

Antioxidant Activities of RoJa Herbal Tea: Combination of Rosella Flower (*Hibiscus sabdariffa* L.) and Ginger Rhizome (*Zingiber officinale* Rosc.)

Wahyu Widowati^{1*}, Teresa Liliana Wargasetia¹, Teddy Marcus Zakaria¹, Michael Sebastian Gunadi¹, Nathanael Halim¹, Sherly Santiadi¹, Hanna Sari Widya Kusuma², Nida Firyal Fauziyah³

¹Faculty of Medicine, Maranatha Christian University, Bandung, West Java, Indonesia

²Aretha Medika Utama, Bandung, West Java, Indonesia

³Faculty of Mathematics and Natural Sciences Education, Universitas Pendidikan Indonesia, Bandung, West Java, Indonesia

ABSTRACT

Herbal teas and plant-based medicines have been widely used worldwide for their therapeutic and healing qualities. Rosella (*Hibiscus sabdariffa* L.) has various benefits in preventing digestive problems, liver disease, fever, and others. Ginger (*Zingiber officinale* Rosc.) or known as *jahe* in Indonesia is believed to benefit numerous clinical conditions linked to oxidative stress, including pancreatitis, hypertension, diabetic kidney disease, Alzheimer's disease, and tumor development. They have been utilized in traditional medical practice to treat conditions such as fever, nausea, and headaches. In this study, we aimed to determine the antioxidant activities of rosella flower tea, ginger tea, and a combination of both, namely RoJa (Rosella-*Jahe*) tea. Antioxidant activities were analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H₂O₂), 2-2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities, and Ferric Reducing Antioxidant Power (FRAP) assay, as well as by measuring the amount of total phenolic and flavonoid content. Rosella tea had the greatest activity in the DPPH assay, with an IC₅₀ value of 0.412 mg/ml. Ginger tea had the greatest activity for the ABTS, FRAP, and H₂O₂ tests, with IC₅₀ values of 12.595 mg/ml, 0.494 mg/ml, and 1.323 mg/ml, respectively. Ginger tea also had the highest amount of total phenolic (3.61 µg GAE/g sample) and flavonoid content (11.81 µg QE/g). In conclusion, ginger tea showed the highest antioxidant activities, as well as total phenolic and flavonoid content, compared to rosella and RoJa tea.

Keywords: antioxidant; flavonoid; ginger; herbal tea; rosella

ARTICLE HISTORY

Received: February 2023

Revised: August 2023

Accepted: August 2023

*corresponding author
Email: wahyu_w60@yahoo.com

INTRODUCTION

The term "herbal tea" refers to infusions or decoctions made with herbs (other than *Camellia sinensis* L.) (Fu et al., 2018). Tisanes are infused beverages from different plants, such as herbs, flowers, or berries (Shannon et al., 2017). Herbal teas differ from those commonly consumed teas from *C. sinensis* (Ravikumar, 2014). In general, herbal teas help increase stamina, aid healing, induce relaxation, treat stomach or digestive problems, help to provide cleansing properties to the body, also boost the immune system (Ravikumar, 2014; Winston, 2019). Herbal formulations can also be custom-made according to the functions needed.

Rosella (*Hibiscus sabdariffa* L.) is indigenous to Sri Lanka and has been cultivated for centuries. Rosella wood produces edible dark red calyx with a pleasant

sweet-bitter taste. The calyx of *H. sabdariffa* has become the focus of many studies since it was identified to contain several active compounds consisting of polyphenol acids, delphinidin-3-O-sambubioside, flavonoids, cyanidin-3-O-sambubioside, and anthocyanins delphinidin-3-O-glucoside (Hapsari & Setyaningsih, 2021). Rosella calyx infusion is used in conventional medicine as a diuretic and treats gastrointestinal issues, liver disease, fever, hypercholesterolemia, and high blood pressure (Riaz & Chopra, 2018). Extracts from this plant also have therapeutic, including antihypertensive, antiseptic, and astringent. Rosella extract also helps to reduce the diuretic effects of fever, cancer, abscesses, coughs, dysuria, and scurvy (Thakuria et al., 2018). It also has other activities including antiaging, antioxidants (Widowati et al., 2017), and antidiabetic particularly to α -glucosidase and α -amylase inhibition activities.

Ginger (*Zingiber officinale* Rosc.) is a medicinal herb consumed for centuries to treat numerous diseases. Ginger is not only one of the species widely used to improve the flavor and taste of food worldwide, but also carries a wide variety of bioactive contents with biological and pharmacological benefits. Many clinical disorders linked to oxidative stress have been claimed to be treated by consuming ginger, regarding its benefit in diabetes-related kidney and pancreas problems, hypertension, tumor development, and Alzheimer. It has also been used to treat various conditions in conventional medicine including nausea, headaches, fever, colds, rheumatic diseases, arthritis, and muscle pain. Ginger also has androgenic qualities, anti-glycation potential, anti-cancer, anti-inflammatory, hepatoprotective, and renoprotective effects (Saainin et al., 2020; Tohma et al., 2017), antioxidant activities including 2,2-Diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activities, and Ferric Reducing Antioxidant Power (FRAP) assay (Widowati, et al., 2022a).

Plants produce polyphenolic compounds as secondary metabolites, giving them antioxidant properties (Tohma et al., 2017). Antioxidants are substances that can mitigate the harmful effects of free radicals, which form naturally during oxidative metabolism. Various scientific studies suggest that antioxidants can help prevent chronic illnesses, such as cancer and coronary heart disease. The process parameters used in preparing herbal teas can affect their antioxidant capacity. Preparation steps such as drying at high temperatures and extended drying times can reduce the antioxidant activity of the dried material. Our research aimed to improve the taste and visual appeal of tea by blending dried rosella flowers and ginger, resulting in a beverage known as RoJa tea. As a result, red-colored tea is expected to be obtained with a fresher but slightly sour, spicy, and hot flavor. Our study focuses on analyzing the antioxidant properties of rosella flower tea, ginger tea, and RoJa tea. To achieve this, we conducted research to evaluate the total flavonoid and phenolic contents, and the antioxidant activities of these teas using various methods such as DPPH, H_2O_2 , ABTS, and FRAP assay. In addition, we used RoJa tea, a combination of rosella and ginger, as well as ginger tea and rosella flower tea, as anti-scavenging agents.

MATERIALS AND METHODS

Materials

The rosella flowers (*H. sabdariffa*) were obtained from Kediri, East Java, Indonesia, and the rhizomes of ginger (*jahe*) (*Z. officinale*) were obtained from Pasar Sederhana, Bandung, West Java, Indonesia. The materials used in

this study were 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).

Preparation of Herbal Tea

Both materials were washed and dried in a food dehydrator (50°C, 36 hours) to reduce the water content. Three herbal tea formulations were used in this study: ginger (*jahe*) tea, rosella tea, and a combination of ginger and rosella called RoJa tea. To make single ginger and rosella tea, each 2 g of dry ginger or rosella was brewed with 200 ml of boiled water for 5 minutes. Then, 2 g rosella and 2 g dried ginger were combined and brewed with 200 ml of boiled water to make the RoJa tea, it was equivalent to 10 mg/ml. The mixture was then left to stand for 5 minutes for further assay (Widowati et al., 2022a; 2023).

Antioxidant Activity Assay in Herbal Tea Formulations

In this study, the herbal teas were tested for their antioxidant properties using various assays such as DPPH, H_2O_2 , ABTS, and FRAP. Additionally, the total phenolic and flavonoid content of the teas were measured at concentrations of 100%, 75%, 50%, 25%, and 12.5%.

Scavenging Activity Assay by DPPH

The DPPH scavenging approach was performed based on previous studies (Widowati et al., 2022a; 2022b; 2022c; 2023). First, samples were made in five final concentrations (20, 15, 10, 5, and 2.5%) and 50 μ l of each sample were added with DPPH (0.077 mmol; 200 μ l) (Sigma Aldrich, D9132) into the sample well. For the blank well, double distilled water (ddH_2O) (250 μ l) and DPPH (0.077 mmol; 250 μ l) were added into the control well. For the incubation, the plate was kept in the dark at room temperature for 30 minutes, after which the absorbance was measured using a microplate reader at 517 nm (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, USA). The Inhibitory concentration 50 (IC_{50}) was then determined, and the calculation of DPPH scavenging was done using the formula below (1):

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

A: The absorbance of control

B: The absorbance of sample

H₂O₂ Scavenging Activity Assay

The H₂O₂ scavenging method was adapted from the previous studies performed by Widowati et al. (2022a; 2022b; 2022c; 2023). A total of 60 µl various samples (40, 30, 20, 10, 5%), H₂O₂ (3 µl; 5 mM) (Merck 1.08597.1000), ferrous ammonium sulfate (12 µl; 1 mM) (Sigma Aldrich; 7783859), and 1,10-phenanthroline (75 µl; 1 mM) (Merck 200-629-2) were added into the sample well. On the other hand, ddH₂O (150 µl) was added for the blank well, while ferrous ammonium sulfate (12 µl; 1 mM), ddH₂O (63 µl), and 1,10-phenanthroline (75 µl) were added to the control well. The 96-well plate was incubated for 10 minutes at room temperature in the dark. A microplate reader was used to measure the absorbance at 510 nm wavelength, and the calculation of H₂O₂ scavenging activity was performed using the following formula (2):

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = (\text{A}-\text{B})/\text{A} \times 100 \dots (2)$$

A: The absorbance of sample

B: The absorbance of control

ABTS Scavenging Activity Assay

ABTS-scavenging method was referring to previous studies (Widowati et al., 2022a; 2022b; 2022c; 2023). ABTS (198 µl) (Sigma Aldrich; A1888) were added after 2 µl various concentrations of samples (1, 0.5, 0.25, 0.125, and 0.0625%). The well of control received ABTS reagents (200 µl) and the blank well received ddH₂O (200 µl). The microplate was incubated at 37°C for 10 minutes. In order to measure the absorbance at 745 nm wavelength, a microplate reader was used. The formula below was used to calculate the scavenging activity of ABTS (3):

$$\text{ABTS scavenging activity (\%)} = (\text{A}-\text{B})/\text{A} \times 100 \dots (3)$$

A: The control's absorbance

B: The sample's absorbance

FRAP Assay

FRAP assay method was conducted based on previous studies (Widowati et al., 2022a; 2022b; 2022c; 2023). First, acetate buffer (300 mM; 10 ml), ferric chloride hexahydrate (20 mM; 1 ml) (Sigma Aldrich, 1039430250), 2,4,6-Tris-(2-pyridyl-5-triazine) (TPTZ) (10 mM) (Sigma Aldrich, T1253) were combined. Then, 7.5µl various concentrations of the sample (5, 3.75, 2.5, 1.25, 0.63 %) were combined with FRAP reagent (142.5 µl) in a well plate and incubated for 30 minutes (37°C). In order to measure the absorbance at 760 nm wavelength, a microplate reader was used.

The following equation is used to calculate the percentage of scavenging activities (4):

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

A: The control's absorbance

B: The sample's absorbance

Total Phenolic Content

The total phenolic content was measured using the colorimetric method previously used in similar studies (Widowati et al., 2022a; 2022b; 2022c; 2023). Briefly, the sample well received the mixture of 15 µl sample, Na₂CO₃ (60 µl; 7.5%) (Merck A897992745), and Folin-Ciocalteu reagent (75 µl; 10%) (Merck; 1.090.010.500). The blank solution consists of 135 µl distilled water and 15 µl of samples. The samples were incubated at 50°C for 10 minutes, then the absorbance was measured at 760 nm using a microplate reader. The total phenolic content was calculated using the linear standard equation of GA (gallic acid) in 6 concentration levels (500, 250, 125, 62.5, 31.25, 15.63 µg/ml) (Sigma Aldrich; 398225). The Gallic Acid Equivalent (GAE) in µg GAE/g sample is utilized to measure the total phenol content of herbal tea.

Total Flavonoid Content

A colorimetric method was employed to determine the flavonoid content using the technique used in earlier studies (Widowati et al., 2022a; 2022b; 2022c; 2023). Briefly, 15 µl quercetin (Sigma Aldrich, Q4951) solution in 6 concentrations level (100, 50, 25, 12.5, 6.25, 3.13 µg/ml) and herbal tea (RoJa, ginger, rosella tea in concentration of 10, 5, 2.5 mg/ml) were added and followed by AlCl₃ (75 µl; 2%) (Merck; 449598) into well. The absorbance was examined at 415 nm wavelength using a microplate reader. Total flavonoid was calculated based on linear regression of quercetin equivalent (QE) (µg QE/g).

Statistical Analysis

SPSS software (IBM Statistics, 20.0) was utilized to conduct the statistical analysis, using One-way ANOVA and Tukey's HSD post-hoc analysis to reveal significant variations between the groups (P<0.05).

RESULTS AND DISCUSSION

Antioxidant is a substance that can slow down or prevent the oxidation of a substrate even when used at a low concentration (Santos-Sánchez et al., 2019). Because of its chemical stability, the free radical can receive an electron and be neutralized, which lessens its ability to cause harm. To interrupt the damaging chain reaction, these antioxidants can interact with free radicals in a safe way (Lobo et al., 2010). Based on this study, the three herbal teas showed a high antioxidant effect in a concentration-dependent manner significantly (P<0.05) (Figure 1-4).

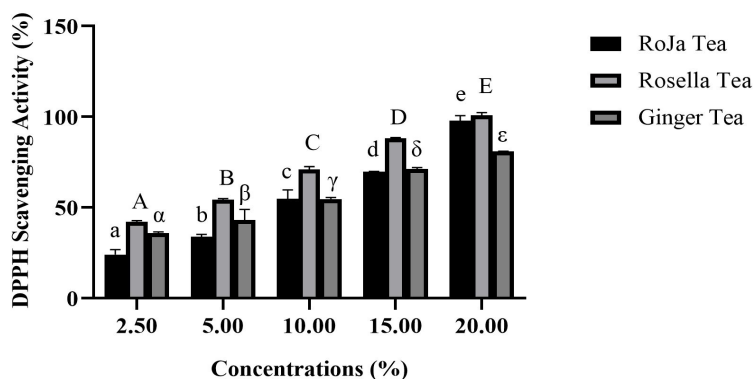


Figure 1. Effect of various concentrations of roja, rosella, and ginger tea toward DPPH scavenging activity

*The mean and standard deviation of the data are displayed (n=3). Significant variations in treatment are manifested as various signs for RoJa Tea (a,b,c,d,e); different Rosella Tea signs (A,B,C,D,E); different signs for Ginger Tea ($\alpha,\beta,\gamma,\delta,\epsilon$) according to the Tukey HSD post hoc test (p<0.05). The information about ginger tea was obtained from an earlier study (Widowati et al., 2022a; 2023).

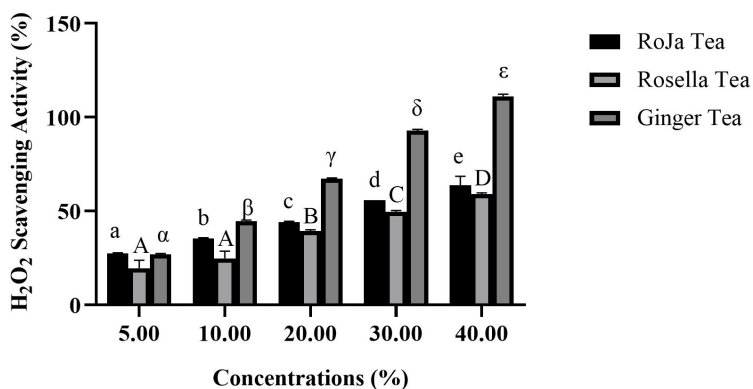


Figure 2. Effect of various concentrations of roja, rosella, and ginger tea toward H₂O₂ scavenging activity

*The mean and standard deviation of the data are displayed (n=3). Significant variations in treatment are manifested as various signs for RoJa Tea (a,b,c,d,e); different Rosella Tea signs (A,B,C,D); different signs for Ginger Tea ($\alpha,\beta,\gamma,\delta,\epsilon$) according to the Tukey HSD post hoc test (p<0.05). The information about ginger tea was obtained from an earlier study (Widowati et al., 2022a; 2023).

Based on all antioxidant assays, the highest antioxidant activities were rosella tea for DPPH and ginger tea for ABTS; FRAP; H₂O₂ in 0.01 g/ml sample concentration. This formula tea had the highest DPPH scavenging activity with the IC₅₀ value of 0.412 mg/ml; ABTS scavenging activity with the IC₅₀ value was 12.595 mg/ml; H₂O₂ scavenging activity with the IC₅₀ value was 1.323 mg/ml; FRAP assay with the IC₅₀ value was 0.494 mg/ml sample concentration. This outcome aligned with the earlier study, which demonstrated ginger tea's highest level of antioxidant activity in DPPH, H₂O₂, and ABTS methods compared to telang flower tea (*Clitoria ternatea* L.) (Widowati et al., 2022a). The antioxidant activity value is primarily due to the high phenol content in ginger such as paradol, shogaol, and zingerone.

High phenolic content in the sample will affect its antioxidant activity. Samples with high phenolic content have great potential as natural antioxidants in reducing free radicals (Abdul, 2020). Some of these compounds are phytochemicals that have been studied extensively and are responsible for their antioxidant, anti-inflammatory, antiemetic, and gastroprotective activities. Moreover, it is well recognized that the antioxidant pathways, induction of apoptosis, free radical activity, and changes in gene expression, which all contribute to the decrease of tumor start, development, and progression, are the leading causes of ginger's cancer-protective impact (Tohma et al., 2017). Therefore, the ginger tea sample has the highest antioxidant activity among other teas.

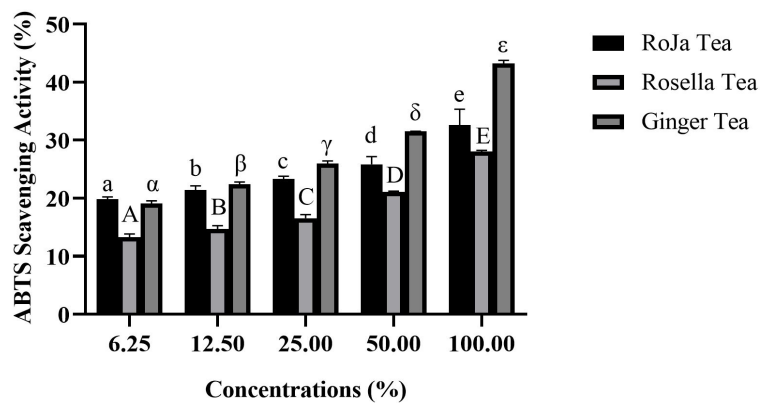


Figure 3. Effect of various concentrations of roja, rosella, and ginger tea toward ABTS scavenging activity

*The mean and standard deviation of the data are displayed (n=3). Significant variations in treatment are manifested as various signs for RoJa Tea (a,b,c,d,e); different Rosella Tea signs (A,B,C,D,E); different signs for Ginger Tea (α,β,γ,δ,ε) according to the Tukey HSD post hoc test (p<0.05). The information about ginger tea was obtained from an earlier study (Widowati et al., 2022a; 2023)

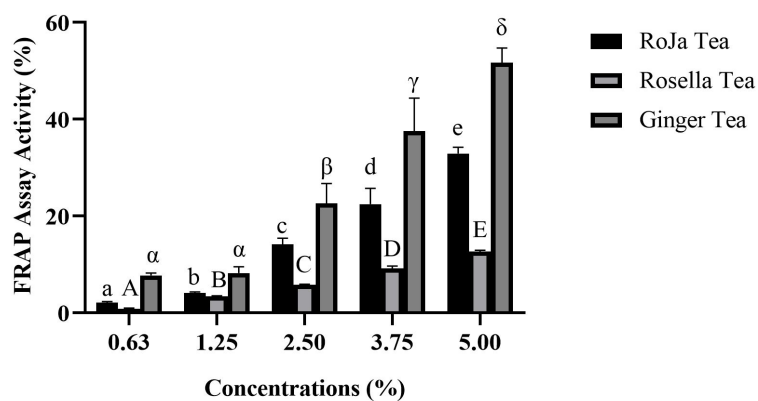


Figure 4. Effect of various concentrations of roja, rosella, and ginger tea toward FRAP assay

*The mean and standard deviation of the data are displayed (n=3). Significant variations in treatment are manifested as various signs for RoJa Tea (a,b,c,d,e); different Rosella Tea signs (A,B,C,D,E); different signs for Ginger Tea (α,β,γ,δ) according to the Tukey HSD post hoc test (p<0.05). The information about ginger tea was obtained from an earlier study (Widowati et al., 2022a; 2023)

Rosella flower tea showed the lowest antioxidant activities according to the FRAP assay and scavenging activities for H₂O₂, DPPH, and ABTS. The DPPH scavenging activity's IC₅₀ value of Rosella tea was 0.412 mg/ml; ABTS scavenging activity was 23.882 mg/ml; H₂O₂ scavenging activity was 3.099 mg/ml; FRAP assay was 1.937 mg/ml. Additionally, the total phenols and flavonoid content in rosella tea were also the lowest when compared to ginger tea and RoJa tea formulation. However, the antioxidant activity of rosella tea remains at a high level. According to Hirunpanich et al. (2015) and Widowati et al. (2017), rosella contains flavonoid compounds consisting of flavanol pigments and anthocyanin compounds (Hirunpanich et al., 2005), myricetin, β-carotene (Widowati et al., 2017). Flavanols serve as organic antioxidants and may lower the risk

of heart or cardiovascular disorders (Alspach, 2007). Anthocyanins function as antioxidants in the body so that they can prevent the occurrence of atherosclerosis, and coronary artery disease. By oxidizing unhealthy lipids in the body, specifically low-density lipoproteins (LDL), anthocyanins may prevent the development of atherogenesis. Then, the endothelial cells that line the walls of blood vessels are protected by anthocyanins, preventing damage (Syarif et al., 2020).

The IC₅₀ value of RoJa tea for DPPH scavenging activity was 0.902 mg/ml; ABTS scavenging activity was 23.179 mg/ml; H₂O₂ scavenging activity was 2.558 mg/ml; FRAP assay was 0.748 mg/ml which is presented in Tables 1-4.

Table 1. The IC₅₀ values of DPPH scavenging activity of roja, rosella, and ginger tea

Sample	Linear Equation	R ²	IC ₅₀ (mg/ml)
RoJa tea	$y = 4.0767x + 13.237$	0.99	0.902
Rosella tea	$y = 3.3391x + 36.24$	0.99	0.412
Ginger tea	$y = 2.6189x + 29.684$	0.99	0.776

The assays were carried out three times. The IC₅₀ of each sample and the coefficient of regression (R²) were calculated based on linear regression.

Table 2. The IC₅₀ values of ABTS scavenging activity of roja, rosella, and ginger tea

Samples	Linear Equation	R ²	IC ₅₀ (mg/ml)
RoJa tea	$y = 0.1315x + 19.519$	0.99	23.179
Rosella tea	$y = 0.1562x + 12.697$	0.99	23.882
Ginger tea	$y = 0.2472x + 18.864$	0.99	12.595

The assays were carried out three times. The IC₅₀ of each sample and the coefficient of regression (R²) were calculated based on linear regression.

Table 3. The IC₅₀ values of FRAP assay of roja, rosella, and ginger tea

Samples	Linear Equation	R ²	IC ₅₀ (mg/ml)
RoJa tea	$y = 7.1704x - 3.6624$	0.99	0.748
Rosella tea	$y = 2.6096x - 0.4856$	0.99	1.937
Ginger tea	$y = 10.576x - 2.1975$	0.99	0.494

The assays were carried out three times. The IC₅₀ of each sample and the coefficient of regression (R²) were calculated based on linear regression.

Table 4. The IC₅₀ values of H₂O₂ scavenging activity of roja, rosella, and ginger tea

Samples	Linear Equation	R ²	IC ₅₀ (mg/ml)
RoJa tea	$y = 1.0252x + 23.78$	0.99	2.558
Rosella tea	$y = 1.1512x + 14.321$	0.99	3.099
Ginger tea	$y = 2.3899x + 18.388$	0.99	1.323

The assays were carried out three times. The IC₅₀ of each sample and the coefficient of regression (R²) were calculated based on linear regression.

Table 5. Total phenolic and flavonoid content of roja, rosella, and ginger tea

Samples	Total Phenolic (µg GAE/g sample)	Total Flavonoid (µg GAE/g sample)
RoJa tea	2.33	5.40
Rosella tea	0.85	4.33
Ginger tea	3.61	11.81

Gallic Acid Equivalence (GAE); Quercetin Equivalence (QE)

The IC₅₀ value revealed that the antioxidant activity value of RoJa tea is quite good. Based on previous studies, the IC₅₀ value of <200 ppm or <200 µg/ml is considered strong antioxidant activity (Sukandar et al., 2017). According to the findings of this study, RoJa has high antioxidant activity, specifically in the DPPH, H₂O₂, and FRAP assay, which includes high antioxidant activity, and in the ABTS test, which includes moderate

antioxidant activity. However, the value of antioxidant activity in RoJa is lower than in ginger (Table 1-4). This can be seen from the IC₅₀ value of ginger, which is smaller in the ABTS, FRAP, and H₂O₂ tests. Another study also reported that the combination formulation of pandan and ginger had lower activity than single ginger (Widowati et al., 2023). The total phenolic compound data revealed that ginger herbal tea (3.61 µg GAE/g sample) and

RoJA tea (2.33 µg GAE/g sample) had the highest total phenolic content, while the lowest total phenolic content was found in the rosella tea (0.85 µg GAE/g sample). The total phenolic content indicates these compounds are excellent antioxidants or have broad biochemical activities, including anticarcinogenic, antimutagenic, and modifying gene expression to cure various oxidative stress diseases. The composition, placement of hydroxyl groups, quantity, and substitution characteristics of phenolic content all affect its antioxidant action on aromatic rings (Prahastuti et al., 2020). Due to the polyphenols content, such as shogaols, catechins, and gingerols, ginger has health potential. Ginger has been acknowledged as a medicinal herb with pharmacological activity. Gingerols, contained in ginger rhizomes, have biological effects that include anti-inflammatory and antioxidant properties (Saanin et al., 2020). Ginger had a higher phenolic content than rosella extract, and this finding was supported by earlier research. In a study by Oboh et al. (2012), the quantity of phenols in ginger extract overall was 95.34 mg GAE/g, meanwhile, rosella extract was 23.85 mg GAE/g.

Based on the analysis of total flavonoid content, it was found that ginger tea had the highest amount of 11.81 µg QE/g, followed by Roja tea with a moderate content of 5.40 µg QE/g sample, and rosella tea with the lowest content of 4.33 µg QE/g sample. Flavonoids play a vital role as biological reaction modifiers, and they have various functions such as antihistamines, antimicrobials, memory enhancers, and mood enhancers (Prahastuti et al., 2020). Therefore, ginger tea can be an excellent source of flavonoids for those looking to benefit from their properties. In contrast, RoJa tea and rosella tea may have a lesser impact due to their lower flavonoid content. Table 5 provides a summary of the flavonoid contents of the different tea formulations. Previous research has found that rosella extract has a moderate amount of total flavonoids (++) (Widowati et al., 2017). The total phenol and flavonoid content in this study is similar to earlier studies that used JaTe, a blend of telang and ginger (*jahe*) tea, as the herbal tea (Widowati et al., 2022a). The antioxidant activity of JaTe was found to be moderate, with ginger tea having the highest total phenol content, followed by JaTe and then telang tea (Widowati et al., 2022a). Other studies that combined pandan tea and *jahe* or ginger tea to create PanJe also supported these findings, showing moderate antioxidant properties with *jahe* tea having the highest total phenol content, followed by panje and pandan tea (Widowati et al., 2023).

CONCLUSION

From the results of this study, ginger tea has the highest activity of antioxidant through H₂O₂, ABTS scavenging activity, FRAP assay, total phenolic and total flavonoid

content, while rosella tea has the highest antioxidant through DPPH assay. In addition, Rosella tea has the lowest total phenol and flavonoid content, followed by RoJa tea. This research is expected to provide additional information related to innovation for mixed herbal tea products and in the future, they can be produced commercially.

CONFLICT OF INTEREST

The authors said they had no competing interests.

ACKNOWLEDGEMENT

We would like to express our gratitude to the Ministry of Education and Culture of the Republic of Indonesia for the funding through the Independent Policy Research Program Learning Independent Campus with Research Result-Based Community Service, and PTS Prototype 2021; Faculty of Medicine at Universitas Kristen Maranatha and Faculty of Mathematics and Natural Sciences Education at Universitas Pendidikan Indonesia for their cooperation in this research; also UMKM ARUMA and POKJA Bunda Kreatif organizations in Bandung who also provided funding for this study. Aretha Medika Utama-Biomolecular and Biomedical Research Center, Bandung, Indonesia also provided laboratory facilities for this study as well.

REFERENCES

- Abdul, A. (2020). Uji aktivitas antioksidan ekstrak etanol daun adas (*Foeniculum vulgare* Mill) dengan metode DPPH dan FRAP. *Pharmed: Journal of Pharmaceutical Science and Medical Research*, 3(2), 43. <https://doi.org/10.25273/pharmed.v3i2.7456>
- Alspach, G. (2007). The truth is often bittersweet...: chocolate does a heart good. *Critical Care Nurse*, 27(1), 11–15.
- Fu, Y., Yang, J., Cunningham, A. B., Towns, A. M., Zhang, Y., Yang, H., Li, J., & Yang, X. (2018). A billion cups: The diversity, traditional uses, safety issues and potential of Chinese herbal teas. *Journal of Ethnopharmacology*, 222, 217–228.
- Hapsari, B. W., & Setyaningsih, W. (2021). Methodologies in the analysis of phenolic compounds in roselle (*Hibiscus sabdariffa* L.): Composition, biological activity, and beneficial effects on human health. *Horticulturae*, 7(2), 1-41.
- Hirunpanich, V., Utaipat, A., Morales, N. P., Bunyapraphatsara, N., Sato, H., Herunsalee, A., & Suthisang, C. (2005). Antioxidant effects of aqueous

- extracts from dried calyx of *Hibiscus sabdariffa* Linn. (Roselle) in vitro using rat Low-Density Lipoprotein (LDL). *Biological and Pharmaceutical Bulletin*, 28(3), 481–484.
- Lobo, V., Patil, A., Phatak, A., & Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*, 4(8), 118–126.
- Prahastuti, S., Hidayat, M., Hasiana, S. T., Widowati, W., Widodo, W. S., Handayani, R. A. S., Rizal, R., & Kusuma, H. S. W. (2020). The ethanol extract of the bastard cedar (*Guazuma ulmifolia* L.) as antioxidants. *Pharmaciana*, 10(1), 77–88.
- Ravikumar, C. (2014). Review on herbal teas. *Journal of Pharmaceutical Sciences and Research*, 6(5), 236–238.
- Riaz, G., & Chopra, R. (2018). A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine & Pharmacotherapy*, 102, 575–586.
- Saanin, S. N., Wahyudianingsih, R., Afni, M., Afifah, E., Maesaroh, M., & Widowati, W. (2020). Suppression of pro-inflammatory cytokines and mediators production by ginger (*Zingiber officinale Roscoe*) ethanolic extract and gingerol in lipopolysaccharide-induced RAW 264.7 murine macrophage cells. *Indian Journal of Natural Products and Resources*, 11(4), 260–266.
- Santos-Sánchez, N. F., Salas-Coronado, R., Villanueva-Cañongo, C., & Hernández-Carlos, B. (2019). Antioxidant compounds and their antioxidant mechanism. *Antioxidants*, 10, 1–29.
- Shannon, E., Jaiswal, A. K., & Abu-Ghannam, N. (2017). Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: A kinetic study. *Food Research*, 2(1), 1–11.
- Sukandar, D., Nurbayti, S., Rudiana, T., & Husna, T. W. (2017). Isolation and structure determination of antioxidants active compounds from ethyl acetate extract of Heartwood Namnam (*Cynometra cauliflora* L.). *Jurnal Kimia Terapan Indonesia*, 19(1), 11–17.
- Syarief, O., Fauziyah, R. N., Suparman, S., Gurid, G., & Hendriyani, H. (2020). The efficacy of Fermented Glutinous Black Rice (FGBR) snack to improve lipid profile among dyslipidemia subjects: A novel finding. *International Medical Journal*, 25(8), 2929–2937.
- Thakuria, P., Nath, R., Sarma, S., Kalita, D. J., Dutta, D. J., Borah, P., Sharma, R., Barman, C., & Hussain, J. (2018). Phytochemical screening of medicinal plants occurring in local area of Assam. *Journal of Pharmacognosy and Phytochemistry*, 7(3), 186–188.
- Tohma, H., Gülçin, İ., Bursal, E., Gören, A. C., Alwasel, S. H., & Köksal, E. (2017). Antioxidant activity and phenolic compounds of ginger (*Zingiber officinale* Rosc.) determined by HPLC-MS/MS. *Journal of Food Measurement and Characterization*, 11(2), 556–566.
- Widowati, W., Rani, A. P., Hamzah, R. A., Arumwardana, S., Afifah, E., Kusuma, H. S. W., Rihibiha, D. D., Nufus, H., & Amalia, A. (2017). Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. *Natural Product Sciences*, 23(3), 192–200.
- Widowati, W., Wargasetia, T. L., Zakaria, T. M., Marthania, M., Gunadi, M. S., Halim, N., Dewi, N. S. M., & Santiadi, S. (2022a). Antioxidant activities of ginger (*Zingiber officinale*) and telang flower (*Clitoria ternatea* L.) combination tea. *Majalah Kedokteran Bandung*, 54(3), 154–160.
- Widowati, W., Wargasetia, T. L., Zakaria, T. M., Marthania, M., Akbar, R. A. T. P. P., Gunadi, M. S., Halim, N., & Santiadi, S. (2022b). Antioxidant activity of TEMON (*Clitoria ternatea* and *Citrus* sp.) as an infused herbal tea. *Majalah Obat Tradisional*, 27(1), 29–32.
- Widowati, W., Wargasetia, T. L., Marthania, M., Hanifa, T. S., Zakaria, T. M., Gunadi, M. S., Halim, N., & Santiadi, S. (2022c). Antioxidant properties of tenan herbal tea formulation “Telang (*Clitoria ternatea*) and Pineapple (*Ananas comosus*).” *Jurnal Kedokteran Brawijaya*, 32(2), 87–93.
- Widowati, W., Wargasetia, T. L., Zakaria, T. M., Gunadi, M. S., Halim, N., Santiadi, S., Marthania, M., & Ziyad, A. R. (2023). Antioxidant activity of panJe tea combination of pandan (*Pandanus amaryllifolius*) and jahe (*Zingiber officinale*). *Pharmaciana*, 13(1), 25–37.
- Winston, D. (2019). *Adaptogens: herbs for strength, stamina, and stress relief*. Simon and Schuster.