ANTIOXIDANT ACTIVITIES USING DPPH, FIC, FRAP, AND ABTS METHODS FROM ETHANOLIC EXTRACT OF LEMPUYANG GAJAH RHIZOME (*Zingiber zerumbet* (L.) Roscoeex Sm.)

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Abstract

One of the Zingiber species that has long been used as traditional medicine by local Indonesian people is lempuyang (Zingiber ssp.). In its use as traditional medicine, it is more often used than other types of lempuyang, namely the lempuyang gajah (*Zingiber zerumbet* (L.) Roscoeex Sm.). Therefore, this study focused on the *Zingiber zerumbet* (L.) Roscoeex Sm. Most of the biological activities reported for this plant are attributed to phenolic contents and volatile principles. Hence, a detailed investigation of antioxidant activity, flavonoid, and phenolic content of *Zingiber zerumbet* (L.) Roscoeex Sm. rhizome was carried out. The purpose of this study was to determine the total flavonoid, phenolic, and antioxidant activity of *Zingiber zerumbet* (L.) Roscoeex Sm. extract in several methods of reducing free radicals DPPH, FIC, FRAP, and ABTS. The extraction method used is maceration extraction with 70% ethanol solvent and concentrated. The total phenolic content was determined by the Folin-Ciocalteu method with gallic acid as the standard, while the total flavonoid content was determined by the quercetin method. Ascorbic acid was used as a positive control of antioxidant activity. The *Zingiber zerumbet* (L.) Roscoeex Sm. plant used was obtained from the village of Tegal Bulu Banyuwangi. This type of research is an experimental study with the concentration of the extract used, namely 20, 40, 60, 80, and 100 mg/L. The results showed that the ethanol extract of *Zingiber zerumbet* (L.) Roscoeex Sm. rhizome contained total phenolic and flavonoid content of 23.58±0.25 mgGAE/g and 12.21±0.03 QUE/g extract, respectively. Antioxidant activity with IC_{50} value in the DPPH free radical reduction method of 11.40 ± 0.23 which is included in the very strong category, FIC of 121.46±2.93 which is included in the medium category, FRAP of 19.38±0.14 which is included in the very strong category, and ABTS of 89.32±0.15 which is included in the strong category. Phenolics and flavonoids are thought to have an important role in the antioxidant activity of *Zingiber zerumbet* (L.) Roscoeex Sm. rhizome.

Keywords*: antioxidant, DPPH, FIC, FRAP, ABTS, ascorbic acid, total flavonoids, total phenolic, Zingiber zerumbet (L.) Roscoeex Sm*

Introduction

One of the Zingiber species that has long been used as traditional medicine, especially as herbal ingredients by local Indonesian people is lempuyang (Zingiber ssp.) (Burkill, 1996; de Guzman and Siemonsma, 1999) to treat asthma, pneumoniae, anti-inflammatory, antiviral, antibacterial, immunomodulators, and others (Jantan et al., 2008; Kaewchoothong, 2009; Iswantini et al., 2011). In its use as traditional medicine, it is more often used than other types of lempuyang, namely the elephant lempuyang. Therefore, this study focused on the *Zingiber zerumbet* (L.) Roscoeex Sm.).

Zingiber zerumbet is known to have certain medicinal properties such as anti-inflammatory (Nhareet & Nur, 2003),

anti-tumor (Huang, et al., 2005), antiallergic (Tewtrakul & Subhadhirasakul, 2007), anti-pyretic (Somchit, et al., 2005), anti-platelet aggregation activity (Jantan & Jalil, 2005), antibacterial (Kader, et al., 2011). This plant is reported to contain sesquiterpenoids, flavonoids, aromatic compounds, vanillin, kaempferol derivatives and other polyphenolic compounds (Jang, et al., 2004; Jang & Seo, 2005; Chien, et al., 2008). Polyphenol compounds are reported to have several biological effects including antioxidant activity (Kähkönen, et al., 1999).

Previous studies on antioxidant properties (AOP) of rhizome extracts from various species of *Alpinia, Curcuma* and *Zingiber* were reported to have antioxidant activity comparable to or stronger than *α-tocopherol* and butylated hydroxytoluene (BHT) (Jitoe, et al., 1992; Habsah, et al., 2000; Zaeoung, Plubrukan, Keawpradub., 2005; Vankar, et al., 2006). Screening 18 ginger species from five species from Taiwan, *Curcuma zedoaria* and *C. longa* rhizomes had the highest phenolic content, and *C. longa* and *H. coronarium* rhizomes had the strongest antioxidant capacity, radical scavenging and reducing power (Chen, et al., 2008). AOPs of different chemical constituents isolated from ginger rhizomes have also been studied. Antioxidants from the rhizomes include compounds related to gingerols and diarylheptanoids in *Z. officinale*, and curcuminoids in *C. longa*, and *Zingiber cassumunar* (Kikuzaki & Nakatani, 1993; Sreejayan, 1996; Song, et al., 2001; Masuda & Jitoe, 1994; Jitoe, 1994). Masuda, & Mabry, 1994). From the rhizomes of *Z. officinale*, gingerol, shogaol, gingerdiol, gingerdion, dihydrogingerdion, hexahydrocurcumin and octahydrocurcumin have been identified (Nakatani & Kikuzaki, 1994). The related compounds gingerols and diarylheptanoids can be classified into four groups, namely, 5-hydroxy-3-one, 4-en-3-one, 3,5-diol and 3,5-diacetate (Kikuzaki, Kawasaki, & Nakatani, 2001). Depending on the substitution pattern of the side chain and benzene ring, and on the length of the side chain, its AOP may be stronger than that of *α-tocopherol*. From the rhizomes of *C. longa* and *Z. cassumunar*, curcuminoids curcumin, demethoxylated curcumin, cassumunin and cassumunarin have been identified (Jitoe, Masuda, & Mabry, 1994). From the rhizome of *Alpinia nutans*, 5,6-dehydrokawain and (-)-pinocembrin have AOPs comparable to -tocopherol (Habsah, et al., 2003).

Research by Nag, Bandtopadhyay, & Mukherjee (2013) showed significant radical scavenging activity of *Z. zerumbe*t against DPPH and hydroxy radicals. While in the research of Budin, et al. (2013), the antioxidant properties of *Z. zerumbet* ethyl acetate extract were compared using in vitro antioxidant methods such as the DPPH radical and the FRAP test showed that the *Z. zerumbet* ethyl acetate extract showed antioxidant activity and radical scavenging at different potency levels although the effect was small on the BHT. The results obtained in this study indicate that the rhizome of *Z. zerumbet* in the concentration range of 6.25 g/ml-50 g/ml is a potential source of natural antioxidants.

Research conducted by Bavesh, Nayak, & Jayashree (2013) on the ethyl acetate extract of *Zingiber zerumbet* rhizome (ZZE) obtained from India, showed that the results of the phytochemical investigation of ZZE contained the presence of phenols, flavonoids and terpenoids. ZZE has a total phenolic content equivalent to 331.93±1.23 mg/ml gallic acid and a total flavonoid content equivalent to 198±2.65 mg/ml quercetin. ZZE also showed significant total antioxidant activity (86.04±0.98 mg/ml ascorbic acid equivalent). Furthermore, ZZE-driven DPPH and ABTS radicals with IC_{50} were

117.65±1.45 and 78.72±1.12 g/ml, respectively.

Previous research by Swargiary, et al. (2021) found that antioxidant studies using FRAP, DPPH, ABTS, and TBARS tests revealed that the five plants contained considerable free radical scavenging activity. C. fistula showed the strongest free radical scavenging activity while C. grandis rind extract showed poor activity. The overall antioxidant activity of plants such as TAC, FRAP, DPPH, ABTS, and TBARS could be regulated by decreasing activity as *C. fistula* > *Z. zerumbet*>*L. crustacea* > *S. myosuroides* > *C. grandis*. All five plants were obtained from India.

However, the antioxidant activity of the elephant lempuyang plant from the village of Tegal Bulu Banyuwangi has not been tested with other free radical methods. Therefore, the antioxidant activity, flavonoid, and phenolic content of *Z. zerumbet* from Tegal Bulu Banyuwangi village requires further study.

Research Methods

Materials and Instrumentation

The materials used in this study include: rhizome of lempuyang (*Zingiber zerumbet*) obtained from the village of Tegal Bulu Banyuwangi., ethanol (technical), DPPH (1,1-Diphenyl-2-picrylhydrazyl), $FeSO_4.7H_2O$, Ferozin, ABTS, absolute MeOH, absolute ethanol, $FeCl_3$, 1% potassium hexacyanoferrate, phosphate buffer pH 6.6 (0.2 M), iron (II) chloride tetrahydrate 1 mM, trichloroacetic acid 10%, quercetin, gallic acid, Folin Ciaocalteu, Aluminum (III) chloride, sodium carbonate, sodium acetate, aquades, and aquabides. All materials used are materials with pure analysis qualifications unless otherwise stated. The tools used are grinder, sieve, tray, rotary vacuum evaporator (Buchi), analytical balance, UV-Vis spectrophotometer VWR 1600PC, filter paper, and glassware.

Procedure

Rhizome extraction method of Lempuyang Gajah

The *Zingiber zerumbet* (L.) Roscoeex Sm. rhizome was extracted by maceration method which was carried out with an amount of 800 grams of lempuyang gajah powder weighed and added with ethanol solvent in a ratio of 1:2 then stirred with a stir rod. The maceration extraction process was carried out for 24 hours. The mixture was then filtered and obtained the filtrate from maceration. The lempuyang gajah powder residue was macerated again until a clear filtrate was obtained. The filtrate obtained was then concentrated using rotary vacuum evaporator.

Total flavonoid and phenolic method

The total flavonoid extract of *Zingiber zerumbet* (L.) Roscoeex Sm. determined using quercetin as standard (Chang, Yang, & Chern., 2002). Preparation of a standard solution of quercetin was carried out by weighing as much as 5 mg of quercetin and dissolved in 25 ml of ethanol as a standard solution of 100 ppm quarcetin. Then made a series of standard solution concentrations of 5, 10, 15, 20, and 25 ppm. As much as 0.5 ml of standard solution of quarcetin was added 1 ml of 2% aluminum (III) chloride, 1 ml of 0.12 M sodium acetate and homogenized. Incubated for 60 minutes, then one concentration of standard solution was taken, the absorbance was measured at a wavelength of 430-440 nm. Each concentration was measured at the maximum wavelength. And the quercetin standard curve was made by connecting the concentration of the quercetin standard solution with the absorption results obtained from measurements using a UV-Vis spectrophotometer at a wavelength of 435 nm. Determination of the total flavonoid content of the extract was carried out by weighing 5 mg of the sample and dissolved in 50 ml of ethanol

until a concentration of 100 ppm was obtained, 0.5 ml of the test sample was added with 1 ml of 2% aluminum. (III) chloride, 1.0 ml of 0.12 M sodium acetate and homogenized. Incubated for 60 minutes, then the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 435 nm. Total flavonoids were calculated using a linear regression equation from the previously measured quercetin calibration curve. Calculation of flavonoid content with the equation (1) (Sayuti & Yenrina, 2015).

$$\text{Total flavonoid} = \frac{\text{C} \times \text{V} \times \text{fp}}{\text{g}} \qquad (1)$$

where C = flavonoid concentration (x value), V = extract volume (mL), fp = dilution factor, and g = weight of the sample used.

The total phenolic test begins with determining the maximum wavelength of gallic acid by making a gallic acid solution with a concentration of 40 ppm in methanol. The solution was pipetted 0.5 ml and put into a cuvette filled with 2.5 ml of Folin Ciaocalteu and 2 ml of 5% Na2CO3 then allowed to stand for 30 minutes. Then read using a UV-Vis spectrophotometer with a wavelength of 760-770 nm. The standard curve for gallic acid was made by connecting the concentration of the standard solution of gallic acid with the absorption results obtained from measurements using a UV-Vis spectrophotometer at a wavelength of 750 nm. Then the determination of total phenolic was carried out by weighing the extract as much as 5 mg dissolved in 50 mL of methanol (100 ppm). Then 0.5 ml pipette was added and 2.5 ml Folin Ciocalteu reagent (10%) and 2 ml Na_2CO_3 (5%) were added and then incubated for 5 minutes at room temperature. The absorbance was measured at a maximum wavelength of 765 nm by UV-Vis spectrophotometry method. Samples were made in 5 repetitions for each analysis and the average value of absorbance was obtained (Sedjati et al., 2017). The absorption yield was calculated using a linear regression equation from the standard gallic acid curve and the result was expressed as a percent gallic acid equivalent. Calculation of total phenolic content with the following equation (2).

$$\text{Total fenolik} = \frac{\text{C} \times \text{V} \times \text{fp}}{\text{g}} \qquad (2)$$

where C = flavonoid concentration (x value), V = extract volume (mL), fp = dilution factor, and g = weight of the sample used.

DPPH method

The antioxidant test method against DPPH free radicals used in this study is a modification of several procedures reported by Brand-Williams et al. (1995), Sharma & Bhat (2009), Panda (2012) and Dolatabadi et al. (2014). Ethanol extract of *Zingiber zerumbet* (L.) Roscoeex Sm. as much as 0.01 grams diluted with methanol in a 100 ml volumetric flask. Subsequently, the concentration of the sample stock solution was varied (20, 40, 60, 80, and 100 mg/L). The preparation of the DPPH solution was carried out by weighing 0.004 grams of DPPH crystals and diluted with methanol in a 100 ml volumetric flask. Furthermore, all solutions were put into a test tube, then incubated at room temperature for 30 minutes, calculated from the addition of DPPH solution to the sample. The absorption was measured at the maximum wavelength of DPPH (517 nm). The comparison solution used was ascorbic acid. Measurement of ascorbic acid absorption was carried out using the same method as the sample measurement. Each measurement was repeated 5 times. The inhibition of each standard is denoted by % inhibition, and is calculated by equation (3).

$$\text{Antioxidant power} = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\ \% \quad (3)$$

FIC method

In this study, the FIC antioxidant test method used is a modification of the procedure reported by Lai & Lim (2011) and Panda (2012). A total of 0.5 mL of samples (20, 40, 60, 80, and 100 mg/L) were put into a test tube, then 0.5 mL of 1 mM FeCl2 and 0.5 mL of 2.5 mM ferrozine were added and then homogenized. The solution was then incubated for 10 minutes at room temperature (25°C) which was calculated from the addition of ferrozine solution to the sample. The sample absorption was measured at a wavelength of 562 nm. The chelating effect of Fe2+ ion is calculated by equation (4).

$$\text{Chelating effect (\%)} = \left(1 - \frac{As}{Ak}\right) \times 100 \quad (4)$$

where As is the extract absorbance and Ak is the control absorbance.

FRAP method

The antioxidant test method with FRAP free radicals used in this study is a modification of the procedure reported by Panda (2012), namely by making a standard curve of Fe^{2+} from iron (II) sulfate heptahydrate with as much as 12.20 mg $FeSO_4.7H_2O$ weighed and dissolved. with distilled water in a 100 mL volumetric flask to mark the limit. Each aliquot is 1.0; 0.5; 0.25; 0.125; and 0.063 mL, were put into a 100 mL volumetric flask and diluted to the limit mark, so that the Fe^{2+} concentration was 0.88, respectively; 0.44; 0.22; 0.11 and 0.05 M. Then for the measurement of the extract using FRAP, 0.5 mL of *Zingiber zerumbet* (L.) Roscoeex Sm. extract (20, 40, 60, 80, and 100 mg/L) was taken and put into a test tube. Then added with 0.5 mL of phosphate buffer pH 6.6 (0.2 M) and 0.5 mL of 1% potassium hexacyanoferate solution. The solution mixture obtained was then incubated for 20 minutes at 50°C and added 0.5 mL of 10% TCA solution (if two layers were formed, they were separated by centrifugation). The top layer was taken as much as 0.5 mL and put into a cuvette, then 0.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ were added. The mixture was then incubated for 5-10 minutes at room temperature (25°C). The absorbance of the solution was measured at a wavelength of 700 nm and calculated as the concentration of Fe^{2+} (μM/g) based on the standard curve Fe^{2+}.

ABTS method

The test procedure was carried out based on Arnao (2000). ABTS powder was weighed as much as 7.1 mg and potassium persulfate powder as much as 3.5 mg. Each ingredient was then dissolved in 5 mL of ethanol. Then both solutions were incubated in the dark for 12 hours. After incubation, the two solutions were mixed and the volume was made up to 25 mL with ethanol. The sample solution of lempuyang elephant extract with concentrations of 20, 40, 60, 80, and 100 mg/L into a test tube was added to a 1:1 ratio of ABTS solution and then homogenized. The mixture was then measured its absorption at a wavelength of 520 nm.

Statistical analysis

All experiments were carried out with at least 5 variations of sample concentration, each repeated 5 times and UV-Vis absorption measured 5 times. The antioxidant activity value of each sample is reported in the form of the average value (x) of all measurement values with standard deviation (sd) and total population data (n) and written in the format x ± sd (n). The correlation coefficient between the data generated by each analytical method was calculated using the SPSS program (version 16.0). The results of the extract free radical scavenger were analyzed using linear

regression analysis. Then, the linear regression equation generated in this process is used to determine *Median Inhibitory Concentration* 50 (IC_{50}).

Results and Discussion

One of the Zingiber species that has long been used as traditional medicine by local Indonesian people is lempuyang (Zingiber ssp.). In its use as a traditional medicine, it is more often used than other types of lempuyang, namely the elephant lempuyang (*Zingiber zerumbet* (L.) Roscoeex Sm.). Therefore, this study focused on the elephant lempuyang (*Zingiber zerumbet* (L.) Roscoeex Sm.). In this study, elephant lempuyang was studied for its total flavonoid and phenolic content as well as its antioxidant properties by various test methods.

Total flavonoids and phenolics

Polyphenol compounds in this case phenolics and flavonoids act as natural antioxidants (Yao et al. 2010) that modulate metabolic activity (Hanhineva et al. 2010) and improve oxidative stress-related diseases such as inflammatory conditions and complications of diabetes (Yang et al. 2012). The total flavonoid content was determined by colorimetric method using $AlCl_3$. Meanwhile, the total phenolic content was carried out using Folin-Ciocalteu reagent with gallic acid as standard. The total phenolic content of Zingiber zerumbet (L.) Roscoeex Sm. was 23,580.25 mgGAE/g extract, as determined by the Folin-Ciocalteu method, determined by reference to a standard curve and reported as gallic acid equivalent ($y = 0.0055x + 0.1345$, $R^2 = 0.97$). The total flavonoid content of *Zingiber zerumbet* (L.) Roscoeex Sm. was 12210.03 mgQUE/g extract determined by reference to the standard curve and reported as quercetin equivalent ($y = 0.0061x + 0.2814$, $R^2=0.95$) (Table 1). The antioxidant potential of phenolic compounds is due to the presence of hydroxyl groups present in phenolic compounds. This hydroxyl group acts as a hydrogen atom donor through an electron transfer mechanism when reacting with radical compounds so that it can inhibit the oxidation process (Miguel-Chávez, 2017). The total flavonoid and phenolic content of high and low are influenced by several factors, including the type of lempuyang plant, the place of collection, age, temperature, climate, radiation levels, season, herbivore density, and availability of nutrients (Djapiala et al., 2011; Suparmi and Sahri, 2009).

Antioxidant activites of Zingiber zerumbet (L.) Roscoeex Sm. on various methods

Antioxidants are compounds that help the body to combat oxidative stress associated with tissue damage and the activity of other highly reactive free radicals. After that, the supply of exogenous antioxidants from food can effectively enhance the innate antioxidant system by increasing the production of antioxidant enzymes and play a protective role against excessive accumulation of free radicals (Kozarski, et al., 2015). The rhizome extract of *Zingiber zerumbet* (L.) Roscoeex Sm. in this study is a research subject whose antioxidant activity will be determined by in vitro free radical scavenging methods of DPPH, FIC, FRAP, and ABTS.

DPPH test method

The antioxidant activity test model with the free radical DPPH (1,1-*diphenyl*-2-*picrylhydrazyl*) has been widely accepted because the analytical method is fast, simple, sensitive to samples even with small concentrations and is easy (Karadag, 2009). The reaction in this test is based on the reduction of the unpaired electron on the nitrogen atom by the hydrogen atom of the antioxidant and forms a yellow hydrazine group. In this method, changes in the absorption of DPPH that have not reacted with the remaining DPPH that have reacted with antioxidants can be measured (Kedare and

Singh, 2011). Several studies on members of the Zingiberaceae family showed significant antioxidant activity of DPPH, but some were also known to have low antioxidant activity of DPPH (36). Although data on the antioxidant activity of *Z. zerumbet* are not widely available, our study revealed that the DPPH scavenging activity of Lempuyang Gajah extract was relatively strong (Table 1).

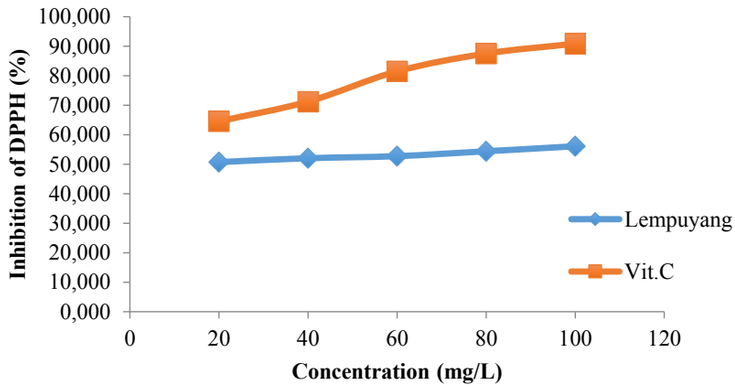

Figure 1. Percentage of DPPH radical inhibition

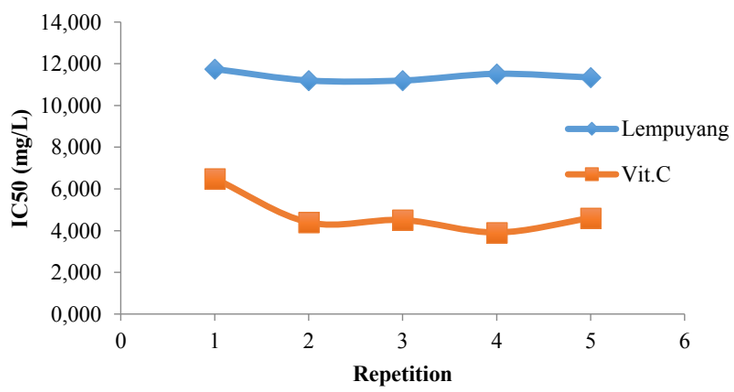

Figure 2. Free radical antioxidant activity of DPPH

The hydrogen atom donor (H^+) from the substance tested on the DPPH radical becomes a non-radical compound DPPH which is indicated by the occurrence of a color change, which causes a purple color bleaching of the DPPH free radical. The ability of antioxidant molecules to scavenge DPPH free radicals can be measured quantitatively spectrophotometrically from the color changes that occur. Variation of concentration of *Zingiber zerumbet* (L.) Roscoeex Sm extract. on the % inhibition of antioxidants in Figure 1, it is obtained that the higher the concentration, the higher the percentage of inhibition. This is because the higher the concentration, the higher the content of metabolites in the extract. The metabolite compounds in the extract donate hydrogen atoms to DPPH free radicals so as to form a more stable bond (DPPH-H) (Sayuti & Yenrina, 2015). The number of DPPH-H bonds increases, the intensity of the absorption color decreases (Mabruroh, 2015). As a result, absorption will be low so that the percentage of antioxidant power will be higher (Sayuti & Yenrina, 2015). The same is true for ascorbic acid. Ascorbic acid has 2 hydrogen atoms so it has the

ability to scavenge free radicals more stable and is a secondary antioxidant. Therefore, the color of the solution will turn yellow and the absorbance of ascorbic acid will be low as a result of the increasing number of DPPH-H bonds formed. The lower the absorption value, the greater the percentage of antioxidants (Mabruroh, 2015). So that the extract of *Zingiber zerumbet* (L.) Roscoeex Sm. showed a very strong effect in inhibiting DPPH free radicals at a significance level of $p<0.05$ with an IC50 value of 11,400.23 mg/L and ascorbic acid 4,7840.99 mg/L (Table 1, Figure 2).

FIC test method

Fe^{2+} has been known to accelerate the formation of hydroxyl radicals through the Fenton reaction, which leads to the occurrence of many diseases (Lim, Lim, & Tee., 2007; Juntachote and Berghofer, 2005). It was reported that chelating agents which form bonds with metal ions are effective secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of metal ions (Kumar, Ganesan, & Rao., 2008). In the FIC test, ferrozine acts as a chelating agent and forms a purple complex ion with Fe^{2+} giving the maximum absorbance at a wavelength of 562 nm. From the measurement results obtained a correlation between the concentration of the ethanol extract of Lempuyang Gajah (*Zingiber zerumbet* (L.) Roscoeex Sm.) and the value of % chelating ability. Figure 3 shows the higher the concentration of the Lempuyang Gajah ethanol extract, the higher the metal chelating activity shown.

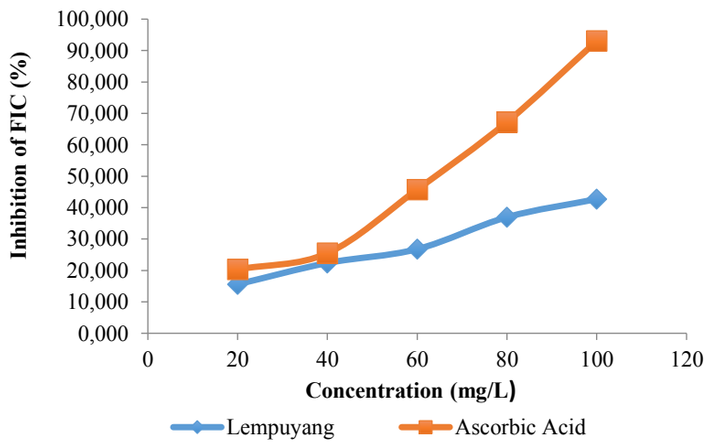

Figure 3. Percentage of FIC radical inhibition

Based on the IC_{50} value, the extract's chelating power to Fe^{2+} ions (Table 1, Figure 4) was below 50%. This value is made possible by the weak coordination bonds of polyphenol compounds to ferrous metals as a result of the resonance of the aromatic groups. In addition, it is also possible due to the electronegativity of the oxygen atom of the phenol group which holds the PEB (lone electron pair) strong enough from the oxygen atom. In general, this is in accordance with that reported by Andjelkovic et al. (2006). It was reported that polyphenolic compounds from natural ingredients have relatively weak chelating power to Fe^{2+} compared to EDTA (Berker et al. 2010). So that the antioxidant activity test with the FIC method is considered relatively less sensitive than other test methods. Lempuyang Gajah ethanol extract at a significance level of $p<0.05$ had an average IC_{50} value of 121.462.93 mg/L which was included in the weak category and ascorbic acid was 47.330.11 mg/L

which was included in the strong category (Table 1).

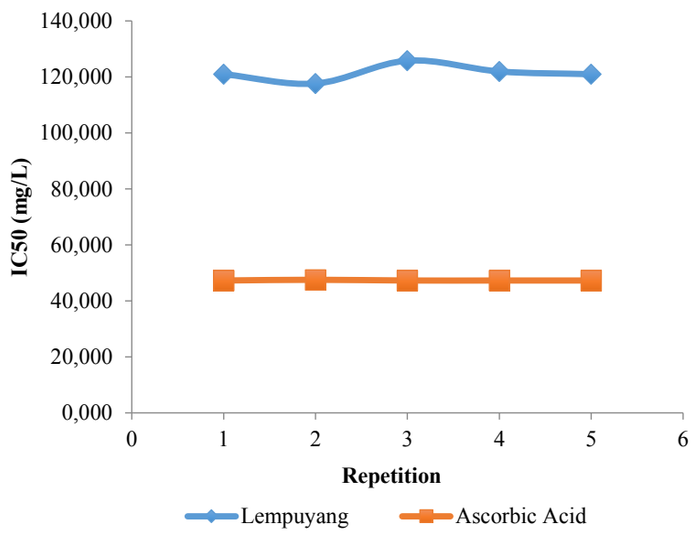

Figure 4. Free radical antioxidant activity of FIC

FRAP test method

Antioxidant activity test using the FRAP method is a fast and simple method. This method is based on electron donors from antioxidant compounds so that the yellow Fe^{3+} (Potassium hexacyanoferat) complex compound in an acidic environment undergoes a reduction reaction to a bluish-green Fe^{2+} complex compound. An indicator of the potential for a compound to be an antioxidant is based on the reducing power of the compound as measured by its ability to convert Fe^{3+} into Fe^{2+} (Kim, et al., 2011). This is possible because antioxidant compounds can donate electrons or hydrogen atoms to stabilize radical compounds. The model of the antioxidant activity test method using the FRAP method was carried out by measuring the absorbance of the Fe^{2+} complex formed using a UV-Vis spectrophotometer at 700 nm (Panda, 2012). Based on the comparison of the data from the FRAP test to the *Zingiber zerumbet* (L.) Roscoeex Sm. and ascorbic acid in Figure 5 shows that ascorbic acid generally has a slightly stronger reducing power to Fe^{3+} than *Zingiber zerumbet* (L.) Roscoeex Sm extract. The concentration ranges of the standards which are relatively different from each other as in the DPPH test method are adjusted to the reducing power of each standard to Fe^{3+}. The results of measurements of absorbance and antioxidant activity values of Lempuyang Gajah (*Zingiber zerumbet* (L.) Roscoeex Sm.) ethanol extract (Table 1) so that the average value of the Lempuyang Gajah (*Zingiber zerumbet* (L.) Roscoeex Sm.) ethanol extract sample was obtained. 19,380.14 mg/L and ascorbic acid 15,050.09 mg/L (Figure 6) at a significance level of $p<0.05$.

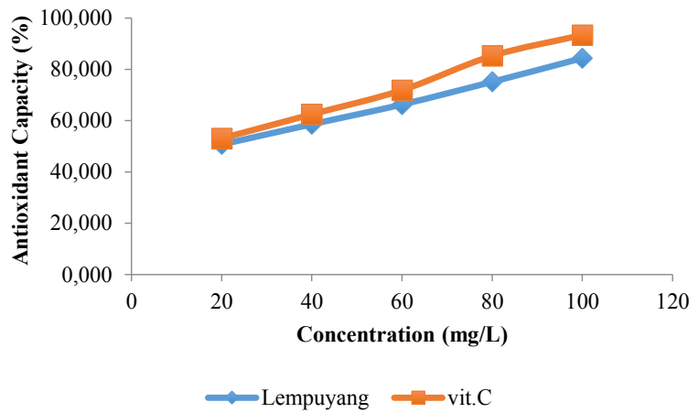

Figure 5. Percentage of FRAP radical antioxidant capacity

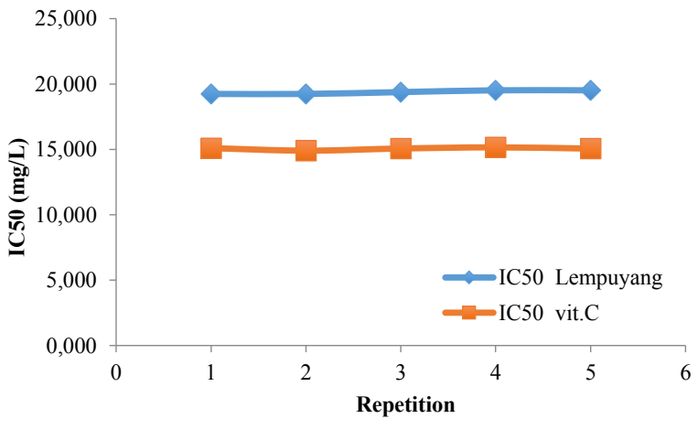

Figure 6. Antioxidant activity of FRAP radicals

ABTS test method

The antioxidant activity test model of the ABTS free radical method is a rapid test model based on specific absorption in visible light which is characterized by the loss of blue color due to ABTS being reduced by antioxidant compounds. The reduced intensity of the blue color in this test method is associated with the ability of antioxidant compounds to donate hydrogen atoms which in this ABTS test method the intensity of the blue color is measured at 752 nm (Karadag, 2009). Ethanol extract *Zingiber zerumbet* (L.) Roscoeex Sm. has antioxidant activity with an IC_{50} value of 89.320.15 mg/L (Table 1) while the IC_{50} value of ascorbic acid as a comparison is 52.460.59 mg/L at a significance level of $p<0.05$. The smaller the IC_{50} value, the stronger the antioxidant power. This shows that the ethanol extract of *Zingiber zerumbet* (L.) Roscoeex Sm. has moderate antioxidant activity with antioxidant intensity in the range of IC_{50} 50-100 mg/L.

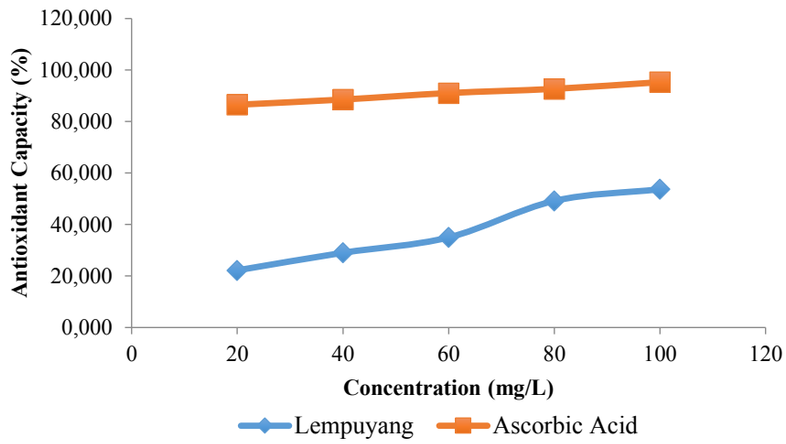

Figure 7. Percentage of ABTS radical antioxidant capacity

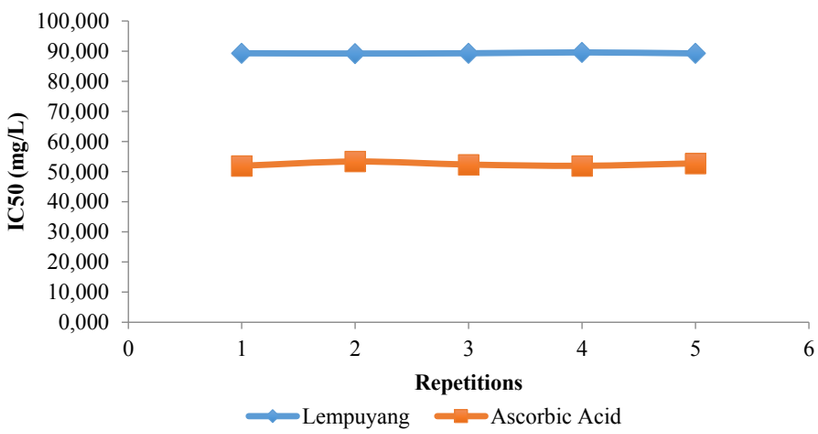

Figure 8. Antioxidant activity of ABTS radicals

The four antioxidant activity test methods were tested for their correlation to the obtained antioxidant activity. The correlation results show that there is a significant correlation in all test methods (R>0.317), especially between the FRAP and DPPH test methods. The value of this correlation coefficient indicates that the four test methods are related to the ability of reduction and oxidation reactions and free radical stabilization by antioxidant compounds through phenol aromatic resonance. In addition, chelating power can also prevent radicals that are formed, although they do not play a dominant role.

Table 1. Extraction yield, total phenolic, total flavonoid, and antioxidant activities of *Zingiber zerumbet* (L.) Roscoeex Sm.

Extraction yield (%)	Total Phenolic (mgGAE/g ekstrak) [mean±SD]*	Total Flavonoid (mgQUE/g ekstrak) [mean±SD]*	IC_{50} (mg/L) radical activity of DPPH [mean±SD]*	IC_{50} (mg/L) radical activity of FIC [mean±SD]*	IC_{50} (mg/L) radical activity of FRAP [mean±SD]*	IC_{50} (mg/L) radical activity of ABTS [mean±SD]*
9.44	23.58±0.25	12.21±0.03	11.40±0.23	121.46±2.93	19.38±0.14	89.32±0.15

*mean of five repetitions; SD = standard deviation

Conclusions

The ethanolic extract of *Zingiber zerumbet* (L.) Roscoeex Sm. rhizome contained total phenolic and flavonoid content of 23,580.25 mgGAE/g and 12,210.03 QUE/g extract, respectively. Antioxidant activity with IC_{50} value in the DPPH free radical reduction method of 11.400.23 which is included in the very strong category, FIC of 121.462.93 which is included in the medium category, FRAP of 19.38 0.14 which is included in the very strong category, and ABTS of 89.320.15 which is in the strong category. Phenolics and flavonoids are thought to have an important role in the antioxidant activity of elephant lempuyang rhizome. Based on the IC_{50} value, the most effective antioxidant activity test method is the DPPH method, while the least effective method is the FIC method because its chelating power is less than 50%. The four test methods have sufficient correlation (R> 0.317).

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