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Antioxidant activity and total phenolic content of three varieties of Ginger (*Zingiber officinale*) in decoction and infusion extraction method

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Abstract. Ginger is one of the plants that is rich with phenolic compounds. This research was aimed at determination of the total phenolic content and antioxidant activity in the rhizome of ginger. However, there is only few information available about the comparison of phenolic compounds and antioxidant activity in the three varieties of ginger. This research employs a descriptive quantitative research using extracted dried gingers on two types of extraction processes, i.e. infusion and decoction. The phenolic compound analysis is conducted by using the Folin-C method, while antioxidant activity was conducted by using DPPH and measured by using Spectrophotometer. Based on ANOVA test result, the highest phenolic was red ginger 12.2533 mg GAE/g (infusion) and 22.9767 mg GAE/g (decoction) followed by emprit and elephant ginger. The highest antioxidant activity by infusion process was found in red ginger of 79.83 % followed by 70.43 % and 61.70% in emprit ginger and elephant ginger. Conversely, the highest antioxidant activity by decoction was found 78.76 % in emprit ginger, followed by 70.56 % and 60.93% for red ginger and elephant ginger. Ginger have sufficient antioxidant activity on extraction by infusion or decoction and the red ginger have a higher phenolic content.

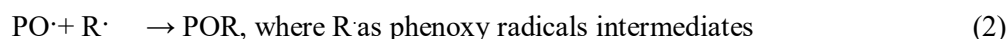
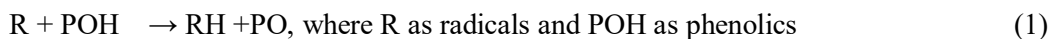
1. Introduction

Cardiovascular disease (CVD) is the number one cause of death in the world, including Indonesia [1]. Oxidative stress may play important role in the pathogenesis of CVD like atherosclerosis. Phenolic compounds exist in large quantities of plants, fruits, and beverages, as it has antioxidant properties, and has a real ability to prevent oxidative stress [2, 3]. The compounds have an anti-cancer characteristic, anti-inflammatory, and other biological characteristics [4, 5]. Epidemiological studies show that consuming lots of fruits and vegetables may decrease some degenerative diseases such as cardiovascular, cancer, cataracts, etc. [6, 7].

Phenolic content is the largest secondary metabolites. They are the result of the synthesizing process of fruit, vegetables, tea, cocoa, and other plants [4]. Phenolic content is characterized as having aromatic rings with one or more groups of hydroxyl attached and range from simple to highly polymerized molecules [8, 9]. The chemical structure owned by the phenolic content consists of the number of the hydroxyl position groups and the nature of the substitution of the aromatic rings that are closely related to the potential antioxidant in phenolic compounds [8].



Dai and Mumper outlined the potential of phenolic compounds as an antioxidant and the potential is described in the following reaction:



Based on these reactions, phenolic compounds have a significant role as natural antioxidants [3].

Phenolic compounds are known to possess antioxidant in vitro capacity and shown to have a higher potential than vitamin C, E, and carotenoids. The consumption of fruits, vegetables, and cereals showed a positive correlation towards a reduced risk of various diseases related to oxidative stress such as cardiovascular, cancer, osteoporosis, and aging-related disorder [3, 10].

Ginger is one of many herbs that has been used for over 2000 years by Polynesians for diabetes, hypertension, body immune, and so on. Ginger has been widely used as a medicinal plant by Chinese Ayurvedic and Tibb Unani populations [11]. Ginger becomes traditional medicine for Indian Ayurvedic as well as traditional drink substance such as in Indonesia. India, China, and Indonesia are the three largest ginger-producing countries in the world [12].

Ginger pharmacological effect mainly caused by 6-gingerol, 6-zogaol, and zingerone compounds besides phenolic compounds and another flavonoid [13]. According to Srinivasan's pharmacological activity of ginger rhizome due to a non-volatile compound of phenolic such as gingerol [14]. There are three varieties of ginger that grow in Indonesia, those: *Zingiber officinale var roscoe* (big white or elephant ginger), *Zingiber officinale var amarum* (small or emprit ginger) and *Zingiber officinale var rubrum* (red ginger) [15, 16].

Research on the total of phenolic content and the activity of antioxidant in ginger have been reported previously through maceration extraction method by using several solvents, those are: elephant ginger and red ginger in methanol [17], red ginger in ethanol [18], as well as elephant ginger in ethanol, acetone and distilled water [11]. Purnomo also worked on both total phenolic content and activity of antioxidant of elephant ginger by roasting and boiling (decoction) [19]. However, the research for comparing the three varieties in Indonesia those are red ginger (*Zingiber officinale var rubrum*), emprit ginger (*Zingiber officinale var amarum*), elephant ginger (*Zingiber officinale var roscoe*) with extraction method by decoction compared with infusion have never been reported. Therefore, the aim of this research are determining of the total phenolic content and activity of antioxidant in *Zingiber officinale var amarum*, *var rubrum* as well as *var roscoe* by decoction and infusion extraction method.

2. Research Methods

2.1. Chemicals

The chemicals used in this research are Folin–Ciocalteu phenol reagent (Merck, Germany), Ethanol (Smart Lab, Indonesia) Na_2CO_3 (Merck, Germany), Gallic Acid (Sigma Aldrich, Germany), and DPPH (1,1 diphenyl,2 picrylhydrazyl) from Sigma Aldrich, Germany.

2.2. Plant materials and preparation

Rhizomes of elephant ginger (*Zingiber officinale var roscoe*), emprit ginger (*Zingiber officinale var amarum*), and red ginger (*Zingiber officinale var rubrum*) were obtained from farmers in Batu region, East Java, Indonesia. Before the plants were being used, each ginger was cut, then dried under the sunlight. Dried ginger was further ground to be ginger powder. Four gram of ginger powder was diluted and boiled in 100 mL of water at 100 °C for ± 6 min, following the previous research [18]. On the other side, 4 g of ginger powder was diluted in 100 mL of hot water ± 100 °C water and waited for about 10 minutes [5, 7].

2.3. Determination of total phenolic content

In order to obtain total phenolic compounds, the following test steps were performed which refers to the theory of Saeed [19]. The steps were defined as follows: 1 mL of sample was added into 1 mL Folin–Ciocalteu phenol reagent, and then incubated for 5 min. The produced solution was added with 10 mL of Na_2CO_3 7 %, and 13 mL of deionized water. Then, the solution shaken and incubated at the dark room and set the temperature at 23 °C. The total phenolic content was measured with Spectrophotometer UV-VIS at 750nm (Shimadzu, UV 1800). The total of phenolic content was then determined by comparing with a standard curve of Gallic acid where a total of phenolic acid was indicated by the value of mg GAE/g of dry ginger.

2.4. Determination of antioxidant activity

Determination of antioxidant activity was also conducted based on Saeed's [20] worked with little modification, as follows: liquid solution of 24 mg DPPH in 100 mL of methanol was prepared and stored at a temperature of 20 °C before use. DPPH can be used by dissolving DPPH in methanol to obtain 0.98 ± 0.02 DPPH absorbance at 517 nm with a spectrophotometer. Three milliliters of DPPH were added into 100 μL of sample. It was further mixed until homogenate and incubated in the dark room for ± 15 min 25 °C.

3. Result and Discussion

3.1. Total phenolic content

Dai [3] asserted that the determination of phenolic compounds in plants was influenced by several factors, those are chemical nature of the analyte as well as assay method, selection of standards and presence of interfering substances. The level of phenolic content from three varieties of *Zingiber officinale* is shown in Figure 1. Decoction extraction method has higher total phenolic content than that extracted by infusion method $p < 0.01$. The phenolic content extracted by decoction was about two times higher than those extracted by infusion. The highest of phenolic content in infusion extraction method was found on red ginger with 12.2533 ± 0.13 mgGAE/g followed by emprit ginger with 9.5033 ± 0.35 mgGAE/g and elephant ginger with 7.6400 ± 0.21 mgGAE/g, likewise in decoction extraction method, red ginger has highest total phenolic content with 22.96767 ± 0.22 mgGAE/g followed by emprit ginger with 17.6500 ± 0.56 mgGAE/g and elephant ginger with 15.4533 ± 0.53 mgGAE/g.

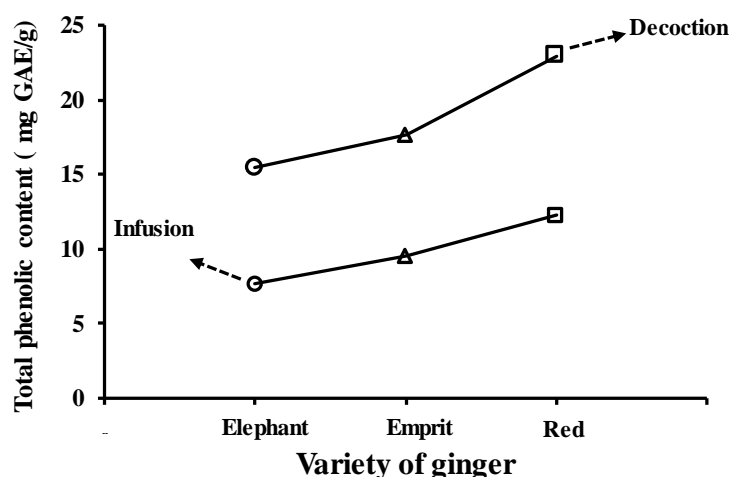


Figure 1. Total content of phenolic compounds of three varieties of ginger by infusion and decoction method.

Based on ANOVA test, the total phenolic compounds on three varieties of ginger significantly different at α (0.01) where *var rubrum* (red ginger) showed the highest phenolic content on decocting as well as on infusing based on Tukey HSD test. The difference of total phenolic compounds on three varieties of ginger was probably caused by genetic variation.

The levels of phenolic content and antioxidant activity generated from previous research have been shown mixed results. Many factors have been influenced by the outcome of total phenolic compounds and antioxidant activity. The results of previous researches [17] from two varieties of ginger have shown the total phenolic compounds; approximately around ± 10.1 mg GAE/g for elephant ginger (Haliabentong Malaysia) and ± 13.4 mg GAE/g for red ginger (Haliabara Malaysia). The result from the previous research showed the same result with our research, where the phenolic compounds in red ginger were higher than elephant ginger, but this research had a higher total phenolic compound of 22.9767 mgGAE/g from red ginger by decoction and 12.5333 mgGAE/g in infusion process. This fact is due to heating that causing an increase in solubility of active compounds. The increasing on solubility of active compounds was occurred because of decomposition of the cell wall and the entry of the solution into the cell [19, 21]. Ali [22] showed that the phenolic compounds on extraction by chloroform and methanol (C: M) 1:1 was around 60 mg GAE/g. The result was having higher total phenolic compounds compared to Gazhemzadeh [17] and the current research. The difference was presumably caused by the ecological condition.

3.2. Antioxidant activity

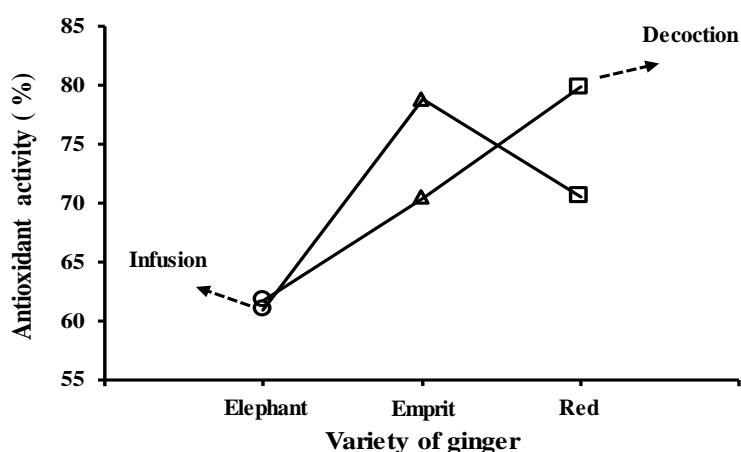


Figure 2. Antioxidant activity of three varieties of ginger by infusion and decoction process.

Based on DPPH method The highest of Antioxidant activity in infusion extraction method is found on red ginger with 70.4333 ± 0.25 % followed by emprit ginger with 70.4333 ± 0.25 and elephant ginger with 61.7000 ± 1.51 , but in decoction extraction method, emprit ginger has the highest antioxidant activity with 78.7667 ± 2.11 followed by red ginger with 70.5667 ± 2.68 and elephant ginger with 60.9333 ± 3.3 .

Based on ANOVA test as shown in Figure 2, it concluded that the antioxidant activity on three varieties of ginger significantly different at α (0.01), where red ginger showed the highest antioxidant activity in infusion extraction method. The different of total phenolic content on three varieties of ginger perhaps genetic variation', but in decoction, the highest antioxidant activity was emprit ginger.

Elephant ginger in previous research by Purnomo [19] had higher antioxidant activity as well as total phenolic content that is 30.8 mg GAE/g compared to 15.45 mg GAE/g and antioxidant activity 84.21% compared to 60.93% as shown in Figure 2. The difference value between Purnomo's and this work is possibly caused by the age of the ginger. The elephant ginger being researched in this current research was 8 months old while the ginger being researched by Purnomo was 10 months old.

This research showed that the antioxidant activity of red ginger was higher than elephant ginger as well as in total phenolic content in extraction by infusion and decoction. These result was similar to the research by Purnomo [19] with extraction by methanol. As the infusion of ginger have an oxidant activity, the result showed almost the same result with extraction by methanol or another solvent. Accordingly, extraction of ginger by infusion can be an alternative an increasing antioxidant in the body for decreasing degenerative diseases.

In general, phenolic content has positive correlated with antioxidant activity, however, this research found different results. This research extraction by decoction method on red ginger has higher total phenolic content than emprit ginger, on the contrary, emprit ginger has higher antioxidant activity than red ginger. However, the higher antioxidant activity of emprit ginger compared to red ginger in decoction extraction method is not well-known and needed for further research. One reason that might be the reason that is: the source of phenol compounds can come from active compounds such as essential oils and non-essential oil. Essential compound in red ginger higher than in emprit ginger that is 3.9 % compared to 3.5 %. Unfortunately, essential oil and chemical compound in essential oil has vulnerability to increasing temperatures. Temperatures from 85°C to 100°C can cause decrease in the active compound content which is 45.12 % in essential oil. Decrease of essential oil induces the decrease of antioxidant activity [23].

The decrease in antioxidant activity derived from these essential oils is possible causes emprit ginger although it has a lower total phenol than red ginger but has a higher antioxidant activity, however, validation of the result is needed as the further research.

4. Conclusion

Extraction by infusion has sufficient antioxidant activity in three varieties of ginger as well as extraction by decoction. The decoction has total phenolic compounds higher than infusion, but has no difference on antioxidant activity. Emprit ginger have higher antioxidant activity than red ginger in the decoction but not in the infusion. The cause of higher antioxidant activity in emprit ginger needs to be further research.

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