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Antioxidant activity of panJe tea combination of pandan (*Pandanus amaryllifolius*) and jahe (*Zingiber officinale*)

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ABSTRACT

Herbal tea is popular because of its potential as medicine, a combination of herbal ingredients promises to produce new colors, flavors, and tastes. This study aimed to determine the antioxidant activities of an herbal tea combinations (pandan and jahe) called PanJe and compared it to the antioxidant single herbal tea namely Pandan tea (0.5 g dried pandan) and Ginger tea (2 g dried ginger). The PanJe tea was a combination of 0.5 g pandan tea and 2 g ginger tea. Pandan tea, ginger tea and PanJe tea are soaked for 5 minutes in 200 mL of boiling water. The antioxidant activities were assayed namely 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenger, and Ferric Reducing Antioxidant Power (FRAP) assay. The flavonoid content used Quercetin Equivalent (QE), while the total phenol content used Gallic Acid Equivalent (GAE). DPPH, H_2O_2, ABTS scavenging activities and FRAP assay of PanJe tea was presented in median inhibitory concentration (IC_{50}) value respectively 14.69, 27.70, 4.11 and 7.09%, while pandan tea resulted in IC_{50} value of 23.63, 27.19, 4.54 and 12.86%, and ginger tea resulted in IC_{50} value of 7.76, 13.23, 1.26 and 4.94%, respectively. These assays proved ginger tea had the highest antioxidant activities compared to PanJe tea and pandan tea which had the lowest antioxidant activities. Total phenolic and flavonoid of PanJe respectively were 0.035 µg GAE/mg and 0.006 µg QE/mg of PanJe tea meanwhile pandan tea respectively contain 0.002 µg GAE/mg, 0.0003 µg QE/mg of pandan tea and ginger tea contained 0.118 µg GAE/mg and 0.036 QE/mg of ginger tea. Compared to PanJe tea and pandan tea, ginger tea has the highest total phenol and flavonoid content. Conclusion ginger tea is higher antioxidant activities also a higher phenol and flavonoid content than PanJe tea.

Keywords: antioxidant, herbal tea, *Pandanus amaryllifolius*, total flavonoid, total phenol, *Zingiber officinale*

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INTRODUCTION

Damage to cell structures such as lipids, membranes, proteins, DNA, and others can occur due to increased oxidative stress caused by free radicals that accumulate in excess (Prahastuti et al., 2020). Antioxidants are known to play a part in reducing oxidative stress in the human body. Antioxidants are naturally found in several plants such as fruits, vegetables or herbal plants which have the potential to neutralize free radical (Widowati et al., 2021). Antioxidants convert them into harmless molecules even with the scavenger properties of reactive free radicals, these substances are able to prevent other molecules from oxidizing, and have a health-promoting in preventing degenerative diseases (Prahastuti et al., 2020).

Commonly consumed herbal tea is made from the roots, fruit, leaves, stems, blossoms and seeds of the species *Camellia sinensi*s L., It has been applied for a long time for therapeutic purposes and the prevention of illness (Zhao et al., 2013). Eastern countries have used herbal medicine for centuries to cure illnesses and infections. Herbal treatments are frequently consumed as a tea, which is made by soaking dried plant parts in boiling water. Most herbal teas usually consist of one primary herb or a blend of herbs that promise to impart new color, taste, flavor, freshness, and disease prevention activities (Mancini et al., 2015). A viable substitute for synthetic antioxidants is the use of herbs and spices as antioxidants in beverages or processed foods. Spices can be made into tea which functions as herbal medicine. Natural antioxidants seem to be safer and healthier in this situation than synthetic antioxidants. Therefore, there is increasing interest in the research, purification, and natural sources characterization and safe natural antioxidants for food and pharmaceutical applications (Elmastas et al., 2018). The habit of consuming *Camellia sinensis* plant black and green tea dates back several thousand years. According to archeological research, tea and other wild plant leaf infusions may have been consumed for more than 500,000 years. Health food stores provide a wide variety of herbal teas. "Herbal teas" are rich in compounds and can play a significant part in providing chemicals and nutrients to supplement a poor diet. Flavonoids are $C_6C_3C_6$ compounds that have been used in recent years. It's of particular interest because of its potential protective effect on human health. It has antioxidants, anti-carcinogenic, antiviral, anti-inflammatory, hepatoprotective, and anti-allergic properties (Omar et al., 2011). This has increased attention to the phytochemical content and possible health benefits of pandan (*Pandanus amaryllifolius*) and ginger or jahe (*Zingiber officinale*). Pandan leaves and ginger rhizome herbal tea is used as antioxidant bioactive components. Pandan and ginger have been studied and found to have antioxidant activity (Ghasemzadeh & Jaafar, 2013; Laksmitawati et al., 2021; Widowati et al., 2022a).

Pandan which belongs to the Pandanaceae family, screw pine, is a herbaceous tropical plant with an aromatic scent. The sole Pandanus genus with fragrant leaves is owing to the chemical 2-acetyl-1-pyrroline (2AP), which gives off this odor (Bhuyan & Sonowal, 2021). In addition, on the leaves are also found other alkaloids (such as pandanamine, pandamerilactones) with a pyroline derivative structure (Bhuyan & Sonowal, 2021).

Ginger which belongs to the Zingiberaceae family has been consumed as herbal medicine and a spice for a long time. Many studies show that ginger has several biological activities, such as neuroprotective, respiratory protective, anti-inflammatory, antioxidant, antiobesity, anticancer, antimicrobial, antiemetic, cardiovascular protective, antidiabetic, and anti-nausea (Mao et al., 2019). There are numerous active components in ginger, including phenolics, flavonoids (Widowati et al., 2022a) and terpene compounds. Ginger's primary phenolic compounds are gingerol, shogaol, and paradol (Prasad & Tyagi, 2015).

The primary objectives of this study were to ascertain phenol and flavonoid contents, antioxidant activities including 2,2-diphenyl 1-pichylhydazy (DPPH), 2,2'-Azinobis-(3-ethylbenzothiazoline- 6-sulfonic acid (ABTS), H_2O_2 scavenging activity, Ferric Reducing Antioxidant Power (FRAP) activity of PanJe tea (combination of pandan & ginger tea), pandan tea, and ginger tea.

MATERIALS AND METHODS

Materials

Pandan leaves and ginger rhizomes were obtained from Sederhana traditional market, Bandung, West Java, Indonesia. DMSO (Merck; 1.090.010.500), DPPH (Sigma Aldrich; D9123), H_2O_2 (Merck; 7722-84-1), Folin-Ciocalteu reagent (Merck; 1.090.010.500), sodium carbonate (Merck A897992745), Gallic Acid (Sigma Aldrich; 398225), $AlCl_3$ (Merck; 449598), Quercetin (Sigma Aldrich; Q4951) 1,10-phenanthroline (Sigma Aldrich; 131377), ABTS•+ (Sigma Aldrich; A1888), ferrous ammonium sulfate (Sigma Aldrich; 7783859), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (Sigma Aldrich; T1253).

Methods

Preparation of dried sample and herbal tea formulation

Pandan leaves and ginger rhizomes were washed, and after that cut into small pieces and dry with food dehydrator (Well Known) to reduce water content material for 72 h at 50℃. Then, the dried pandan and dried ginger were formulated into PanJe (Pandan-Jahe) herbal tea. The PanJe formulation was made by steeping 0.5 g of "dried pandan" + 2 g of "dried ginger" for 5 mins as a 100% concentration of the sample. Pandan tea was made by steeping 0.5 g of dried pandan for 5 min in boiling water as a 100% concentration of the sample and ginger tea was made by steeping 2 g dried of ginger for 5 mins in boiling water as a 100% concentration of the sample (Widowati et al., 2022a; 2022b).

DPPH scavenging activity

This modified method of DPPH scavenging activity was used (Widowati et al., 2018; 2022a; 2022b). Briefly on the plates of 96 wells containing 50 µL of samples at varied concentrations added 200 µL 0.077 mmol DPPH. For the blanks, 250 µL of sample solvent (ddH_2O) was used and for control, 250 µL of 0.077 mmol DPPH was used. Absorbance measurements were carried out at a wavelength of 517 nm after incubated for 30 mins at room temperature and in a dark environment. DPPH scavenging activity is computed using the Formula (1):

$$\text{DPPH scavenging activity (\%)} = (A\text{-}B)/A \times 100 \quad (1)$$

A: control absorbance
B: sample absorbance

ABTS scavenging activity

ABTS-scavenging activity assay was carried out based on a slight modification method (Laksmitawati et al., 2021; Prahastuti et al., 2020; Widowati et al., 2017a; 2022a). Briefly, on plate 96 wells were added 2 µL from various concentration samples were followed by 198 µL of ABTS reagents released to the sample wells and 200 µL of the ABTS reagents into control wells, 200 µL ddH2O was loaded to the empty wells. The microplate was incubated for 10 mins at 37°C. 745 nm was used as the wavelength to measure the absorbance. Utilizing the following Formula (2), the percentage of the ABTS reduction is calculated:

$$\text{ABTS reduction activity (\%)} = (A\text{-}B)/A \times 100 \quad (2)$$

A: control absorbance
B: sample absorbance

H_2O_2 scavenging activity

H_2O_2 scavenging was evaluated using the procedure outlined by Laksmitawati et al., 2021; Prahastuti et al., 2020; Widowati et al., 2022a; 2022b. Therefore, 60 µL of various concentrations sample, 12 µL of ferrous ammonium sulfate 1 mM, and 3 µL of H_2O_2 (5 mM) into the sample wells. Briefly 150 µL ddH_2O was used for the blanks, whereas 12 µL of the ferrous ammonium sulphate 1

mM and 63 µL ddH_2O were used for the control. Additionally, the well-plate containing the samples, controls, and blanks was incubated for 5 minutes at room temperature in the dark. The incubation was repeated for 10 mins after adding 75 µL of 1,10-phenanthrolines. Absorbance measurements were carried out at a wavelength of 510 nm. The following equation can be used to get the H_2O_2 scavenging activity percentage:

$$H_2O_2 \text{ scavenging activity } (\%) = A)/B \times 100 \quad (3)$$

A: sample absorbance
B: control absorbance

FRAP activity

Preparation the FRAP reagent was conducted using the modified method (Laksmitawati et al., 2021; Prahastuti et al., 2020; Widowati et al., 2017;2018;2022a;2022b). Briefly 300 mM acetate buffer of 10 mL, 20 mM ferric chloride hexahydrate of 1 mL dissolved in distilled water, while 2,4,6-Tris-(2-pyridyl-5-Triazine) (TPTZ) 10 mM and 40 mM HCl of 1 mL were dissolved. In a 96-well microplate (TPP, 92096), 7.5 µL of multiple sample concentrations were inserted along with 142.5 µL of FRAP reagent, and the mixture was then mixed and allowed to sit for 30 min at 37 °C. The absorbance was calculated at 760 nm using a microplate reader. Utilizing the following formula (3), percentage of scavenging activities is calculated:

$$\text{FRAP activity } (\%) = (A)/B \times 100 \quad (4)$$

A: sample absorbance
B: control absorbance

Total phenolic compound

The total phenol concentration was measured by a colorimetric technique developed from previous investigations (Laksmitawati et al., 2021; Prahastuti et al., 2020; Widowati et al., 2017, 2022a;2022b). Using the Folin-Ciocalteu reagent, the amount of total phenol was determined. In the total phenol assay, a sample of 15 µL was included in the sample well and 60 µL of 7.5% Na_2CO_3 and 10% Folin-Ciocalteu reagent were added in the amount of 75 µL. Well samples were incubated for 10 minutes at 50°C. Absorbance measurements were carried out at a wavelength of 760 nm. The total phenolic value was calculated via the standard linear equation of Gallic Acid. GAE or Gallic Acid Equivalent in µg phenol/mg sample is used to measure phenol content.

Total flavonoid compound

A colorimetric assay is used to measure the total flavonoid content (Laksmitawati et al., 2021; Prahastuti et al., 2020; Widowati et al., 2017, 2018, 2022b). Briefly 75 µL quercetin standard solution in 6 concentrations level (500, 150, 125, 62.5, 31.25, 15.63 µg/mL) and herbal tea (PanJe, pandan, ginger tea) were introduced in the microplate and mixed with 75 µL of $AlCl_3$. The absorbance was determined at 415 nm. Linear regression equivalent (QE) in flavonoid in µg of flavonoid per mg sample used in measuring the total flavonoid content.

Data Analysis

SPSS was used to conduct the statistical analysis (version 20.0). Post hoc testing (Tukey's Honest Significant Difference test and Dunnet T3 and Analysis of Variance) and FRAP assay processing are employed (One Way ANOVA). The median inhibitory concentration 50 (IC_{50}) value for each experiment is determined using a linear regression standard curve.

RESULT AND DISCUSSION

Teas made from herbal infusions are popular all around the world. In order to extract bioactive chemicals from the leaves, an infusion or decoction procedure is commonly used. The most prevalent type of phytochemical in herbal teas, phenols may have anti-bacterial, anti-carcinogenic, and antioxidant properties, and prevalent remedies in traditional medicine. For example, freshly cut ginger rhizomes are used to make ginger tea a (Almajano et al., 2008; Tipduangta et al., 2019; Widowati et al., 2022a; 2022b). The DPPH, H_2O_2, ABTS scavenging activity, and FRAP assays were used in this work to evaluate the antioxidant properties of PanJe, pandan, and ginger teas. This study examined the antioxidant capacities of PanJe, pandan, and ginger teas using the ABTS, DPPH, FRAP, and H_2O_2 assay.

DPPH scavenging activity

The effectiveness of herbal teas for scavenging DPPH was evaluated using a DPPH assay. The most common techniques include the production of free radical species and the use of substances known as antioxidants to prevent them. An unpaired electron created DPPH, a stable free radical. A change in hue from deep purple to yellowish or pale yellow indicates the presence of an active antioxidant in DPPH free radical scavenging (Prahastuti et al., 2020; Widowati et al., 2016; 2022a).

A common substrate for determining antioxidant activity is the radical DPPH. An antioxidant's 50 % DPPH free radical neutralization concentration, or IC_{50} value, indicates how active the antioxidant is. A lower IC_{50} value indicates a more active antioxidant. Table I and Figure 1 shows the results of the DPPH scavenging activity. According to the study's findings (Table 1), the IC_{50} values for PanJe, pandan and ginger tea were 14.69 %, 22.63 %, and 7.76 %/100% concentration of the sample, respectively. The smaller IC_{50} value of ginger indicates that ginger has the highest antioxidant activity compared to PanJe and pandan tea.

In this study the highest DPPH scavenging activity with the lowest IC_{50} value of DPPH scavenging activity in PanJe, pandan and ginger teas respectively were 7.76%, 14.69% and 23.63% (Table 1). Based on the another study, ginger extract has DPPH scavenging activity with IC_{50} value of 4.25 mg/mL and also ABTS-reducing activity (0.40 mg/mL) (Mošovská et al., 2015). This result also in line with (Makanjuola, 2017) study, ginger tea powder extract has higher total phenol content and its may cause has the DPPH, ABTS, and peroxide scavenging activity (Makanjuola, 2017). Pandan tea has a lower DPPH scavenging activity than ginger or PanJe tea. This result was consistent with other research, that ethanolic ginger extract had high antioxidant activity on DPPH scavenging activity with IC_{50} 6.77 µg/mL more active compared to *kencur* (*Kaempferia galanga*) extract (Laksmitawati et al., 2021), and ginger tea extract with IC_{50} 6.77 µg/mL was categorized highly active antioxidant (Marjoni & Zulfisa, 2017; Widowati et al., 2021). Ethanolic, aqueous extract of pandan had IC_{50} values on DPPH scavenging activity respectively 129.32; 265.75 µg/mL (Quyen et al., 2020), the IC_{50} of pandan ethanolic extract, pandan aqueous extract respectively categorized moderate, weak antioxidant (Marjoni & Zulfisa, 2017). The data of DPPH scavenging activity (Table 1, Figure 1) was consistent with previous research that the combination of ginger tea and telang tea (JaTe) and ginger tea has the most active of antioxidants compared with telang tea and JaTe tea (Widowati et al., 2022a).

Table 1. DPPH scavenging activity of PanJe tea, Pandan tea and Ginger tea

Samples	Linear Equation	R^2	IC_{50} (%)
PanJe tea	y = 3.2405x + 2.3911	0.99	14.69 ± 0.78
Pandan tea	y = 2.2706x - 3.6471	0.99	23.63 ± 3.00
Ginger tea	y = 2.6189x + 29.684	0.99	7.76 ± 0.60

The data on ginger DPPH scavenging activity were obtained from previous studies (Widowati et al., 2022a).

The statistical analysis showed that the highest concentration of the sample (20%) was significantly highest than other concentrations ($p < 0.05$).

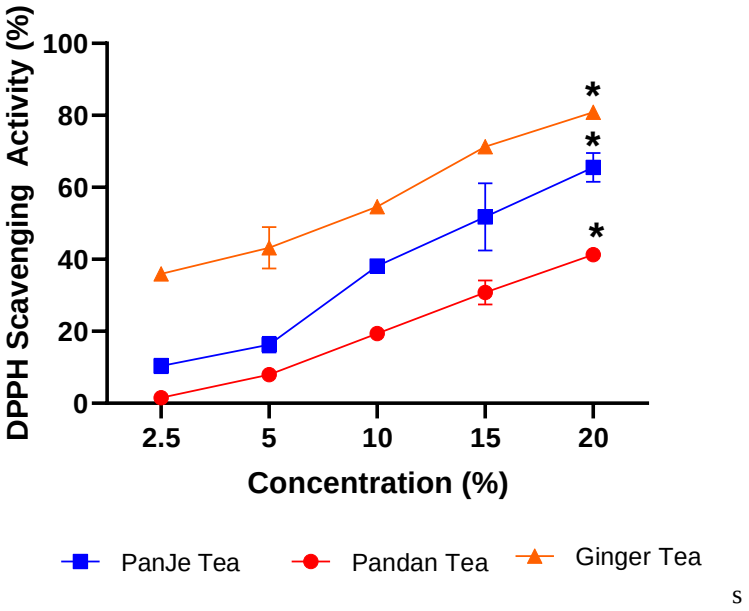

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Figure 1. Effect various concentratios of PanJe tea, Pandan tea, and Ginger tea toward DPPH scavenging activity

*To obtain the final concentrations, ddH_2O was used to dilute each sample. Each sample concentration in this experiment was done in triplicate. Data are presented in mean ± STD. The asterisk symbol (*) shows a significant difference among concentrations of samples toward DPPH scavenging activity based on the Tukey HSD post hoc test. ($p < 0.05$). The data on ginger DPPH scavenging activity were obtained from previous studies (Widowati et al., 2022a).

ABTS scavenging activity

An interaction between ABTS salt and strong oxidants generated ABTS. By using a hydrogenation antioxidant, the ABTS radicals in the turquoise solution were lowered (Widowati et al., 2016), and a long-wave absorption spectrum was examined. Ginger tea has a lower IC_{50} value than PanJe tea and pandan tea, which were 1.26 %, 4.11 %, and 4.54 %, respectively (Table 2).

Table 2. ABTS scavenging activity of PanJe tea, Pandan tea and Ginger tea

Samples	Linear Equation	R^2	IC_{50} (%)
PanJe tea	y = 8.2159x + 16.193	0.99	4.11 ± 0.63
Pandan tea	y = 9.29x + 7.7792	0.99	4.54 ± 0.31
Ginger tea	y = 24.72x + 18.864	0.99	1.26 ± 0.02

The data on ginger ABTS scavenging activity were obtained from previous studies (Widowati et al., 2022a).

Figure 2 shows that, among all the concentrations in each sample, the value of the percentage of ABTS reduction activity that had the highest activity ($p<0.05$) was the sample with a concentration of 1%. Based on these data, the highest concentration had the highest activity of PanJe tea at 24.3%, pandan tea at 17.05% and ginger tea at 43.23%. Based on another study, kombucha tea from ginger has antioxidant and antitumor activity by regulating several antioxidant parameters in breast cancer models (Salafzoon et al., 2018). The total flavonoid of tea and ginger has correlated with ABTS radical activity. The combination of tea and ginger has moderate antioxidant activity and showed synergistic effects (Alsahli et al., 2021). According to (Makanjuola, 2017), the results also showed that the extracts of tea-ginger mixes had synergistic effects and may have the ability to scavenge ABTS and DPPH radicals (Makanjuola et al., 2015). The IC_{50} of ethanolic ginger extract on ABTS reducing

activity was 67.33 µg/mL as an active antioxidant (Marjoni & Zulfisa, 2017), meanwhile the IC_{50} of ethanolic pandan extract on ABTS-reducing activity was 104.31 µg/mL as moderate antioxidant and aqueous pandan extract 204.99 µg/mL as weak antioxidant (Marjoni & Zulfisa, 2017; Quyen et al., 2020). The data of ABTS scavenging activity (Table 2, Figure 2) was consistent with previous research that a combination herbal tea of ginger tea and telang tea (JaTe) had the highest antioxidant compared to ginger tea, and telang tea (Widowati et al., 2022a).

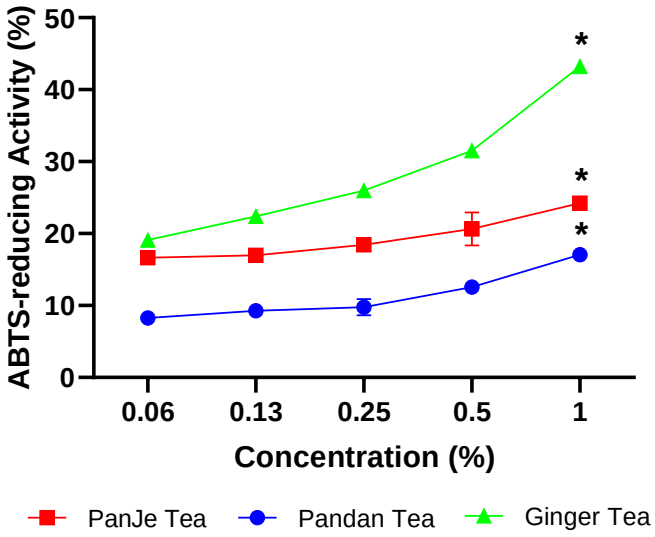

Figure 2. Effect various concentrations of PanJe tea, Pandan tea, and Ginger tea toward ABTS-reducing activity

*To obtain the final concentrations, ddH_2O was used to dilute each sample. Each sample concentration in this experiment was done in triplicate. Data are presented in mean ± STD. The asterisk symbol (*) shows a significant difference among concentrations of each sample toward ABTS-reducing activity based on Tukey HSD ($p < 0.05$). The data of ginger ABTS scavenging activity were obtained from the previous studies (Widowati et al., 2022a).

FRAP activity

Current investigations suggest that FRAP activity depends on concentrations at which higher concentrations increase FRAP activity (Liana et al., 2019; Widowati et al., 2017; 2022a). This study (Table 3) showed that ginger tea compared to PanJe tea and Pandan tea had the highest antioxidant activity based on FRAP antioxidant tests. The IC_{50} value of ginger tea is 4.94%, PanJe tea is 7.09% and pandan tea is 12.86%. The highest concentration of each sample had the highest FRAP activity ($p<0.05$). The highest concentration (5%) of FRAP activity in PanJe tea 34.97%, pandan tea 19.62%, and ginger tea 51.71% (Figure 3). Ginger tea exhibited the highest FRAP activity compared to a combination of pandan and ginger tea (PanJe) and pandan tea. In a previous study we evaluated the antioxidant properties of tea combination using ginger tea and telang tea (JaTe). Furthermore, ginger tea has a higher FRAP activity compared to the tea combination (JaTe) (Widowati et al., 2022a). The leaves and rhizomes of ginger methanolic extract from two varieties have good free radical scavenging abilities such as FRAP activity and DPPH scavenging activity compared to BHT and α-tocopherol (positive control) (Ghasemzadeh & Jaafar, 2013). The data of FRAP activity (Table 3, Figure 3) was consistent with previous research that a combination herbal tea of ginger tea and telang tea (JaTe) had slightly lower antioxidant in FRAP activity compared to the highest ginger tea (Widowati et al., 2022a).

Table 3. FRAP activity of panJe tea, pandan tea and ginger tea

Samples	Linear Equation	R^2	IC_{50} (%)
PanJe tea	y = 7.0945x - 0.3075	0.99	7.09 ± 0.36
Pandan tea	y = 3.865x + 0.2808	0.99	12.86 ± 0.82
Ginger tea	y = 10.576x - 2.1975	0.99	4.94 ± 0.26

The data on ginger FRAP activity were obtained from previous studies (Widowati et al., 2022a)

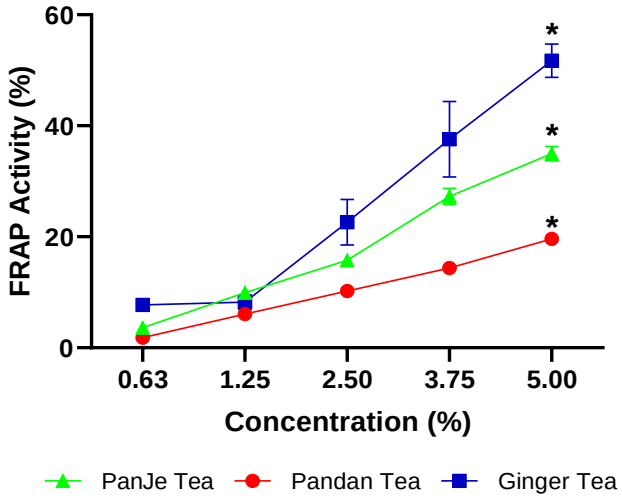

Figure 3. Effect various concentrations of PanJe tea, Pandan tea, and Ginger tea toward FRAP activity

*To obtain the final concentrations, ddH_2O was used to dilute each sample. Each sample concentration in this experiment was done in triplicate. Data are presented in mean ± STD. The asterisk symbol (*) shows a significant difference among concentrations of each sample toward FRAP activity based on the Tukey HSD ($p < 0.05$).

H_2O_2 scavenging activity

A by-product of normal aerobic metabolism is H_2O_2 while infectious diseases, training sessions, and stressful conditions are created and proliferate (Prahastuti et al., 2020). Table 4 depicts the IC_{50} value for H_2O_2 scavenging activity, the IC_{50} of PanJe tea, pandan tea, and ginger tea respectively was 27.70 %, 27.19 % and 13.23 %. The lowest IC_{50} value was ginger tea indicating that ginger had the highest H_2O_2 scavenging activity compared to PanJe tea and pandan tea. With increasing concentration, the percentage of H_2O_2 scavenging activity grew significantly in a dependent manner ($p<0.05$) (Figure 4).

Alsahli et al., 2021 study, gingerol compound in ginger rhizome exhibited H_2O_2 reduction activity by 600 µg/mL. The H_2O_2 reduction activity of ginger may be due to its electron-donating activity. Ginger methanol and ethanol extracts showed higher scavenging activity on H_2O_2 with values of 63.6% and 66.7%, respectively compared to BHT and BHA (25.0% and 39.9%). Both extracts may scavenge H_2O_2 due to their phenolic content, which may donate electrons to H_2O_2 (Yesiloglu et al., 2013). Ginger ethanolic extract was categorized active antioxidant in H_2O_2 scavenging activity with IC_{50} 52.42 µg/mL (Laksmitawati et al., 2021; Marjoni & Zulfisa, 2017). The data of H_2O_2 scavenging activity (Table 4, Figure 4) was consistent with previous research that a combination herbal tea of ginger tea and telang tea (JaTe) had moderate antioxidants with H_2O_2 scavenging activity from highest to lowest is ginger tea, JaTea tea, and then telang tea (Widowati et al., 2022a).

Table 4. H_2O_2-scavenging activity of panJe tea, pandan tea and ginger tea

Samples	Linear Equation	R^2	IC_{50} (%)
PanJe tea	y = 1.6359x + 4.691	0.99	27.70 ± 1.12
Pandan tea	y = 1.8623x - 0.6366	0.95	27.19 ± 0.14
Ginger tea	y = 2.3899x + 18.388	0.99	13.23 ± 0.08

*The data on ginger H_2O_2 scavenging activity were obtained from the previous studies (Widowati et al., 2022).

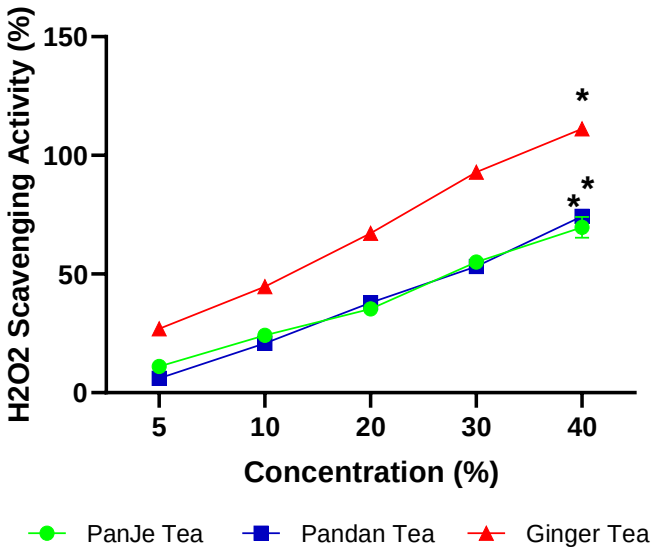

Figure 4. Effect various concentrations of panJe tea, pandan tea, and ginger tea toward H_2O_2 scavenging activity

*To obtain the final concentrations, ddH_2O was used to dilute each sample. Each sample concentration in this experiment was done in triplicate. Data are presented in mean ± STD. The asterisk symbol (*) shows a significant difference among concentrations of each sample toward H_2O_2 scavenging activity based on the Tukey HSD. ($p < 0.05$). The data on ginger H_2O_2 scavenging activity were obtained from previous studies (Widowati et al., 2022a).

Phenolic content

This study showed phenol content of 0.035 µg GAE/mg PanJe tea, 0.002 µg GAE/mg pandan tea, and 0.118 µg GAE/mg ginger tea. Table 5 presented that pandan tea contained the lowest amount of phenols, whereas ginger tea had the highest. The phenolic content of rhizome ginger extract had a value of 60.34 mg GAE/g and it has antioxidant activity with an IC_{50} value of 8.29 µg/mL. The pandan tea showed the lowest value in phenolic content, there may cause the drying methods to cause losses in the phenolic content of dried leaves (Chan et al., 2009; Jusril et al., 2016). Ginger is believed to have health advantages since it contains polyphenols. Gingerols, shogaols, and catechins are some of the ginger polyphenols. Ginger has been recognized as a pharmacologically active herbal medicine (Shirin & Jamuna, 2011). Gingerols an active phenolic compound found in ginger rhizomes has been demonstrated of biological activities as an antioxidant and anti-inflammatory agent (Wang et al., 2014). Ginger had high phenolic content compared to pandan extract, this result data was validated with previous research that the total phenolic content (TPC) of ginger extract respectively 95.34 mg GAE /100 g (Oboh et al., 2012), ethanolic pandan extract with TPC 38.12 GAE mg/g meanwhile pandan aqueous extract with TPC of 10.97 mg GAE/g (Quyen et al., 2020). The data TPC (Table 5) was consistent with previous research that a combination herbal tea of ginger tea and telang tea (JaTe)

had moderate antioxidants with TPC from highest to lowest in ginger tea, JaTe tea, telang tea (Widowati et al., 2022a).

Flavonoid content

This result of flavonoid content can be seen in Table 5. PanJe tea contained 0.006 µg QE/mg, Pandan tea contained 0.0003 µg QE/mg sample, and ginger tea contained 0.036 µg QAE/mg. The highest flavonoid concentration was found in ginger tea, followed by PanJe which had a moderate concentration, and pandan, which had the lowest (Table 5). Thus result in line with another study, the total flavonoid of pandan leaves collected from different locations had values of 1.87, 1.32, and 1.12 mg/g DW (Ghasemzadeh & Jaafar, 2013). The total flavonoid content (TFC) of pandan ethanolic extract is 11.79 mg QE/g, and pandan aqueous extract is 3.56 mg QE/g (Quyen et al., 2020). The tea-ginger extract combination had the highest total of flavonoid content with a value of 886.7 mg catechin equivalent (CE)/L (Makanjuola et al., 2015). The wide range in the secondary metabolite content and antioxidant properties of plants may be caused by a number of variables, such as height, temperature, age, climate, plant variety, and geographic areas (Ghasemzadeh & Jaafar, 2013). The data on TFC (Table 5) was consistent with previous research that a combination herbal tea of ginger tea and telang tea (JaTe had moderate antioxidants with TFC ranging from highest to lowest in ginger tea, JaTe and lemon tea (Widowati et al., 2022a).

Table 5. Phenol and flavonoid content of PanJe tea, Pandan tea, Ginger tea

Samples	Flavonoid Content (µg QE/mg Sample)	Phenolic Content (µg GAE/mg Sample)
PanJe tea	0.006	0.035
Pandan tea	0.0003	0.002
Ginger tea	0.036	0.118

*The data on ginger H_2O_2 scavenging activity were obtained from the previous studies (Widowati et al., 2022a).

CONCLUSION

Based on the antioxidant activity assay, ginger tea has been proven to have the highest antioxidant activities in DPPH, ABTS, H_2O_2 scavenging activities, and FRAP activity. PanJe tea has moderate meanwhile pandan tea has the lowest antioxidant activities. Ginger tea contains the highest phenol and flavonoid content meanwhile PanJe tea contains moderate and pandan tea contains the lowest phenol, and flavonoid content.

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