

Total Phenolic and Flavonoid Compound of Crude and Purified Extract of Green Tea Leaves (*Camellia sinensis*) from Makassar-Indonesia

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Article info	Abstract
<p>History Submission: 30-09-2022 Review: 02-11-2022 Accepted: 24-12-2022</p> <p>*Email: muammar.fawwaz@umi.ac.id</p> <p>DOI: 10.33096/jffi.v9i3.916</p> <p>Keywords: green tea, phenolic, flavonoid, gallic acid</p>	<p><i>Polyphenols are a class of chemical compounds most commonly found in plants, and these compounds have many health benefits associated with the character it has. The antioxidant activity of natural materials connected by polyphenol content, which can counteract free radicals. This study aims to determine the levels of total phenolic and flavonoid in crude extracts and purified extracts of green tea leaves (<i>Camellia sinensis</i>). Determination of total phenolic content was done using standard gallic acid by the Folin-Ciocalteu method. Total flavonoid assay using a standard reference of quercetin by Chang method. The instrument used to measure the absorbance of the reaction is a UV-Visible spectrophotometer. The results obtained after the measurement indicates that the sample of crude extract and purified extract of tea leaves contained total phenolic levels of 40.5 and 43.9 mgGAE/g, respectively, while the levels of total flavonoids contained respectively by 2.77 and 3.14 mgQE/g. From the results obtained it can be concluded that green tea leaves have a total phenolic content is greater than the total flavonoid content, thus it has great potential as an antioxidant.</i></p>

I. Introduction

Polyphenols are a form of bioflavonoids with several phenol groups (Abbas et al., 2017; Shahidi & Ambigaipalan, 2015). Polyphenols in green tea are catechins, including epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin (Smith, 2011). Polyphenol compounds can act as catchers of hydroxyl free radical (OH) that does not oxidize lipids, proteins, and DNA in the cell. When polyphenols are eaten on a daily basis, they protect against cognitive deterioration in community-dwelling older women (Sakurai et al., 2020). Green tea's chemical composition promotes health benefits such as anticarcinogenic, anti-inflammatory, cardioprotective, antiviral, and glucose metabolism regulation (Chacko et al., 2010; Kochman et al., 2021).

Flavonoids are natural phenolic compounds in almost all plants (Mutha et al., 2021). Flavonoids are phenolic compounds included in the class with the chemical structure of the C₆-C₃-C₆, flavonoid skeleton consisting of one aromatic ring A, one B aromatic ring, and a middle ring in the form of oxygen-containing heterocyclic ring and the oxidized form is used as the basis flavonoid division into sub groups (Aneklaphakij et al., 2021). Some medicinal plants that contain flavonoids have been reported to have antioxidant, antibacterial, antiviral,

anti-inflammatory, allergy, and anticancer (Ullah et al., 2020). The catching of free radicals causes the antioxidant effect of these compounds through donor the hydrogen atom of the hydroxyl group of flavonoids (Kasote et al., 2015).

Previous studies have shown that green tea from the Gambung Tea Plantation, West Java, Indonesia has a high polyphenol content based on determining the total phenolic content using standard gallic acid. Furthermore, green tea extract has potent antioxidant activity based on the results of free radical scavenging tests using the DPPH method (Trinovani et al., 2022). In this study, green tea (*Camellia sinensis*) leaves extract is to be analyzed the content of total phenolic and flavonoid compounds using UV-Visible spectrophotometry.

II. Research Method

II.1 Chemicals and Sample

Gallic acid and quercetin were obtained commercially (Sigma Chemie GmbH Aldrich, Germany). Pro analysis grades of ethanol, aluminum chloride (AlCl₃), potassium acetate (CH₃COOK), reagent of Folin-Ciocalteu, sodium bicarbonate (Na₂HCO₃), were purchased from Merck, filter paper no.1 (Whatman). The stock solution (c = 1000 µg/mL) was prepared by dissolving 10 mg of gallic acid and quercetin standard with 100 mL of distilled



water, respectively. *Camellia sinensis* were collected from Makassar, Indonesia.

II.2 Extraction

The extraction of the sample was performed in the Natural Product Laboratory. Green tea leaf powder of as much as 10 kg was put in a maceration container with water of as much as 30 L. The sample was heated in the microwave for 15 minutes. The liquid extract was filtered and collected. The residue was re-macerated to perfect the extraction is complete. The liquid extract is dried using a freeze-dryer.

II.3 Purification

The purification was performed following the previous study with a modification (Altemimi et al., 2017). The crude extract of green tea leaves in the purification uses various solvents from petroleum ether, chloroform, ethyl acetate, and ethanol. The aqueous extract of green tea leaves was added to the solvent n-hexane and placed in the flask funnel. Added petroleum ether, shaken, allowed to stand, and filtered. Purification is then performed sequentially with chloroform, ethyl acetate, and ethanol. The water layer was collected and thickened, then dried with freeze-drying.

II.4 Total Phenolic Determination

Total phenolic was determined by the Folin-Ciocalteu colorimetric method using gallic acid as the standard reference. Gallic acid was made by 4, 6, 8, 10, 12, 14 $\mu\text{g/mL}$ from stock solution 1000 $\mu\text{g/mL}$. The sample was prepared by diluting 10 mg into 10 mL ethanol; then, it was diluted again until getting 200 $\mu\text{g/mL}$. Briefly, 100 μL of standard and samples were added to 1 mL of 10% Folin-Ciocalteu reagent and left on for three minutes. The mixture was then added to 3 mL of Na_2HCO_3 7.5%. The mixture was shaken for five minutes and then incubated at 37°C for 15 minutes, followed by incubation in the dark for 1 hour (Fawwaz, Nurdiansyah, et al., 2017; Siddiqui et al., 2017). Absorbance was then measured at 759.75 nm by UV-Visible Spectrophotometry, which distilled water as a blank. Gallic acid was used to construct a standard curve. The amount of total phenol content was calculated as mg/g gallic acid equivalent (GAE).

II.5 Total Flavonoid Determination

The total flavonoid content of the extract was measured by Chang method with some modifications (Baba & Malik, 2015; Fawwaz, Muliadi, et al., 2017). Standard quercetin made by 6, 8, 10, 12, 14 $\mu\text{g/mL}$ from stock solution 1000 $\mu\text{g/mL}$. Briefly, 1 mL of each standard and sample was poured into a centrifuge tube, respectively; this

was followed by the addition of 0.2 mL of AlCl_3 10% and 0.1 mL of CH_3COOK 1M. The content of the centrifuge tube was mixed thoroughly with a vortex mixer for two to three minutes and allowed to stand for 30 minutes at room temperature. Absorbance was then measured at 435 nm by UV-Visible spectrophotometry. Quercetin was used as a standard reference for the quantification of total flavonoids. Total flavonoid content is expressed in each extract's grams of quercetin equivalent (QE).

II.6 Data analysis

A calibration standard curve was obtained by running on a UV-Visible spectrophotometer and then plotting the absorbance against concentrations. The best fit of the line curve was calculated by the equation of a line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept, and slope of the calibration curve were calculated. The best fit of data was determined by linear regression using the following equation $y = bx + a$ where, y = absorbance, b = slope, x = concentration, and a = intercept.

III. Results and Discussion

Green tea has been used extensively throughout the territory of Indonesia in the form of beverages as well as herbal medicine. Basically, the use of green tea is associated with catechins, its chemical components, and has high antioxidant activity (Lorenzo & Munekata, 2016). Additionally, it is believed also contain other components of phenolic and flavonoid compounds, thus the need to explore the scientific basis of the levels of both classes of these compounds (Mutha et al., 2021; Sakurai et al., 2020).

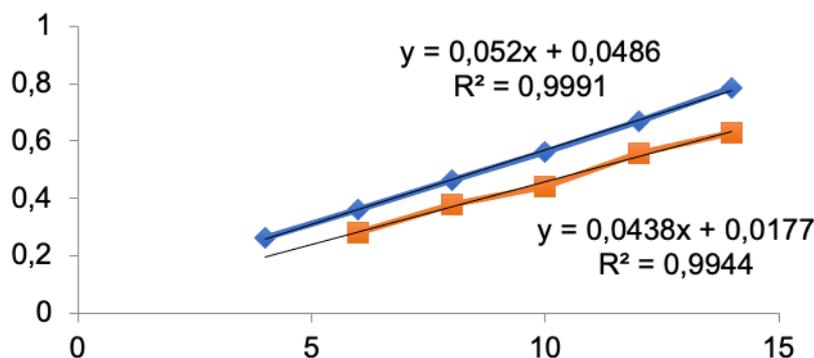
Determination of total phenolic and flavonoid using standard reference as a comparison in the measurement by spectrophotometer. Gallic acid and quercetin are the standard references of total phenolic and flavonoid, respectively. These standards were made in the series concentration of parts per million ($\mu\text{g/mL}$). The absorptions were measured at a maximum wavelength, and the results are provided in the Table 1.

Table 1. The absorbance of standard series

Concentration ($\mu\text{g/mL}$)	Gallic acid (759.75 nm)	Quercetin (435 nm)
4	0.261	-
6	0.360	0.278
8	0.464	0.378
10	0.562	0.442
12	0.667	0.555
14	0.785	0.628

Based on the absorbances of both standard references, we obtained the equation of linear regression. The line graph (Figure 1) shows that there is a linear relationship between concentration and absorbance. The linearity is not only evidenced

by the line formed but also by looking at the value of R^2 nearing 1. This proves that the methods used in the measurement can be declared valid.

**Figure 1.** The calibration standard curve of gallic acid (blue) and quercetin (orange)

Samples of crude and purified extract of green tea leaves were performed in the Natural Products Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia. The extract was dissolved in ethanol and reacted with each reagent. The solution was measured at a wavelength of maximum absorbance in accordance with standard

reference. Determination of the total phenolic compound was conducted three times. The results showed high levels of the phenolic total in both crude and purified extracts, as shown in **Table 2**. The data exhibited that purified extract has a high concentration of total phenolic than crude extract, although this difference is not significant.

Table 2. Total phenolic content of the green tea leaves extract

Sample (200 $\mu\text{g/mL}$)	Absorbance (759.75 nm)	Concentration ($\mu\text{g/mL}$)	Total Phenolic (mgGAE/g)
Crude Extract	0.460	8.10	40.5
	0.486		
	0.464		
Purified Extract	0.511	8.78	43.9
	0.463		
	0.542		

Determination of the total flavonoid compound was conducted three times. The data exhibited that between crude and purified extract has similar concentration of total flavonoid level (Table 3), indicating that the purification process utilized

was successful in keeping the overall flavonoid content in the purified extract.

Table 3. The results of the total flavonoid content of the green tea leaf extract

Sample (1000 µg/mL)	Absorbance (435 nm)	Concentration (µg/mL)	Total Flavonoid (mgQE/g)
Crude Extract	0.144	2.77	2.77
	0.133		
	0.141		
Purified Extract	0.163	3.14	3.14
	0.150		
	0.153		

The determination of both total phenolic and flavonoid content between crude and purified extract showed not significant level. This demonstrates that the solvent used in the extract's purification is capable of extracting the maximum amount of total phenolic and flavonoids from the crude extract. Besides, the levels of total phenolic in both samples are much higher than the levels of total flavonoid content. This result is in line with the previous study, which showed that total phenolic levels dominated the green tea extract (Anesini et al., 2008; Smith, 2011).

IV. Conclusions

The total phenolic level of green tea leaves was greater than the total flavonoid level. Thus, developing green tea leaves as herbal medicine should consider the phenolic compound. The levels between the crude and purified extract are not significantly different in both total phenolic and flavonoid compounds.

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