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Determination of Catechin Content in Green and Black Tea (*Camellia sinensis* L.) by UV-Vis Spectrophotometric Method

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Abstract

Tea is one of the most commonly consumed beverages around the world. The chemical compounds contained in tea have a good effect on health because they contain polyphenolic compounds, catechins. This study examined the catechin compound content of black tea and green tea from Karanganyar (A) and Malang (B). This research aims to determine the differences in catechin levels in black and green tea types in Malang, East Java, and Karanganyar, Central Java plantations. The research method used was experimental. Identification of catechin content was done using UV-Vis Spectrophotometry method. Tea is extracted by brewing, 4 grams of green tea is brewed using distilled water for 6 minutes, while 5.6 grams of black tea is brewed using distilled water for 10 minutes with the comparison used is pure catechin solution. The results obtained for green tea Karanganyar (A) had a catechin content of 3.61±0.083%, green tea Malang (B) had a level of 2.97±0.037%, black tea Karanganyar (A) had a level of 3.14±0.065%, and black tea Malang (B) had a level of 1.60±0.009%. It is concluded that the catechin content of green tea is higher than black tea, and of the two samples, the high catechin content comes from Karanganyar (A).

Keywords: catechins, green tea, black tea, malang, karanganyar

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1 Introduction

Tea is one of the most widely consumed beverages after water and is the most popular drink in the world because of its taste, aroma, and health benefits. Tea comes from the genus Camellia and the species Camellia sinensis (L.) which is processed from the young leaf shoots of the plant. Tea is divided into three main groups, namely green tea, oolong tea, and black tea, which are distinguished by the level of oxidation and fermentation. Green tea is a type of tea that is processed without going through a fermentation process, oolong tea is processed through partial fermentation, and black tea is processed through full or perfect fermentation [1]. The three types of tea come from the same plant but have significant differences in their bioactive compounds, namely polyphenols.

Polyphenolic compounds have health benefits, especially flavonoids which are the largest class of compounds that have cardioprotective effects, namely strong antioxidants. One of the flavonoid compounds that has high antioxidant activity is catechins. Catechin is a secondary metabolite that has antioxidant properties. Catechin is a polyphenol compound that belongs to the flavonoid family [2]. Catechins can function as scavengers of free radicals such as singlet oxygen compounds, hydroxyl radicals and peroxynitrite [3]. Catechins are the main constituent of tea leaves. Catechin is a bioactive compound with a flavan-3-ol framework and is the main compound that determines quality and can give a distinctive bitter taste to tea [4].

Catechins are known to be effective in reducing the risk of cardiovascular disease, diabetes, weight loss, anti-inflammatory, antiviral and antibacterial. The highest catechin content, (-)-epigallocatechin-3-gallate (EGCG), was found to be strongly associated with reduced risk of metabolic diseases by increasing fat oxidation and improving energy utilization. The bioactive content of different types of tea is influenced by plant varieties, growing conditions, and brewing methods [5].

Brewing is the process of separating one or more components using a water solvent. The brewing technique is quite useful to produce maximum antioxidant compounds. Green tea brewing is done by weighing 4 grams of tea brewed in a glass with 220 mL of boiling water and waiting for 10 minutes [6], while the black tea brewing process by weighing 5.6 grams brewed in a glass with 280 ml of boiling water and waiting for 6 minutes [7].

Research on the determination of catechin levels on commercial green tea (Camelia sinensis L.) extracted in two ways, namely maceration using 70% ethanol solvent and by brewing, obtained the highest catechin content in extraction by brewing, namely 6.01 ± 0.06% [8]. Another study determined the catechin content of black tea (Camelia sinensis L.) by maceration from local Balinese plantations, resulting in catechin levels of 8.37 ± 0.13% [9].

Based on this background, this study was conducted to determine the differences in catechin levels in black and green tea types in Malang, East Java and Karanganyar, Central Java plantations. From this study, it is expected that scientific information can be obtained about catechin levels from the various tea plantations.

2 Methods

2.1 Sample Collection

Samples of green and black tea leaves were obtained from tea plantations in Malang and Karanganyar. The samples taken were in the form of dried simplicia from tea plants. This tea sample was taken directly from the tea producer.

2.2 Simplicia Tea Water Content Test

2 grams of dried simplicia is placed on an aluminum foil plate (special) then inserted into the Halogen Moisture Analyzer, so that the

water content of the tea sample can be determined.

2.3 Extraction by Brewing

Brewing green tea is done by weighing 4 grams of tea leaves, then brewing it in a glass with 220 mL of boiling water and waiting covered for 10 minutes [6]. Meanwhile, the process of brewing black tea is done by weighing 5.6 grams, then brewing it in a glass with 280 mL of boiling water and waiting covered for 6 minutes [7]. The brewed tea is filtered using filter paper to obtain a filtrate. Each brewing was replicated 3 times.

2.4 Qualitative Test of Catechins

2 mL of each filtrate was taken and put into a test tube then added with 10% $FeCl_3$ (ferric chloride) solution, if a purple green or black color was formed then the result was positive for containing catechin. Next, in the same way, using Stiasny's reagent (formaldehyde 37% and concentrated HCl 2:1) is added to the test tube containing each sample solution and heated. If a red-orange precipitate forms then the sample is positive for containing gallocatechin [10].

2.5 Determination of Catechin Levels by UV-Vis Spectrophotometry

2.5.1 Determination of Catechin Standard Solutions

Weighed 10 mg of pure catechin, dissolved it using ethanol p.a. solvent in a 10 mL volumetric flask to obtain a pure catechin solution concentration of 1000 ppm, then diluted to 100 ppm by taking 1 mL of 1000 ppm pure catechin solution and dissolved it with ethanol p.a. in a 10 mL flask. . After that, a concentration series of 20, 30, 40, 50, 60, 70, and 80 ppm was made in a 5 mL flask up to the limit mark [9].

2.5.2 Determination of Maximum Wavelength

Determination of the maximum wavelength of catechins was carried out by measuring a standard solution of 100 ppm catechins using a spectrophotometer at a wavelength of 220-400 nm [8].

2.5.3 Determination of Catechin Levels

Each of the steeping solution and tea extract solution is made to a concentration of 10,000 ppm in 100 mL. Then diluted to 5000 ppm by measuring 12.5 mL and increasing the volume to 25 mL. The absorption of the sample solution was measured using a UV-Vis spectrophotometer at the maximum wavelength [10].

2.6 Data analysis

From the results obtained, data analysis was carried out for measuring catechin levels using linear regression equations and statistical tests.

2.7 Calculation of Catechin Levels

The research data were analyzed based on the linear regression equation 1.

$$y = ax + b \quad \text{(Equation 1)}$$

Information:
y = absorbance
a = intercept/intersection of the line with the vertical axis
b = slope/gradient
x = concentration

3 Results and Discussions

3.1 Tea Product Samples

Green tea and black tea products are obtained from two production sites, from tea plantations in Ngargoyoso Village, Karanganyar Regency, Central Java and tea plantations in Lawang, Malang Regency, East Java.

Green tea and black tea products are tested for water content in order to provide a maximum limit or range for the amount of water content in the ingredients. The water content in a simplicia can be a determining factor in the quality of the simplicia it self. High water content allows the simplicia to grow by fungi which can damage and affect the quality of the simplicia. Water content testing was carried out using a Moisture analyzer by weighing 2 grams of each product and obtained water content results that met the requirements, namely ≤ 10% (Tabel 1) [11].

Table 1. Tea Water Content

Sample	Water Content (%)
Green tea A	3.01
Green tea B	4.12
Black tea A	3.65
Black tea B	9.92

Note:
A : Karanganyar Plantation, Central Java
B : Malang Plantation, East Java

3.2 Extraction of Green Tea and Black Tea using the Brewing Method

The choice of brewing method is because catechins can be dissolved using polar compounds but using a heating process because the nature of catechins is that they do not dissolve in ordinary water [12]. Apart from that, people consume tea by brewing it using water.

The brewing process is carried out by brewing, because tea is enjoyed by brewing it. From the brewing results, the color of the brewing water, taste and aroma of black tea and green tea can be seen in the table 2.

The color of the brewing water for green tea A and B can be described as reddish yellow and quite bright. with elements of taste assessment stated as moderate (fairly good) to delicious (good) [13]. The color of the water for steeping green tea A and B is different, where B (Malang) is softer, this happens because the typical color of each tea is different because there are differences in where it grows [14]. The color of the brewed tea will be dark and thick, which indicates that the fermentation process took a long time. The clearer the color of the tea, the thinner the distinctive taste of the tea will be [15].

Based on the parameters, it was found that the color of the brewing water for black tea A (Karanganyar) and B (Malang) was red and quite bright with the taste assessment element being stated as fair to good [7].

Based on the type of tea, black tea and green tea, the color density black tea is darker than green tea. The darker the color of the brewed tea, the lower the tannin content, which is due to several factors, one of which is that if the tannin compounds are exposed to light and air for longer, the color will change to dark. Tea processing also uses high heat which will reduce the tannin compounds [8].

Table 1. Organoleptics of Tea Brewing

Parameter	Black Tea A	Black Tea B	Green Tea A	Green Tea B
Brewing water color	red and quite bright	red and quite bright	reddish yellow and quite bright	reddish yellow and quite bright
Flavor	fresh, has an astringent taste but not bitter	fresh, has an astringent taste but not bitter	fresh, has an astringent taste but not bitter	fresh, has an astringent taste but not bitter
Aroma	Characteristics of black tea	Characteristics of black tea (softer than black tea A)	Characteristics of green tea	Characteristics of green tea (softer than black tea A)

Note: A : Karanganyar Plantation, Central Java B : Malang Plantation, East Java

Table 3. Tea Brewing Qualitative Test Results

Sample	Reagent $FeCl_3$		Result	Reagent *Stiasny*		Result
	Previous Color	After Colour		Previous Color	After Colour	
Green Tea A	The reddish yellow is quite bright	Blackish green	+	The reddish yellow is quite bright	Yellowish white precipitate	-
Green Tea B	The reddish yellow is quite bright	Blackish green	+	The reddish yellow is quite bright	Yellowish white precipitate	-
Black Tea A	Red and quite bright	Blackish green	+	Red and quite bright	Yellowish white precipitate	-
Black Tea B	Red and quite bright	Blackish green	+	Red and quite bright	Yellowish white precipitate	-

Note: A : Karanganyar Plantation, Central Java B : Malang Plantation, East Java

3.3 Qualitative Test of Tea Brewing

Qualitative tests are carried out with the aim of determining the presence of compounds in the material to be tested, namely tea brewing. The qualitative tests carried out were phenolic tests and tests using Stiasny reagent. The presence of phenolic compounds can be seen

from the change in color of the test solution to blackish green after adding 10% $FeCl_3$ (ferric chloride). Because $FeCl_3$ reacts with the hydroxyl groups in phenol compounds. Meanwhile, the test using Stiasny reagent was carried out to determine the presence of gallocatechins, characterized by the formation of an orange-red precipitate after heating [8]. Based on the results of research that has been carried out, it is known that brewed green tea and black tea contain phenolic compounds but there are no gallocatechins. In other research, tea brew contains gallocatechins which are characterized by the presence of red-orange precipitates [16]. This difference could be due to the lack of concentration of the tea brewed so that the test results do not show a significant change in color. Factors that influence test results include growing location, extraction method, temperature and time [17].

3.4 Determination of Maximum Absorption Wavelength

Before calculating sample levels on a UV-Vis spectrophotometer, the maximum wavelength is first determined with the aim that the sample absorbance is at the maximum wavelength so that maximum results are obtained. The solvent used to determine this maximum wavelength is ethanol p.a. Results The maximum wavelength obtained for catechin was 280 nm. Wavelengths between 278.4-280 nm are the maximum wavelengths commonly used to detect catechins [18].

3.5 Catechin Standard Curve

Determination of the standard curve was obtained by reading the absorbance value of the standard solution of catechin at several concentrations, 20, 30, 40, 50, 60, 70 and 80 ppm.

Table 4. Measurement Results of Catechin Standard Solutions

No	Concentration (ppm)	Absorbance
1	20	0,299
2	30	0,354
3	40	0,455
4	50	0,529
5	60	0,630
6	70	0,684
7	80	0,819

In this test, a blank solution of ethanol p.a. was used. Ethanol p.a. was used as a blank solution, because catechin was dissolved with ethanol p.a. A blank solution is used to eliminate the absorption of compounds that do not need to be analyzed. Catechin is dissolved in ethanol p.a because catechin is slightly insoluble in cold water but soluble in hot water, soluble in alcohol and ethyl acetate, almost insoluble in benzene chloroform and ether [19].

3.6 Results of measuring catechin levels in green tea and black tea

Table 5. Results of Tea Brewing Catechin Levels

Sample	Absorbance	Catechin Levels	% Catechin Levels (%)
Black tea A	0,797	7,315	3,210
	0,771	7,073	3,089
	0,775	7,111	3,107
Black tea B	0,449	4,086	1,589
	0,453	4,123	1,607
	0,452	4,114	1,603
Green tea A	0,888	8,159	3,634
	0,898	8,252	3,680
	0,863	7,927	3,517
Green tea B	0,747	6,851	2,977
	0,738	6,767	2,935
	0,754	6,916	3,009

Note:
A : Karanganyar Plantation, Central Java
B : Malang Plantation, East Java

Comparison of Black Tea and Green Tea

Table 6. Comparison of Tea Types

Sample	Average Catechin Content (%)
Green tea	3,29 ± 0,35
Black tea	2,37 ± 0,84

Green tea catechin levels are higher than black tea catechin levels. This is because green tea is a tea product that does not undergo a fermentation process during processing [9]. The fermentation process is avoided by inactivating the oxidase/phenolase enzyme found in fresh tea leaves which is done by heating or steaming using hot steam or by panning, so that the enzymatic oxidation process of catechin compounds can be prevented. Meanwhile, the black tea processing process is carried out by complete fermentation by utilizing enzymatic oxidation of the tea polyphenol content. The

enzyme that plays a role in the oxidation process is the polyphenol oxidase enzyme which is assisted by oxygen in the air which will cause the polyphenol compounds contained in tea to oxidize to orthoquinone [20] which then condenses to form black tea pigments, namely teaflavin and tearubigin, which have less active hydroxyl groups, resulting in reduced polyphenol content in black tea [21].

Comparison of Karanganyar and Malang Plantation Tea.

Table 7. Comparison of Tea Growing Places

Sample	Average Catechin Content (%)
Tea A	3,37 ± 0,268
Tea B	2,29 ± 0,752

Note:
A : Karanganyar Plantation, Central Java
B : Malang Plantation, East Java

Based on the data, it shows that the place where the tea is grown can make a difference in catechin levels. Tea from the Karanganyar plantation (A) has a higher catechin content compared to tea from the Malang plantation (B). Where the tea is grown affects catechin levels [10].

The requirements for tea plants to grow well and have high productivity are:

1. Grows well at altitudes above 700 meters above sea level.
2. The expected rainfall is around 2500-3500 mm per year, with a minimum rainfall of 1100 - 1400 mm per year.
3. The temperature where the tea plants grow is 14 – 25°C.
4. Good soil that suits the needs of tea plants is soil that is quite fertile with a high organic matter content, is not rocky and has a pH of between 4.5-6.0.
5. A good harvest time is morning until noon from 5 to 9 am or 10 to 12 noon. Because the air is cool and there are not many contaminants [22].

4 Conclusions

Catechin levels in tea brews from tea plantations in Karanganyar and Malang showed that black tea and green tea from Karangnyar plantations had higher catechin levels than black tea from Malang plantations. Green tea has higher levels of catechins than black tea.

5 Declarations

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5.3 Authors Contributions

The names of the authors listed in this journal contributed to this research.

5.4 Conflict of Interest

The authors declare no conflict of interest

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