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Comparison of Antioxican Tea Potential (*Camellia Sinensis*) between Green Tea and Black Tea in Datingradical 2,2“Diphenyl-1-Picrylhydrazyl”(Dpph)

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ABSTRACT

At present, two types of tea drinks are most popular in the world namely black tea and green tea. The main difference between the two types of tea is whether or not the fermentation process is carried out. Because the manufacturing process is not the same, the taste, aroma, and ingredients are also different. This study aims to compare the antioxidant potential between 2 packs of green tea and black tea by calculating IC50 values against DPPH radicals. Measurement of antioxidant activity using the DPPH method (2,2 diphenyl-1-picrylhydrazyl) with positive control used as ascorbic acid. Radical capture activity was measured as a decrease in absorbance of DPPH with a microplate reader at a wavelength of 520 nm..The results of phytochemical screening in the flavonoid and phenolic tests showed positive results in both samples, namely giving a red color change in the flavonoids and a green color in the phenolic test. As for the IC50 value (µg / mL) of green tea and black tea and positive ascorbic acid control obtained respectively:10,804; 25.79; 2.61. The results of the antioxidant potential of the green tea IC50 value is better because it is smaller in value than black tea because the smaller the IC50 value, the stronger the potential of sambal in counteracting free radicals.
Keywords: Tea packaging, DPPH, Phytochemicals, Antioxidants

PRELIMINARY

Scientific advances have found that many factors cause premature aging, one of which is genetic, lifestyle, environmental, gene mutase, immune system damage and free radical damage. (Zuhra, et al., 2008). This is supported by the increasingly severe

environmental conditions and changes in food consumption patterns that occur in the community. The change is a change from traditional food consumption patterns that contain starch (complex carbohydrates) and fiber into modern consumption patterns with high protein, fat, sugar, and salt content but low in fiber content. As a result, various types of diseases arise including obesity and obesity, coronary heart disease, hypertension, diabetes mellitus, and cancer that the number of sufferers from time to time is increasing (Ananda,

Public awareness of the emergence of various diseases above raises the attitude to re-improve their consumption patterns to maintain health. The slogan "return to nature" (back to nature) is currently one alternative that is sought after and consumed by the public in general (Ananda, 2009). One product that can overcome the presence of free radicals in the body, ranging from beverage products to food supplements. Manufacturers in addition to using basic ingredients that contain antioxidants also use a combination of several ingredients that contain antioxidants.

Tea (Camellia sinensis) is a beverage that is widely consumed by people throughout the world, including in Indonesia. About 20–22% of the tea produced and consumed worldwide is green tea and black tea because it contains a bioactive component called polyphenols. Phenol compounds can prevent oxidation. In general, polyphenols in plants consist of flavonoids. Flavonoids are the largest class of polyphenols which are also very effectively used as antioxidants (Kusmiyati., Et al, 2015)

Currently, various types of processed tea products have been widely circulating in the market, including ready-to-drink beverages in packaging. Green tea and black tea are now widely produced in the form of packaging to make it more practical with a variety of brands on the market. Green tea and black tea drinks in an instant form are one of the diversification efforts of tea products that have been developed at this time. The advantages of instant green tea and black tea are more practical both in terms of packaging, easy way of serving, and also this tea is more durable because it is in the form of powder.

This study uses samples of green tea and black tea packaging of a particular brand obtained at one of Pekanbaru supermarkets and will be tested by comparing their activities using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, with the hope of providing information to the wider community of the potential between both in counteracting free radicals, in this case using DPPH as a source of radicals, the reason for using this method is because it is fast, easy, and has a high level of accuracy. in addition to this method also uses samples in small or small amounts.

The plant (taxonomic) classification of tea is:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Ericales
Family : Tehaceae
Genus : Camellia
Species : *Camellia sinensis*

Tea Plants(Source: Shah, 2006)

Based on the above background the authors are interested in conducting research that is a comparison of the antioxidant potential of Tea (*(Camellia sinensis)* between types of green tea and black tea in counteracting the radical 2,2 diphenyl-1-picrylhydrazyl "(DPPH).

MATERIALS AND METHODS

Material

The sample used in this study was 2 packets of tea namely green tea and black tea from two different brands obtained from one of the Riau Street Pekanbaru supermarkets. The materials used are methanol, ascorbic acid, distilled water, aluminum foil, HPLC grade methanol. The tools used are analytical balance and an LB-941 model berthold reader.

Method

A. Phytochemical Test of Tea Samples

1. Phenolic Test

Three drops of a sample are added to the drops and 1-2 drops of 1% iron (III) chloride solution are added. If a strong green, red, purple, blue or black color means that there are phenolic compounds (Khotimah, 2016).

2. Flavonoid Test

A sample of five drops is put into a test tube then added 1-2 small pieces of metal magnesium and a few drops of concentrated hydrochloric acid. The color change indicates the presence of flavonoid compounds(Marliana, 2005).

B. Antioxidant Test

The antioxidant activity test was carried out using a microplate reader using the 2,2-diphenyl-1-picrylhidrazyl (DPPH) method at a wavelength of 520 nm. A sample of 2 mg was dissolved in 2 mL of methanol so that the sample concentration became 1000 µg / mL. Row A is sampled as much as 100 µL (plates consist of row A - H each totaling 12 wells). A total of 50 µL of methanol was added to each well in lines B - F. Row A was piped as much as 50 µL and put into line B, line B was pipetted 50 µL put into line C and the same was done until row F, row F was pipetted 50 The µL was then discarded so that the concentration of 1000 µg / mL, 500 µg / mL, 250 µg / mL, 125 µg / mL, 62.5 µg / mL and 31.25 µg / mL. Whereas specifically on the G-H line filled with 50 µL methanol, specifically on the H line filled with only 1-6 wells. Row A - G was added as much as 80 µL DPPH with a concentration of 40 µg / mL, then incubated for 30 minutes. Radical capture activity was measured as a decrease in absorbance of DPPH with a microplate reader, as a comparison namely ascorbic acid with a concentration of 50 µg / mL. (Almurdani, et al, 2013).

RESULTS AND DISCUSSION

Results

A. Phytochemicals:

Based on the research that has been done, the following results are obtained: phytochemical tests are carried out namely the flavonoid test and phenolic test results that can be seen in table 4.1:

Table 1. Phytochemical Screening Results

Srining Phytochemistry	Reactor	Sample	Discoloration	Results
Phenolic	FeCl3 1%	Green Tea	Green	+
		Black Tea	Green	+
Flavonoids	Concentrated Mg + HCl powder	Green tea	Red	+
		Black Tea	Red	+

B. Antioxidant

Antioxidant Activity Test Results from samples for methanol extract of green tea leaves and black tea packaging as shown in the following table:

Table 2% of Obstacles to Green Tea

Extract Concentration (µg / mL)	Ln Concentration (X)	% Of Obstacles (Y)
1000	6,908	94.0748
500	6,215	87,7339
250	5,521	79.2100
125	4,828	74,0125
62.5	4,135	68,9189
31.25	3,442	59,1476

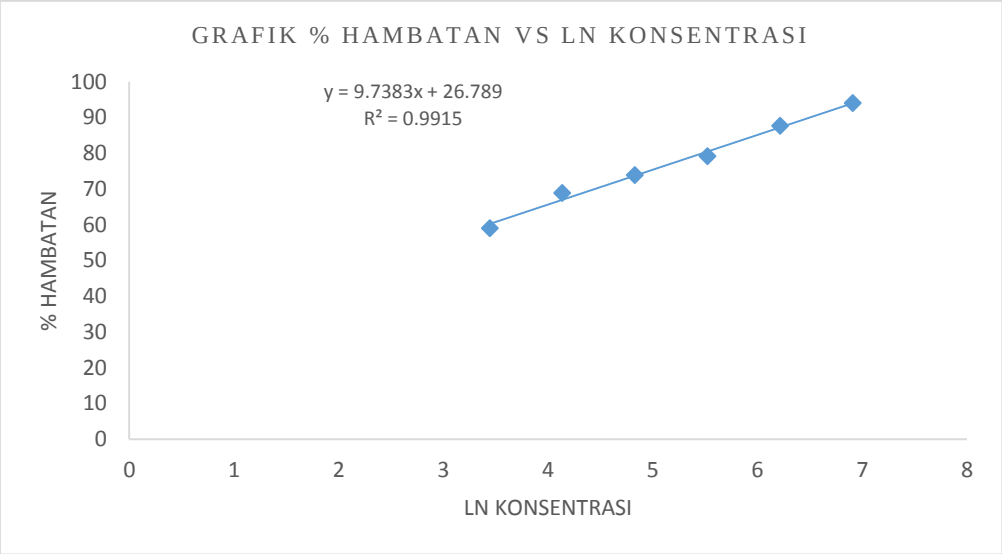

Figure 1. Graph of the relationship of percent inhibition and Ln concentration

IC calculation50
Linear regression equation:
Y = 9.7383X + 26.789
50 = 9.7383 Ln X + 26.789
LnX = 2.38
X = 10,804
IC50 = 10.804 µg / mL
Table 3.% Obstacles vs Ln Concentrations in Black Tea

Extract Concentration (µg / mL)	Ln Concentration (X)	% Of Obstacles (Y)
1000	6,908	90,2993
500	6,215	83,4881
250	5,521	74,2002
125	4,828	68.9370
62.5	4,135	60.4747
31.25	3,442	51.0836

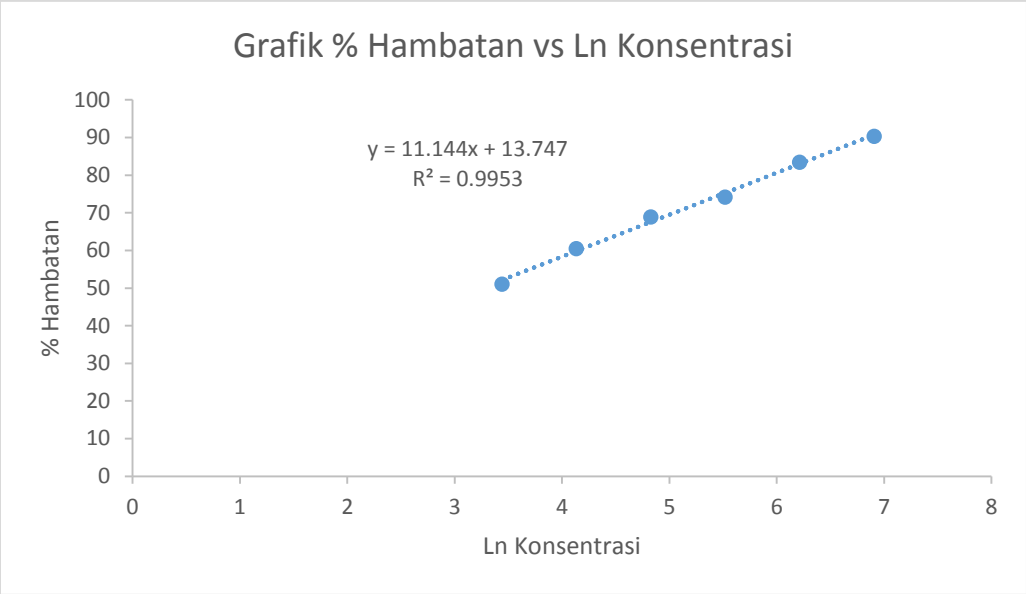

Figure 2. Graph of the relationship of percent inhibition & Ln concentration of Black tea

IC calculation50
Linear regression equation:
Y = 11,144X + 13,747
50 = 11,144LnX + 13,747
LnX = 3.25
X = 25.79
IC50 = 25.79 µg / mL

Table 4.% Obstacles vs Ln Concentration of Ascorbic Acid

Extract Concentration (µg / mL)	Ln Concentration (X)	% Of Obstacles (Y)
100	4,605	99,5776
50	3,912	93,5586
25	3,219	86.2724
12.5	2,526	81,0982
6.25	1,833	74,4456
3,125	1,138	69,0601

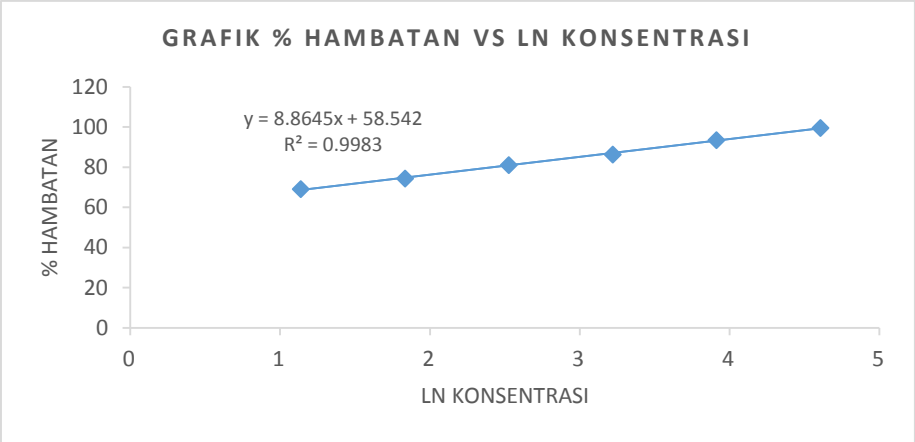

Figure 3. Graph of the relationship of percent inhibition and Ln ascorbic acid concentration

IC calculation50
Linear regression equation:
Y = 8.8645X + 58,542
50 = 8.8645Ln X + 58.542
LnX = 0.96
X = 2.61
IC50 = 2.61 µg / mL

To see the potential and strength of antioxidants seen in the following table:

Table 5. The level of antioxidant strength (Jun M, 2006)

Antioxidant intensity	IC50 value (µg / mL)
Very strong	<50
Strong	50-100
Is	100-250
Weak	250-500
Not active	> 500

Discussion:

The sample used in this study was packaged tea from 2 types of tea namely green tea and black tea obtained from supermarkets. Stages of sample preparation include weighing, to get the weight we want, the process of dissolving using methanol solvent then soaked for 2X24 hours. Samples that have been left standing are then filtered and then the extraction process is continued using a rotary evaporator. Tea extraction process uses the maceration method. The maceration method in this study uses methanol solvent pa. The extracted result is concentrated with a rotary evaporator with the principle of the

process of separating the extract from the solvent. Phytochemical test is a qualitative test to determine the active compounds contained in the sample. The basic principle of a color testing reaction with a color reaction.

Phytochemical analysis is carried out, namely phenolic test and flavonoid test. In the phenolic test using FeCl reagents3 1% will change the color of the sample to green which indicates the presence of phenolic in the packaged tea sample. In the flavonoid test with concentrated Mg + HCl powder reagents there was a color change in the sample to a red color indicating the presence of flavonoids in packaged tea samples (Putranti, 2013).

The phytochemical screening results from the 2 packages are as shown in Table 1. The results show positive results showing the color formed for phenolic giving green color while the flavonoid test gives red color. One of the most commonly used methods to test antioxidant activity is to use the free radical 2.2 diphenyl-1- picryhydrazil (DPPH). Measurement of antioxidants using the DPPH method is a method of measuring antioxidants that is simple, fast and does not require a lot of reagents. The measurement of anti-oxidant activity of the DPPH method uses spectrophotometry with a wavelength of 520 nm (Putranti, 2013).

The sample concentrations used were made in several concentrations namely 1000 µg / mL, 500 µg / mL, 250 µg / mL, 125 µg / mL, 62.5 µg / mL and 31.25 µg / mL. The presence of antioxidant activity will be marked by a change in color from purple to yellow. Measuring uptake can be observed using spectrophotometry so that the free radical reduction activity by the sample can be determined (Putranti, 2013). IC value50 is a concentration of antioxidant compounds that give the picture that with certain concentrations can inhibit free radicals by as much as 50%. The positive control used was pure ascorbic acid obtained by IC50 at 2.61 µg / mL. Whereas if compared. Its antioxidant activity is very strong because it is a pure compound.

In the sample of tea packaging as seen in the IC50 calculation also gives very strong results namely: for the packaging of green tea it has IC50 = 10.804 µg / mL, whereas for black tea IC50 = 25.79 µg / mL of the value of both packages has a very

strong antioxidant level as well, the antioxidant strength when measured from the IC50 value is as shown in Table 5 that both tea samples both green and black tea samples have very strong antioxidants. However, if compared between 2 packages the green tea is stronger than Black tea because the smaller the IC50 value, the stronger the sample is because with a small IC50 value in accordance with its definition that even a small concentration can inhibit free radicals by 50%.

Another reason why black tea has lower antioxidants compared to green tea is because black tea is processed from the leaves of the roasted tea plants and dried in the sun first and then carried out the fermentation process. Therefore, causing its antioxidant content to be reduced when compared to green tea it is done without drying so that the antioxidant levels of green tea remain intact and well preserved this is when compared to the antioxidant levels of the two types of tea but when compared to other benefits between the two having compound content another which is good for health.

CONCLUSION AND SUGGESTION

Conclusion

Based on the results of this study, it can be concluded that:

1. IC50 Green Tea: 10.804 µg / mL
2. IC50 Black Tea: 25.79 µg / mL
3. IC50 ascorbic acid for: = 2.61 µg / mL
4. Both of these tea packages have very strong antioxidant potential.

Suggestion

Other content tests are needed for both types of tea packaging and producers can also list the IC50 values of their packaging products.

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