribute to the health of the human body. The purpose of this study is to evaluate the effect of blanching treatment and extraction methods (maceration and ultrasonic) on total phenolic, total flavonoid, and antioxidant activity of kencur extract. The kencur samples were divided into five treatments; control (without any treatment), fresh by maceration extraction, blanching by maceration extraction, fresh by ultrasonic extraction, and blanching by ultrasonic extraction then analyzed the pH value, total phenolic, total flavonoid, and antioxidant activities using the 2,2-diphenyl-1 method-picrylhydrazyl (DPPH). The results of this study indicate that the kencur extract sample has a pH value ranging from 6.2 to 6.5. The total phenolic sample of kencur extract by blanching and ultrasonic extraction (67.32 mgGAE/L) was higher than control (41.66 mgGAE/L). Total flavonoid sample of kencur extract by blanching and ultrasonic extraction (452.76 mgEK/L) was higher than control (282.87 mgEK/L). The antioxidant activity sample of kencur extract by blanching and ultrasonic extraction (56.20% RSA) was higher than control (22.1% RSA). This study concludes that the combination of blanching treatment with ultrasonic extraction showed a significant increase in total phenolic, total flavonoid, and antioxidant activity of kencur extract.

1. Introduction
Kencur (Kaempferia galanga L.) is a type of plant that is cultivated as a native plant of Indonesia and is a local plant that is widely used because it has bioactive content that is beneficial to health. In the food sector, kencur is the main ingredient in making kencur rice herbal medicine. Kencur rice herbal drink is a typical Indonesian traditional drink and can be classified as a functional beverage because it is rich in bioactive compounds which have various health benefits. The rhizome of kencur has phenolic content of 19.347mgGAE/100gr and flavonoid content namely: catechin 1.071mg/100g, epicatechin 570mg/100g, quercetin 624.98mg/100g, myricetin 184.82mg/100g, kaempferol 68.74mg/100g, naringenin 43.79mg/100g, luteolin 37.60mg/100g, and apigenin 404.36mg/100g [1]. The antioxidant...
activity of kencur is equivalent to 77±7 mg ascorbic acid equivalent antioxidant capacity and has a total phenolic content equivalent to 146±9 mg gallic acid [2]. The phenolic and flavonoid content found in kencur can contribute to various biological activities in the food sector, especially Antioxidant activity [1], and antimicrobial activity [3]. In the field of pharmacology, kencur has a variety of biological activities such as analgesic and anti-inflammatory [4], nematicidal activity [5], larvicidal activity [6], Vasorelaxant activity [7], Antineoplastic activity [8].

Treatment using heat, like blanching, is thought to be able to increase antioxidant activity. The results of several commodity studies indicate that blanching can increase antioxidant activity. In the processing of kencur extract conducted using heat shows that the use of this heat can reduce the total bioactive components of polyphenols present in kencur extract. Heat treatment can reduce the number of bacteria up to 4.1x10³ CFU/ml. However, the higher use of heating temperatures causes a decrease in antioxidant activity, a decrease in total phenolic and flavanoid, and a decrease in total FRAP (Ferric Reducing Power) [9].

The extraction process can also affect the bioactive components extracted from the ingredients. The process of extraction in kencur is done by two methods, namely maceration extraction, and ultrasonic extraction. Ultrasonic extraction is one method of extraction with the help of ultrasonic waves which can accelerate the extraction process [12]. Ultrasonic extraction using cavitation can produce cell wall material breakdown mechanically to increase the yield of compounds extracted from the material. Ultrasonic extraction uses ultrasonic waves that are formed from micro cavitation around the material to be extracted so that heating occurs on the material and will release components of the compound to be extracted. The ultrasonic extraction method is used with two objectives produced, namely the heating of the extracted sample so that diffusion occurs in the sample extract, and the breakdown of the cell wall material thereby freeing the contents of the compounds contained in it [13]. Cavitation bubbles on walls or surfaces use kinetic energy in the form of ultrasonic waves to increase mass transfer between the material and the solvent. The advantages of using the ultrasonic extraction method, in addition to increasing penetration of liquid into the cell membrane wall, also support the release of compound components into the extract and increases mass transfer [14]. Other advantages with this ultrasonic method are greater extraction efficiency, faster extraction time, and faster mass transfer rate compared to conventional extraction using Soxhlet [15].

The purpose of this study is to evaluate the effect of blanching and non-blanching treatments as well as maceration and ultrasonic extraction methods on total phenolic, total flavonoid, and antioxidant activity of kencur extract.

2. Materials and methods

2.1. Preparation of kencur extract
The Kaempferia galanga rhizome was peeled, washed, drained, then weighed. Kencur rhizome added with distilled water with a ratio of 1:2. The kencur extraction process was focused by using water solvents given their use in food products. The kencur rhizome was then crushed with a blender for 3 minutes. The kencur extract was then separated into 5 treatment variations:
1) Control (C): Fresh kencur extract without blanching treatment and without extraction methods
2) Fresh maceration extraction (FM): Fresh kencur extract was extracted using maceration for 180 minutes.
3) Blanching with maceration extraction (BM): Kencur extract was extracted with blanching treatment temperature of 80°C for 5 minutes and extraction using maceration for 180 minutes.
4) Fresh by ultrasonic extraction (FU): Fresh kencur extract was extracted using ultrasonic for 30 minutes.
5) Blanching with ultrasonic extraction (BU): Kencur extract was extracted with blanching treatment temperature of 80°C for 5 minutes and extraction using ultrasonic for 30 minutes.
2.2. Determination of the pH value
The pH value was determined in a sample of 50-70 ml, which would be measured using a pH meter. Calibration with a buffer solution of pH 4.0 and pH 7.0 was first performed to ensure that the pH meter used was appropriate.

2.3. Determination of total phenolic
Total phenolic was measured using the Folin-Ciocalteu method with gallic acid which was used as a standard. This method was carried out with a slight modification. 0.4 ml sample solution was put into a 10 ml measuring flask and then added 0.4 ml of the Folin-Ciocalteu reagent. The incubation time was 5 minutes, after five minutes 4 ml 7% Na2CO3 (w/v) was added, and distilled water was added so that it reached a volume of 10 ml then incubated again at 23°C for 90 minutes. Absorbance was measured in a standard solution of gallic acid with a wavelength (λ) of 750 nm using a double beam UV-VIS spectrophotometer.

2.4. Determination of total flavonoids
Total flavonoid was determined by a method based on Aluminum Chloride Colorimetry. A total of 1 ml of the sample solution was pipetted and then put into a 10 ml measuring flask containing 4 ml of ion-free distilled water and added 0.3 ml of 5% NaNO2 (b/v), and incubated for 5 minutes at 25°C. After five minutes, then added 0.3 ml AlCl3 10%(b/v), and after incubation for 1 minute, it was added 2 ml of NaOH 1 M. The addition of ion free distillation water was done to precisely 10 ml. The absorbance of standard solution (+)-quercetin was measured at wavelength (λ) using a UV-VIS spectrophotometer.

2.5. Determination of the antioxidant activity of the DPPH method
Antioxidant activity was measured by the capacity of free radical capture which was carried out with a slight modification using the slightly modified 2-2-diphenyl-1-picrylhydrazyl (DPPH) method. A sample of 0.1 ml was put into a measuring flask and then added with 2.9 ml of a 0.1 mM DPPH solution, and incubated at 25°C in a dark room to be incubated for 30 minutes. Absorbance was measured by a UV-Vis spectrophotometer at a wavelength (λ) of 517 nm. The free radical capture power was expressed in % RSA.

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\text{Radical Scavenging Activity} = \frac{\text{Abs blank} - \text{Abs Sample}}{\text{Abs blank}}
\]

2.6. Statistical Analysis
The experimental design in this study used a completely randomized design (CRD). The total sample of kencur extract used in this study were five samples with three replications and two analyzes. Correlation between total phenolic and total flavonoid with the antioxidant activity of kencur extract was carried out using the Pearson method which was used to show a correlation between total phenolic and total flavonoid with antioxidant activity of kencur extract. In this study, the research data obtained were subsequently analyzed statistically by the one-way analysis of variance (ANOVA) method. If there were differences between treatments, then they proceed with a real difference test using the Duncan's Multiple Range Test (DMRT) analysis at a significance level of \( p = 0.05 \).

3. Results and Discussion

3.1. The PH value of kencur extract
The measurement of the pH value of kencur extract was carried out to determine the change in pH value by blanching and extraction methods. In Table 1, it can be seen that the pH value of kencur extract in blanching treatment increased compared to control. The increase in pH in blanching and ultrasonic extraction showed no significant difference. It shows that the pH value of kencur extract with blanching
treatment and the treatment of maceration and ultrasonic extraction methods did not change the pH value of kencur extract without treatment (control). The pH value of kencur extract ranged from 6.23 to 6.46.

Table 1. pH values of kencur extract with various treatments

| Sample                  | pH Value |
|-------------------------|----------|
| Control                 | 6.30     |
| Fresh + Maceration      | 6.27     |
| Fresh + Ultrasonic      | 6.23     |
| Blanching + Maceration  | 6.46     |
| Blanching + Ultrasonic  | 6.45     |

3.2. Total phenolic of kencur extract

Total phenolic was determined based on phenolic compounds contained in the ingredients. Phenolic compounds have many types, and there are phenolic compounds with simple groups to phenolic compounds with complex groups that can bind to glucose groups as glycols. Flavonoids are a group of phenolic compounds that contribute as antioxidants. Total phenolic was determined in the sample to find out how much antioxidant activity was contained in the extract of the sample tested.

Phenolic compounds produce reactions in the presence of Folin-Ciocalteu reagents which in turn will produce more complex compounds, namely the tungsten molybdenum complex. It is because the Folin Ciocalteu reagent contains sodium tungstate and sodium molybdate to produce complex compounds. The results of the reactions between these compounds produce a blue color that indicates the presence of phenolic compounds in the sample. Factors that also affect phenolic compounds, namely the presence of structural variations and non-phenolic reducing agents so that the results of the analysis that can be measured are the relative results of phenolic compounds.

The total phenolic analyzed was determined using the Folin-Ciocalteau method using gallic acid standards so that the identified phenolic total was determined as milligrams equivalent to mg GAE/L gallic acid. Gallic acid is a general reference for measuring the number of phenolic compounds contained in an ingredient (16). Total phenolic extract of kencur can be seen in Figure 1.

Figure 1. Total phenolic content of kencur extract with various treatments

Chemical compounds that have an aromatic ring with one or more hydroxyl groups in a spectrum of various types of compounds that are called phenols. Plants produce phenol compounds through secondary metabolic products that take place in plants. Phenolic compounds in plants have an important role for growth, antipathogenic compounds, play a role in the formation of plant pigments, as well as a role in the oxidation stability and safety of food microbiological contamination. Phenolic compounds in food have been shown to have biological activities and antioxidant activities that are beneficial to health.
Total phenolic is analyzed by measuring the intensity of the color change to blue that occurs due to the reaction between metal oxides which are reduced by antioxidant polyphenols, for example gallic acid and catechins commonly used as standard phenolic compounds. The intensity of the deep blue color indicates the greater levels of total phenols.

Fig 1 shows that an increase in phenolic compounds extracted in the material in the presence of blanching treatment and the use of ultrasonic methods. This happens because phenolic compounds from plants will be extracted in considerable amounts if given heat treatment. The use of this heat will help the phenolic compounds to be separated from the material so that the total phenolic in the extract will also increase and antioxidant activity will increase too. The relationship between total phenolic content (mg GAE/L sample) on antioxidant activity based on several research results showed that 1) The total phenolic content of mangosteen has a very strong correlation to antioxidant activity with a correlation value of 84% (17); 2) The correlation value between polyphenol content and antioxidant activity is 99% in steady-leaf plants [18]; and 3) The total phenolic content contributes 77% to the antioxidant activity of the peperomia pellucida plants [19]. The total phenolic content in the kencur ethanol extract in its flower section was significantly higher than the ethanol extract of the kencur rhizome. The content of compounds that have antioxidant activity such as flavonoids is found in the galanga rhizome [20].

3.3. Total flavonoid of kencur extract

The most significant polyphenol compounds are a class of flavonoid compounds. Flavanoid compounds consist of many compounds including flavonols, flavones, flavonoids, anthocyanidins, catechins, isoflavonoids, and others. Total flavonoids were analyzed using the principle of analysis based on aluminum chloride colorimetry. The aluminum chloride reaction will form a stable acid complex with groups of keto C-4, C-3 or C-5 hydroxyl groups of flavones and flavonoids. Aluminum chloride will form a labile acid complex with an ortho-dihydroxyl group in the A- or B-rings of flavonoids [21].

Flavonoids formed in plants derived from aromatic amino acids phenylalanine and tyrosine, and malonates through the shikimate pathway [22]. Total flavonoids were analyzed to determine the content of flavonoids that contribute as antioxidants. Analysis of total flavonoids was using the method of visible spectroscopy (UV/Vis) with AlCl3 and NaOH as reagents and quercetin as a standard. Total flavonoids are expressed in mg of quercetin equivalent per liter (mg EK/L). Flavonoid compounds have been extensively studied, showing their contribution to antioxidant activity that is beneficial to health. These flavonoid compounds are naturally found in many vegetables and fruits.

Various treatments were carried out in this study to produce kencur extract with a high content of flavonoid compounds. Total flavonoids of kencur extract can be seen in Figure 2. The results of this study, blanching treatment using the ultrasonic method had a higher total flavonoid compared to other treatments. It is in line with the total phenolic that has been tested and gives results that the blanching and ultrasonic treatment is the best treatment in the extraction process of phenolic and flavonoid compounds that contribute as antioxidants in food.

Flavonoid compounds act as antioxidants through the donation of hydrogen atoms and/or through their ability to chew metal, and it is because flavonoid compounds are a form of glucoside containing glucose or flavonoid side chains in a free form called aglycone (23). The form of glycoside flavonoids will be hydrolyzed into aglycones. Hydrolytic glycoside compounds into aglycones are proven in
bilberry extract which is given a heating treatment [24]. It shows that the use of heat is effective in extracting flavonoid compounds in kencur.

![Figure 2](image.png)

**Figure 2.** Total flavonoid content of kencur extract with various treatments

Increased antioxidant activity was equivalent to an increase in total flavonoids in kencur extract after experiencing blanching. The blanching treatment using heat has the effect of increasing total flavonoids, which also simultaneously increases antioxidant activity. Flavonoids function as antioxidants because flavonoid compounds can play a role in capturing free radicals by releasing hydrogen atoms from their hydroxyl groups. The hydrogen atom of the phenolic compound released will then bind to free radicals, so free radicals become neutral so that free radicals that have been stabilized will stop carrying out chain reactions to prevent damage to lipids, proteins, or DNA. Flavonoid compounds which lose hydrogen atoms due to free radical scavenging will experience resonance from the hydroxyl group so that it will remain stable even though its activity is reduced.

The content of secondary metabolites of *Keampferia galanga* that has efficacy as an antibacterial includes essential oils, sesquiterpenoids, flavonoids, phenolic compounds or polyphenols and alkaloids. *Kencur* extract (*K. galangal*) can inhibit growth by forming inhibitory zones in various bacteria, such as *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Klebsiella pneumonia*. Natural flavonoid compounds such as kaempferol, morin, myricetin, and quercetin have antioxidant activity. Kaempferol and morin are less effective than myricetin and quercetin [25].

### 3.4. Antioxidant activity of kencur extract

Antioxidants are compounds that can donate electrons or so-called reductants. These antioxidant compounds can prevent the formation of free radicals that can be harmful to health. Antioxidant activity is analyzed to find out how much antioxidants in a substance can inhibit free radicals. Free radicals are often used in the analysis of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. The method of analyzing antioxidant activity using DPPH is a simple, effective, and easy method to analyze how much antioxidant activity is in counteracting free radicals. Antioxidant activity of kencur extract [26]. DPPH is a stable organic radical with a deep purple color.

Measurement using the DPPH method on the antioxidant activity of a foodstuff, seen based on changes in purple to yellow. The higher the antioxidant activity, the more concentrated the yellow, and the more faded the purple. It shows that the higher the capture of purple free radicals, the greater the antioxidant activity of a sample. Antioxidant activity was analyzed using standard ascorbic acid (vitamin C) so that the unit of measurement was stated as AEAC (Ascorbic Acid Equivalent Antioxidant Capacity). Vitamin C is often used because it is an example of a compound that has high antioxidant activity. Pinen, kampen, carvon, benzene, eucalyptol, borneol, methyl sinnamate, pentadecan, and ethyl-p-methoxynamic are compounds that are proven to exist in the galanga rhizome and have the ability as antioxidant activity [27]. The results of testing the antioxidant activity of kencur extract can be seen in
Figure 3, which shows an increase in antioxidant activity in the kencur extract sample with blanching treatment. It is because blanching at 80°C for 5 minutes can inactivate the polyphenol oxidase enzyme.

![Figure 3. The antioxidant activity of kencur extract with various treatments](image)

It is consistent with the research of several commodities which show that blanching can increase antioxidant activity. Antioxidant activity on the cogwheel samples carried out by blanching showed an increase of 9% compared to cabbage without blanching [10]. Blanching on corn also affects the increasing total phenolic content [11]. The increase in antioxidant activity during the blanching process is determined by the type of antioxidant component in the form of a phenolic component in the material. In previous studies, it was mentioned that blanching could increase antioxidant activity in corn, and tomatoes which were analyzed by DPPH method. Blanching method is effective in increasing antioxidant activity [11]. Increased antioxidant activity is also caused by heating which can increase the antioxidant component, namely total phenol levels. Blanching in nuts can increase antioxidant activity, as well as broccoli blanching [28].

Ultrasonic extraction methods can also increase the antioxidant activity of kencur extract. It is because ultrasonic waves are formed from ultrasonic waves from micro cavitation in food that will be extracted so that the cell wall breaks in the material. It causes the release of compounds into the extract. The more components of the content that are extracted, the more antioxidant activity of kencur extract is increased. The essential oil component of simplicia kencur was analyzed using GC-MS, namely ethyl cinnamate 43.47%, ethyl methoxy cinnamate 31.36%, penta dekana 3.35%, borneol 3.35%, delta 3-karen 2.86%, β-pinene 2.47%, kamfen 2.22%. Kaemgalangol is a new compound that is found and can be isolated from the galanga rhizome to inhibit the growth of cancer cells [29]. In the kencur rhizome, there are ethyl p-methoxy cinnamon compounds, which are one of the compounds of cinnamic acid derivatives, these compounds have various biological activities such as antioxidants and antibacterial activities. Diarylheptanoids contained in kencur have anti-inflammatory activity, which functions as an anti-inflammatory for health products [30]. Antiinflammation in kencur can also be useful to prevent pain caused by inflammation, such as strep throat.

Based on the results of previous studies, in vivo testing was conducted to determine that the antioxidant compounds contained in kencur extract. It can reduce abnormal conditions in the pancreas damaged by chemical exposure. In the research results, it can be seen that there was a decrease in blood glucose levels in the sample given kencur extract. This occurs because of the suspected presence of saponin compounds, flavonoids, phenolics, terpenoids, and polysaccharides in the form of amylose found in the extract. Saponin compounds reduce blood glucose levels by inhibiting glucose transport in the digestive tract and stimulating insulin secretion in pancreatic β cells [31].
3.5. Correlation of total phenolic and total flavonoids with antioxidant activity of kencur extract

The correlation of total phenolic and total flavonoids on the antioxidant activity of kencur extract is shown in Table 2. The correlation results show that total phenolic and total flavonoid correlate significantly with the antioxidant activity of DPPH kencur method because the bioactive compounds in plants are in the form of phenolic compounds. It shows that the correlation between phenolic compounds and flavonoids has high antioxidant properties, thus forming a strong correlation between the two [32]. Strong correlation also occurs in grape juice containing polyphenols and added compounds quercetin and resveratrol, produce a synergistic effect between the two to significantly increase antioxidant activity into antioxidant-rich functional drinks [33]. The higher the correlation between total phenolic and total flavonoids, the higher antioxidant in kencur extract which is directly proportional.

Table 2. Correlation of total phenol and total flavonoid with antioxidant activity of DPPH method in kencur extract

| Phenolic component | Antioxidant Activity (DPPH method) |
|-------------------|-----------------------------------|
| Total phenol       | 0.913**                           |
| Total Flavonoids   | 0.942**                           |

**The level of significance is 0.05

4. Conclusions

The results of this study concluded that the extract of kencur extracted using a combination of blanching treatment with ultrasonic extraction showed a significant increase in total phenolic, total flavonoid, and antioxidant activity of kencur extract. The combination of these two treatments is effective in increasing the antioxidant activity of kencur extract. The use of blanching treatment and extraction using ultrasonic produces a dual effect, namely ultrasonic waves, causes splitting of the kencur cell wall. Then, it will release phenolic, and flavanoid compound contents and blanching treatment with the use of heat which will increase diffusion so that many phenolic and flavonoid components are dissolved into the extract can increase the antioxidant activity of kencur.

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