

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

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ABSTRACT

Polyphenols are a class of chemical compounds most commonly found in plants, these compounds have many health benefits associated with its character. The antioxidant activity of natural materials related to the polyphenol content, which can counteract free radicals. This study aims to determine the levels of total phenolic and flavonoid in crude and purified extracts of the green tea leaves (*Camellia sinensis*). Determination of total phenolic content was used standard gallic acid by the *Folin-Ciocalteu* method. Total flavonoid assay using standard reference of quercetin by Chang method. 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 containing total phenolic levels 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 the green tea leaves have a total phenolic content is greater than the total flavonoid content, thus it has great potential as an antioxidant.

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INTRODUCTION

Polyphenols are a form of bioflavonoids with several phenol groups. Polyphenols in green tea are catechins. Polyphenol compounds can act as catcher of hydroxyl free radical (OH) that does not oxidize lipids, proteins and DNA in the cell. The ability of polyphenols catches free radicals 100 times more effective than vitamin C and 25 times more effective than vitamin E (Khan & Mukhtar, 2018). One important type of polyphenols is flavonoids. Flavonoids consist of various types, such as flavonols, flavones, isoflavones, flavonem, anthocyanin and catechin. As the bioactive substance, anthocyanin and catechin serves catch free radicals that can inhibit

the occurrence of damage to the cell membrane (Chaturvedula & Prakash, 2011). This mechanism is more effective than vitamin C and E (Heim et al., 2002).

Flavonoids are natural phenolic compounds contained in almost all plants (Saeed et al., 2012). 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 middle ring in the form of oxygen-containing heterocyclic ring and the oxidized form is used as the basis flavonoid division into sub-subgroups (Abdi, 2010). Several medicinal plants that contain flavonoids have been reported to have

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antioxidant, antibacterial, antiviral, anti-inflammatory, allergy, and anticancer. The antioxidant effect of these compounds caused by the catching of free radicals, through donor the hydrogen atom of the hydroxyl group of flavonoids (Saeed et al., 2012).

The previous research provides that water extract of green tea leaves has the highest phenolic content of seven types of tea; decaffeinated green tea bag (dGTB), green tea bag (GTB), black tea bag (BTB), decaffeinated BTB, black tea leaves and oolong tea leaves (OTL) (Yang & Liu 2013). In this study, green tea leaves crude and purified extract to be analyzed content of total phenolic and flavonoids compounds using UV-Visible spectrophotometry, where the sample is obtained from the Natural Product Laboratory, Universitas Muslim Indonesia, Makassar.

EXPERIMENT

Chemicals and Standard Solution

Gallic acid and quercetin were obtained from Sigma Chemie GmbH Aldrich. Pro analysis grades of ethanol, aluminium chloride (AlCl_3), potassium acetate (CH_3COOK), reagent of Folin-Ciocalteu, sodium bicarbonate (Na_2HCO_3), was purchased from Merck, filter paper no.1 (Whatman). The stock solution ($c = 1000 \mu\text{g/ml}$) was prepared by dissolving 10 mg of gallic acid and quercetin standard with 100 ml of distilled water, respectively (Fawwaz et al., 2017).

Plant Materials

Camellia sinensis were collected from Makassar, Indonesia. The extraction of sample was performed in Natural Product Laboratory, and then we analyse crude extract and purified extract from that's process.

Extraction

Green tea leaf powder weighed as much as 10 kg, then put in a maceration container with solvent water added as much as 30 L. Then microwave for 15 minutes, done 3 times. Filtered, the liquid extract is collected. The residue was extracted back to perfect the extraction is complete. The liquid extract is dried using freeze dry.

Purification Extract

The crude extract of green tea leaves in the purification by using a variety of different solvents from petroleum ether, chloroform, ethyl acetate, and ethanol. The aqueous extract green tea leaves added to the solvent n-hexane was placed in the flask funnel and was added petroleum ether, shaken, and 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 dry.

Total Phenolic Determination

Total phenolic were determined by the Folin-Ciocalteu colorimetric method that using gallic acid as standard reference. Gallic acid was made by 4, 6, 8, 10, 12, 14 $\mu\text{g/ml}$ from stock solution 1000 $\mu\text{g/ml}$. Sample was prepared by dilution 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 then added 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 (Končić et al., 2010; McDonald et al., 2001; Nabavi et al., 2008; Adedapo et al., 2009). Absorbance was then measured at 759.75nm 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) (Chang et al., 2002).

Total Flavonoid Determination

The total flavonoid content of the extract was measured by Chang method with some modifications. 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 (Dogara et al., 2025). The content of the centrifuge tube was then mixed thoroughly with a vortex mixer for two to three minutes and allowed to stand for 30 minutes at room temperature (Adedapo et al., 2009; Ebrahimzadeh et al., 2009; Ordonez et al., 2006). Absorbance was then measured at 435nm by UV-Visible spectrophotometry. Quercetin was used as a standard reference for the quantification of total flavonoids. Total flavonoid content is expressed in grams quercetin equivalent (QE) of each extract (Fawwaz et al., 2017).

Data Analysis

A calibration standard curve was obtained by running on UV-Visible spectrophotometer and then plotting absorbance against concentrations. The best fit of the line curve was calculated by equation of line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept and slope of 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.

RESULTS & DISCUSSION

Indonesia is rich in biodiversity that has been used for a long time, formerly only as a source of food such as soybeans as a source of protein, but with the improvement of science it is found that fermented soybeans such as tempeh are able to overcome the symptoms of osteoporosis (Fawwaz et al., 2014) as well as other flora including green tea that has now been widely studied. Green tea (*Camellia sinensis*) is a plant that 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

Table 1

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 |

The same table is shown on the standard reference of quercetin as a comparison in determining the levels of flavonoids. The absorbance was measured at 435 nm, which is the maximum wavelength of quercetin on the results of running the lambda max. Wavelength selection in a very important measurement given sample will absorb light maximally at these wavelengths. Based on the measurement data of both standard references, obtained the equation of linear regression. It clearly seen that there is a linear relationship between concentration and absorbance, according to the equation of a line graph in Figure 1.

associated with catechins its chemical components and has a high antioxidant activity. Additionally, it 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.

Assay of total phenolic and flavonoid use standard reference as a comparison in the measurement in the spectrophotometer. Gallic acid as a standard of total phenolic was made in the series concentration of parts per million ($\mu\text{g/ml}$) measured absorption at a maximum wavelength of 759.75nm. This wavelength is determined based on the results of running maximum wavelength using one of the standard series. The data is shown in Table 1.

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. Samples of crude and purified extract of green tea leaves was performed in Natural Products Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia, then dissolved in ethanol as an organic solvent, capable of dissolving completely the samples. Once the solution is formed and reacted with each reagent, we conducted measurements at a wavelength of maximum absorbance in accordance with standard measurement reference.

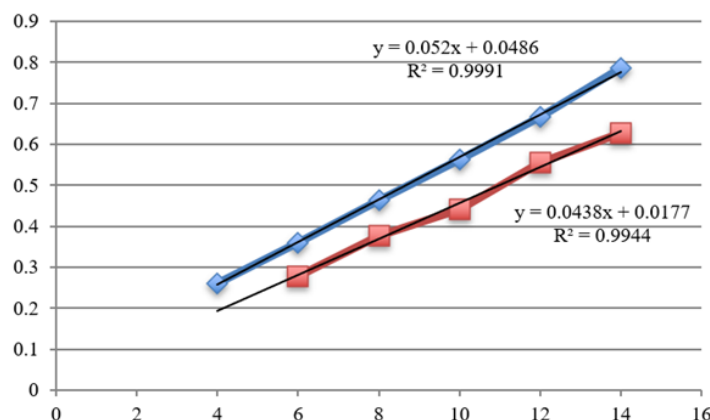


Fig. 1. The calibration standard curve of gallic acid (blue) and quercetin (red)

In the determination of total phenolic conducted at three times, obtained high levels of each crude

and purified extracts, in accordance with Table 2. In the table clearly seen purified extract has higher

concentration than crude extract, but this difference is not significant.

Table 2

The results of the total phenolic content of the green tea leaves extract

| Sample 200 µg/ml | Absorbance | Concentration (µg/ml) | Total Phenolic (mgGAE/g) |
|------------------|-------------------------|-----------------------|--------------------------|
| Crude Extract | 0.460 0.486 0.464 | 8.10 | 40.5 |
| Purified Extract | 0.511 0.463 0.542 | 8.78 | 43.9 |

Data in the Table 3 is the results of the determination of total flavonoids showed levels were not significantly different between crude extracts and purified, where the levels of total flavonoids of the extract is much lower than the levels of total phenolic contains, whereas the concentration of the samples used in the testing of flavonoids 5 times greater than the concentration of total phenolic testing samples in which only 200 ppm. The other study in Sabah-Malaysia shows that ethanolic extract of green tea leaves have different

Table 3

The results of the total flavonoid content of the green tea leaves extract

| Sample 1000 µg/ml | Absorbance | Concentration (µg/ml) | Total Flavonoid (mgQE/g) |
|-------------------|-------------------------|-----------------------|--------------------------|
| Crude Extract | 0.144 0.133 0.141 | 2.77 | 2.77 |
| Purified Extract | 0.163 0.150 0.153 | 3.14 | 3.14 |

The method of extraction of green tea also influences the content of total phenolic and flavonoid. Intact and pulverized leaves were used for extraction by methanol or acidified methanol, and determined by spectrophotometric methods. This research found that extracts obtained by extraction of pulverized green tea leaves with acidified methanol had the higher phenolic content (3.823 g/kg) than in methanol extracts. Flavonoid and tannin contents followed the same tendency as phenol content (Kopjar et al., 2015).

CONCLUSION

From the result, it can be concluded that the total phenolic levels in green tea leaves are greater than the levels of flavonoids. Thus, the development of green

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concentration based on the parts of the leaves. The shoot contained significantly higher total phenolic content, followed by the young and matured leaves ($p < 0.05$). The same trend was also observed for antioxidant activity as assessed using FRAP (ferric reducing/antioxidant power), DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and ABTS (2,2'-azinobis(3-ethylbenzthiazoline)-6-sulphonic) radical scavenging assays (Izzreen & Fadzelly, 2013).

tea leaves as herbal medicine should consider the phenolic compound. If the review of type of extracts in the test, the comparison levels between crude extract and purified extract is not significance different for both total phenolic and flavonoid assay.

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Competing Interest

The authors had no competing interests.

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