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Test for Determination of Tannin Characteristics of Oolong Tea Leaf Extract (*Camellia sinensis* (L.) Kuntze) from Kemuning Tea Plant Karanganyar

Anita Agustina Styawan*, Fatikhatun Nissa' Khairiyah

Diploma of Pharmacy, Faculty of Technology and Health, Universitas Muhammadiyah Klaten,Indonesia

*Corresponding author: Anita Agustina Styawan | Email: anita@umkla.ac.id

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Abstract: The oolong tea plant protects the body's cells from the damaging effects of free radicals by acting as an antioxidant. The content of oolong tea leaves contains tannin compounds which have a good effect on the body and the more tannin content in the tea, the greater it is its antioxidant activity. The purpose of this study is to use UV-visible spectrophotometry to ascertain the tannin content of the oolong tea leaf extract from the Kemuning tea plantation. The observational research method is being used. A sample of dried oolong tea leaves is used in this investigation. 1% $FeCl_3$ was used to assess the sample qualitatively, and UV-Vis spectrophotometry was used to analyze the sample quantitatively. The solvent used was ethanol 96%%. The study's conclusions show that the sample contains tannins, which are qualitatively distinguished by a shift in color to a greenish-black tint. The oolong tea leaf extract has a quantifiable tannin concentration of 12.26% mg TE/g. Indicates that tannins can give a bitter and astringent taste, and can give a pleasant flavor to tea.

Keywords: tannin, oolong tea, maceration, UV-Vis spectrophotometry

1. INTRODUCTION

With a vast geographical area, tremendous biodiversity, and ideal natural and climatic conditions, Indonesia is an agricultural country [1]. One of the widespread and rapidly growing agricultural products in Indonesia is tea (*Camellia sinensis* (L.) Kuntze) [2]. Tea is a beverage and/or dried leaf stalks of the plant (*Camellia sinensis* (L.) Kuntze) [3]. Oolong tea is tea that has been partially oxidized during the manufacturing process. To make oolong tea, the tea leaves are dried or aerated and then filtered to oxidize the tea leaves to the desired degree [4]. The oxidized tea is dried and processed to give it a unique shape characterized by curled leaves [5]. There is one more drying operation to perform. The antioxidant content of oolong tea is lower than green tea but higher than black tea due to partial oxidation [6].

Tannins are large molecular weight phenolic compounds that combine with proteins and other macromolecules to produce strong and useful complexes [7]. They are characterized by hydroxyl groups and other related groups, such as carboxyl. Plant tannins have several functions, one of which is to defend against herbivorous animals [8]. Another name for tannins is anti-nutrients. Condensed tannins and hydrolyzable tannins are the two main categories of tannins [7]. Plants contain both types of tannins, but condensed tannins are more common [9]. While hydrolyzable tannins are produced

by esterification reactions between phenolic acids and sugars (glucose), condensed tannins are produced by polymerization (condensation) reactions involving flavonoids [10].

Two different light sources are used in UV-Vis spectrophotometric methods: a UV light source and a visible light source [11]. In UV-visible spectrophotometry, wavelengths are expressed as horizontal axes in the form of spectral bands and absorption correlations [12]. UV-Vis spectral bands are produced by different energy transitions, which cause electronic excitation [13]. The reason researchersuse the UV-Vis spectrophotometric method is because tannins have conjugated double bonds which can be analyzed using UV-Vis spectrophotometry [14].

Extraction procedures can be used to remove tannins from compounds [15]. Using solvents, extraction entails the separation of liquids and solids . Solubility of the solvent is very important for this process [16]. Maceration is the extraction method used. Maceration is used because dry Simplisia is easier to wet first or macerate for a certain period of time [17] . Maceration is a way of soaking Simplisia in a filter at room temperature or heating to extract Simplisia compounds. This maceration method is more practical and relatively easy for dry simplisia [18]. The tea plant is advantageous for traditional medicine in treating headaches, diarrhea, diabetes, shrinking stones, cholesterol and glycerides, fighting high blood pressure, gastrointestinal infections, cancer, and thickening and blackening hair [1]. Consuming excess tea is also not good for the heart, it can cause sleeplessness and upset stomach [19].

Based on the benefits of oolong tea and the side effects of oolong tea, research was conducted with extraction using maceration methods that minimize damage to tannin compounds [20]. So that it is known whether or not there are tannin compounds in order to avoid the side effects of these compounds if consumed in excess [21].

2. MATERIALS AND METHODS

2.1. Materials

The type of research conducted was descriptive observational research. This study used the UV Vis spectrophotometric method. In this study, 200 grams of oolong tea leaves (*Camellia sinensis* (L.) Kuntze) were used as samples taken from the "Gambyong" tea cultivation site in Kemuning Village, Ngargoyoso District, Karanganyar Regency.

Analytical scales, maceration devices, test tubes, UV-Vis spectrophotometer (*Shimadzu*), beakers (*Gilson*) , porcelain cups (*Gilson*), volumetric flasks (*Gilson*), micropipettes, and volumetric pipettes are some of the instruments used.

The materials used are dry simplisia of oolong tea leaves, gallic acid (*Merck*), aquadestilata , 96% ethanol (*Merck*), 15% Na_2CO_3 solution (The Merck Index), 2% NaCl (*Merck*), $FeCl_3$ (Merck), folin ciocalteu (material safety data sheet), and folic acid (*Merck*).

2.2. Research Procedures

2.2.1. Determination

This identification ensures that the leaves used in the study are genuine oolong tea leaves (*Camellia sinensis* (L.) Kuntze), thus avoiding sampling errors. Determination was carried out at the Ahmad Dahlan University Integrated Research Laboratory.

2.2.2. Sample preparation

Dried simplisia samples of oolong tea (*Camellia sinensis* (L.) Kuntze) were obtained from the "Gambyong" tea cultivation area from Kemuning Village, Ngargoyoso District, Karanganyar Regency. Oolong tea leaves to be analyzed are 200 grams in a dry state.

2.2.3. Extract Preparation

Dry 200 grams of oolong tea leaf powder (*Cammelia sinensis* (L.) Kuntze) is put into a container or maceration vessel, soaked with 96% ethanol as much as 2 liters and shaken for about 10 minutes, then let stand for three times for twenty-four hours occasionally shaking at the same time, namely every 8 hours, after which the extraction of oolong tea leaves is filtered. The extraction results are evaporated into a waterbath to obtain a thick extract of oolong tea [22].

2.2.4. Qualitative Test for Tannins

How to do a qualitative test of ethanol extract from simplisa oolong tea leaves added with $FeCl_3$ reagent 1% as much as 1 ml in the sample solution. To determine the presence or absence of tannins marked with green, blue to blackish color [23].

2.2.5. Quantitative Test for Tannins [24].

a. UV-Vis Spectrophotometry Extraction Procedure
   1) Preparation of *folin Ciocalteu* reagent

      Take 1 ml of *folin ciocalteou* solution, add aquabidest to a volume of 10 ml, and sonicate until homogeneous.
   2) Preparation of Na_2CO_3 solution

      Weigh as much as 7.5 grams of Na_2CO_3 plus 80 ml of aquabidest, sonicator until completely dissolved. Let stand for 24 hours, then filtered pour into a 100 ml volumetric flask and add aquabides to the marked line to make it uniform and homogeneous.
b. Preparation of 1000 ppm tannin Master Solution

   Tannin standard weigh 10 mg dissolved to the limit into a 10 ml volumetric flask with aquabidest (1000 μg/mL).
c. Determination of maximum Wavelength.

   Pipette 250 μL of tannin 1000 solution into a 5 mL volumetric flask, fill with distilled water and mix 300 μL of tannin solution, add 1.5 mL of Folin Ciocalteau reagent, shake and leave for 3 minutes.

   Add 1.2 mL, 75% Na_2CO_3 to the solution, shake and homogenize. Incubate for one hour at room temperature and measure the absorbance. The wavelength was measured in the range of λ 400-800 nm.
d. Determination of *operating time*

   In ensuring complete mixing, 250 μL of 1000 ppm tannin solution was pipetted into a 5 mL volumetric flask. Distilled water was then added up to the calibration mark. The tannin solution was then combined with 1.5 mL of Folin-Ciocalteu reagent in 300 μL, stirred, and left for three minutes. The mixture was then stirred and homogenized after 1.2 mL of 75% Na_2CO_3 was added, and the absorbance was measured after 30 minutes.

e. Determination of Standard Curve

A series of standard solutions of 20 ppm, 30 ppm, 40 ppm, 50 ppm, and 60 ppm were prepared to build a standard curve.

Pipette 1 mL of standard solution into a 10 mL volumetric flask, add 1 mL of folin-ciocalteu reagent, shake, and let stand for 5 minutes. Then add aquabidest up to the mark and shake until a homogeneous mass is formed.

Observe the absorbance at the maximum wavelength and use 2 mL of gallic acid standard solution, 1 mL of folin-ciocalteu reagent, and 2 mL of 15% Na_2CO_3 as a blank [25].

f. Determination of tannin content

1) About 100 mg of tannin extract is used in 10 milliliters of aquabidest. Sonicate for 15 minutes, and then turn it off. Pipette with 0.3 mL solution was placed in a 10 mL beaker, and then mixed until the mixture was uniform.
2) The sample solution was taken as much as 1000 µL, added 1 mL of Folin-Ciocalteu reagent, then gojog and allowed to stand for a duration of 3 minutes.
3) Add 2 ml of 75% Na_2CO_3 solution to the above solution and homogenize. Leave until the stable time range is reached. The absorbance of the extract was observed at the maximum wavelength. The concentration was repeated twice. Total tannin content was calculated as gallic acid equivalent.

3. RESULTS AND DISCUSSION

3.1. Determination of Tea Plants

Oolong tea was used in dry form as much as 100 grams. The samples were then determined at the Laboratory of the Faculty of Applied Science and Technology, Ahmad Dahlan University, Yogyakarta. The identification results of the tea plant, *Camellia sinensis* O.K.var. assamica (Mast.) and its synonym *(Camellia sinensis* (L.) Kuntze) and its identification key are as follows:

1b – 2b – 3b – 4b – 5b – 6b – 7b – 9b – 10b – 11b – 12b – 13b – 14a – 15a – 109b – 119b – 120b – 128b – 129b – 135b – 136b – 139b – 140b – 142b – 143b – 146b – 154b – 162a – 163b – 167b – 169b – 171b – 177b – 179a – 180b – 182b – 183b –184a Theaceae 1 Camellia

Camellia sinensis O.K. var. *assamica* (Mast.)
Synonyms : *Camellia sinensis* (L.) Kuntze

3.2. Oolong Tea Leaf Extraction Results

Using maceration method and 96% alcohol as solvent, 200 grams of dried oolong tea leaves (*Camellia sinensis* (L.) Kuntze) were extracted for three days. 24.3 grams of concentrated extract was produced by this process, resulting in a yield of 12.15%.

Extraction was carried out using the maceration method, which is the process of immersing the sample in a solvent at room temperature without heating. A total of 200 grams of dried oolong tea leaves (*Camellia sinensis* (L.) Kuntze) was used as raw material. The solvent used was ethanol with a concentration of 96%, which is known to be effective in extracting active compounds from plants. The maceration process lasted for three days, with periodic stirring to increase extraction efficiency. After the process was complete, the mixture was filtered and the solvent was evaporated using a rotary

evaporator until a concentrated extract was obtained. From this process, 24.3 grams of concentrated extract was obtained, which showed a yield of 12.15% of the initial weight of dry simplisia.

3.3. Qualitative tannin test results

The qualitative tannin test was conducted to determine whether dauh tehoolong contains senawa tannin. This test uses FeCl3 coloration. The results of the qualitative test are presented in Table 1.

Table 1. Qualitative Test for Tannins

Replication	Reagents	Results	Description
1	$FeCl_3$ 1%	Blackish blue	(+) Tannin
2	$FeCl_3$ 1%	Blackish blue	(+) Tannin
3	$FeCl_3$ 1%	Blackish blue	(+) Tannin

Description: (+) tannins cause brown to blue-black color.

The test results indicate the presence of tannin, which is indicated by a change in the color of the solution from brown to blue-black. This color change is a typical indicator of tannin reaction with $FeCl_3$ reagent, which forms a dark complex as evidence of the presence of phenolic compounds. The result is positive for tannin, marked by a change in color from brown to blackish blue.

3.4. Maximum wavelength determination

When measuring the wavelength of maximum absorption, determining the maximum wavelength will result in maximum absorption. Based on the results of monitoring the wavelength and maximum absorption, the maximum absorption of tannin compounds is 780 nm. With absorbance of 0.6332. Details of the maximum wavelength data results can be seen in Table 2.

Table 2. Maximum Absorption Wavelength

Maximum wavelength (nm)	Absorbance
780.0	0.6332

3.5. Determination of Operating Time (OT)

The determination of Operating Time (OT) is based on a predetermined wavelength of 780 nm at 60 minutes.

3.6. Results of Standard Curve Preparation

The preparation of the standard curve aims to determine the relationship between tannin concentration and sample absorbance. The results of the concentration and absorbance of the standard solution are shown in Table 3.

Tannin standard solution was prepared in 6 concentrations of 10; 20; 30; 40; 50; and 60 ppm, with absorbance of 0.1135; 0.2231; 0.3228; 0.4329; 0.5379; and 0.6332, respectively. This concentration variation shows that the absorbance value increases as the concentration increases. Based on these data, a linear regression equation $y = 0.0104x + 0.0159$ was obtained with $R^2 = 0.9995$. The results of the tannin standard curve are shown in Figure 1.

Table 3. Standard curve solution

Concentration (ppm)	Absorbance
10	0.1135
20	0.2231
30	0.3228
40	0.4329
50	0.5379
60	0.6332

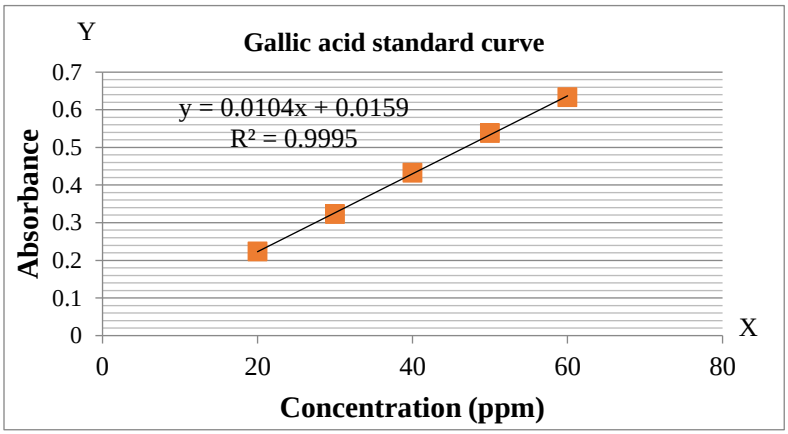

Figure 1. Standard Curve Graph

In this study, the absorbance obtained at a concentration of 10 ppm did not match the good absorption range. The concentration of 10 ppm was not included in the standard curve plot because the good absorption range is between 0.2-0.8.

3.7. Results of Level Determination

The results of the determination of tannin levels in oolong tea leaves (*Camellia sinensis* (L.) Kuntze) were carried out twice and the results of tannin compound levels are presented in Table 4.

Table 4. Tannin Content Determination Result

Replication	Volume (ml)	Concentration	Sample weight (mg)	Abs	Levels (%) b/b
1	10	34.210	100	0.3690	12.3350%
2	10	33.774	100	0.3644	12.1877%
	X ± SD				12.26 ± 0.074

Researchers only did 2 replications because the research site could only do 2 replications. The number of samples was calculated as y = 0.0104x + 0.0159. where y is the absorption value of the sample measurement and x value is the sample concentration value (ppm). Based on Table 4 for iterations, the average tannin content of oolong tea leaves is 12.26 ± 0.074% mgTE/g.

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that:

1. Qualitative tests conducted on oolong tea leaf extract (*Camellia sinensis* (L.) Kuntze) contain tannins characterized by a blackish blue color.

2. Analysis of tannin content using UV-Vis spectrophotometric method obtained total tannin content of oolong tea leaves (*Camellia sinensis* (L.) Kuntze) which is 12.26% mg TE/g.

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Conflict of Interest: Author declare there is no conflict of interest

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