Antioxidant activity and total phenolic content in Red Ginger (Zingiber officinale) based drinks

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Abstract. Indonesia is a rich spices country, both as a cooking spice and medicine. One of the most abundant commodities is red ginger, where it still less in application. On the other hand, the level of pollution is higher, so antioxidants are needed to protect the body cells from the bad effects of free radicals. The body can not naturally produce antioxidants as needed, so we need to consume foods with high antioxidant content. The purpose of this study is to know the antioxidant activity and total phenolic content in red ginger (Zingiber officinale) based drinks. Research design with complete randomized design (RAL) with factorial pattern 3 x 3, as the first factor is red ginger extract and water ratio (1: 1, 1: 2 and 1: 3) and second factor is the type of sugar used (cane sugar, palm sugar and mixed sugar). The results of this study indicate that red ginger extract and water ratio of 1: 3 give higher antioxidant. The highest antioxidant obtained in red ginger extract and water ratio of 1: 3 and using mixed sugar. That antioxidants value is 88.56%, it is not significant decreased compared to the antioxidant of pure ginger extract that is 91.46%. For higher phenol total content obtained on syrup that uses palm sugar. The highest phenol total content obtained in red ginger extract and water ratio of 1: 1 and using palm sugar. That total phenol content value is 6299 ppm.

Keywords : Antioxidant activity, phenolic content, red ginger, based drinks

1. Introduction

One of the most abundant commodities in Indonesia is red ginger (Zingiber officinale), and it still less in application. It is an herb plant that has high antioxidant activity. In big cities, the level of pollution is higher, so antioxidants are needed to protect the body cells from the bad effects of free radicals. Free radicals can be obtained naturally and from outside the body. Naturally produced by food digestion. While from outside that is caused by cigarette smoke, pollution and radiation. Free radicals that can not be handled by the body, it can trigger chronic diseases, such as cancer and heart disease. The body can not naturally produce antioxidants as needed, so we need to consume foods with high antioxidant content. The benefit of this study is to know the antioxidant activity and total phenolic content in red ginger (Zingiber officinale) based drinks.

In this research was made a drink based from red ginger which rich in antioxidant Antioxidant supplements may not bring about as much as antioxidant benefits in fresh foods. Even some
supplements pose a risk. Antioxidant supplements in high doses can bring health risks. More risk will be given if it taken with other drugs. In smokers supplements of high levels beta-carotene can cause lung cancer. High doses of vitamin E may increase the risk of stroke and prostate cancer.

From the previous studies [1], performed supercritical extraction of ginger tubers in which potential compounds were produced. The active compounds are 6-gingerol as much as 238.94 ± 0.79 and 170.50 ± 0.45 mg / g extract, total phenolics of 183.96 ± 1.25 and 0.4 ± 0.72 mg gallic acid / gram extract. Compounds in ginger oil are very large potential in the health / pharmacology field. The pharmacological effects of red ginger are able to strengthen the efficacy of other ingredients that are mixed in the process of drug manufacture [2, 3, 4]. In this study, we were analysis performance and activity of antioxidant in red ginger as a healthy drinks which is the red ginger is mixed with the other spices.

In her study, [5] used ginger extract as an anti-fungal Candida albicans. [6] has also conducted a study showing ginger extract as an anti-mutant in mice. [7] conducted the study and looked at the potential of ginger oil as an antioxidant especially gingerol. [8] and [9] used ginger extract as a potential compound as anti-cancer. In this study we would know which is the best condition to extract the antioxidant from the red ginger to get the maximum phenolic compound and to know the effects of adding sugar into the red ginger extract to antioxidant content.

Ginger oil is an essential oil produced from ginger through the distillation process. Characteristics of ginger oil have to fix as the quality standard of EOA (The Essential Oil Association of America). Ginger oil contains two main component groups, namely essential oil and fixed oil. Essential oils in dried ginger root are about 1-3% and these compounds cause a distinctive smelling ginger. The main components in ginger oil are zingiberen and zingiberol, which causes a fragrant odor. The compounds are n-nonialdehyde d-camphene, d-α-phellandrene, metal heptenone, sineol, borneol and geraniol, lineol, acetate, caprylate, citral, chaviol, limonene, and phenol [10, 11, 12]. According to [13] research, red ginger has starch content (52.9%), essential oil (3.9%) and alcohol-soluble extract (9.93%) higher than ginger emprit (41.48 , 3.5 and 7.29%) and elephant ginger (44.25, 2.5 and 5.81%).

2. Methods

2.1. Material
In this study used red ginger (Zingiber officinale) as the main raw material. In addition, used of side materials such as: sugar, temulawak, lemongrass, cloves, nutmeg, cinnamon and water. As one of the variable, used cane sugar and palm sugar as a sweetener. Water as solvent, cinnamon and lemongrass as a scent maker, while nutmeg, temulawak and cloves as a source of additional antioxidants. Red ginger used is obtained from farmers in the Batang area, central java. While other materials obtained from one of the local traditional market in Semarang.

2.2. Variables
The controlled variables in this research are temulawak (1 gr), lemongrass (1 branch), cloves (2 pcs), nutmeg (0.5 gr) and cinnamon (2 cm). The response variable in this study is the antioxidant and phenol total of syrup product. The independent variables in this study were ginger-water ratio (1:1; 1:2; and 1:3) and type of sugar used (cane sugar, palm sugar and mix sugar).
Table 1. Experiment optimization

| Red ginger extract (ml) | Water (ml) | Cane Sugar (gr) | Palm Sugar (gr) | Responses       |
|------------------------|------------|----------------|----------------|----------------|
| 150                    | 150        | 100            |                | 1. Antioxidant |
| 100                    | 200        | 100            |                |                |
| 75                     | 225        | 100            |                | 2. Phenol Total |
| 150                    | 150        |                | 100            |                |
| 100                    | 200        |                | 100            |                |
| 75                     | 225        | 50             | 50             |                |
| 150                    | 150        |                | 100            |                |
| 100                    | 200        | 50             | 50             |                |
| 75                     | 225        | 50             | 50             |                |

2.3. Syrup production
Washed ginger with water, then peeled it. All ginger is smoothed using a scar machine. Separating the ginger juice from the pulp by filtering. Then separate the dissolved starch by depositing it for a moment. Heat the water and add other ingredients such as cinnamon, nutmeg, cloves and lemongrass to boiling. After that, enter water ginger juice until it boil and drain. Separate from the dregs that still carry after it cold. Then reheat it by adding the sugar until it dissolves and smiles. Take it from the stove and wait until room temperature. Fill in it into the bottle.

2.4. Total Phenolic Determination

2.4.1. Galat Acid Calibration Curves Manufacture. Making galat acid standard curves based on method that Developed by Rungruang and Suwannee (2010). Galat acid solution is made with concentrations of 100, 200, 300, 400 and 500 mg/L. From each concentration is taken 1 mL. Added 10 mL of distilled water and 5 mL Folin-Ciocalteu reagents are whipped up homogeneous. Then the solution is kept for 8 minutes. After that, 4 mL of solution Na₂CO₃ 20% is added, and Shake until homogeneous and keep for 30 minutes at room temperature. The absorbance of each solution is measured at Wavelength 760 nm. Make Calibration curve of the relationship between Concentration of gallic acid with absorbance.

2.4.2. Permination of Total Content Phenolic Compounds. 1 ml of sample is taken and it was added with 10 mL of distilled water And 5 mL of Folin-Ciocalteu reagents. Then the solution is shaken and allowed to stand for 8 minutes. 4 mL of 20% Na₂CO₃ solution added, and shaken Until homogeneous. Keep it for 30 Minutes at room temperature. Determined absorbance at length Wave 760 nm with UV-Vis Spectrophotometry. Gynecology Total phenolate is expressed as mg amount Gallic acid equivalent each gr extract.

2.5. Antioxidant Activity Test

2.5.1. Determination of DPPH Maximum Absorption Wavelength. 75 μM DPPH solution was prepared with How to dissolve as much DPPH powder 7.393 mg to 250 mL methanol. Take 5 mL of the solution and Put in vials and bottles Added with 0.2 ml of methanol. Solution Shaken until homogeneous than left for 30 minutes in the dark. The solution Absorption was measured by a UV-Vis spectrophotometer at a wavelength of 400-600 nm. Methanol is used as a blank [14].

2.5.2. Antioxidant Activity Test. Samples were made variations Concentrations of 15, 30, 45, 60, and 75 ppm in Methanol. They has been varied The concentration is taken 0.2 ml into the vial and reacted with 5 mL of 75 μM DPPH solution. The mixture is homogenized and Idle for 30 minutes in dark place. Furthermore, absorbance measurements were read using UV-Vis spectrophotometer on Wavelength 516 nm. Ability To reduce DPPH radicals (% inhibition) Calculated using the equation:
\[
\text{% Antioxidant} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}} \times 100\%
\]

Then Calculation of IC50 which is Concentration of samples to be able Muffle 50% of DPPH's radical activity. The value of IC50 is obtained from the intersection Line between 50% inhibitory power with Sample concentration [14].

3. Result and discussions

3.1. Antioxidant

From the diagram can be seen the results that the highest antioxidant found in syrup with the ratio of red ginger and water of 1: 3, then followed by 1: 2 and 1: 1. The results of this study indicate that red ginger extract and water ratio of 1: 3 give higher antioxidant. It obtained in each sugar type variable (cane sugar, palm sugar and mixed sugar). The highest antioxidant obtained in red ginger extract and water ratio of 1: 3 and using mixed sugar. That antioxidants value is 88.56%, it is not significant decreased compared to the antioxidant of pure ginger extract that is 91.46%.

![Figure 1. Antioxidant in red ginger syrup product](image)

In the water extract of red ginger, which acts as an antioxidant is a gingerol compound [15]. Gingerol is a compound that is not soluble in water. This is what causes red ginger ratio addition does not increase the antioxidant content. Suspected dissolved antioxidants in the syrup are generated from additional ingredients used. The ingredients include: sugar, temulawak, lemongrass, cloves, nutmeg, and cinnamon. In palm sugar, antioxidants are produced higher than cane sugar because of the presence of phenol compounds contained therein are flavanoid and benzoquinone compounds which are products of Maillard reaction that occur during the sugar-making process [16].

According to [17], the inhibition mechanism of oxidation by antioxidants in two ways, the binding / capture of free radicals (primary antioxidants) and other mechanisms that do not directly capture free radicals (secondary antioxidants), such as binding or chelating metals, oxygen capture, singlet, the conversion of hydrogen peroxide becomes non radical. Meanwhile, according to [18], phenolic compounds have the capacity as primary antioxidants so that the rapid reaction of DPPH radicals occurs with some phenols, therefore the antioxidant capacity of red ginger rhizome water extract, various types of sugar, in red ginger drink in this study measured by analysis of free radical fishing activity (DPPH) and total phenol content.
Antioxidants are also produced from other material addition used. Some researchers claim that these ingredients contain antioxidants. In the water extract of temulawak, which acts as an antioxidant is a water soluble phenol compound [19] that is xanthorrhizol [20] which is a primary antioxidant. Research on lemongrass stem was also done by [21], using DPPH method they prove that lemongrass stem extract contains antioxidant. They performed it with mortality tests on shrimp larvae (brine shrimp lethality test). The study was also conducted by [22] and [23] on the content of antioxidants in cloves. According to Dian et al (2013), clove flower oil of 55 μL effectively traps free radicals of up to 60% alkyl, 48.57% hydroxyl, and 35.71% peroxyl. Based on research, the active component in clove oil that has antioxidant activity is eugenol. According to [23] clove leaf extract has activity as a catcher of free radicals or antioxidants and possess phenol content. Along with the antioxidants of nutmeg and cinnamon studied by [24], he claims that the spice contains many antioxidant components, which are known to be rich in phenolic components. High levels of antioxidants obtained in the ratio of ginger and water 1:3, it contain less ginger than 1:2 and 1:1. Because there are more solvent in 1:3 variable, so it allow more dissolved antioxidant compounds.

3.2. Total Phenolic Content

![Figure 2. Phenol total content in red ginger syrup product](image)

Based on the research that has been done, from the diagram can be seen that the type of sugar give an effect on the total content of phenols in red ginger syrup. The highest total phenol was obtained on syrup using palm sugar. This occurs in every ginger and water comparison variable (1:1, 1:2 and 1:3). The highest phenol total content obtained in red ginger extract and water ratio of 1:1 and using palm sugar. That phenol total content value is 6299 ppm. It followed by red ginger and water ratio 1:2 and 1:3 in using palm sugar with the phenol total value 4289 ppm and 4047 ppm. High levels of phenol caused by flavanol and benzoquinon compounds in every sugar contained. The content of flavanols and benzoquinone compounds in palm sugar is higher than sugar. Benzoquinon is a carbonyl compound that plays an active role in the degradation of strikers in the formation of melanoidin in sugar production [25]. Both compounds are the result of maillard reactions that occur during the sugar production.

According to [26], the cane sugar is made from the crystals of sugarcane juice which has the composition of sucrose 8.4-13.4%, inverted sugar 0.2-0.5%, organic acids (carbocyclic, amino) 0.15%, organic substances (Protein, starch, wax, fat, phospholipids) 11-19%. Meanwhile, palm sugar is made from palm sugar which has sucrose composition 13.9-14.9%, carbohydrate 11.28%, and protein 0.02%
In the manufacture of sugar through evaporation stage with high temperature where can form maillard products in it [28]. Maillard is a reaction between the amino complex (often amino acids, peptides, or proteins) and carbonyl complexes (usually reducing sugars, including glucose, fructose, or lactose). In addition to the influence of the sap composition, the possibility of purification also affects the decrease of flavanols and benzoquinones in sugars. This is due to the decrease in melanoidin levels by the purification process [26].

Processing of sugar through two stages of purification that is when the formation of raw sugar and after dilution back raw sugar. The process of purifying the raw cane sugar solution through two stages, namely the addition of lime and phosphoric acid solution and the activated carbon addition step to remove the color so that the resulting white crystals [29]. In the process of making palm sugar only through the heating or evaporation stage without going through a special purification process as in the process of making sugar. This affects the decreasing levels of brown pigment in sugars which is melanoidin. Therefore, the total phenol of palm sugar is greater than cane sugar.

4. Conclusions
The results of this study indicate that red ginger extract and water ratio of 1:3 give higher antioxidant. The highest antioxidant obtained on usage mixed sugar that is 88.56%. That value is not significant decreased compared to the antioxidant of pure ginger extract that is 91.46%. For higher phenol total content obtained on syrup that uses palm sugar. It has the same result in the ginger and water ratio variations (1:1; 1:2 and 1:3). The highest phenol total content obtained in red ginger extract and water ratio of 1:1 and using palm sugar. That phenol total content value is 6299 ppm.

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