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Determination of Tannin Levels Using the Boiling and Brewing Method in Green Tea (*Camellia sinensis L.*) From Households Produced in Batang District

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ABSTRACT
Tea contains tannins, which help protect body cells from free radical damage. The purpose of this study was to determine whether there were tannin levels in tea produced by households in Batang Regency, and to ensure that all samples met the tannin consumption limit, if consuming too much tannin can cause anemia. The method used in the study was the extraction method by boiling and steeping with distilled water. Identification of tannins using color reactions and TLC using ethyl acetate, distilled water, formic acid (18:1:1) as the mobile phase. The tannin determination test used the UV-Vis spectrophotometer method. The results showed that green tea samples contained tannins. The maximum wavelength obtained was 279 nm. The highest tannin content was obtained in S6 as much as 205.24 mg/g tannin content. Samples in S2, S3, S4, and S5 produced tannin levels of 200.76; 167.73; 166.78; 186.78; and 186.71 mg/g. While the lowest tannin content was found in S1, which was 145.29 mg/g. It is concluded that the circulating tea produced by households in Batang Regency meets the requirements for tea consumption limits of less than 1 g so that it does not exceed the dose of 2–4 grams of tannin consumption per day.

Keywords: Content Level; UV-Vis Spectrophotometry; Tannin; Tea

INTRODUCTION
In addition to being a delicious and refreshing drink, tea has many health benefits, such as antioxidants, anti-cholesterol, anti-cancer, anti-diabetic, and anti-inflammatory. Unfermented tea (green tea and white tea), semi-fermented tea (oolong tea), and fermented tea (green tea). All types of tea come from the tea plant (Camellia sinensis) (1).

Caffeine, theobromine, theophylline, tannin, adenine, essential oils, quercetin, naringenin, and natural fluoride can be found in tea leaves. Caffeine is a powerful stimulant that increases breathing and heart activity. Theophylline increases heart function by widening coronary blood vessels and has a strong diuretic effect. Muscle is the main target of theobromine. Tannin has an astringent effect on the digestive tract because of its nature as a polar compound (2).

Tea or Camellia sinensis, is a herbal plant with various benefits. This plant can be recognized by its upright, woody, and branched stems, as well as the tips of the twigs and young leaves that are finely haired. The tea plant has one leaf and a short, alternate stem. The leaf blades are stiff and thin like leather. These leaves are green and have a shiny surface, with a length of 6-18 cm and a width of 2-6 cm. The top part, or peko, combined with two to three young leaves produces high-quality tea (3).

Tannin, a polyphenol, is divided into two types based on its chemical structure: hydrolyzable tannin (known as hydrolyzable tannin) and condensed tannin. Hydrolyzable tannin is a simple sugar ester with one or more carboxylic acid polyphenols. When these compounds are exposed to acids, bases, or enzymes, they tend to be easily hydrolyzed. Hydrolyzable tannin can be hydrolyzed into gallic acid in aqueous solution. Tea has tannin

compounds that are good for the body. However, excessive consumption can reduce iron absorption, especially in pregnant women (4). Decoction is an extraction method using water solvents until boiling. It is cooked in boiling water at a temperature of 100–105 ° Celsius, for 5–30 minutes, and the solvent is water. This is one of the most common extraction methods used by people around the world (5).

Batang Regency tea production is very diverse. Batang people really like tea drinks because of the good quality of the product and its uniqueness. Green tea is the most popular type. This study used UV-Vis spectrophotometry to measure tannin levels in tea produced by households in Batang Regency.

METHODS

Instruments

The equipment used in this study were analytical scales (OHAUS), UV-Vis Spectrophotometry (SHIMADZU UV-Vis 1280), Cuvette (SHIMADZU), glassware (Pyrex), micropipette, silica gel plate 60 F254 (Merck), chamber, elution vessel, elution glass.

Materials

The materials used in the study are materials with pro-analysis quality, namely: Catechin. Materials with technical quality, namely: $FeCl_3$, aquadest, 96% ethanol, Merck TLC plates, $FeCl_3$, Steasny reagent (formaldehyde, hydrochloric acid), sodium acetate, aquabides, ethyl acetate, formic acid and samples of household tea production in Batang Regency.

Experimental Procedure

Sampling

Dry green tea samples were obtained from household production in Batang district.

Extraction by Boiling

5.6 grams of green tea then boiled with 250 ml of distilled water until boiling, then filtered to produce tea extract, then concentrated.

Extraction by Brewing

Weighed 5.6 grams of green tea then brewed with 250 ml of distilled water, brewing was carried out for 10 minutes then filtered to produce tea extract, then concentrated.

Qualitative analysis

Thin Layer Chromatography (TLC)

According to Rustanti (2018) Mix the ethyl acetate, aqueous phaser, and formic acid in a ratio of 18:1:1 and let the TLC (Thin Layer Chromatography) chamber in a clear state with this mixture. Apply the solution to the TLC plate, with the spot position 1 cm from the bottom of the plate. Let the spots dry with a distance between spots of about 1 cm (6).

Color Reaction

2 mL of tea extract is added with 10 ml of distilled water and heated in a water bath. In Filtrate I, add 1% iron (III) chloride (FeCl3) solution. Filtrate II was added with 15 ml of Steasny reagent (20% formaldehyde: 2:1 hydrochloric acid) and heated in a water bath (7).
Filtrate III, add sodium acetate and then 1% iron (III) chloride solution ($FeCl_3$).

Quantitative Analysis

Preparation of Catechin Standard Solution

After weighing 10 mg of pure catechin, water solvent was used in a 100 mL flask to achieve a concentration of pure catechin solution
100 µg / ml.

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Preparation of Series Solutions
1 mL of pure catechin solution was pipetted and mixed with water in a 10 mL flask (concentration 100 µg / ml). After that to make concentrations of 10, 20, 30, 40, 50 and 60 µg / ml.

Wavelength Determination
The absorbance of the solution series of concentrations 10, 20, 30, 40, 50 and 60 µg/ml was measured, using the UV-Vis spectrophotometry method with a wavelength range of 200-400 nm using a UV-Vis spectrophotometer. The maximum wavelength obtained was used for catechin detection on a 279 UV-Vis spectrophotometer.

Preparation of Standard Curve
The absorbance of the solution series of 10, 20, 30, 40, 50 and 60 µg/ml was measured, using UV-Vis spectrophotometry using the maximum wavelength that had been obtained, then the absorption results obtained were recorded and a calibration curve was made with y = bx+a

Preparation of Sample Solution
Approximately 1 gram of sample was dissolved in distilled water, dissolved little by little into a 50 ml measuring flask, 6 tea samples were used. Then the absorbance is measured using UV-Vis spectrophotometry with a predetermined wavelength.

RESULT AND DISCUSSION

The samples used were obtained in Ngadirejo Village, Reban District, Batang Regency. The research used dried green tea material. Plant determination was carried out at the Biology Laboratory of the Faculty of Applied Science and Technology, Ahmad Dahlan University.

In this study, the sample extraction method was used by boiling and brewing, because many people use this method to enjoy tea. The extracted material consisted of a mixture of 5.6 grams of green tea sample in 250 ml of distilled water by boiling and brewing, 5.6 grams of green tea sample in 100 ml of distilled water by boiling and brewing, and 3 grams of tea sample in 250 ml of distilled water by boiling and brewing.

Further research tested qualitative analysis with Thin Layer Chromatography (TLC). The purpose of this study was to find spots and developments on the TLC plate, as well as to determine the Rf value of the comparative material and sample.

The stationary phase used was silica gel 60 GF 254, which was activated by heating in an oven at 105^{o}C for 30 minutes. This activation aims to remove water impurities that may still be present on the TLC plate. The mobile phase used was a mixture of ethyl acetate, water, and formic acid (18:1:1) (Rustanti, 2018). Before use, the chamber was saturated with the mobile phase. Catechin and each sample were spotted on the activated plate, with a distance of 1 cm from the bottom and between spots, then left to dry. Then viewed using UV Light Wavelength 254 and 366 nm.

Based on the results in Table 1, it shows that the samples analyzed by Thin Layer Chromatography at 254 nm UV light will show a fluorescent plate and the sample will appear dark in color. While at 366 nm UV light, the plate will be dark in color and the spots will fluoresce. TLC analysis confirmed the presence of tannins in all six tea samples, with Rf values close to that of catechin (Rf ~0.5). Color reactions further supported the presence of both condensed and hydrolyzable tannins. The Rf value of the catechin standard with the extract sample all

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positively contains tannin, it is said to be positive for containing tannin because the difference between the sample Rf and the standard Rf is ≤0.05 (8). This is because the difference in Rf values does not exceed the specified limit, so that it meets the requirements of the standard Rf value, which is between 0.2 and 0.8 (9).

Table 1. TLC Results of Green Tea Samples

Sample	Spot height (cm)	Rf
B	4.0	0.5
S1	4.1	0.51
S2	4.0	0.5
S3	3.7	0.46
S4	4.0	0.5
S5	3.9	0.48
S6	4.0	0.5

The next qualitative analysis, namely the color reaction test, can be seen from Table 2 showing the color changes that occur in each sample and different reagents. Filtrate I was then tested with $FeCl_3$ solution. The color change in the sample indicates the presence of oxidized phenolic compounds, characterized by a change in the color of the filtrate from brown to black, the addition of $FeCl_3$ in the extract produces various colors such as green, red, purple, blue, or strong black. The blackish color is formed due to the reaction between tannins in the extract with Fe^{3+} ions. Filtrate II was added with the Sterasny reaction (a mixture of 20% formalin and 10:5 hydrochloric acid) as much as 15 ml. This addition is intended to identify the presence of catecholate tannins or condensed tannins. The test results indicate that there are tannins in the green tannin. Because of the collision of white sediment. the sample is brown in color, and produces a brownish yellow color so it is said to be positive (10). Filtrate III was mixed with sodium acetate and $FerCl_3$ solutions. In this study, the sample solution showed a black color, indicating that the hydrolyzed tannins present were gallotannin types (7).

Table 2. Results of Phytochemical Screening

Sample	$FeCl_3$	Steasny Reagent	Na Acetate + $FeCl_3$
B	++	+	++
S1	++	+	++
S2	++	+	++
S3	++	+	++
S4	++	+	++
S5	++	+	++
S6	++	+	++

Before determining the tannin content in the sample, the maximum wavelength must be determined first. This reading is used to reduce the error in reading the absorption to a minimum, because the measurement of the maximum absorption wavelength produces maximum absorption, in the wavelength range of 200-400 nm (11). The maximum absorption wavelength of the pure catechin standard solution was obtained, which was 279 nm. The wavelength can be seen in Figure 1.

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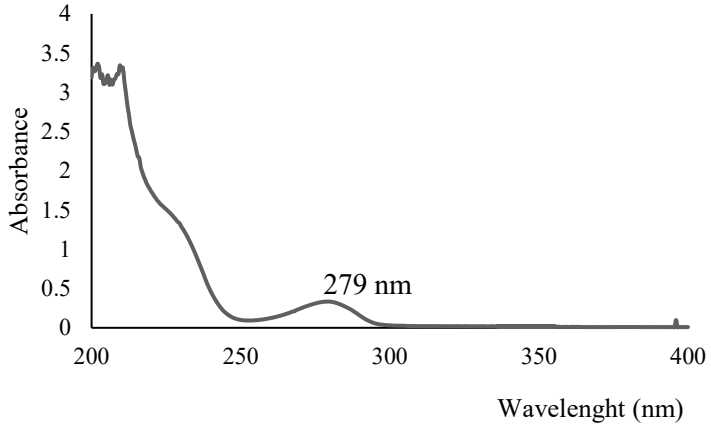

Figure 1. Graph of standard wavelengths of catechins

After determining the maximum wavelength, a standard curve is made, the purpose of making this standard curve is to determine the relationship between catechin concentration and absorption. From a standard solution of 100 µg/mL catechin, a series of solutions with concentrations of 10, 20, 30, 40, 50 and 60 µg/mL are then made. Then the linear regression is sought and a standard curve is made. From the measurement of the calibration curve that has been carried out, a linear regression equation can be made y = 0.109x - 0.0033 with an R2 value (relational coefficient) = 0.9999, the value of the relationship coefficient approaching 1 indicates a linear relationship between the concentration of the reference standard and its absorption can be seen in Figure 2.

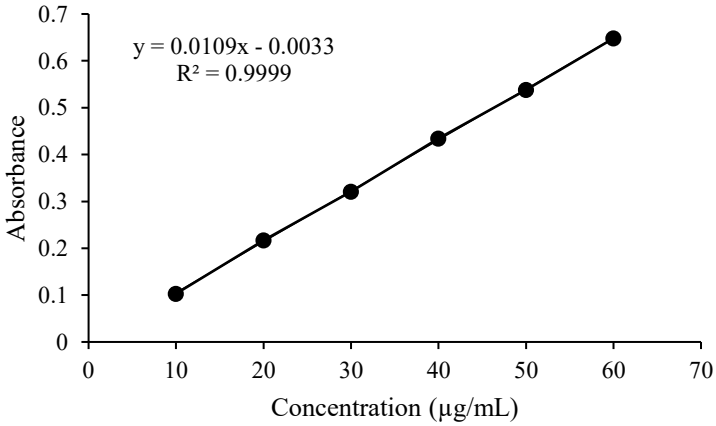

Figure 2. Standard curve of catechin

Based on Figure 3, it can be seen that the average results of the highest tannin content were obtained in S6 as much as 205.24 mg/g tannin content. Samples in S2; S3; S4; and S5 produced tannin levels of 200.76; 167.73; 166.78; 186.78; and 186.71 mg/g, respectively. While the lowest tannin content was found in S1, which was 145.29 mg/g. As in the study conducted by (2), the study stated that the more concentrated the tea solution, the lower the tannin content.

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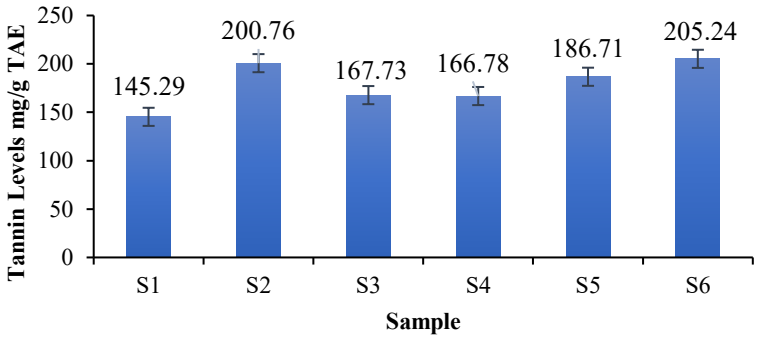

Although statistical analysis such as ANOVA was not performed, the observed variations highlight the need for standardization in household tea processing. Nonetheless, all samples fell below the critical intake level of 1 g per serving, and within the recommended daily tannin intake limit of 2–4 g (12).

CONCLUSION

All analyzed green tea samples from Batang Regency households contained measurable amounts of tannins, with levels ranging from 145.29 to 205.24 mg/g. These values are within the safe limits for daily consumption, indicating the general safety of these household tea products. Future studies should incorporate statistical analyses and explore the effect of different brewing times and temperatures on tannin levels.

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none

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