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Comparison of Tannin Levels in Brewed and Boiled Green Tea (*Camellia sinensis* L. Kuntze) by UV-Vis Spectrophotometric Method

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ABSTRACT

Introduction: *Green tea (Camellia sinensis (L.) Kuntze) has antibacterial and antioxidant properties that are effective in preventing and treating diseases. Green tea contains tannin compounds with antidiarrheal properties, stops bleeding and prevents inflammation of the oral mucosa. To get its properties, the way tea is processed affects the tannin content in tea.* ***Aims****: To determine the tannin content in brewed and boiled green tea using UV-Vis Spectrophotometric method.* ***Method:*** *The research method used was experimental research. The sample used was green tea taken from the cultivation site "Mbok Karti" Kemuning, Ngargoyoso, Karanganyar. Extracted using the reflux method. Determination of levels analyzed by UV-Vis Spectrophotometry method. Qualitative test results using FeCl3 1% that brewed and boiled green tea contains tannins by showing a change in color to blackish green.* ***Result:*** *The results of quantitative tests using UV-Vis Spectrophotometry obtained a comparison of tannin levels in brewed green tea of 0.05163% w/b and boiled ones of 0.04988% w/b. Where in every 200 grams of brewed green tea samples there are 103.26 mg of tannins for boiled green tea samples there are 99.67 mg of tannins. Tannin levels in brewed green tea are greater than those boiled because tannins are damaged at temperatures above 80°C. While when boiling uses temperatures above 80°C. Whereas when boiling uses temperatures above 80°C.* ***Conclusion:*** *So it is concluded that brewed green tea has higher tannin levels than boiled green tea.*

KEYWORDS*:* Brewed green tea, boiled green tea, reflux extraction, tannins, UV-Vis spectrophotometry

INTRODUCTION

Tea (*Camellia sinensis*) is one of the most widely enjoyed beverages in the world. Tea has antibacterial and antioxidant properties that are effective in preventing and treating diseases. Tea is divided into 4, including: fermented tea (black tea), semi-fermented tea (oolong tea), tea without going through the fermentation process (green tea), and tea without fermentation at all (white tea) ((Rohdiana et al., 2005). Tea leaves made without passing through the fermentation process are green tea (*Camellia sinensis* (L.) Kuntze), in green tea leaves contain tannin compounds, tannins as astringents have properties as antidiarrheal, stop bleeding, prevent inflammation in the oral mucosa, and in severe poisoning alkaloids can be used as

antidotes. In addition, due to the presence of phenol groups tannins have properties as antiseptics (Hanani, 2015). However, tannins can bind iron which results in anemia (Besral, 2007).

The method used is UV-Vis spectrophotometry. The wavelength as abscissa consists of UV-Vis spectral bands and absorbance correlations, which are formed by energy transitions that are not similar. These spectral bands cause electronic excitation. Researchers used the UV-Vis Spectrophotometry method because tannins have conjugated double bonds, which allows the analysis of such compounds. In addition, this method has a precision and accuracy that is still well acceptable with a precision value of 0.201% and an accuracy of 121.73% with an RSD of 0.2033% (Ngibad & Herawati, 2019).

Based on the description above, researchers conducted a study comparing tannin levels in brewed and boiled green tea using the UV-Vis Spectrophotometry method. The purpose of this study was to determine the best way to process green tea and to determine the lowest level of tannins to drink. In addition, based on research by (Fajrina et al., 2016) which states that tannin levels are high in green tea compared to black tea because green tea does not go through a long drying method, while black tea goes through high heating and long drying.

MATERIAL AND METHODS

Plant Determination

Determination of tea plants (*Camellia sinensis*) was obtained from the tea cultivation site "Mbok Karti" in Kemuning Village, Ngargoyoso, Karanganyar. Determination was carried out at the Integrated Laboratory of the Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional (B2P2TOOT). Reflux extraction at the Pharmaceutical Analysis Laboratory at Muhammadiyah University of Klaten. UV-Vis Spectrophotometric test was conducted at the Integrated Research Laboratory of Ahmad Dahlan University Yogyakarta.

Materials

The materials used are green tea leaf simplisia, aquadestillata, Folin-Ciocalteau, Tannin standard, calcium carbonate, chloroform, and sodium carbonate.

Sample Preparation

Brewed green tea by weighing 200 grams of green tea samples. Heat 1000 mL of aquadestillata to a temperature of 80°C. The fire was turned off. After that, the beaker glass containing aquadestillata was lowered and then poured into another beaker glass containing green tea samples. Stirred and waited for 8 minutes. Then filtered using filter paper (Annuryanti et al., 2018)

Green tea was boiled by weighing 200 grams of green tea samples. Heat 1000 mL of aquadestillata to a temperature of 100°C. After

that, the green tea sample was included in aquadestillata. Stirred and waited for 8 minutes. Then filtered using filter paper. This process is carried out using medium heat heating (Annuryanti et al., 2018)

Qualitative Test

For brewed green tea simplisia, heat 100 mL aquadestillata, wait until it boils, after boiling, lower it from the top of the electric stove, add as much as 2 grams of green tea, filtered, and obtained filtrate. (Khasanah et al., 2021)

For boiled green tea simplisia, 2 grams of green tea was weighed, then added 100 mL aquadestillata, waited for boiling, then cooled, filtered, and obtained filtrate. (Khasanah et al., 2021)

For brewed and boiled green tea, if a green, purple, or black color is formed after adding 1% iron (III) chloride ($FeCl_3$) solution, the results indicate that it contains positive tannins. (Khasanah et al., 2021)

Quantitative Test

The reflux extraction procedure was carried out by weighing 300 mL of green tea brewing water and 300 mL of green tea cooking water. Then put into a 500 mL beaker glass. Add 200 mL Aquadestillata and 10 grams of $CaCO_3$, then boil for 60 minutes. In a hot state, filter using flannel cloth. Extract the filtrate using 60 mL $CHCl_3$ in a separatory funnel, then shake. Separate the $CHCl_3$ phase (bottom layer). Extract the aqueous phase using 40 mL $CHCl_3$ (Lower layer). Collect the CHCl3 phase. Using a vaporizer cup, the filtrate is evaporated on a waterbath until the chloroform fraction disappears or until it no longer smells of chloroform (Kementerian Kesehatan Republik Indonesia, 2000)

Determination of tannin content using UV-Vis Spectrophotometry was carried out by weighing 50 mg of tannin extract, dissolved in 2 mL of aquabidest. Sonicator for 15 minutes, then filtered. Then put it in a 5 mL measuring flask, added aquabidest until the limit mark and homogenized. A total of 300 µL of sample solution was taken, added 1.5 mL of Folin Ciocalteau reagent, then shake and let stand for 3 minutes. Into the above solution, 1.2 mL of 7.5% Na_2CO_3 solution was added, shake and homogenized. Incubated at the range operating time at room temperature and the absorbance was measured at the wavelength obtained.(Khasanah et al., 2021)

Independent T-Test statistical test, used with the SPSS program, was used to analyze the tannin content of brewed and boiled green tea.

RESULTS AND DISCUSSION

Laboratory determination results showed that the tested sample was *Camellia sinensis* (L.) Kuntze. Qualitative tannin test results on brewed and boiled green tea showed that positive samples contained tannins characterized by the appearance of a blackish green color. Extraction of brewed and boiled green tea tannins was carried out using the reflux extraction method. The yield produced

Table 1 Standard Curve

Concentration	Absorbance
10,000	0,099
20,000	0,195
30,000	0,303
40,000	0,407
50,000	0,507
60,000	0,602
70,000	0,671
80,000	0,775
90,000	0,877

from 200 grams of brewed green tea simplisia sample is 0.096% b/b and in the boiled sample is 0.093% b/b.

The results of the concentration and absorbance of the standard curve solution can be seen in Table 1.

Based on Table 1 tannin standard solution using 9 concentration series. The concentration variation is carried out to determine the difference in absorbance, where the higher the absorbance value is the greater. The absorbance read on UV-Vis Spectrophotometry, namely at 90 ppm at the 90th minute, obtained an absorbance of 0.877 using a maximum wavelength of 750.0 nm.

Based on graph 1, a linear regression y = 0.0095 x + 0.0147 was obtained with a value of R2 = 0.9985.

The results of the determination of tannin levels in brewed and boiled green tea samples can be seen in table 2 below:

The equation used to determine tannin levels in brewed and boiled green tea (Gandjar & Rohman, 2007)

Tannin Content % = (V . X . Df)/SW

Description:

V = Solvent Volume (mL)

Xppm = Concentration (mg/mL)

Df = Dilution factor

SW = Sample Weight (mg)

Reflux method is the extraction method used in this study. In the extraction process, $CaCO_3$ was added to break the tannin bond with other compounds. The separation of tannins in the tea solution was carried out using chloroform, because chloroform is non-polar so that when mixed with water which is polar, chloroform cannot dissolve. In this process, two layers are formed: the lower layer contains the water phase and the upper layer contains tannins in chloroform. This happens because the specific gravity of the two solutions is different (Maylani et al., 2019). The result of tannin extraction in brewed green tea was 0.096% w/b in the form of powder and in the boiled sample was 0.093% w/b in the form of powder. The yield results obtained are not in accordance with the literature, namely tea produces tannin extract around 38.4822% (Endarini, 2019). This is because evaporation is not carried out until the sublimation process to obtain pure tannins in crystal form, but only evaporated until the chloroform fraction disappears. The chloroform fraction disappeared marked by no smell of chloroform/ ether and the color became clear green, in order to facilitate the next process, namely the UV-Vis Spectrophotometry process. The UV-Vis Spectrophotometry method is used because tannins have a double bond that is incorporated where the compound

Table 2. Determination of brewed and boiled tannin

Sample	Mass (mg)	Vol (ml)	Absorbance	Dilution factor	Concentration (ppm)	Content (% w/b)
Brewed	50.19	0.3	0.838±0.1	1	86.380±0.1	0.0516
Boiled	50.06	0.3	0.808±0.3	1	83.242±0.1	0.0498

can be analyzed using UV-Vis Spectrophotometry. The obtained tannin content in brewed green tea averaged 0.05163% w/b and in boiled green tea it was 0.04988% w/b (Table 2). Where in every 200 grams of brewed green tea samples there are 103.26 mg of tannins for boiled green tea samples there are 99.67 mg of tannins. Lower boiled tannin levels are due to brewing temperatures that are too high. This is because tannins are damaged at temperatures above 80°C, while at the time of boiling green tea using a temperature of 100°C, this is in accordance with research conducted by (Raisa Ayu, 2011), namely tannins are not resistant to heating that is too high, so they are not used at temperatures greater than 80°C. So it is said that tea is brewed at a temperature of more than 80°C. So it can be said that brewed and boiled tea produces higher tannin levels. After that, the Independent T-Test was used to analyze the data. The results showed that the tannin content of brewed and boiled green tea had a significant difference, with a significant value of 0.000 < 0.05.

CONCLUSION

The study has shown that green tea (Camellia sinensis (L.) Kuntze) contains tannins. The wavelength of 750.0 nm with absorbance of 0.877 showed the tannin content in brewed green tea was 0.05163 % w/b and boiled green tea was 0.04988 % w/b. There is a significant value of 0.000, which indicates that $p < 0.05$.

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