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Antioxidant Activities of Ginger (*Zingiber officinale*) and Telang Flower (*Clitoria ternatea L.*) Combination Tea

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Abstract

Tea is widely consumed for health purposes to prevent and treat various diseases. This paper reports the antioxidant activities of a herbal tea namely jahe (ginger, *Zingiber officinale*) telang (*Clitoria ternatea L.*), and the combination namely JaTe (jahe and telang). Each of jahe, telang, and JaTe teas were made of 2g ginger, 5 buds dried telang flower, and the combination respectively. The teas were boiled in 200 mL for 5 minutes. The dried ginger, telang, and JaTe formulation were evaluated for antioxidant activities. Total phenolic content was evaluated by Folin-Ciocalteu using the gallic acid equivalent (GAE), while total flavonoid content was evaluated by aluminum chloride colorimetric assay using the quercetin equivalent (QE). The antioxidant activity was measured using 2,2-diphenyl-1picrylhydrazyl (DPPH), and 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-sulfonic acid (ABTS), hydrogen peroxide (H_2O_2) scavenging activities, and ferric reducing antioxidant power (FRAP) assay. The highest phenolic and flavonoid contents were found in ginger tea with 23.62 µg GAE/100% concentration and 7.22 µg QE/100% concentration, followed by telang tea with 16.20 µg GAE/100% concentration and 4.88 µg QE/100% concentration. Ginger tea has the strongest DPPH scavenging activity with IC_{50}=7.76% followed by telang tea 17.07%. JaTe tea has the strongest ABTS scavenging activity with IC_{50}=1.01% followed by ginger tea with IC_{50}=1.26%. Ginger tea has the strongest H_2O_2 scavenging activity with IC_{50}=13.23% and followed by JaTe tea with IC_{50}=14.66%. JaTe tea has the strongest FRAP activity with IC_{50}=3.59%, followed by ginger tea with IC_{50}=4.94%. Overall, JaTe herbal tea formulation has a strong antioxidant activity.

Keywords: Antioxidant, *clitoria ternatea*, herbal tea, *zingiber officinale*

Introduction

Tea is known as one of the favorite beverages within the international, together with coffee and cocoa. Tea has been used as a health product or medicinal drug to prevent and deal with various diseases. Previous studies have proven the benefits of tea, including antioxidant, bacteriostatic, anticancer, and regulation of lipid metabolism.[1] In Indonesia, the teas that are often consumed by the public are green tea, black tea, oolong tea, and white tea. Most of the beneficial effects of tea are mainly to its polyphenolic compounds and their antioxidant activity. The phenolic content in tea is strictly related to the processing steps during the production of different tea. The difference between teas in general and herbal teas is that herbal teas are made from a combination of dry ingredients of leaves, seeds, grass, nuts, bark, fruits, and flowers and that give them the taste of other herbal plants that have pharmacological activity. The chemical composition of tea has been thoroughly studied. The main compound from tea leaves has a high polyphenol content in dry weight.[2,3]

Ginger (*Zingiber officinale*) is a known and widely used species that is rich in numerous chemical compounds, such as phenolic, terpenes, polysaccharides, lipids, and natural acids. The main compound of ginger that has a health benefit is its phenolic compounds, which include

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gingerols and shogaols.[4] A study discovered that dried ginger exhibited the strongest antioxidant activity, higher than that of fresh, stir-fried, and carbonized ginger, respectively. Several research have indicated that ginger was effective for protection against oxidative stress which was the model that is often use to know the antioxidant action.[4]

Clitoria ternatea L. or commonly known as telang originates from the Fabaceae family. The telang flower also possesses properties that are beneficial such as antioxidant, antidiabetic, antimicrobial, anthelminthic, hepaprotective, and antiasthmatic. The major constituents found in *C. ternatea* are phenolic compounds, flavonoids, anthocyanins, alkaloids, ternatins, saponins, tannins, taraxerol, and taraxerone.[5,6]

Both ginger and telang flower petals have great potential for their antioxidant activity which is beneficial for health, but the combination of telang flower and ginger to make tea is not fully known. Thus, in this study we investigated the antioxidant activity from the combination of jahe (ginger) and telang flower tea (JaTe) as potential sources of antioxidants by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-Bis-3-Ethylbenzothiazoline-6-sulfonic acid (ABTS), hydrogen peroxide (H_2O_2) scavenging activities, ferric reducing antioxidant power (FRAP) assay methods compared to ginger tea and telang tea.

Methods

The fresh specimen of *Z. officinale.* rhizome was collected from Pasar Sederhana Bandung, West Java, Indonesia. While the specimen of *C. ternatea* flower was collected from Kampung Herbal Desa Sukolilo, Prigen, Pasuruan, East Java, Indonesia.

A fresh ginger specimen was washed using clean water and then cut lengthwise into small pieces. Peel removed and washed with clean water. Ginger was dried for 72 hours at 50°C, while the telang flower was dried for 72 hours at 50°C on a food dehydrator (Well Known). After all the specimens were fully dried, JaTe tea combination of 2 g dried ginger combined with 5 buds of dried telang flower, 2 g dried ginger, and 5 buds of dried telang flower were infused using 200 mL boiling water and then left for 5 minutes until its ready to be used for assays.[7]

The total phenol content was determined using the Folin-Ciocalteu reagent. In the first step, 15 µL of the sample was inserted into a 96-well microplate. Then, 75 µL of Folin-Ciocalteu reagent (10%) and 60 µL of sodium carbonate (7.5%) were added. The plate was shaken and incubated for 10 minutes at 50˚C. The absorbance value was measured at a wavelength of 760 nm using a microplate reader. Gallic acid equivalence (GAE) in µg/100% concentration sample was used to calculate total phenolic content. This test was repeated three times and the data was performed in mean ± standard deviation.[7]

The total content of flavonoids was measured by aluminum chloride colorimetric assay.[7,8] Briefly, each sample and 250 µL of standard were mixed with 75 µL of 5% $NaNO_2$. After 6 minutes, 150 L of 10% $AlCl_3.H_2O$ solution was added. After 5 minutes, 0.5 mL of 1M NaOH solution was added to the mixture. The final mixture was measured using a microplate reader absorbance at a wavelength of 510 nm. The total flavonoid value was calculated according to the standard Quercetin linear equation. Standard linear equation: y=ax+b. The flavonoid content was expressed in terms of quercetin equivalence (QE) in µg/100 % concentration of sample. This test was repeated three times and the data was performed in mean ± standard deviations.[7,8]

The 2,2-diphenyl-1-picrylhydrazil (DPPH) assay was performed using DPPH (Sigma Aldrich D9132), DMSO (Merck 1029522500), microplate reader spectrophotometer (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The sample concentrations used were 20, 15, 10, 5, and 2.5%. A total of 50 µL of the sample was put into a 96-well plate, then 200 µL of DPPH 0.077 mmol was added to the well containing the sample (well sample). For the well blank, add 250 µL of sample solvent (DMSO). For the well control, add 250 µL of 0.077 mmol DPPH. Then incubated at the room temperature for 30 minutes in the dark. The absorbance value was measured using a microplate reader at a wavelength of 517 nm. Calculation of DPPH scavenging activities is carried out with the following formula:

DPPH scavenging activity (%) = $\frac{A-B}{A} \; x \; 100$

Where A is control solutions absorbance; B is sample absorbance.

The ABTS reduction test was measured using the free radical assay of diammonium salt ABTS+ 2, 2-Azinobis-(3 ethylbenzothiazoline-6-sulfonate).[7-9] The sample concentrations used were 1, 0.5, 0.25, 0.125, and 0.0625%. A total of 2 µL of the test sample was added to the 96-well plate followed by 198 µL of ABTS reagent, 200 µL of ddH_2O was added to the control well and 200 µL of ABTS reagent was added to the

empty well as a negative control, while for the blank well 200µL absolute ddH_2O was used. The microplate was incubated for 10 min at 37°C. The absorbance was calculated with a wavelength of 745 nm using a microplate reader. Calculation of ABTS reduction percentage was carried out by the following formula:

$$\text{ABTS reduction activity (\%)} = \frac{A-B}{A} \; x \; 100$$

Where A is control absorbance; B is sample absorbance.

Hydrogen peroxide (H_2O_2) scavenging assay was performed by using ferrous ammonium sulphate (1 mM, Sigma 7783859), H_2O_2 5 mM (Merck 1.08597.1000), 1,10-phenanthroline 1 mM (Merck 200-629-2). The H_2O_2 test was measured based on the method described by Mukhopadhyay et al.,[13] with slight modifications. Each sample contained 60 µL of sample, 12 µL of ferrous ammonium sulfate (1mM, Sigma Aldrich 7783859), and 3 µL of H_2O_2 (5mM, Merck 1,08597.1000). The sample concentrations used were 40, 30, 20, 10, and 5%. For the negative control, 12 µL ferrous ammonium sulfate and 63 µL ddH_2O were used, while for the blank only 150 µL ddH_2O was used. They were incubated for 10 minutes in a dark room at room temperature. At 510 nm, the absorbance value was measured.[7–9] Calculation of the ability of compounds that have hydrogen peroxide binding activity is calculated using the following formula:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{A-B}{A} \; x \; 100$$

Where A is control absorbance; B is sample absorbance.

A total of 10 mL of 300 mM acetate buffer, 1 mL of ferric chloride hexahydrate, 20 mM dissolved in distilled water, and 1 mL of 2,4,6-Tris-(2-pyridyl-5-Triazine) (TPTZ) 10 mM (Sigma Aldrich T1253) was dissolved with 40 mM HCl then mixed to make FRAP reagent.[7–10] A total of 7.5 µL of the sample was put into a 96-well plate. The sample concentrations used were 5, 3.75, 2.5, 1.25, and 0.63%. Add 142.5 µL of FRAP reagent to the sample well, 200 µL of sample solvent (DMSO) to the well blank, then incubate the plate for 30 minutes in an oven at 37°C. Absorbance was measured using a microplate reader at =760 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific). The standard curve is created using $FeSO_4$, between 0.49 and 62.50 M $FeSO_4$.

The data obtained from the above assays were processed statistically by analysis of variance (One Way ANOVA) and post hoc Tukey's HSD test in the SPSS program (version 20.0). These analyses were aimed to calculate noticeable dissimilarities among the concentrations of the samples. The IC_{50} value of each assay (DPPH, H_2O_2, ABTS and FRAP scavenging) was calculated based on the curve standard.

Results

The total polyphenols and flavonoids were measured using the calibration curve for gallic acid and quercetin. The experiment shows the presence of high phenolic content in the samples and there is a difference in total phenol and flavonoid in the sample. Based on the results of the study, it was found that the total flavonoids in the pure tea ginger have the highest total phenolic and flavonoid, followed by telang sample and JaTe sample as shown in Table 1. The result was compared with phenolic content and flavonoid of telang tea from the previous study respectively were 16.20 µg GAE/100% concentration, 4.88 µg QE /100% concentration.[7]

The highest DPPH scavenging activity was in the pure ginger tea sample which was 80.90%. The data shows the percentage of

Table 1 Total Phenolic and Flavonoid Content of Sample

Sample	Total Phenolic (µg GAE/100% Concentration of Tea Infusion)	Total flavonoid (µg QE/100% Concentration of Tea Infusion)
JaTe tea	10.08±0.50[a]	3.66±0.02[a]
Ginger tea	23.62±1.44[c]	7.22±0.02[c]
Telang tea	16.20±0.63[b]	4.88±0.40[b]

*The data was presented as mean±standard deviation. GAE: gallic acid equivalence; QE: quercetin equivalence. Significant difference among treatment shown as different sign (a, b, c) based on ANOVA and Tukey HSD post hoc. The data of total phenolic and total flavonoid were obtained from a previous study[7]

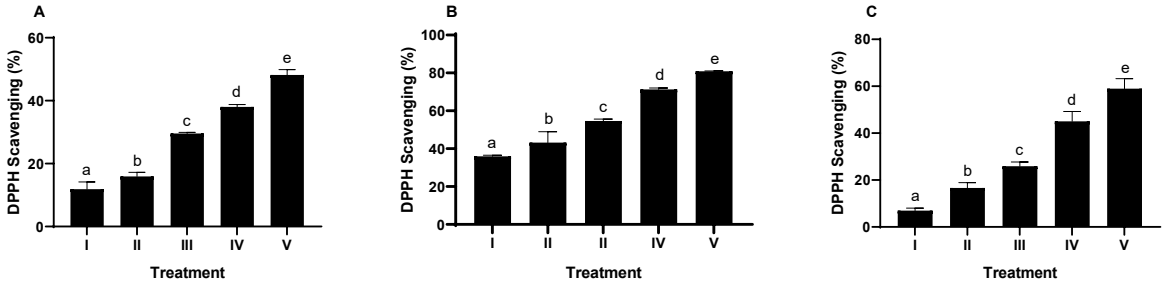

Figure 1 Effects of Various Concentrations of Sample on DPPH Scavenging Activity

JaTe tea (A), Ginger tea (B), Telang tea (C). Sample concentration I: 2.5%, II: 5%, III: 10%, IV: 15%, and V: 20%. *All data are presented in mean±standard deviation. The assay was done triplicate for each treatment. Significant difference among treatment shown as different sign (a, b, c, d, and e) based on ANOVA and Tukey HSD post hoc test ($p<0.05$). The data of telang DPPH scavenging activity were obtained from a previous study[7]

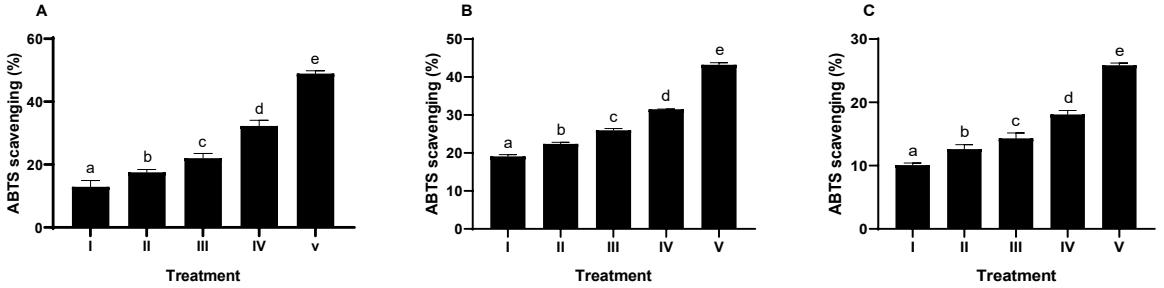

Figure 2 Effects of Various Concentrations of Sample on ABTS Scavenging Activity

JaTe tea (A), Ginger tea (B), Telang tea (C). Sample concentration I: 0.0625%, II: 0.125%, III: 0.25%, IV: 0.5%, and V: 1%. *All data are presented in mean±standard deviation. The assay was done triplicate for each treatment. Significant difference among treatment shown as different sign (a, b, c, d, e) based on ANOVA and Tukey HSD post hoc test ($p<0.05$). The data of telang ABTS scavenging activity were obtained from a previous study[7]

DPPH scavenging activity in the concentration-dependent manner, which means that the higher the concentration has higher the scavenging activity (%) as shown in Figure 1.

The highest ABTS scavenging activity was JaTe tea which was 48.94%. The data shows the percentage of ABTS scavenging activity in a concentration-dependent manner, which

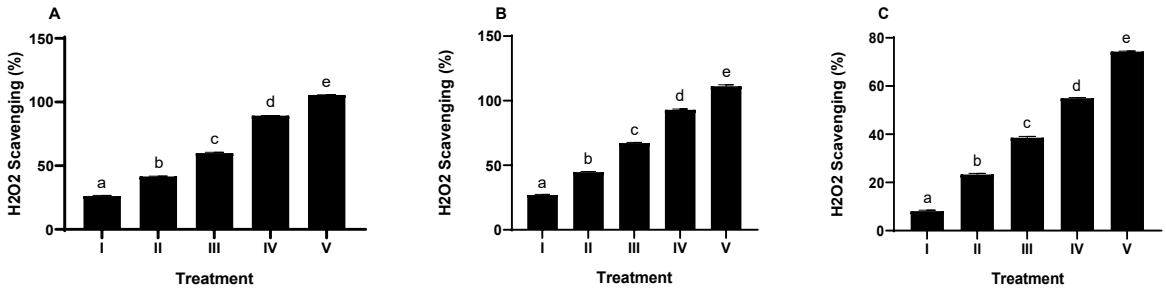

Figure 3 Effects of Different Sample Concentrations on H_2O_2 Scavenging Activity

JaTe tea (A), Ginger tea (B), Telang tea (C)**.** Sample concentration I: 5%, II: 10%, III: 20%, IV: 30%, and V: 40%. *All data are presented in mean±standard deviation. The assay was done triplicate for each treatment. Significant difference among treatment shown as different sign (a, b, c, d, e) based on ANOVA and Tukey HSD post hoc test ($p<0.05$). The data of telang H_2O_2 scavenging activity were obtained from a previous study[7]

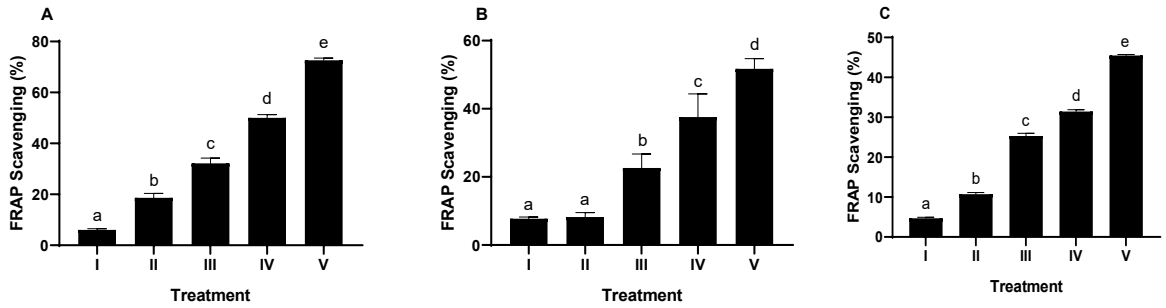

Figure 4 Effects of Different Sample Concentrations on FRAP Activity

JaTe tea (A), Ginger tea (B), Telang tea (C)**.** Sample concentration I: 0.63%, II: 1.25%, III: 2.5%, IV: 3.75%, and V: 5%. *All data are presented in mean±standard deviation. The assay was done triplicate for each treatment. Significant difference among treatment shown as different sign (a, b, c, d, e) based on ANOVA and Tukey HSD post hoc test ($P<0.05$). The data of telang H_2O_2 scavenging activity were obtained from a previous study[7]

means that the higher concentration has higher scavenging activity (%) as shown in Figure 2.

The highest H_2O_2 scavenging activity was ginger tea sample which was 111.18%. The data shows the percentage of H_2O_2 scavenging activity is concentration dependent manner, it means that the higher the concentration have higher scavenging activity (%) as shown in Figure 3.

The highest FRAP scavenging activity was JaTe tea which was 72.60%. The data shows the percentage of scavenging n is concentration dependent, it means that the higher the concentration have higher scavenging activity (%) as shown in Figure 4.

Discussion

The total polyphenols were measured using the calibration curve for gallic acid. The experiment shows the presence of high phenolics content in the pure tea and combination tea, and there is a difference in total phenol and flavonoid in the sample. Based on the results of the study, it was found that the total flavonoids in the JaTe (Jahe Telang), Ginger, and Telang formulations were of 3.66±0.02 µg QE/100%; 7.22±0.02 µg QE/100%; and 4.88±0.40 µg QE/100% respectively. The total flavonoid content of the sample was measured by the aluminum chloride method. Its quantification revealed the presence of a high quantity of phenol in a sample, which total phenol samples of JaTe, ginger, and telang tea were 10.08±0.50 µg GAE/100%; 23.62±1.44 µg GAE/100%; and 4.88±0.40 µg GAE/100% concentration respectively. The highest total of flavonoid content is found in ginger tea, followed by telang tea and the JaTe tea. The high content of total phenolic and flavonoid in the sample is due to ginger having main compounds such as gingerol and shogaols which are one type of phenolic compound.[11]

The DPPH assay is a test that measures the activity of antioxidant compounds in reducing the free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is characterized as a stable free

Table 2 The IC_{50} Values the Antioxidant Activities of Sample

Sample	IC_{50} (%)		
	JaTe Tea	Ginger Tea	Telang Tea
DPPH scavenging activity	20.62±1.67[c]	7.76±2.99[a]	17.07±6.66[b]
ABTS scavenging activity	1.01±4.77[a]	1.26±2.08[b]	2.51±1.00[c]
H_2O_2 scavenging activity	14.66±0.22[b]	13.23±0.19[a]	26.62±0.27[c]
FRAP activity	3.59±0.77[a]	4.94±5.29[a]	5.56±1.29[b]

*The data are presented in mean ± standard deviation. The assay was conducted in triplicate. Significant difference among treatment in DPPH, ABTS, H_2O_2 scavenging activity shown as different sign (a, b, c) based on ANOVA and Tukey HSD post hoc. Significant difference among treatment in FRAP assay (a. b) based on ANOVA and Dunnet T3 post hoc. The data of telang antioxidant activities were obtained from a previous study[7]

radical based on the delocalization of the spare electron above the molecule as a whole, so that the molecule does not dimerize, like most other free radicals. Delocalization also gives rise to a deep purple color, with absorption in ethanol solution at about 520 nm. When an antioxidant compound or substance is reacted with DPPH, the free radicals will be reduced, which is characterized by the fading of the purple color to yellow. In the presence of a hydrogen donor, it becomes paired and reduced the absorption at 517 nm.[12] In this study, from the three samples, there was a significant difference ($p<0.05$) in the average DPPH scavenging activity as shown in Figure 1. The smallest IC_{50} value (the highest DPPH trapping activity) was found in the ginger tea, followed by the telang tea and JaTe tea (Table 2). A small IC_{50} value indicates a strong antioxidant activity. Based on the IC_{50} value, a compound can be said to be a very strong antioxidant if it has an IC_{50} value of <50 µg/mL, categorized as strong with an IC_{50} value of 50–100 µg/mL, moderate with an IC_{50} value of 100–150 µg/mL, and weak with an IC_{50} value of 151–200 g/mL. Among the three samples that had the strongest antioxidant activity, only the ginger tea had an IC50 value of 7.76%. However, the IC_{50} value of DPPH trapping activity for all samples had a strong antioxidant capacity, which was <50 µg/mL.

The ABTS reduction test was measured using the free radical assay of diammonium salt ABTS+ 2, 2-Azinobis-(3 ethylbenzothiazoline-6-sulfonate). In this study, ABTS was produced by the reaction between a powerful oxidizer and ABTS salt. The ABTS radical blue-green-colored solution was reduced by hydrogen-donating antioxidant[12] and analyzed at the spectrum of a long-wave absorption. The results showed that the concentration of the sample was directly proportional to its trapping activity, which means that the greater the concentration of the sample, the higher the rate of the ABTS salt reduction (Figure 2). At a concentration of 100% of JaTe tea, the trapping results were obtained with the highest value of 48.94% compared to ginger tea, which was 43.23%, and the telang flower tea, which was 25.86%. The smallest IC_{50} value was found in the JaTe tea 1.01%, the ginger tea and telang which were 1.26% and 25.1%, respectively (Table 2). It shows that a combination of ginger (jahe) and telang called "JaTe" has the strongest antioxidant activity in ABTS reduction compared to the ginger and telang samples alone.

Amongst the ROS, H_2O_2 is a crucial molecule as although it's not toxic by itself, but is often converted to other even more toxic radicals like OH by Fenton reaction or acid by the enzyme myeloperoxidase.[13] The Median Inhibitory Concentrations (IC_{50}) of JaTe, ginger, and telang in H_2O_2radical scavenging activity was shown in Table 2 and Figure 3. The results showed that the IC_{50} values for JaTe, ginger, and telang tea were 14.66%; 13.36%; 26.62% respectively. Strong antioxidant activity was found in samples of ginger, JaTe, and telang respectively based on the IC50 value.

The higher of sample concentration, the higher inhibition level of the compound in the FRAP test. In the FRAP test, the ability of antioxidant compounds to convert Fe3+ into Fe2+, electrons from antioxidant compounds can stabilize these free radicals.[14] The FRAP activities of JaTe, ginger, and telang can be seen in Figure 4. All three samples showed strong antioxidant activity, indicated by the IC_{50} value <50 µg/mL. The best value was obtained from the JaTe sample IC_{50}=3.59%, followed by the ginger tea IC_{50}=4.94% and the telang tea IC_{50}=5.56% as shown in the table. There is a different result between the FRAP assay and the DPPH assay, wherein the DPPH test the highest activity is shown by the ginger sample, while in the FRAP assay in the JaTe sample, this is because there are differences in the principle of the reaction that occurs between DPPH and FRAP. This is in line with research conducted by Safitri et al.[15] which had different results of DPPH and FRAP assay from the sample.

In a conclusion, herbal JaTe tea has a strong active antioxidant activity on FRAP assay, ABTS, H_2O_2, and DPPH scavenging activity with IC_{50} values of 3.59%, 1.01%, 14.66%, and 20.62%, respectively. The antioxidant activity of JaTe was higher than ginger and telang tea by FRAP assay and ABTS scavenging activity, however, ginger tea has higher antioxidant activity than JaTe and telang tea in DPPH and H_2O_2 scavenging activity.

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