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ANALYSIS OF TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF GREEN TEA (*Camellia sinensis*) WITH VARIATIONS IN TYPE AND BREWING

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ABSTRACT
Free radicals can react to cause damage when they enter the human body. The use of antioxidants can neutralize free radicals in the body. This study aims to determine the total flavonoid levels and antioxidant activity in premium and original green tea with variations in brewing temperature. Green tea produced by Kulon Progo is brewed with aquadest at 75 ℃ and 100 ℃, and then the filtrate obtained is ground by freeze-drying. Identification of flavonoid compounds using the flavanoid-ammonia vapor color complex. The total flavonoid levels analysis using UV-vis spectrophotometry and antioxidant activity was tested by the DPPH (1,1-diphenyl-2-picrylhydrazil) method. Based on the test results, the highest to lowest total flavonoid levels were premium green tea at 100 ℃ original at 100 ℃, original at 75 ℃, and premium at 75 ℃ (155.095 ± 3.158; 153.333 ± 2.788; 151.7181 ± 4.944; and 148.634 ± 2,095 mgQE/gram extract). The highest to lowest potential antioxidant activity, respectively, is quercetin (flavonoid standard), premium green tea at 100 °C, premium at 75 °C, original at 100 °C, and original at 75 °C, with IC_{50} values of 3.0692 ± 1.064; 32.359 ± 7.346; 31.502 ± 6.746; 27.527 ± 7.868; and 27.167 ± 1.817 µg/mL. Analysis of the total flavonoids of green tea showed that the highest levels were in premium green tea at a temperature of 100 °C. An antioxidant activity test with the DPPH reagent produced the highest antioxidant potential in premium green tea at 100 °C. So premium green tea at a brewing temperature of 100 °C is more recommended to consumers.

Keywords: Brewing; *Camellia sinensis* L; Green tea; Flavonoid; Freeze drying

1. INTRODUCTION

Free radicals are unstable compounds and can harm health if they enter the human body (Wahdaningsih et al., 2011). Based on IQAir in 2022, Indonesia ranks 26th out of 131 countries with the highest air pollution and has the worst air pollution in Southeast Asia. Apart from that, the increase in health complaints in Indonesia in 2022 has increased to 29.94% from the previous 27.23% in 2022. Antioxidants are compounds that can neutralize and reduce the negative impact of free radicals on the body. Antioxidants are divided into two, namely enzyme and non-enzyme antioxidants. Enzyme antioxidants include catalase enzymes and glutathione peroxidase enzymes, while non-enzyme antioxidants such as vitamin C, anthocyanidins, and flavonoids (Zulaikhah, 2017). Antioxidants work by donating one electron to an oxidant compound so that the activity of the oxidant compound can be inhibited (Zulaikhah, 2017). Antioxidant compounds are easily oxidized, so the tea brewing procedure will affect the amount of antioxidant compounds extracted. The oxidation process that occurs due to the influence of the tea brewing procedure can affect the amount of antioxidant compounds produced. This is because the free radical scavenging group in the antioxidant compound is damaged.

Green tea (*Camellia sinensis* L.) is a plant that contains phenolic compounds, alkaloids, steroids, tannins, and flavonoids (Arya et al., 2019). In Indonesia, tea has long been known as a drink that is popular with Indonesian people (Tehubijuluw et al., 2019). Tea is divided into four based on processing methods: green tea, white tea, black tea, and oolong tea. of the four types of tea, green tea contains the highest antioxidant compound activity (Fajar et al., 2018). Tea is also rich in flavonoids (Sasmito, 2020). This content is thermolabile and volatile (Gloriana et al., 2023). So, the tea brewing temperature can affect the number of total flavonoids extracted (Sugiyanto et al., 2022).

The method that can be used to analyze total flavonoid levels is UV-Vis spectrophotometry. This is because flavonoids have conjugated aromatic groups and can show strong absorption bands in the UV-Vis region. Besides, flavonoids are polar, which can absorb radiation absorption lengths in the UV-Vis region (Winahyu et al., 2019). Based on this, research is needed to analyze the effect of brewing temperature on total flavonoid levels in premium and original green tea produced by Kulon Progo using the UV-Vis spectrophotometric method. Kulonprogo is one of the districts that has original tea products from the Special Region of Yogyakarta. This research used two variations, namely, brewing temperature and tea quality, with three replications for each variable. The difference between the two tea samples in this study is the premium class with original green tea. The difference between the two samples is based on the materials used. Premium green tea only uses young tea from the top of the plant, while original green tea uses a mixture of shoots and young leaves up to the fourth stalk. Based on the differences in the materials used, knowing their effect on the difference in total flavonoid content as antioxidant activity is necessary.

2. METHODS

2.1. Materials

The raw materials used are premium and original green tea leaf simplicia (*Camellia sinensis* L.), quercetin (Sigma), 2,2- *diphenyl-1-picrylhydrazyl* (DPPH) (Sigma), ethanol 96% (Brand), sodium acetate (Brand), $AlCl_3$ (Brand), and distilled water.

2.2. Sample Extraction

Each premium and original green tea sample was weighed at 10 g each, then brewed using distilled water in a dispenser at 75 °C and 100 mL of boiling water at 100 °C, ultrasonified for 10 minutes. The brewed water is filtered using filter paper until you get a clear filtrate (Chadijah et al., 2021). The clear filtrate is then dried using the freeze-dryer method.

2.3. Yield Test

The yield is obtained by comparing the final weight obtained (dry extract) with the initial weight of the sample (Salamah et al., 2017).

$$\%\, Yield = \frac{Initial\ weight\ of\ simplicia}{Weight\ of\ extract} \times 100\% \quad (1)$$

2.4. Flavanoid Content Test

Premium and original green tea that has been refined weighed 1 gram and dissolved in 10 mL of 96% ethanol. Two drops of green tea extract were dropped on filter paper, and then the filter paper was placed on the mouth of a beaker containing ammonia on a heater. A positive result is indicated by a change in the color of the filter paper to yellow or orange (Arnida et al., 2021).

2.5. Drying Shrinkage Test

Drying loss was carried out by weighing 2 grams of each simplicia sample, placing it on an aluminum foil plate (special), and then placing it in a halogen moisture analyzer at 105 °C for

15 minutes. Drying loss meets the requirements if the drying loss percentage value obtained is <10% (Silverman et al., 2023).

2.6. Ash Content Test

Determination of ash content is carried out using the gravimetric method. Each dry sample was weighed to 2 grams on a silicate crucible that had been tarred and ignited. The steam cup containing the sample is then heated in a furnace at a temperature of 600 °C until ash forms and the weight is constant. Ash content is calculated from the weight of the test material expressed in percent w/w (Silverman et al., 2023).

$$\%\ Ash\ content = \frac{w1 - w2}{w} \times 100\% \quad (2)$$

where, w: Sample weight before kilning (g); w_1: Weight of sample and cup after kiln (g); w_2: Weight of empty cup (g)

2.7. Determination of Total Flavonoids

Each tea sample (freeze-drying result) was weighed at 25 mg, and ethanol p.a was added to a 25 mL volumetric flask. Take 0.25 mL and put it in a 10 mL measuring flask. Add 3 mL of pa ethanol, 0.2 mL of 10% $AlCl_3$, 0.2 mL of 1 M sodium acetate, and distilled water to the mark. Leave it for operating time and measure the maximum absorption length (Silverman et al., 2023; Swandi et al., 2020).

2.8. Antioxidant Activity Test using the DPPH Method

The antioxidant activity test was carried out using the DPPH method and UV-Vis spectrophotometry. The sample (green tea) and standard (quercetin) were dissolved in 96% ethanol with a ratio of 0.15 mM DPPH: sample (2:1). The solution was incubated during OT at room temperature and covered with aluminum foil. The absorbance was measured at 517 nm lambda (corresponding to the DPPH lambda at that time). Absorption readings were carried out three times for each standard and sample. The IC_{50} (Inhibitory Concentration 50%) value, or 50% inhibitory concentration, can indicate antioxidant activity. The IC_{50} value is the antioxidant level that reduces DPPH by up to 50%. In addition, the IC_{50} value is one of the most commonly used parameters when using the DPPH method to test antioxidant activity. Please note that the greater the IC_{50} value, the smaller the antioxidant activity; vice versa; if the IC_{50} value is smaller, the antioxidant activity will be greater (Andriani & Murtisiwi, 2020). Antioxidant activity can be analyzed based on the percentage of antioxidant activity with the formula:

$$\%\ Antioxidant\ activity = \frac{(A\ negative\ control - A\ sample)}{A\ Negative\ control} \times 100\% \quad (3)$$

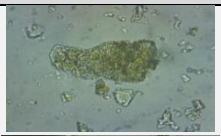
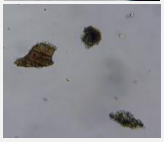
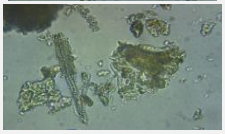
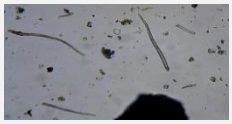
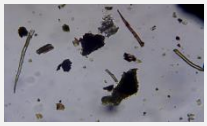
where, A is absorbance value

3. RESULTS AND DISCUSSION

The research began with sample collection; green tea samples were obtained from green tea producers in Padukuhan Suroloyo, Kulon Progo, Yogyakarta. Two types of quality green tea are used, namely premium quality and original green tea. Premium quality green tea only uses young leaves at the top of the leaf, whereas original quality green tea contains a mixture of stems and young green tea leaves. The green tea sample obtained was in the form of dried Simplicia, which was ready to be consumed, so to ensure that the sample taken was green tea, a microscopic test was carried out to look at the parts of the tea leaves in detail. The preparation is examined through a microscope using a water reagent in microscopic testing. Microscopic tests were carried out at the Laboratory of the Faculty of Applied Science and Technology (FAST), Faculty of Biology, Ahmad Dahlan University. They obtained microscopic test results for premium and

original green tea in parenkin tissue, vascular bundles, and trichomes, which can be seen in **Table 1**. This microscopic identification is very important to ensure that the sample used is truly the *Camelia sinensis* L. species because the sample used from the beginning was only a dry green tea Simplicia and not a whole plant. We use the microscopic results to identify the fragments of green tea leaves (Silverman et al., 2023).

Table 1. Microscopic test of premium and original green tea

No	Leaf parts according to the monograph	Sample	
		Premium	Original
1.	Parenkin		
2.	Vascular Files		
3.	Trichomata		

3.1. Sample Preparation

The samples were obtained from freeze drying; the freeze-drying of green tea brewing water obtained the weight of the dry extract, as shown in **Table 2** and **Figure 1**.

Table 2. Green tea yield

No.	Sample	Weight Simplisia (g)	Extract weight (g)	Yield (%)
1.	Premium Green Tea 100 °C	10.093	0.786	7.787
2.	Original Green Tea 100 °C	10.062	1.001	9.948
3.	Original Green Tea 75 °C	10.035	1.195	11.908
4.	Premium Green Tea 75 °C	10.078	1.394	13.832

Premium green tea extract 100 °C | Original green tea extract 100 °C | Original green tea extract 75 °C | Premium green tea extract 75 °C

Figure 1. Freeze drying green tea extract

Based on (Silverman et al., 2023), The required percent yield value of thick tea leaf extract (> 7.8%) means that the yield value of premium and original green tea produced by Kulon Progo is following the requirements.

3.2. Identification of Flavanoid Content

Flavonoid identification was carried out to ensure flavonoid compounds were in Kulon Progo Green Tea. The reagent is ammonia vapor, producing an intensive yellow color change on the filter paper if the sample contains flavonoids. The test results can be seen in **Table 3**. There is an OH- group in the flavonoid structure, which can easily release the H+ atom in the presence of an ammonia base and then combine with ammonia to make the O2- atom have additional free electrons. A quinoid structure is formed when these electrons are added, producing a yellow color. Because there is a large amount of water vapor (H2O) in the air, the H+ atom will be given to the

O2- atom, which has an excess of electrons, so that the color and reaction will quickly return to normal (reversible) (Nugraha et al., 2022).

Table 3. Flavonoid content test

Sample	Reactant	Before being evaporated by ammonia	After being evaporated by ammonia	Description
Quercetin standard	ammonia vapor			(+) There is an intense color change to yellow.
Premium green tea sample	ammonia vapor			(+) There is an intense color change to yellow.
Original green tea sample	ammonia vapor			(+) There is an intense color change to yellow.

3.3. Drying Shrinkage

Determination of drying losses is one of the requirements that must be fulfilled in the standardization of nutritious plants to provide a maximum limit (range) regarding the amounts of compounds lost in the drying process (Najib et al., 2017). The drying loss content of premium green tea was 6.999%, while original green tea was 6.6.45%. Based on (Silverman et al., 2023), the drying loss requirement for tea leaves is not more than 10%, so the drying loss value of premium and original green tea obtained follows the permitted requirements.

3.4. Ash Content Test

The ash content value aims to determine the mineral content in the simplicia or extract. The mineral content in a sample is divided into organic and inorganic salts. Apart from that, determining the ash content is useful for determining the high or low quality of the processing process, the type of material, and the nutritional value parameters of the material (Pine et al., 2015). Ash content is calculated from the weight of the test material expressed in % w/w. The research results showed that the percent ash content of premium green tea was 3.341% and original green tea was 4.403%. Based on the Indonesian herbal pharmacopeia, the limit value for the ash content of tea leaves is no more than 5.6%, so the percent ash content of premium and original green tea in this study is appropriate. Low ash content values indicate minimal mineral and inorganic contamination in the extract. Contamination can occur due to the growing location or an unclean extract manufacturing process. The lower the ash content, the higher the quality of the powder and extract.

3.5. Total Flavanoid Content

The standard quercetin solution was left for 30 minutes, and the absorbance was read at the maximum wavelength, as shown in **Figure 2**. The quercetin absorbance value obtained was then calculated to obtain the equation y = 0.0908x + 0.1406 with squared efficiency (R2) = 0.998, with y as the light absorption and x as the concentration of the quercetin solution. The quercetin standard curve graph can be seen in **Figure 3**. The content of flavonoid compounds in green tea samples is expressed in total quercetin equivalents per gram of green tea (mg EQ/gram extract).

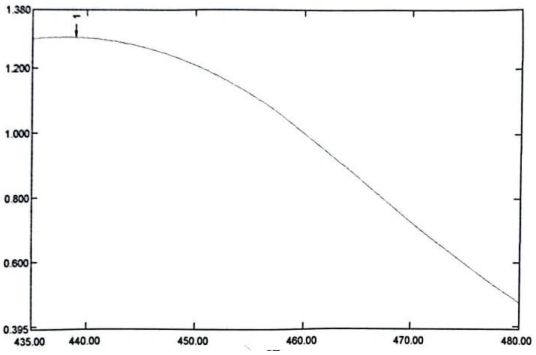


Figure 2. Graph of the maximum wavelength of quercetin 439 nm

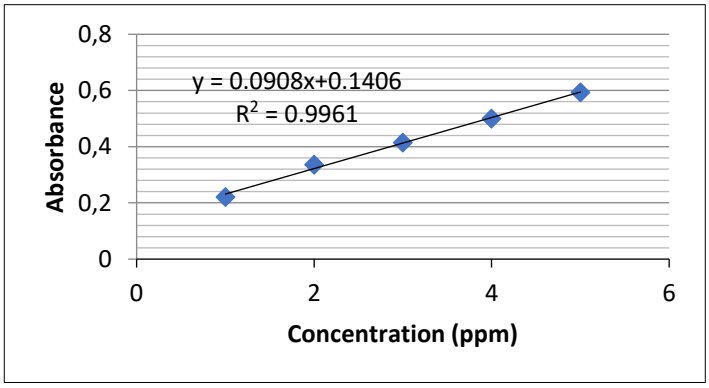


Figure 3. Quercetin standard curve obtained by UV-Vis spectrophotometry

Table 4. Total flavonoid content of green tea

No.	Name	Total Flavanois Levels (mg QE/g)
1.	Premium Green Tea 100 °C	155.095 ± 3.158
2.	Original Green Tea 100 °C	153.333 ± 2.788
3.	Original Green Tea 75 °C	151.718 ± 4.944
4.	Premium Green Tea 75 °C	148.634 ± 2.095

The total flavonoid content values from the research results can be seen in **Table 4**. Respectively, the total flavonoid content of green tea is premium green tea at 100 °C, original green tea at 100 °C, original green tea at 75 °C premium green tea temperature 75 °C. Based on brewing temperature, samples brewed using water at 100 °C produced higher levels of flavonoids than samples brewed in water at 75 °C. This is following research (Chatterjee et al., 2016), which states that the highest total levels of green tea flavonoids were obtained at a temperature of 100 °C with an extraction time of 15 minutes at various experimental temperatures of 60 °C, 80 °C and 100 °C. Meanwhile, regarding the differences in the quality of the samples used, based on the average total flavonoid content of the two samples, original quality green tea was superior to premium quality green tea. A graph of the total flavonoid content of premium and original green tea can be seen in **Figure 4**.

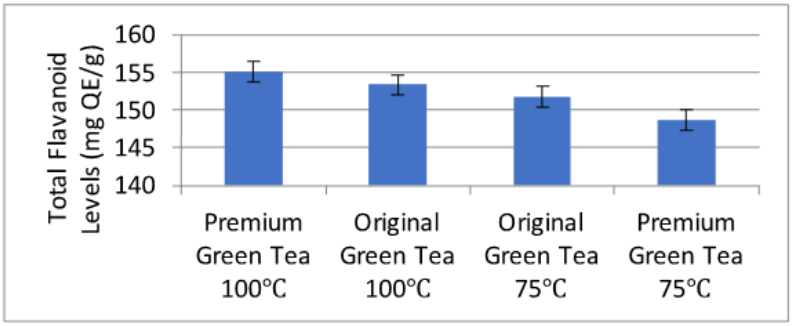


Figure 4. Graph of total flavonoid content of premium and original green tea

3.6. Preliminary Test for Antioxidant Activity

A preliminary test aims to find out whether the sample being tested has antioxidant activity. DPPH is a purple free radical. If DPPH reacts with antioxidant compounds, the intensity of the purple color is reduced. If free radical-reducing compounds react in large quantities or are strong antioxidants, DPPH can change color to yellow. In this test, react between DPPH at a concentration of 0.15 mM with original tea extract at a temperature of 100 ℃ and premium tea at a temperature of 100 ℃ and wait until the color is constant or does not change any more or at least 30 minutes. From this test, it is known that both green tea extracts contain antioxidants.

3.7. Measurement of Antioxidant Activity

The parameter for measuring antioxidant activity is the IC50 (Inhibitory Concentration) value (Purwanto et al., 2017). The percentage of free radical capture is the number of free radicals captured by standards and samples. The sample solution was left for 30 minutes at room temperature, and the DPPH free radical capture value was calculated at the maximum wavelength, as shown in **Figure 5**. The results from the percentage of free radical capture, the IC50 value, was calculated. Data on DPPH free radical capture and IC50 values for quercetin standards and samples can be seen in **Table 5**.

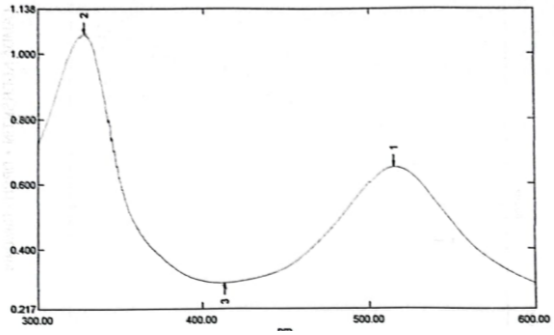


Figure 5. Graph of the maximum wavelength of a green tea sample, 515 nm.

Table 5. Data on % capture of DPPH free radicals and IC_{50} values of quercetin standards and samples

No.	Name	IC_{50}
1.	Standart Quercetin	3.069 ± 1.064
2.	Premium Green Tea 100 ℃	27.167 ± 1.817
3.	Original Green Tea 100 ℃	31.502 ± 6.746
4.	Original Green Tea 75 ℃	32.359 ± 7.346
5.	Premium Green Tea 75 ℃	27.527 ± 7.868

The results of the research show that green tea extract contains antioxidants. The IC_{50} value of standard quercetin is smaller than the IC_{50} value of the four samples, namely standard quercetin, original green tea at 75 ℃, original green tea at 100 ℃, premium green tea at 75 ℃, and premium green tea at 100 ℃. From the four tea extracts, it can be concluded that the smallest IC_{50} is in the premium tea extract at 100 ℃, which means that the premium tea extract at 100 ℃ has the best antioxidant activity. The IC_{50} value graph can be seen in **Figure 6**.

Analysis of the total flavonoid content of green tea with $AlCl_3$ using Uv-Vis spectrophotometry showed that the highest total flavonoid content value was premium green tea at a temperature of 100 ℃. Previous research was conducted by (Chatterjee et al., 2016), which stated that the highest total levels of green tea flavonoids were obtained at a temperature of 100 ℃ with an extraction time of 15 minutes at various experimental temperatures of 60 ℃, 80 ℃ and 100 ℃. The antioxidant activity test with DPPH reagent produced the strongest antioxidant activity with an IC_{50} value of 27.167 ± 1.817 µg/mL, namely premium green tea at 100 ℃. This follows research (Sasmito, 2020) in Sonneratia albapada green tea steeping water; it produces quite high antioxidant activity at a temperature of 100 °C for ten days with an IC_{50} value for DPPH

of 96.5 µg/mL. A compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 µg/ml, a strong antioxidant for an IC_{50} value of 50-100 µg/ml, and a moderate antioxidant if the IC_{50} value is 151-200 µg/ml (Andriani & Murtisiwi, 2020).

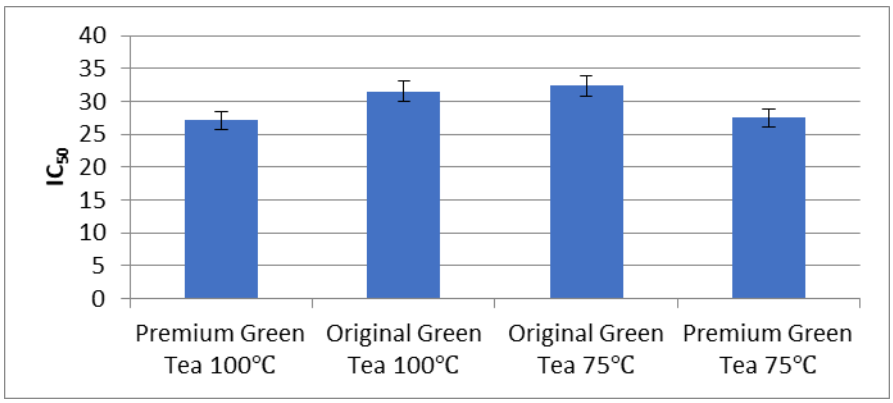


Figure 6. Graph of IC_{50} value for premium and original green tea

4. CONCLUSION

Analysis of the total flavonoids of green tea showed that the highest levels were in premium green tea at a temperature of 100 °C. The antioxidant activity test with DPPH reagent produced the highest antioxidant potential with an IC_{50} value of 27.167 ± 1.817 µg/mL, namely premium green tea at a temperature of 100 °C. So premium green tea at a brewing temperature of 100 is recommended to consumers. Premium quality green tea only uses young leaves at the top of the leaf.

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6. AUTHOR DECLARATION

Authors' Contributions and Responsibilities

The authors made substantial contributions to the conception and design of the study. The authors took responsibility for data analysis, interpretation, and discussion of results. The authors read and approved the final manuscript.

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Availability of Data and Materials

All data are available from the authors.

Competing Interests

The authors declare no competing interest.

Additional Information

No additional information from the authors.

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