

Antioxidant properties of TeNan

by Jkb 22

Submission date: 09-Dec-2022 10:44PM (UTC+0700)

Submission ID: 1976412957

File name: B9._JKB_22,_Antioxidant_properties_of_TeNan.pdf (1.83M)

Word count: 5855

Character count: 31637

Research Article

Antioxidant Properties of TeNan Herbal Tea Formulation "Telang (*Clitoria ternatea*) and Pineapple (*Ananas comosus*)"

Aktivitas Antioksidan Formula Teh Herbal TeNan "Telang (*Clitoria ternatea*) dan Nanas (*Ananas comosus*)"

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ABSTRACT

Herbal teas are widely consumed by people around the world and have high antioxidant activities. The flavor and color combination of specific teas enriches the aroma and appearance of the tea, producing a tea with fresh color and tastier flavor. This study determines the antioxidant activity of telang flower tea (*Clitoria ternatea*), pineapple tea (*Ananas comosus*), and the formula of the combination of telang flower tea and pineapple tea called TeNan. The antioxidant activities of herbal teas were measured by 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Hydrogen Peroxide (H₂O₂), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP) assay. The total phenol measurement used Gallic Acid Equivalent (GAE), while the flavonoid content measurement used Quercetin Equivalent (QE). The DPPH scavenging activities of telang flower tea, pineapple tea, and TeNan tea (IC₅₀: 17.07%, 11.81%, and 22.22%), H₂O₂ scavenging activities (IC₅₀: 26.62%, 41.81%, and 96.22%), ABTS-reducing activities (IC₅₀: 2.51%, 3.39%, and 1.02%), and FRAP activities (IC₅₀: 5.56%, 18.67%, and 7.48%). The total phenolic and flavonoid contents of TeNan tea were lower (9.44 µg GAE/100%; 3.46 µg QAE/100% sample concentration) than those of telang flower tea (16.20 µg GAE/100%; 4.88 µg QAE/100% sample concentration) but higher than those of pineapple tea (0.82 µg GAE/100%; 0.17 µg QAE/100% sample concentration). TeNan tea has the higher in ABTS and FRAP activities but lower in H₂O₂ and DPPH scavenging activities compared to telang flower tea and pineapple tea. In summary, telang tea has stronger antioxidant activity compared to pineapple and TeNan tea in FRAP and H₂O₂ assays.

Keywords: *Ananas comosus*, antioxidant, *Clitoria ternatea*, flavonoids, phenol

ABSTRAK

Teh herbal yang banyak dikonsumsi orang di seluruh dunia mengandung antioksidan tinggi. Penggabungan rasa dan warna untuk memperkaya aroma dan penampilan teh, menghasilkan teh dengan warna yang menarik dan rasa yang lebih enak. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan teh bunga telang (*Clitoria ternatea*), nanas (*Ananas comosus*), dan formula kombinasi bunga telang dan nanas yang disebut Telang-Nanas (TeNan). Penelitian ini bertujuan untuk mengukur aktivitas antioksidan teh buah bunga telang, nanas, dan TeNan. Aktivitas antioksidan teh herbal yaitu dengan melakukan pengukuran pada pemerangkapan 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Hydrogen Peroxide (H₂O₂), 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), Ferric Reducing Antioxidant Power (FRAP) assay, serta kandungan fenol menggunakan *standard gallic acid* dan flavonoid menggunakan quercetin. Aktivitas pemerangkapan DPPH secara berurutan yaitu pada teh bunga telang, nanas, TeNan (IC₅₀: 17,07%; 11,81%; 22,22%), pemerangkapan H₂O₂ (IC₅₀: 26,62%; 41,81%; 96,22%), aktivitas reduksi ABTS (IC₅₀: 2,51%; 3,39%; 1,02%), aktivitas FRAP (IC₅₀: 5,56%; 18,67%; 7,48%). Total fenol secara berurutan yaitu teh bunga telang, nanas, TeNan (16,20; 0,82; 9,44 µg GAE/100% sampel) dan total flavonoid secara berurutan yaitu teh telang, nanas, TeNan (4,88; 0,17; 3,46 µg QAE/100% sampel). Teh TeNan mengandung total fenol dan flavonoid lebih rendah (16,20 µg GAE/100%; 4,88 µg QAE/100% sampel) dibandingkan teh bunga telang dan lebih tinggi dibandingkan nanas (0,82 µg GAE/100%; 0,17 µg QAE/100% sampel). TeNan lebih aktif pada aktivitas reduksi ABTS dan FRAP tetapi lebih rendah pada pemerangkapan H₂O₂ dan DPPH dibandingkan teh bunga telang dan nanas. Teh telang memiliki aktivitas antioksidan yang paling kuat dibandingkan dengan teh nanas dan TeNan pada uji FRAP dan H₂O₂.

Kata Kunci: *Ananas comosus*, antioksidan, *Clitoria ternatea*, flavonoid, fenol

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DOI: <http://dx.doi.org/10.21776/ub.jkb.2022.032.02.3>

INTRODUCTION

Tea ranks amongst the top in terms of beverage consumption after water (1). Tea is produced and consumed on average of three billion kg per year. Statistical data show that tea consumption worldwide was supposed to reach 6.3 billion kg in 2020 (2). Herbal teas, also known as herbal infusions or herbal tisanes tea, are created by steeping plant parts including leaves, flowers, seeds, fruits, branches, or roots. They have been used for health and illness prevention for centuries (3). Herbal teas are popular for their therapeutic and stimulating their characteristics as well as their ability to induce mood positivity (4), especially because Chinese people have historically used herbal teas for preventative and/or medicinal purposes (5). The procedure of drying leaves or other portions of herbal teas becomes crucial when it comes to the composition of chemicals that are beneficial to health (3). In addition, herbal teas are also widely used since they are simple to make, have a mild action, and, in most cases, have few side effects as well as being economical and rich in resources (6).

Tea's potential medical properties have been related to its various phytochemical substances, each of which has its own biological capabilities. Tea polyphenols and their derivatives, which make up of at most 30% of the dry mass of tea, are among these phenolic chemicals (7). Among the phytochemicals contained, this bioactive substance is the principal class of compound components in plants that have free radical scavenging or antioxidant activities (8). The free radicals have detrimental effects on the body, such as destroying cell structure and affecting optimal cell function (9). Apart from antioxidant activity, it has many additional basic biological functions, including anti-inflammatory, antibacterial, antiviral, analgesic, antiaging, and anticarcinogenic functions (8,10). As a result, further research concerning natural antioxidants contained in fruits and veggies is beneficial for human health.

Pineapple (*Ananas comosus*) is a tropical plant that was first widely distributed in South America (11). This pineapple plant's fruit is part of a plant that is frequently consumed by the community. Pineapple is also a sought-after fruit due to its appealing color, scent, flavor, and freshness. Pineapple is utilized as a topic in this study on its biological activities in addition to being a food product. It has been shown to offer various medical benefits, including antioxidant, chemotherapeutic, antibacterial, antimalarial, anti-inflammatory, and antidiabetic properties (12,13). In recent years, pineapple's antioxidant activity and purpose in the prevention and treatment of numerous chronic and degenerative diseases in humans have gained considerable attention.

Clitoria ternatea or "telang" (Fabaceae) is a tropical Asian plant that has just recently gained popularity in Africa, Australia, America, and the Pacific (The Plant Database, USA). The purplish-blue flower is the most exciting feature of the butterfly pea or telang plant since the transparent color is quite appealing. The color appears when the acidity conditions are low, and because of their stunning colors, telang petals are utilized as decorative plants. *C. ternatea* flower is utilized as an antidepressant, sedative, antipyretic, painkiller, anti-inflammatory, and antidiabetic in the health sector (14). It turns out that telang flower has long been used in Ayurvedic medicine to improve brain

function and as an anxiolytic medication. Telang flower also serves as an antioxidant agent due to the presence of total phenolic and anthocyanin content and that is one of the reasons for its high antioxidant capacity (15,16). Telang flower and pineapple both have potential from a pharmacological aspect, one of which contains antioxidants.

It is crucial for human health to conduct a through analysis of natural antioxidants, such as those found in fruits and vegetables. According to research, antioxidant activity has been discovered in *C. ternatea* flower and pineapple and they are used as natural sources of antioxidant bioactive components (17-19). Herbal teas, such as telang flower tea, have been utilized not only for daily consumption at times, but also for therapeutic purposes. Telang flower has a wide range of bioactivity and beautiful blue color. However, it is less palatable or unpleasant, but the combination of unusual hue and health advantages will promote telang flower tea as a functional drink. To increase the taste, freshness, various color, we combined two dry ingredients: dried telang flower and dried pineapple to make a TeNan herbal tea formulation, which had a purple hue, a little sour taste, and a fresh taste. This strength of Tenan herbal tea which has a visual aspect or an attractive appearance is able to increase enthusiastic to consume it, this will increase the selling value of the product if it is commercialized.

This research was performed to evaluate the phenolic and flavonoid contents and antioxidant activities including ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) reduction, DPPH (2,2-diphenyl 1-piclylhydazy) activity, FRAP (Ferric Reducing Antioxidant Power) activity, and H₂O₂ scavenging activity of telang flower tea, pineapple tea, and TeNan tea, which was a combination of telang and pineapple tea.

METHOD

Preparation of Dried Sample and Herbal Tea Formulation

The dried butterfly pea flower used was taken from Kampung Herbal Desa Sukolilo, Prigen, Pasuruan, East Java, Indonesia, and the pineapple was taken from Pasar Sederhana, Bandung, West Java. The pineapple fruit used was washed and dried to reduce the water content using a food dehydrator (Well Known) for 36 hours at a temperature of 50°C. Then, the dried ingredients were formulated into TeNan (Telang-Pineapple) herbal tea. The following formulation is used to brew herbal teas: 200 mL hot water for 5 buds of "telang" + 2 g of "pineapple" for 5 minutes as a 100% concentration of sample. Telang tea was made by soaking 5 buds of telang flower in hot water for 5 minutes as a 100% concentration of sample and pineapple tea was made by soaking 2 dried pineapples in hot water for 5 minutes as a 100% concentration of sample (20).

Total Phenolic Content Assay

The Folin-Ciocalteu reagent (Merck 1.090.010.500) was used to determine the total phenol content and the process was carried out in a 96-well plate. The sample was added to each well as much as 15 µL, and then the 75 µL of Folin-Ciocalteu reagent 10% and 60 µL of sodium carbonate (Merck A897992745) 7.5% were added. The plate was incubated at 50°C for 10 minutes. Ciocalteu used the Gallic Acid (Sigma Aldrich 398225) calibration curve

and total polyphenols were measured (20,21). The Gallic Acid standard linear equation was used to get the phenolic total value with $y=ax+b$ as a standard linear equation. The phenolic content was measured in Gallic Acid Equivalent (GAE) in microgram phenol per 100% sample concentration. The absorbance value was obtained using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific) at a wavelength of 760nm (20,21).

Total Flavonoid Content Assay

Colorimetric assay was used to determine the total content of flavonoid. In a nutshell, the 15µl of each sample and standard was combined with 75µl $AlCl_3$ (Merck 449598) 2%. The well blank contained the sample (well sample) and 150µl sample solvent was added. The absorbance of the finished combination was measured using a microplate reader at 415nm. The standard Quercetin (Sigma Aldrich Q4951) linear equation was used to get the total flavonoid value with $y=ax+b$ as a standard linear equation. The flavonoid content was measured in Quercetin Equivalent (QE) in microgram flavonoid per 100% sample concentration. Three replications of the experiment were carried out (20,21).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay

DPPH (Sigma Aldrich D9123) 200µl of 0.077mmol was added into a 96-well plate consisting of 50µl of samples with various concentrations. The well blank was added with 250µl of sample solvent (ddH_2O) and the control well was added with 250µl of 0.077 mmol DPPH. After 30 minutes of incubation at room temperature in the dark, the absorbance was measured using a microplate reader at a 517nm. Below is the formula to determine the DPPH scavenging activity (20-23):

$$DPPH \text{ scavenging activity (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) Reduction Assay

The sample was added for 2µl to the 96-well plate followed by 198µl of ABTS reagent (Sigma Aldrich A1888) while for the negative control, 200µl of ABTS reagent was added and for the blanks, 200µl ddH_2O was added. The microplate was incubated at 37°C for 10 minutes and afterward, the absorbance was measured at 745nm using a microplate reader. The following formula was used to calculate the ABTS scavenging activity of the sample (20-23):

$$ABTS \text{ reduction activity (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

Hydrogen Peroxide (H_2O_2) Scavenging Assay

This H_2O_2 scavenging method was based on a previous study by Lister *et al.*, (22,23) with slight modification. The 60µl of sample, 12µl of ferrous ammonium sulphate 1mM (Sigma Aldrich 7783859), and 3µl of H_2O_2 5mM (Merck 108597.1000) were added to the 96-well plate. The 12µl of ferrous ammonium sulfate and 63µl of ddH_2O were used as negative control and the 50µl of ddH_2O was used as blank. The mixture was then incubated for 5 minutes in a dark environment at a room temperature. 75µl of 1,10-phenanthrolines (Sigma Aldrich 131377) were added to the sample and the control well, and then incubated for another 10 minutes at a room temperature in a dark environment. At a wavelength of 510nm, the absorbance

value was measured. The H_2O_2 scavenging activities of the sample were calculated using the following formula:

$$H_2O_2 \text{ scavenging activity (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\%$$

Ferric Reducing Antioxidant Power (FRAP) Reduction Assay

FRAP reagent was prepared with a mixture of 10 mL of 300 mM acetate buffer, 1 mL of 2,4,6-Tris-(2-pyridyl)-5-Triazine(TPTZ)(Sigma Aldrich) 10 mM, 1mL of ferric chloride hexahydrate 20 mM (Merck 10943.0250), and 40 mM HCl. In the 96-well plate, 7.5µl of sample was mixed with 142.5µl of FRAP reagent and incubated at 37 °C for 30 minutes. A microplate reader was used to measure the absorbance value at a wavelength of 593nm (22,23).

Statistical Data Analysis

The DPPH scavenger, H_2O_2 scavenger, ABTS reduction, FRAP activity assays, and the median Inhibitory Concentration (IC_{50}) were calculated based on linear regression.

RESULT

Total Phenolic Content

This study showed a considerable amount of phenolic content in herbal tea, with 9.44µg GAE/100% concentration for TeNan tea and 0.82µg GAE/100% concentration for pineapple tea. The result was compared with phenolic content of telang tea from our previous study which was 16.20µg GAE/100% concentration (21). The data can be seen in Table 1. Telang tea had a moderate phenolic content, whereas pineapple tea had the lowest phenolic content, and telang tea had the highest phenolic content (Table 1).

Total Flavonoid Content

This result quantified that each sample had a considerable concentration of flavonoids. TeNan tea had 3.46µg QE/100% concentration and pineapple tea had 0.17µg QE/100% concentration. The result was compared with flavonoid content of telang tea from our previous study which was 4.88µg QE/100% concentration (21). TeNan tea had a moderate flavonoid content, while pineapple tea had the lowest flavonoid content, and telang tea had the highest flavonoid content (Table 1).

Table 1. Total phenolic and flavonoid contents of TeNan tea, telang tea, pineapple tea

Samples	Phenolic Content (µg GAE/100% concentration of tea)	Flavonoid Content (µg QE/100% concentration of tea)
TeNan tea	9.44	3.46
Telang tea*	16.20	4.88
Pineapple tea	0.82	0.17

*Data were obtained from a previous study (21)

DPPH Scavenging Activity

The IC_{50} values of the TeNan and pineapple tea samples were 22.22µg/100% concentration and 11.81µg/100% concentration. The result was compared with IC_{50} of telang tea from our previous study which was 17.07µg/100% concentration (21) (Table 2). Based on IC_{50} result, the pineapple sample showed the smallest value with the highest antioxidant activity against DPPH free radical.

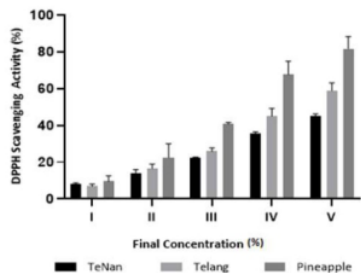


Figure 1. Effect of herbal tea formulation TeNan, telang and pineapple toward The DPPH scavenging activities. Each sample was diluted with ddH₂O to create the final concentrations. I : Each sample concentration of 2.5%; II : Each sample concentration of 5.0%; III : Each sample concentration of 10.0%; IV : Each sample concentration of 15.0%; V : Each sample concentration of 20.0%.

Note: The data of telang antioxidant activity were obtained from a previous study (21)

ABTS Reduction Activity

It was found that the ABTS reduction activities in the TeNan and pineapple tea samples had high percentages at the final concentration of 100%. The result was compared ABTS reduction activity of telang tea from our previous study (21). The percentages of ABTS reduction activity starting from the highest were TeNan tea, telang tea, and pineapple tea; the figure of ABTS reduction activity can be seen in Figure 2. The results are in line with the IC₅₀ value in Table 2.

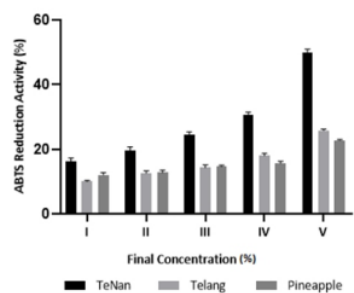


Figure 2. Effect of herbal tea formulation TeNan, telang and pineapple toward the ABTS reduction activities. Each sample was diluted with ddH₂O to create the final concentrations. I : Each sample concentration of 0.06%; II : Each sample concentration of 0.13%; III : Each sample concentration of 0.25%; IV : Each sample concentration of 0.50%; V : Each sample concentration of 1.00%.

Note: The data of telang antioxidant activity were obtained from a previous study (21)

H₂O₂ Scavenging Activity

All samples with the final concentrations showed high antioxidant activities against H₂O₂ reduction (Figure 3). The antioxidant activities for each sample by H₂O₂ scavenging method were TeNan tea < pineapple tea < telang tea based on the IC₅₀ value, where the sample of telang tea (26.62%) (21) had the strongest scavenging activity compared to pineapple tea (47.81%) and TeNan tea (96.22%). The H₂O₂ scavenging activities of TeNan tea, pineapple tea, and telang tea can be seen in Table 2.

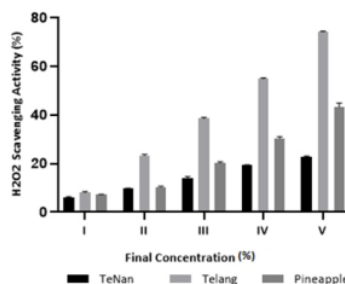


Figure 3. Effect of herbal tea formulation TeNan, telang and pineapple toward the H₂O₂ scavenging activities. Each sample was diluted with ddH₂O to create the final concentrations (5.0%, 10.0%, 20.0%, 30.0%, and 40.0%). I : Each sample concentration of 5.0%; II : Each sample concentration of 10.0%; III : Each sample concentration of 20.0%; IV : Each sample concentration of 30.0%; V : Each sample concentration of 40.0%.

Note: The data of telang antioxidant activity were obtained from a previous study (21)

Table 2. The IC₅₀ values and antioxidant activities of TeNan, telang and pineapple teas in various assays

Assays	Samples	Linear Equation	IC ₅₀ (% sample concentration)	R ²	The highest average of antioxidant activity (%)
DPPH Scavenging Activity	TeNan tea	y = 2.1261x + 2.7497	22.22	0.99	45.12±1.09
	Telang tea	y = 2.9396x - 0.1648	17.07	0.98	58.98±4.24
	Pineapple tea	y = 4.2042x + 0.3468	11.81	0.99	81.79±6.56
ABTS Reduction Activity	TeNan tea	y = 34.503x + 14.865	1.02	0.99	49.79±1.17
	Telang tea	y = 16.021x + 9.9844	2.51	0.99	25.86±0.34
	Pineapple tea	y = 10.976x + 11.355	3.39	0.99	22.77±1.08
H ₂ O ₂ scavenging activity	TeNan tea	y = 0.4729x + 4.499	96.22	0.99	22.76±0.39
	Telang tea	y = 1.8076x + 1.8895	26.62	0.99	74.29±0.18
	Pineapple tea	y = 1.0309x + 0.7077	47.81	0.99	43.30±1.66
FRAP Reduction Activity	TeNan tea	y = 7.1704x - 3.6624	7.48	0.99	47.76±3.14
	Telang tea	y = 8.9464x + 0.2577	5.56	0.99	45.47±0.24
	Pineapple tea	y = 2.2615x + 7.7779	18.67	0.99	19.56±2.82

Notes: The data was presented in the form of a mean and standard deviation. The tests were carried out in triplicate. Linear regression was used to compute the coefficient of regression (R²) and the IC₅₀ of each sample. The data of telang antioxidant activity were obtained from a previous study (21)

FRAP reduction activity

According to the result, the radical reduction activities of the samples were on the order of pineapple tea < TeNan tea < telang tea. The IC₅₀ values of the samples required to reduce FRAP are shown in Table 2. The IC₅₀ values of TeNan tea, telang tea, and pineapple tea were 7.48 %, 5.56 %, and 18.67%, respectively.

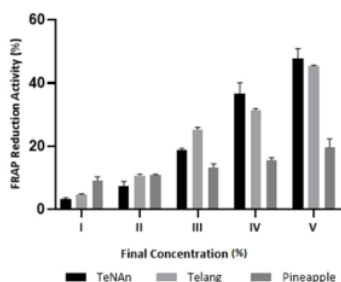


Figure 4. Effects of various concentrations of herbal tea formulations of TeNan, telang and pineapple teas toward the FRAP reduction activities. Each sample was diluted with ddH₂O to create the final concentrations (0.63%, 1.25%, 2.5%, 3.75%, and 5.0 %).

Note: The data of telang antioxidant activity were obtained from a previous study (21)



Figure 5. A. "Telang" Herbal Tea, B. "TeNan" Herbal Tea Formulation

DISCUSSION

The action of preventing the production of free radicals has been linked to phenolic and flavonoid compounds as phytochemical components with antioxidant characteristics (24,25). Entangling phenolic chemicals with a blue complex generated unanimously by decreasing Folin-reagent led to this discovery. The measurement of total flavonoids was carried out using the Aluminum Chloride Colorimetric method (26). The addition of aluminum chloride (AlCl₃) developed acid-stable complexes with C-4 keto groups and C-3 or C-5 hydroxyl groups of flavones and flavonols as do acid-labile complexes with ortho-dihydroxy groups of flavonoids (27). The calibration curve used was quercetin as a standard material.

The most frequent approaches for evaluating the antioxidant activity of a chemical or plant extract are ABTS, DPPH, FRAP, and H₂O₂ assays. The mechanism of

antioxidant activity includes the production of free radical species and their neutralization. DPPH is a free radical of the hydrogen radical group and it is often used in measuring antioxidant activity. The DPPH assay is generally used to predict antioxidant activity with antioxidant mechanisms as agents to inhibit lipid oxidation. DPPH free radicals will capture hydrogen atoms from samples with antioxidant components that reduce targets (28). In this way, the free radical scavenging capacity will be determined with maximum absorption at 517 nm (22). In addition to DPPH, the free radical commonly used to assess antioxidant activity is 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) which is often referred to as ABTS. ABTS reagent contains a potent oxidizing agent (e.g., potassium permanganate or potassium persulfate) which reacts with the ABTS salt to generate a solution. The ABTS assay, like the DPPH assay, involves the reduction of colored oxidants and includes an antioxidant activity test based on electron transfer (29). The H₂O₂ scavenging activity was determined using a slightly modified reaction technique involving ferrous ammonium sulfate and phenanthroline, which will form an orange Fe²⁺-tri-phenanthroline complex. Complexes are not formed if there is H₂O, that does not react with antioxidant compounds (30). The antioxidant potential of an extract with FRAP method is measured by its ability to decrease Fe³⁺-tripyridyltriazine to Fe²⁺-tripyridyltriazine. The assay is based on electron transfer processes in which potassium ferricyanide, a ferric salt, is utilized as an oxidant. The reduction of ferric 2,4,6-tripyridyl-s-triazine to the colorful ferrous form is the reaction mechanism (28).

Phenolic substances are potent antioxidants that are also safer to use than synthetic antioxidants. The antioxidant potential of the phenolic component is due to the presence of a benzene ring structure (31). In action, the mechanism of antioxidant activity that may occur in phenolic compounds can be through the mechanism of Hydrogen Atomic Transfer (HAT) (32). The results of the study revealed that telang tea had a high phenolic and flavonoid content, while pineapple and TeNan has a low phenolic and flavonoid content. This finding matched prior research, which found that telang flowers with a volume of 300 g in 1 l of ddH₂O contained 53 mg GAE/g sample total phenolic content, 11.2 mg CE/g sample total flavonoid content, and 1.46 mg cyanidin-3-glucoside equivalents/g sample total anthocyanins (33). Telang flower blue color anthocyanins are one anthocyanin source that contains stable blue color polyacrylated anthocyanins. Anthocyanins of telang flower improved its functional qualities, such as antioxidant and antibacterial properties (21,34). The research data also show that pineapple tea contains low phenolic and flavonoid contents (Table 1) and has low antioxidant activity (Table 2). Based on present study, the result was in accordance with previous studies which found that the total phenolic content of pineapple tea was 467.44 mg GAE/100 g fresh extract, 269.89 mg GAE/100 g dried extract (35), and 39.4 mg QE/g extract total flavonoid content (36).

In the present study, TeNan tea has the antioxidant activity due to ABTS and FRAP reduction activities in addition to having a good appearance (Figure 5). TeNan tea has the highest percentage in ABTS and FRAP reduction activities in the highest concentration compared to other treatments tea (Figure 2 and 4), and telang has the highest activity in H₂O₂ scavenging activity (Figure 3). The pineapple tea has

the highest percentage in DPPH scavenging activity (Figure 1), this result in line with previous studies which stated that the antioxidant capacity of pineapple extract using the phosphomolybdenum measurement method was 612.1% equivalent to ascorbic and 28.7-51.8% inhibition of bleaching of *-carotene* (37).

Telang has the lowest IC₅₀ in H₂O₂ and FRAP reducing activities compared to pineapple and TeNan tea (Table 2). The results were in line with prior studies which found that telang petals had an antioxidant activity with IC₅₀ values of 195.5 g/ml in the DPPH scavenging activity and 42.9 g/ml in the ABTS test, which protected human keratinocytes from H₂O₂-induced cytotoxicity (37). Based on Jeyaraj *et al.*, study, telang extract has the IC₅₀ value was 1.18mg/ml in the DPPH radical scavenging activity, and 19.8mg of GAE/g in the FRAP assay (38). However, telang tea is effective as an antioxidant (21) compared to TeNan formulation and pineapple, this may be due to pineapple has a high bromelain enzyme compared to telang. Bromelain has a broad specificity for protein cleavage and is stable over a broad pH range (pH 4–8). It generally cleaves the protein sites, but the specificity of cleavage may be altered by a change in pH. Bromelain in pineapple enhanced the total phenolic and flavonoid content and also antioxidant activities (39,40). Therefore, bromelain

enzyme in pineapple and TeNan tea may can suppressing of antioxidant activities.

From this study, it can be concluded that telang tea has the most total phenols and flavonoids (21), followed by TeNan and pineapple. TeNan tea has low-high antioxidant activity as indicated by the result of the DPPH, H₂O₂ scavenging activities, ABTS, and FRAP capacity. However, telang tea has stronger antioxidant activity (21) compared to pineapple and TeNan tea in FRAP and H₂O₂ assays.

ACKNOWLEDGEMENT

We thank profusely for the financial support from the *Bantuan Pendanaan Program Penelitian Kebijakan Merdeka Belajar Kampus Merdeka dan Pengabdian Masyarakat Berbasis Hasil Penelitian dan Purwarupa PTS 2021* Funding from the Ministry of Education and Culture of the Republic of Indonesia. This research was supported by Biomolecular and Biomedical Research, Aretha Medika Utama, Bandung, Indonesia. We would like to thank Medical Research Center, Faculty of Medicine, Maranatha Christian University and Faculty of Mathematics and Natural Sciences Education, Indonesian Education University which have assisted in carrying out this research well.

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