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The Antioxidant Test And Determination OfPhenolic Content In Packaged Green Tea Using The FRAP Method

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Abstract: The tea plant is one of the plants belonging to the Camellia genus which is widely spread in Southeast Asia. There is an assumption in the community that packaged green tea is good for consumption since green tea does not undergo fermentation, consequently it has a higher antioxidant activity. Polyphenols present in tea provide an antioxidant activity that can be used to prevent cardiovascular disease, cancer, inflammation, and diabetes. This study aims to examine the antioxidants and determine the phenolic content of packaged green tea using the FRAP method. This research was carried out using a simplified assay for phenolic content determination and using a UV-Visible spectrophotometer with folic ciocalteu reagent and using gallic acid as a comparison. Antioxidant activity was tested using the FRAP method with vitamin C as a comparison, which was measured using a UV-Visible Spectrophotometer. The total phenolic content contained in green tea extract A packaged was 0.2604 mg GAE/g green tea B packaged was 0.2656 mg GAE/g green tea extract C packaged was 0.2556 mg GAE/g. Packaged green teaextract A, packaged green tea B, and packaged green tea C had antioxidant capacities equivalent to ascorbic acid, namely green tea extract A 77.761 mg AAE/g, green tea extract B packaged 76.809 mg AAE/g and green tea extract C packaged which is equivalent to ascorbic acid, namely 74.904 mg AAE/g extract. The highest antioxidant value was green tea extract A packaged at 77.761 mg of Ascorbic Acid Equivalent/ gram of extract.

Keywords: Antioxidants, Green Tea, Phytochemical Screening, FRAP

INTRODUCTION

The tea plant is one of the plants belonging to the Camellia genus which is widely spread in Southeast Asia. The content of polyphenols contained in green tea has the potential as an antioxidant that can protect the body from free radical attacks. Free radicals are a form of reactive compounds, which are generally known as compounds that have unpaired electrons in their outermost shell (Winarsi, 2007). Free radicals play an important role in the occurrence of atherosclerosis, coronary heart disease, stroke, cancer, kidney failure, and the aging process in humans (Kumalaningsih, 2006; Youngsn, 2005).

The antioxidant potential of tea is said to be stronger than the antioxidants in vegetables and fruits. Where the content of polyphenol compounds that have the potential as antioxidants in vegetables and fruit is only about 0.25% and these components are generally present in a bound state or conjugated with sugar compounds, while the composition of polyphenol compounds in green tea consists of flavanol compounds, flavanols, flavanoids, and tamarind. Phenolic acid is estimated to be about 30% of the dry weight of green tea leaves. Polyphenols present in tea provide an antioxidant activity that can be used to prevent cardiovascular disease, cancer, inflammation, and diabetes (Tepphakorn *et.al*., 2013). There is an assumption in the community about packaged green tea is good for consumption related to the

availability and ability of antioxidant compounds in packaged green tea. Unfermented green tea has higher antioxidant activity. According to Daniells (2008), green tea contains 30-40% polyphenols.

The FRAP method is a method used to test antioxidants in plants. The advantages of the FRAP method are that it is inexpensive, the reagents are easy to prepare, and are quite simple and fast. This method can determine the total antioxidant content of a material based on the ability of antioxidant compounds to reduce Fe^{3+} ions to Fe2+ so that the antioxidant strength of a compound is analogous tothe reducing ability of that compound (Halvorsen *et.al*., 2002). The principle of the FRAP test is the electron transfer reaction from antioxidants to Fe^{3+} compounds. The Fe^{3+} compound itself representsan oxidizing compound that may be present in the body which can damage cells (Halvorsen *et.al*., 2002).

Based on the above information, an experiment was carried out to prove whether there were significant differences in antioxidant activity between several brands of packaged green tea using the FRAP method and its correlation with the phenolic content contained in them using Folin Ciocalteu reagent.

METHODS

Materials

The main ingredient used in this study was packaged green tea which was obtained around the city of Solo. Other materials used in this study were distilled water, ascorbic acid, 1% oxalic acid, 10% trichloroacetic acid (TCA), 0.1% FeCl3, phosphate buffer (0.2M pH 6.6), 1% potassium ferricyanide, K_3FE(CN) 6%, ethanol 96%.

Mathode

The samples were sorted between the leaves and stems of green tea and cut into small sizes, then weighed. Furthermore, the green tea is dried in the oven at 40 C for 10 minutes to dry. Packaged green tea that has dried can be marked by squeezing, where the dried leaves will be brittle. The dried Simplicia was weighed. Then it was blended into a rather fine powder and then sieved using mash 40 and then put in a tightly closed container and stored at room temperature 25 C (Winangsih, 2013)

Green tea extraction process Camellia sinensis sp. Packaging is done using the maceration method. The maceration method is a simple extraction method that is carried out by soaking simplicia in solvent for several days at room temperature protected from light (Damayanti and Fitriana, 2012). The advantage of this method is that the equipment used is quite simple.

Packaged green tea (Camellia sinensis sp.) powder of as much as 275 grams is carefully put into a maceration vessel with 2000 ml 96% ethanol solvent made with a ratio of 1:10, namely in 1 part of simplicia is included in 8 parts, then the extracted liquid is left for 5 days with stirred once a day. The macerate results were filtered using a flannel cloth. The filtered results were added again with 750 ml of 96% ethanol and allowed to stand for 2 days, this treatment is referred to as the maceration process. After that, the filtrate of packaged green tea (Camellia sinensis sp.) ethanol extract was obtained, moreover the extract was concentrated with a vacuum rotary evaporator at 80 rpm at 40 °C to obtain a thick extract.

Phytochemical screening of packaged green tea extract using Thin Layer Chromatography method. The analysis using TLC was carried out by applying the sample to the stationary phase (silica gel G60) that had been prepared. The bottling is done little by little until the dot is thick enough.

Identification of Alcaloid Compounds

The mobile phase used to identify alcaloid compounds was n-Hexane : ethyl acetate (6:4). A positive reaction is shown by some alcaloids giving blue or yellow fluorescence.

Identification of Flavonoid Compounds

The mobile phase-Hexane is used to identify Flavonoid compounds : ethyl acetate (6:4). A positive reaction is indicated by the formation of a yellow-brown stain after evaporation with ammonia in visible light and a blue color at UV 366 nm which indicates the content of flavonoids.

Identification of Terpenoid Compounds

The mobile phase used to identify terpenoid compounds is n-Hexane: ethyl acetate (6:4). With Liebermann Burchard reagent stain remover. A positive reaction is indicated by the formation of purple and green stains.

Identification of Phenolic Compounds

The mobile phase used to identify phenolic compounds was by using n-Hexane:ethyl acetate (6:4) solvent, then observed for spots in a UV lamp and sprayed with iron (III) chloride ($FeCl_3$) reagent. Positive for phenol if the stain is strong green, red, purple, blue, or black. Phytochemical screening of packaged green tea extract, using a test tube. The total phenolic content of packaged green tea was determined using the Folin-Ciocalteu reagent, a pipette for each green tea sample that had been diluted as much as 0.5 ml was added with 0.4 ml of Folin-Ciocalteau reagent, shaken and allowed to stand for 4-8 minutes, add 4.0 ml of 7% Na_2CO_3 solution, shake until homogeneous. Add sterile distilled water up to 10 ml and let stand for 2 hours at room temperature. Measure the absorbance at the maximum absorption wavelength of 750 nm which will give a blue complex. Repeat 3 times therefore the phenolic content is obtained as mg gallic acid equivalent/100 mg fresh sample. The total phenolic content in packaged green tea extract is expressed as mg of gallic acid equivalent per g of green tea extract. The absorbance value of the test solution is entered into the standard curve equation for gallic acid.

Identification of Saponin Compounds

Test using a tube, weigh 0.5 g of green tea extract and then dilute it with 5 ml of hot 96% ethanol solution. Placed in a test tube containing distilled water and then shaken to produce 3 cm high foam after adding 2 drops of 2 N hydrochloric acid, the foam did not disappear with a height of 2 cm for 30 seconds. The foam that is formed is due to the saponin compound having physical properties which are easily soluble in water and will cause foam when shaken.

Antioxidant Test with FRAP Method

The strength of antioxidant compounds in ferric-reducing packaged green tea was determined by the method that has been used. A total of 10 mg of sample was dissolved in 10 ml of 96% ethanol (1000 ppm), then pipetted 1 ml, added 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% K_3Fe (CN)6 after that, incubated for 20 minutes with a temperature of 50°C. After incubation, 1 ml of TCA was added and centrifuged at 3000 rpm for 10 minutes. After centrifuge, 1 ml of the top layer was pipetted into a test tube and added 1 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. The solution was allowed to stand for 10 minutes and the absorbance was measured by UV-Visible Spectrophotometry at 720 nm. As a blank, a mixture of oxalate solutions was used. FRAP value is expressed in mg equivalent of ascorbic acid (AAE)/ grams (g) of extract. In calculating the FRAP value obtained from the standard curve of Vitamin C (Ascorbic Acid).

RESULT AND DISCUSSION

Phytochemical Screening of Green Tea Extract

Phytochemical screening using the Thin Layer Chromatography (TLC) method. The analysis with TLC was carried out by spotting the sample on the stationary phase (silica gel G60) which had been prepared. The TLC test was carried out by spotting packaged green tea samples on a saturated silica gel plate. The plate was then put into the chamber and waited for it to be completely eluted. After being eluted, the plate was removed from the chamber and observed under visible light, UV 254 nm and UV 366 nm. for the Mobile Phase n-Hexane: ethyl acetate (6:4, % v/v) was used to test the content of Alcaloids (using Dragendorf's color reagent) and Flavanoids (using Cytoborate's reagent), Terpenoids (using Liebermann Burchard's reagent) and Phenolic (using $FeCl_3$ color reagent). The results of the phytochemical screening test for packaged green tea extract showed on **Table 1** for the same results:

Table 1. Phytochemical Screening of Green Tea Extract

Screening	Reagent	Result	Sample	Eluen	Rf Value	Note
Alcaloid	Dragendrof	Orange	A	3,8	0,63	Positive (+)
			B	3,8	0,63	Positive (+)
			C	4,6	0,76	Positive (+)
Flavanoid	cytoborat	Yellow Raddish	A	3,8	0,63	Positive (+)
			B	3,6	0,60	Positive (+)
			C	4,7	0,78	Positive (+)
Terpenoid	Liebermann Burchard	Green Bluish	A	3,8	0,61	Positive (+)
			B	3,7	0,63	Positive (+)
			C	3,3	0,55	Positive (+)
Fenolic	FeCl	Blue Blackish	A	3,8	0,63	Positive (+)
			B	3,7	0,61	Positive (+)
			C	3,5	0,58	Positive (+)
Phytochemical	Test Tube					
Saponin	Aquadest Acid 2N Chloride	Foam Appears	A	-	-	Positive (+)
			B	-	-	Positive (+)
			C	-	-	Positive (+)

* **To give ideal results, the compound to be determined must have an Rf value between 0.2-0.8.**

Alcaloid test results using Thin Layer Chromatography Mobile Phase n-Hexane: ethyl acetate (6:4, % v/v) green tea sample A with Rf 0.63, green tea sample B with Rf 0.63, green tea sample C with Rf 0.76, obtained an Rf value that showed positive in the Dragendorf reagent which was characterized by the formation of an orange precipitate and the formation of an orange precipitate in the Dragendorf reagent, it is estimated that nitrogen in the alcaloids will form coordinate covalent bonds with K^+ metal ions from potassium tetraiodobismutat to form a precipitated potassium alcaloid complex.

Test results for flavonoids using Thin Layer Chromatography Mobile Phase n-Hexane: ethyl acetate (6:4, % v/v) green tea sample A with Rf 0.63, green tea sample B with Rf 0.60, green tea sample C with Rf 0.78, which shows positive due to a yellow color change caused by a reaction with cytoborate. Reduction with magnesium and concentrated hydrochloric acid gives a reddish- yellow color.

Terpenoid test results using Thin Layer Chromatography Mobile Phase n-Hexane : ethyl acetate (6:4, % v/v) green tea sample A with Rf 0.61, green tea sample B with Rf 0.63, green tea sample C with Rf 0.55, which shows a positive result marked with a bluish green ring. The formation of bluish- green at the boundary of the two solvents is a positive result for the presence of steroids with the addition of Liebermann Burchard's reagent. This color change is caused by an oxidation reaction in the steroid group through the formation of conjugated double bonds.

Phenolic test results using Thin Layer Chromatography Mobile Phase n-Hexane : ethyl acetate (6:4, % v/v) green tea sample A with Rf 0.63, green tea sample B with Rf 0.61, green tea sample C with Rf 0.58, which showed positive which was indicated by the presence of a blue-black color change with the addition of $FeCl_3$ reagent. The color change caused by phenol reducing Fe^{3+} to Fe^{2+} is marked with a blue-black color (iron (III) hexacyanoferrate) (Hanani, 2015).

The results of the saponin test were positive because the sample formed foam. Using distilled water and 2 N hydrochloric acid. The highest foam index is green tea A 240 and the second is green tea B 220 and the third is green tea C 220. A Saponin is a form of glycoside which has the ability to form foam in water which shows a positive result.

The **Table 1** above results show, that Green Tea A, Green Tea B, and Green Tea C contain alcaloids, flavonoids, tannins, terpenoids, and saponins. All green tea packages A, B, and C have good rf values, namely, green tea also has potential as an antioxidant in the presence of compounds that have potential as antioxidants, namely flavonoids and phenolics. These compounds act as free radical scavengers because the hydroxyl groups they contain can donate hydrogen to free radicals therefore free radicals can be changed to non-radicals (Silalahi, 2006).

Total Phenolic Compound Test with UV-Visible Spectrophotometry Using Folin-Ciocalteu Method

Phenolic compounds contribute to antioxidant activity. The potential of phenolic compounds as antioxidants is caused by the presence of hydroxyl groups in phenolic compounds. The hydroxyl group can function as a hydrogen atom donor when reacting with radical compounds through an electron transfer mechanism moreover the oxidation process can be inhibited. Therefore, the estimation of total phenolic content is intended to determine the number of phenolic compounds in packaged green tea extracts that have antioxidant activity (Duh, Tu, and Yen, 1999).

Phenolic compounds will undergo oxidation to form phenolic ions, while the Folin-Ciocalteu reagent will be reduced to form phosphotungstate-phosphomolybdate complexes and finally form molybdenum blue complexes. The darker the blue color formed, the more reduced the phosphotungstate-phosphomolybdate complex. The whole reaction takes place in an alcaline condition which is obtained by adding sodium carbonate. The total phenolic content was expressed as the mass equivalent of gallic acid. Gallic acid was chosen because it is a pure and stable substance. In addition, gallic acid is a phenolic compound with three phenolic hydroxy groups which are known to have antioxidant activity.

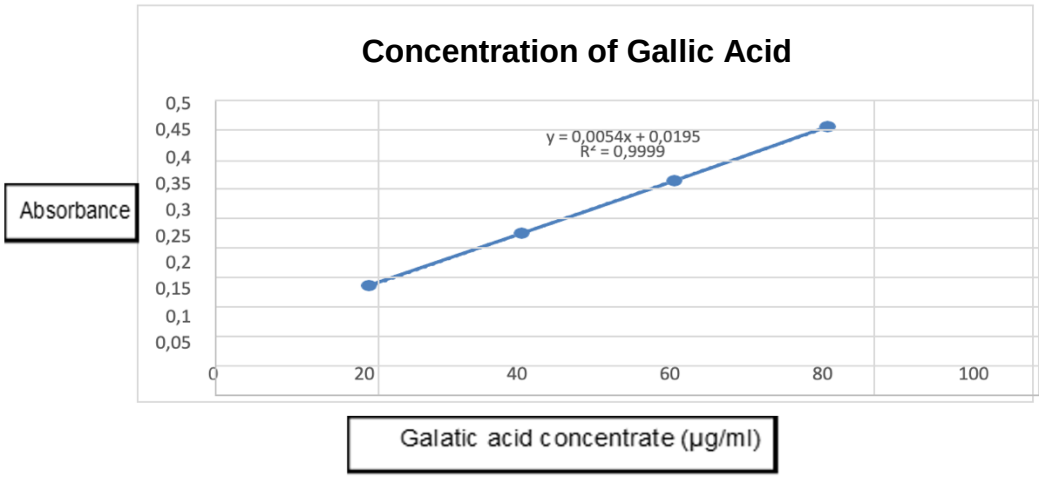

Figure 1. Selected standard curve for the determination of total phenolic content of gallic acid

Preparation of gallic acid standard curve (Figure 4.1). The linear regression equation used is the replication equation y = 0.0054x + 0.0195 r = 0.9999 with the best r-value, so that the average total phenolic content of Green Tea extract (Tables 2.8, 2.9, 2.10) is 0. 2656 ± 0.51 mg gallic acid equivalent (GAE) per gram Green Tea extract A of 0.2556 ± 0.50 mg gallic acid equivalent (GAE) per gram Green Tea extract B, amounting to 0.2538 ± 0.50 mg equivalent gallic acid (GAE) per gram of Green Tea extract C.

Table 2. Results of Determination of Total Phenolic Amount of Green Tea A Wavelength 750nm

Replication	absorbance	Content Phenolic (µg/ml)	Content Total Phenolic (mg)	$\bar{x}$± SD
1	0.173	28,42	0.2651	0.2656 ± 0.51
2	0.173	28,42	0.2651	
3	0.174	28,61	0.27	

Table 3 Results of Determination of Total Phenolic Amount of Green Tea B Wavelength 750nm

Replication	absorbance	Content Phenolic (µg/ml)	Content Total Phenolic (mg)	$\bar{x}$± SD
1	0.168	27.50	0.2486	0.2556 ± 0.50
2	0.171	28.05	0.2631	
3	0.172	28,24	0.2553	

Table 4. Results of Determination of Total Phenolic Amount of Green Tea C Wavelength 750nm

Replication	absorbance	Content Phenolic (µg/ml)	Content Total Phenolic (mg)	$\bar{x}$± SD
1	0.170	27,87	0.2510	0.2538 ± 0.50
2	0.172	28,24	0.2544	
3	0.173	28,42	0.2560	

Total Phenolic Compound Test with UV-Visible Spectrophotometry Using FRAP (Ferric Reducing Antioxidant Power) Method

FRAP is an analytical method commonly used to measure the strength of antioxidants in reducing Fe^{3+} to Fe^{2+} and the color changes from yellow to blue. Fe^{2+} it self is colorant and Fe^{3+} is a free radical. The standard solution used is ascorbic acid which is used as a comparison since it functions as a secondary antioxidant, namely capturing free radicals and preventing chain reactions. The addition of TCA so that the potassium complex precipitates. The addition of Fe^{3+} also aims to form green to blue complexes.

1. Wavelength Determination
   Next, the maximum absorption wavelength was determined using vitamin C at a concentration of 60 µg/ml (60 ppm), then mixed with 1 ml of 0.2 M phosphate buffer (pH 6.6) and1 ml of 1% potassium ferricyanide, the mixture was incubated at 50ºC for 20 minutes. After completion of incubation, 1 ml of trichloroacetic acid solution was added, then centrifuged at 3000 rpm for 10 minutes. Take the top layer of the solution as much as 1 ml then add 1 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$, the aim

is to find out at what absorption the substance is read by the UV spectrophotometer optimally resulting in maximum absorption of 720 nm. The results of measuring the maximum absorption of vitamin C can be seen in **Figure 2**.

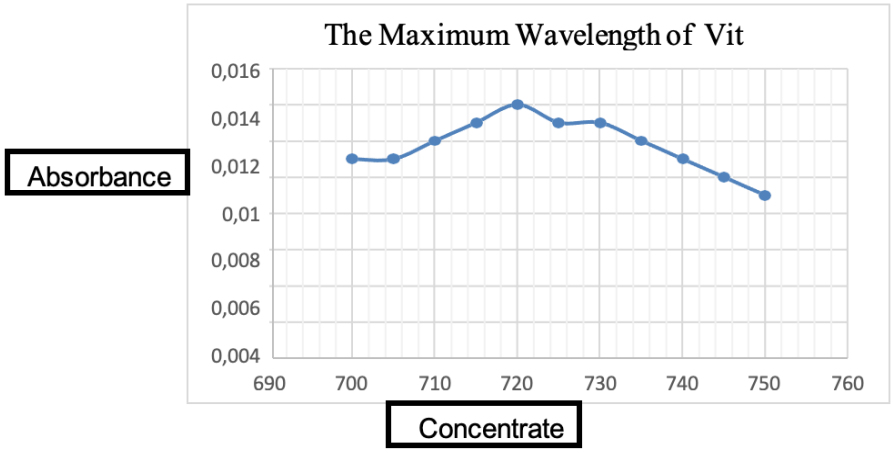

Figure 2. The Maximum Wavelength of Vitamin

2. Determination of Operating Time
   Next, determine the maximum operating time using vitamin C at a concentration of 60 µg/ml (60 ppm) with 1 ml of the solution taken and then mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide 1 %, the mixture was incubated at 50°C for 20 minutes.

Table 5. Maximum Wavelength Curve of Vitamin C (720 mm)

t (minute)	absorbance
1	0.009
2	0.010
3	0.012
4	0.013
5	0.014
6	0.015
7	0.016
8	0.017
9	0.018
10	0.017
11	0.017
12	0.016

After completion of incubation, 1 ml of trichloroacetic acid solution was added, then centrifuged at 3000 rpm for 10 minutes. Take the top layer of the solution as much as 1 ml and then add 1 ml of distilled water and 0.5 mL of 0.1% $FeCl_3$, which aims to determine the stable measurement time (optimal stability). The stability of the product compound is known by observing the absorbance starting from the time it is reacted until a stable absorption is achieved. In this study, the time interval used was 12 minutes using a wavelength of 720 nm. The measurement results obtained operating time in the 9th minute.

3. Determination of Calibration Curve
Determination of the calibration curve was carried out by measuring the absorbance of vitamin C at a concentration of 60 µg/ml; 70µg/ml; 80µg/ml; 90µg/ml; and 100 µg/ml at a maximum wavelength of 720 nm. The results of the vitamin c calibration curve can be seen in **Figure 3**.

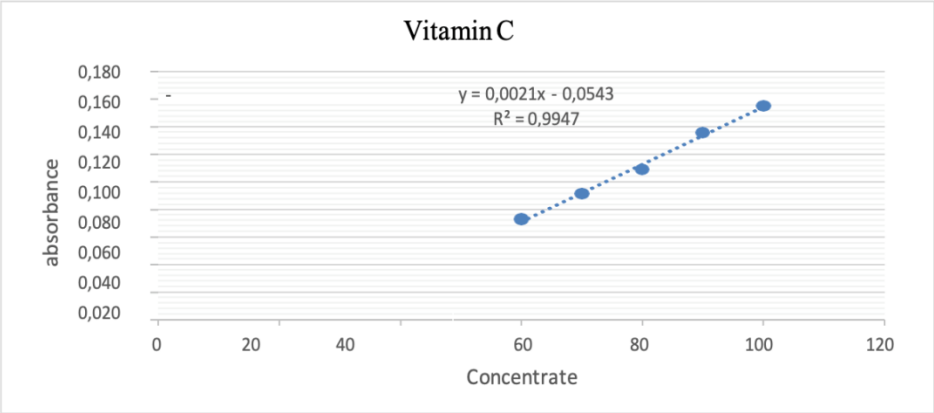

Figure 3. Calibration Curve of Vitamin C

From the results the obtained r value is 0.9947 with the linear regression equation y = 0.0021x + 0.0543. The next step is to calculate the antioxidant activity expressed in mg equivalent of ascorbic acid/g extract (AAE). The results of determining the levels of Antioxidants (Ascorbic acid Equivalent Antioxidant Capacity) can be seen in **Table 6, 7 and 8**.

Table 6. The Data on AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) Value of Green Tea A

Sample	Absorbance	Antioxidant Activity (mgAAE/g Sample	X ±SD
Etanol Extract	0,108	77,285	77.761±8.81
	0,109	77,761	
	0,110	78,238	

Table 7. The Data on AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) Value of Green Tea B

Sample	Absorbance	Antioxidant Activity (mgAAE/g Sample	X ±SD
Etanol Extract	0,106	76,333	76.809±8.76
	0,107	76,809	
	0,108	77,285	

Table 8. The Data on AEAC (Ascorbic Acid Equivalent Antioxidant Capacity) Value of Green Tea C

Sample	Absorbance	Antioxidant Activity (mgAAE/g Sample	X ±SD
Etanol Extract	0,102	74,428	74.904±8.65
	0,103	74,904	
	0,104	75,380	

4. Preparation of Standard Solutions
The standard solution used is ascorbic acid. Ascorbic acid is used as a comparison because it functions as a secondary antioxidant, which captures free radicals and prevents chain reactions from occurring. Vitamin C is a secondary antioxidant that can counteract various extracellular free radicals. This is since vitamin C has free hydroxy groups which act as free radical scavengers and if it has polyhydroxy groups it will increase antioxidant activity.

Measurement of antioxidant activity using this FRAP test with an ascorbic acid solution as a standard. The addition of TCA aims to precipitate the potassium ferrocyanide complex.

The addition of $FeCl_3$ also aims to form green to blue complexes (Berlin blue). Reduction power is an indicator of the potential of an antioxidant compound. Reducing power in this case is measured by the ability of an antioxidant to convert Fe^{3+} to Fe^{2+}.The reaction that occurs:

$K_3Fe(CN)_6$ (Potassium Ferrycyanide) $\longrightarrow$ $K_4Fe(CN)_6$ (Potassium Ferrocyanide)

$Fe^{3+} + e^- \longrightarrow Fe^{2+}$(Reduction)

Compounds that have reducing power may act as antioxidants because they can stabilize radicals by donating electrons or hydrogen atoms therefore the radical compounds become more stable. The ability to reduce power (Fe^{3+}) with the FRAP method which can describe antioxidant activity has limitations in the FRAP method in general it will describe high antioxidant activity in compounds that are polar compared to those that are nonpolar

5. Antioxidant Test with FRAP method
   After getting the linear regression equation y = 0.0021x + 0.0543. This absorption curve is made by plotting the absorbance value on the Y-axis and the concentration on the X-axis. For the parameter of a linear relationship, the correlation coefficient r is used in linear regression: y = ax + b To calculate the antioxidant activity capacity, the absorbance value of the sample is entered into the vitamin C linear regression equation. The FRAP value is expressed in mg ascorbic acid equivalent (AAE)/g extract.

CONCLUSION

Based on the phytochemical screening results of green tea extract A, green tea B, and green tea C, secondary metabolites contained in packaged green tea extract showed a positive (+) presence of phenolic content. The total phenolic content contained in Packaged Green Tea Extract A was 0.2604 mg GAE/gram, Packaged Green Tea Extract was 0.2656 mg GAE/gram, and Packaged Green Tea Extract was 0.2556 mg GAE/gram. The highest total phenolic value was Green Tea Extract A Pack of 0.2538 mg GAE/gram. Antioxidant levels contained in green tea extract A was 77.761 mg AAE/gram extract, green tea extract B was packaged at 76.809 mg AAE/gram extract and green tea extract C was packaged at 74.904 mg AAE/gram extract.

AUTHOR CONTRIBUTION

The author are greatful to all of laboratory assistance. Special thanks to my research team and Duta Bangsa University for the support and assistance to finalize this project

CONFLIC OF INTEREST

The declare that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest

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Be delivered saying thanks to researcher and passion researching for more other themes interesting in the future.

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