

## Red Ginger Effect on Yield Percentage and Antioxidant Activity in Red Ginger–Angkak Combination

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### ABSTRACT

Red ginger and angkak are often combined in Chinese cuisine to maintain health. Both of these ingredients are known to have potent antioxidant activity respectively, but the combination of red ginger and angkak has never been studied. The purpose of this research is to see the effect of the amount of red ginger on the percentage of yield and antioxidant activity in the combination of red ginger and angkak. Red ginger and angkak extracts are combined, each ingredient is extracted singly. The amount of red ginger was varied, namely 10, 20, 30, and 40 g. In comparison, the number of angkak remains at 55 g. Both materials were extracted by kinetic heat at a temperature of 60°C and a stirring speed of 800 rpm for 120 minutes. The yield percentage of the viscous extract obtained was calculated, and its antioxidant activity was analyzed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. In addition, the chromatogram profile test of red ginger and angkak was also carried out. Increasing the amount of red ginger in the red ginger - angkak extract did not have a significant effect ( $p>0.05$ ) on the yield percentage but had a significant effect ( $p<0.05$ ) by increasing the antioxidant activity of the red ginger - angkak extract. The combined extracts' antioxidant activity was less than the red ginger extract. The ginger chromatogram profile shows ten different spots, while angkak has three different spots.

**Keywords:** angkak; antioxidant activity; Red ginger; yield percentage

### INTRODUCTION

Free radicals are compounds with one or more unpaired electrons in their outer orbit. The presence of unpaired electrons causes the compound to become reactive, looking for a partner by attacking and binding electrons around it. The effects of free radical reactivity include autoimmune diseases, cell or tissue damage, cancer, and degenerative diseases. Antioxidants reduce the negative impact of reactive oxygen compounds in the body. The way antioxidants work is by donating one electron to free radical compounds so that their activity can be inhibited (Zulaikhah, 2017).

Red ginger (*Zingiber officinale* var. Rubrum) is a plant often consumed as a spice and herbal medicine. Red ginger has been found to have biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and anti-cancer activities. Many bioactive compounds in red ginger have been identified, such as phenolic compounds and terpenes. Phenolic compounds, especially gingerols, shogaols, and paradols, explain the various bioactivities of ginger, especially its contribution to the antioxidant activity (Mao et al., 2019).

Angkak is a fermented product of rice seeds by the Monascaceae, *Monascus purpureus*, which

turns rice into reddish purple seeds due to its pigmentation ability. Angkak is known to have many biological properties with hypolipidemic, anti-atherosclerotic, anti-cancer, anti-obesity, immunomodulatory, anti-inflammatory, antihypertensive, and antimicrobial activity. Chemical analysis has revealed that angkak contains monacolin compounds, pigments (e.g., monascin, rubropunctatin, and rubropunctamine), organic acids, amino acids, sterols, decalin derivatives, flavonoids, lignans, coumarins, terpenoids, and polysaccharides (Zhu et al., 2019). Monacolin K and pigment are one of the components of angkak, which has antioxidant activity. Monacolin K shows antioxidant activity through several tests, such as the ability to inhibit peroxidation of linoleic acid, reducing power, ability to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, ability to capture OH- and ability to chelate Fe<sup>2+</sup> (Ardiani et al., 2019; Kraboun et al., 2019). In addition, the natural pigment of angkak, which contains anthocyanin, also acts as an antioxidant (Nabila & Hendriani, 2018; Wanti Suratika, Andriani MAM, 2015).

Red ginger has good antioxidant activity. Previous research has shown that red ginger rhizome extract has robust antioxidant activity with an IC<sub>50</sub> value of 57.14 ppm (Herawati & Saptarini, 2020). Meanwhile, angkak has a vigorous antioxidant activity of 2.60 ppm extracted by maceration with 96% ethanol

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(Peranginangin, 2018). Antioxidant activity in red ginger is correlated with the content of polyphenolic compounds such as gingerol and shogaol, whereas in angkak, the monacolin K compounds, pigments, and flavonoids contribute as antioxidants (Ardiani et al., 2019; Hasim et al., 2020). The two substances are combined in extraction, hoping to produce maximum yield and antioxidant activity in the presence of a combination of secondary metabolites. The amount of dry ginger powder used by Chinese herbalists in medicine ranges from 10 - 40 g (Supu et al., 2019), while angkak ranges from 14 - 55 g (Zubaidah & Sriherfyna, 2015). The simplicia extraction ratios were randomly selected by determining the numbers of angkak, namely 55 g. In comparison, the amount of red ginger varied from 10, 20, 30, and 40 g.

## MATERIALS AND METHODS

### Materials

The materials used in this study were red ginger powder (*Zingiber officinale* var. *Rubrum*) (Agradaya with P-IRT 5103404040184-27 and production code 5CM02220886), angkak (SU Brand with BPOM RI code ML 219309046124), DPPH powder (2,2-diphenyl-1-picrylhydrazyl) (Tokyo Chemical Industry CO. LTD D4313), ascorbic acid (CSPC WeiSheng Pharmaceutical (Shi Jia Zhi Jiang) Co. Ltd), 96% ethanol (Merck 1.00983.2500), toluene (Smart-Lab, Batch No. 210121001), acetone (Smart-Lab, Batch No. 180121001), acetic acid (JTBaker, Batch No. 0000204534), ethyl acetate (Bratachem Batch No. 62111B012), food-grade vanillin (Koepoe-Koepoe, No. Batch P31.16010), and sulfuric acid (Smart Lab, Batch No. 080720005), silica gel plate 60 F<sub>254</sub> (Supelco Serial No. 1.05554.0001).

The tools used in this study were a hot plate (stirrer) (DLAB Serial No. Ms H280 Pro), UV/Vis spectrophotometer (Shimadzu type 2450), rotary evaporator (Heidolph Serial No. 031311186), micropipette (Dragonlab Serial No. YE187AL0045815), chamber, UV lamp (CAMAG), TLC stain spotting sprayer, analytical balance (Ohaus® Serial No. B334704032).

### Methods

#### Extraction

The extraction solvent used was 96% ethanol, with a sample and solvent ratio of 1:10 for each extraction. Red ginger and angkak simplicia were carefully weighed, respectively, as shown in Table I. Then, the simplicia was extracted on a hot plate (stirrer) set at a speed of 800 rpm and a temperature of 60°C for 120 minutes. After the extraction process, the extract

is filtered with Buchner, and the filtrate is taken, then evaporated with a rotary evaporator to obtain a thick extract. After that, the extract was dried in an oven at 50°C for 3 days. Extraction with each variable was repeated three times.

The extract yield was obtained by weighing the total weight of the viscous extract with the total simplicia weight and then calculating the % yield.

$$\% \text{ Yield} = \frac{\text{Weight of Dry extract (g)}}{\text{Weight of Dry plant material (g)}} \times 100\%$$

#### TLC profile testing

The TLC profile test was carried out by activating the TLC plate in an oven at 110°C for 30 minutes. The TLC plate used was 6.5x2 cm of silica gel and made a baseline at the bottom, about 0.5 cm from the bottom end of the plate, and the finish line at the top. The activated TLC plate was stained with the extract at baseline. The spot was then dried, and each plate was eluted in a TLC elution chamber containing the mobile phase of toluene:acetone (9:1) for the red ginger chromatogram profile and ethyl acetate:methanol:distilled water (7:1:1) and one drop acetic acid for the red ginger chromatogram profile, each 5 mL. The elution was stopped when the swelling limit was reached, and the plates were allowed to air dry. The spots are then examined by observing the spot in 366 nm UV light (Wagner & Blatt, 1996), as well as visuals directly to observe the pigment angkak. The retention factor (Rf) value is also calculated (Courtney, 2012; Yuliani et al., 2022).

$$Rf = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent front}}$$

#### Antioxidant activity testing

DPPH solution was prepared in a concentration of 45 ppm. A stock solution of vitamin C concentration of 50 ppm was prepared and diluted in 5 concentrations (3, 5, 7, 9, 11 ppm), and a stock extract solution of 500 ppm was made and diluted in 2 concentrations (120 and 150 ppm). Each series of extract and vitamin C was then 3 mL and added 6 mL of DPPH and homogenized and incubated in dark conditions for 30 minutes. The maximum wavelength of DPPH was then determined beforehand to be used in measuring the absorbance of DPPH blanks, ethanol blanks, and each series of samples and vitamin C concentrations that had been incubated. % Inhibition is calculated by the formula (Juniarka et al., 2011):

$$\% \text{ Inhibition} = \frac{(\text{Control Abs} - \text{sample Abs})}{\text{Control Abs}} \times 100\%$$

**Table I. The amount of materials used in the study**

Material	P1	P2	P3	P4	P5	P6
Angkak (g)	30	0	55	55	55	55
Red ginger (g)	0	30	10	20	30	40
Ethanol 96%	300 mL	300 mL	650 mL	750 mL	850 mL	950 mL

**Table II. Yield Percentage Results**

No.	Extract Variations	Yield (%)
1	P1	11.77 ± 6.28
2	P2	12.38 ± 3.19
3	P3	12.24 ± 5.43
4	P4	13.01 ± 1.98
5	P5	12.27 ± 2.21
6	P6	12.78 ± 2.78

Then, a plot is made between ln concentration and % inhibition, and the equation  $y = bx + a$  is obtained. The formula calculates  $IC_{50}$ :

$$x = \frac{(y - a)}{b}$$

### Statistic analysis

Data on yield % yield and inhibition percentage of extracts obtained were analyzed using SPSS 25.0. The statistical homogeneity test was carried out with the Levene Test ( $\alpha > 0.05$ ), and the normal distribution was carried out with Shapiro Wilk ( $\alpha > 0.05$ ). Data on yield and percentage of inhibition were included as parametric data so that it was continued with the ANOVA test to see whether there was significance, i.e., if  $p < 0.05$ . The Excel application obtained SD and RSD values for the sample's yield percentage, inhibition percentage, and  $IC_{50}$ .

## RESULTS

### Extract Yield

Details regarding yield percentage results are provided in Table II.

### TLC Profile Test

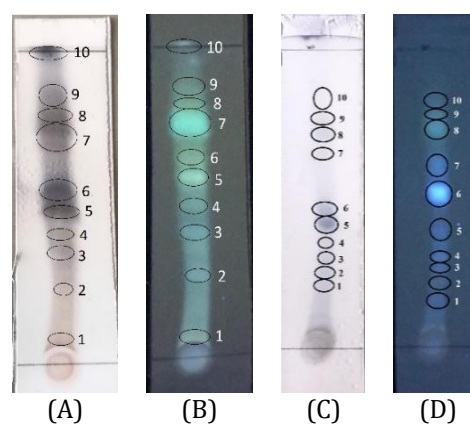
The supporting evidence for the result of red ginger chromatogram pattern from TLC test is illustrated in Figure 1.

### Antioxidant Activity Testing

The results of the antioxidant activity testing are presented in Table III and illustrated in Figure 3.

## DISCUSSION

The percentage of extract yield is in the range of 11.77–13.01 %. The results of the extract yield test obtained parametric data with overall



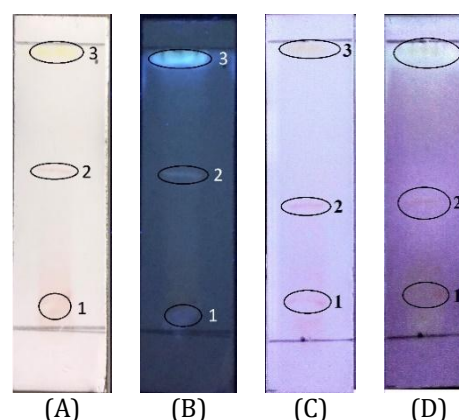
**Figure 1. Red Ginger Chromatogram Pattern from TLC Test Results**

Information: Stationary phase = Silica Gel 60 F<sub>254</sub>; Mobile phase = Toluene:acetone (9:1); (A) Plate after being sprayed with sulfuric acid vanillin (Red ginger extract); (B) Observation results under UV light 366 nm (Red ginger extract); (C) Plate after being sprayed with sulfuric acid vanillin (Combined extract); (D) Observation results under UV light 366 nm (Combined extract)

data values that fulfilled homogeneity, and the ANOVA test results showed that there was no significant relationship between the increase in the amount of ginger and the percent yield ( $p > 0.05$ ), which means that the increase in the amount of ginger did not affect the percent yield. The percentage of extract yield is because the treatment for the extraction process is the same, which is related to the extraction method, type of solvent, temperature, simplicia and solvent ratio, simplicia particle size, stirring, and extraction time. These things affect the percentage yield (Wewengkang & Rotinsulu, 2021; Wijaya et al., 2016). So that the resulting yield does not show a significant difference. Based on Table II, the largest yield was obtained in the P4 extract, namely 13.01±1.98 %.

The results of the chromatogram pattern (Figure 1) showed the presence of different spots observed after being sprayed with vanillin sulfuric acid and 366 nm UV light. Spraying vanillin sulfuric acid to identify phenolic compounds, terpenoids, steroids, and essential oil groups (Jork, 1990; Wagner & Bladt, 1996). Positive results were indicated by changes in the color of the spots to blue-purple after heating. This chromatogram pattern indicates the presence of phenolic compounds, terpenoids, steroids, and essential oil groups in the red ginger extract. Red ginger contains the main phenolic compounds, namely gingerol, shogaol, and paradol, and contains steroids and essential oils (Mao et al., 2019). Research also supports this by showing that phenolic compounds, essential oils, and terpenoids were positively identified in red ginger (Herawati & Saptarini, 2020; Kusnadi & Tivani, 2017). There were ten different spots under observation using 366 nm UV light. In the red ginger extract, the Rf values were 0.08, 0.24, 0.36, 0.42, 0.5, 0.56, 0.72, 0.8, 0.88, and 1. In the combination extract (C), the Rf values were 0.2, 0.28, 0.32, 0.38, 0.41, 0.5, 0.68, 0.74, 0.8, and 0.86. While on plate (D), the Rf values were 0.14, 0.2, 0.28, 0.32, 0.4, 0.52, 0.62, 0.74, 0.8, and 0.86. However, some stains are visible at 366 nm UV light but do not give color when sprayed with vanillin sulfate. For example stains 1(D), 6(D), and 7(D), it is suspected that these stains are numeric pigments. Angkak pigment belongs to the group of anthocyanin and flavonoid compounds, so it does not produce color after being sprayed with sulfuric acid vanillin (Nabila & Hendriani, 2018; Wanti Suratika, Andriani MAM, 2015). Some stains gave a purple color after being sprayed with sulfuric acid vanillin but were not visible at 366 nm UV light, namely stains 4(C), 6(C), and 7(C). It was suspected that these stains were steroid compounds in ginger, which were not detected under UV light at 366 nm. UV light 366 nm detects lignans, alkaloids, flavonoids, and triterpenoids (Anwar, 2011).

The results of the chromatogram pattern (Figure 2) showed that there were different spots in the angkak extract, which were observed by direct visualization and 366 nm UV light. There are three spots in direct visual observation (A) and observation under UV light 366 nm (B). The three spots have Rf values (0.1, 0.58, and 0.98). The visible stain is suspected to be a red pigment belonging to the flavonoid group, where 366 nm UV light can detect lignans, alkaloids, flavonoids, and triterpenoids (Anwar, 2011). In contrast, the chromatogram pattern of the combination extract (red ginger-angkak), which was observed by



**Figure 2. Chromatogram pattern of TLC test results**

Information: Stationary phase = Silica Gel 60 F<sub>254</sub>; Mobile phase = ethyl acetate:methanol:distilled water (7:1:1) and 1 drop of acetic acid; (A) Observation in visible light (Angkak extract); (B) Observation results under UV light 366 nm (Angkak extract); (C) Observation in visible light (Combined extract); (D) The plate after being sprayed with DPPH (Combined extract)

direct visual (C), and the plate, after being sprayed with DPPH (D), had three stains. The three spots have Rf values (0.12, 0.46, and 0.96). The resulting Rf value is similar to the chromatogram pattern of the single angkak extract. So, it can be assumed that the combined extract contains red and yellow pigment compounds in angkak. Spraying the plate with DPPH resulted in a slightly pale yellow discoloration around the stain, which indicated that the pigment in angkak had antioxidant activity, but the intensity was weak (Amany et al., 2020; Kang et al., 2014). The red pigments in angkak are monascorubramine and rubropunctamin, and the yellow pigments in angkak are monascin and anklafavin (Hasim et al., 2018).

The absorbance of the solution was measured after incubation with a wavelength of 517.5 nm. The DPPH wavelength is absorption at 517 nm from the range of 515-520 nm (Kristiningrum et al., 2018; Santos et al., 2019). The results of the absorbance data are used to calculate the IC<sub>50</sub> value. The IC<sub>50</sub> measurement of vitamin C is categorized as a powerful antioxidant with a value of 4.64 ppm. The analysis results (Table III) showed that angkak Tunggal (P1) extract at a concentration of 120 ppm gave % inhibition of 38.93±1.39 % and at 150 ppm, 38.68±3.12 %. However, based on studies that have been conducted, angkak at 2.6 ppm provides 50% inhibition, which is categorized as intense antioxidant activity. This study also used the DPPH method (Warrior, 2018). Material factors can cause low antioxidant activity. The angkak

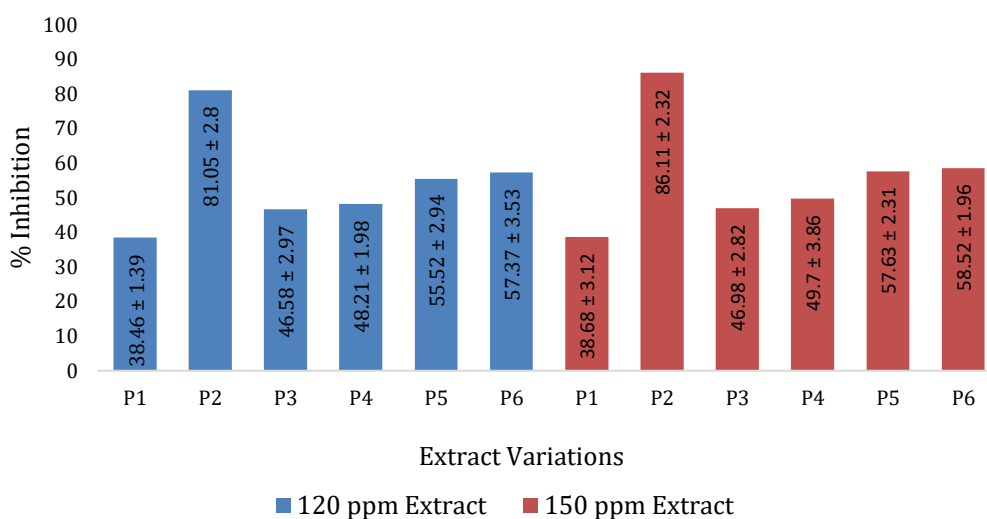


Figure 3. Antioxidant Activity of Various Extract

Table III. Test Results for Extract Antioxidant Activity

Extract Variation	Percent Inhibition (%)		P value
	120 ppm Extract	150 ppm Extract	
P1	38.46 ± 1.39	38.68 ± 3.12	> 0.05
P2	81.05 ± 2.8	86.11 ± 2.32	< 0.05
P3	46.58 ± 2.97	46.98 ± 2.82	> 0.05
P4	48.21 ± 1.98	49.7 ± 3.86	> 0.05
P5	55.52 ± 2.94	57.63 ± 2.31	> 0.05
P6	57.37 ± 3.53	58.52 ± 1.96	> 0.05

used in this study was imported from China, which may allow for a decrease in the quality of the ingredients during shipping. The specific process of making angkak is also not known. There may be an excessive heating process that can destroy compounds that have antioxidant activity. The type of *Monascus purpureus* biotype, temperature, inoculum content, type of rice, and fermentation time also affect the levels of compounds that contribute to antioxidants in red yeast rice (Hamdiyati et al., 2016; Marič et al., 2019; Saithong et al., 2019). In addition, product storage also affects compounds that contribute to antioxidants in angkak, where pigments in angkak are sensitive to light and temperature. In other studies that have been conducted, the antioxidant activity of angkak rice was found with the DPPH method of 1.716±0.036 mg AEE/g, with the FRAP method of 0.023±0.002 mmol Fe<sup>2+</sup>/g and with the hydroxyl radical scavenging activity method of 134.398±10.301 mg AEE/g (Huang et al., 2017). It can be seen in the results of the TLC of angkak sprayed with DPPH, which shows a change in the color of the DPPH, which is not concentrated. The DPPH incubation results and the concentration series of angkak extract also showed a slight

color change. Extracting single ginger (P2) at 120 and 150 ppm concentrations produced % inhibition of 81.05±2.8 % and 86.11±3.32 %. Another study also found that red ginger has potent antioxidant activity at a concentration of 57.14 ppm resulting in 50% inhibition, while at an extract concentration of 100 ppm, it produces 54.91% inhibition. The red ginger extract contains flavonoids, tannins, and alkaloids, which have hydroxyl groups that can donate hydrogen to interact with DPPH radicals to produce DPPH-H (Herawati & Saptarini, 2020). Based on the results obtained in the DPPH test, the percent inhibition of the combination extract at a concentration of 120 ppm is presented in Table III and Figure 3. There was an increase from the P3 extract, namely 46.58±2.97 %; extract P4 48.21±1.98 %; P5 extract 55.52±2.94 %; and P6 extract 57.37±3.53 %. Whereas at a concentration of 150 ppm, the combination extract also experienced an increase in the percentage of inhibition, namely P3 extract 46.98±2.81 %; extract P4 49.7±3.86 %; extract P5 57.63±2.31 % and extract P6 58.52±1.96 %. The increase in the percentage of inhibition in the extract indicates that the greater the amount of red ginger in the combination extract and the

concentration of the extract, the greater the inhibition percentage. This is because the percentage inhibition of free radical activity will also increase with increasing concentration (Damanis et al., 2020). In addition, phenolic compounds such as gingerols and shogaols in red ginger have good antioxidant activity. Extraction at 60°C can also convert gingerol into shogaol, where increased shogaol levels correlate with increased antioxidants (Hargono, Fitra Pradhita, 2013). The results of comparative testing of the antioxidant activity of the combination extract and single extract showed that the DPPH radical scavenging ability of the red ginger extract was stronger than the combination. This combination can be caused by antagonistic reactions (negative interactions) between compounds, which can cause a decrease in antioxidant activity in reducing DPPH free radicals. Flavonoid (in red ginger) and anthocyanin (in angkak) compounds in the combined extract are known to cause strong antagonistic reactions. Therefore, it is possible that in this interaction, hydrogen bonds can occur between compounds, thereby reducing the availability of hydroxyl groups, which can reduce the possibility of interaction with DPPH (Hidalgo et al., 2010; Zhang et al., 2022; Zhu et al., 2019). The combination of soursop leaf extract and papaya leaf extract, in a ratio (of 1:2), gives a slightly antagonistic effect, which was analyzed by the CompuSyn application (Seta Rikantara et al., 2022). The antagonistic effect is due to the interaction between the chemical compounds in each extract. In plants, apart from the main compounds that are the most influential, there are still other compounds that might affect the expected response (Sambodo, 2019).

The results of the percentage inhibition analysis of the 120 ppm and 150 ppm extracts were statistically parametric because they fulfilled the data normality test using the Shapiro-Wilk test, which showed a  $p > 0.05$  value so that the data were normally distributed. Suppose the data normality test and data were normally distributed that the statistical analysis was continued by using the ANOVA parametric test and the Post Hoc Test. The results of the ANOVA test for the two extract concentrations showed significant differences in the various extracts ( $p < 0.05$ ). The difference in each treatment group at 120 ppm extract was followed by a Post Hoc test which produced a single angkak extract (P1) significantly different from the extracts P2, P3, P4, P5, and P6 ( $p < 0.05$ ). Single ginger extract (P2) was significantly different from extracts P3, P4, P5

and P6 ( $p < 0.05$ ). The P3 extract was significantly different from the P5 and P6 extracts ( $p < 0.05$ ) and not significantly different from the P4 extract ( $p > 0.05$ ). The P4 extract differed significantly from the P5 and P6 extracts ( $p < 0.05$ ). The P5 extract was not significantly different from the P6 extract ( $p > 0.05$ ). The difference in each treatment group at 150 ppm extract was followed by a Post Hoc test which produced a single angkak extract (P1) significantly different from the extracts P2, P3, P4, P5, and P6 ( $p < 0.05$ ). Single ginger extract (P2) was significantly different from extracts P3, P4, P5 and P6 ( $p < 0.05$ ). The P3 extract significantly differed from the P4, P5, and P6 extracts ( $p < 0.05$ ). The P4 extract was insignificantly from the P5 and P6 extracts ( $p < 0.05$ ). The P5 extract was not significantly different from the P6 extract ( $p > 0.05$ ). The difference in each treatment group at 150 ppm extract was followed by a Post Hoc test which produced a single angkak extract (P1) significantly different from the extracts P2, P3, P4, P5, and P6 ( $p < 0.05$ ). Single ginger extract (P2) was significantly different from extracts P3, P4, P5 and P6 ( $p < 0.05$ ). The P3 extract significantly differed from the P4, P5, and P6 extracts ( $p < 0.05$ ). The P4 extract differed significantly from the P5 and P6 extracts ( $p < 0.05$ ). The P5 extract was not significantly different from the P6 extract ( $p > 0.05$ ). The difference in each treatment group at 150 ppm extract was followed by a Post Hoc test which produced a single angkak extract (P1) significantly different from the extracts P2, P3, P4, P5, and P6 ( $p < 0.05$ ). Single ginger extract (P2) was significantly different from extracts P3, P4, P5 and P6 ( $p < 0.05$ ). The P3 extract significantly differed from the P4, P5, and P6 extracts ( $p < 0.05$ ). The P4 extract differed significantly from the P5 and P6 extracts ( $p < 0.05$ ). The P5 extract was not significantly different from the P6 extract ( $p > 0.05$ ).

The % value of antioxidant activity obtained did not correlate with the yield percentage, where the addition of red ginger significantly affected the % inhibition value but did not affect the yield percentage. This percentage of inhibition is because the yield is negatively correlated with antioxidants (Braga et al., 2016). Antioxidant levels depend on the number of hydroxyl groups of the metabolites contained (Do et al., 2014). In addition, the affinity of different materials for the polarity of the solvent also affects the extraction results and the antioxidant activity contained in the extract (Taoreh et al., 2015).

## CONCLUSION

Increasing the amount of red ginger in the red ginger extract did not have a significant effect ( $p>0.05$ ) on the yield percentage. However, it had a significant effect ( $p<0.05$ ) by increasing the antioxidant activity of the red ginger extract.

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