Research article

Antioxidant Activities of Green Tea (Camellia Sinensis L.) Leaves From Ciwidey, West Java

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Abstract.
It has been reported that polyphenols-rich diets provide some protection against the development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Exogenous antioxidants are abudantly found in plants. Tea (*Camellia sinensis* L.; family *Theaceae*), which is the most popular beverage in Southeast Asia, is known for its medicinal properties. In this study, we evaluated antioxidant activity as well as phenol and flavonoid content in green tea leaves from PPTK Gambung Ciwidey in West Java. Antioxidant activity was observed through measuring 2,2- diphenyl-1 picrylhydrazyl (DPPH) scavenging activity. The total phenolic content was determined by the Folin Ciocalteu method. The green tea leaf extract showed the highest DPPH-scavenging activity at a concentration of 25 ug/ml (DPPH = 94.55%;IC_{50} = 0.54 µg/mL). Furthermore, the total phenolic and flavonoid contents of the green tea leaf extract were 36.64 and 8.43 mg QE/mg extract, respectively. These findings showed that the green tea leaf extract exhibited antioxidant activity.

Keywords: *Camellia sinensis* L, flavonoid, antioxidant

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1. Introduction

Free radicals are biochemical responses in the body which further promotes cancer, ischemic heart disease, inflammation, diabetes, aging, atherosclerosis, immunosuppression, and neurodegenerative disorders. [1-3] Antioxidant system in human body is usually responsible to scavenge the radicals. However, this might be interferred by excessive *reactive oxygen species* (ROS) and *reactive nitrogen species* (RNS) due to the exposure of cigarette smoking, alcohol, radiation, or environmental toxins. [2, 4, 5] Exogenous antioxidants are considered to restore such event by inhibiting oxidative chain reaction [6].

The exogenous antioxidants are mostly derived from food and medicinal plants [7-10]. These natural antioxidants are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) [6]. Polyphenols and carotenoids exhibit many biological

activities, such as anti-inflammatory, antibacterial, antiviral, anti-aging, and anticancer properties. [11-13]

Antioxidant activities have been widely studied on numerous plants. Tea (*Camellia sinensis* L.; family Theaceae), a well-known beverage in Southeast Asia, has been acknowledged for its medicinal properties [14-16]. White, green, oolong, black, and pu'erh teas are the major tea types sourced from leaves and buds of the tea plant [17]. These tea types also differ based on the variety of *C. sinensis* used in their production. Some studies support that among all tea types, green teas contain the highest amount of catechins, a group of polyphenolic flavan-3-ol monomers and their gallate derivatives [18]. The major catechins include (–)-epicatechin (EC), (–)- epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG). These compounds are responsible for its various biological functions, including antioxidant activities, anti-inflammatory activities, anti-melanogenic effects, and hepatoprotection [19-23]. Similar polyphenolic content among green tea varieties can provide more options of healthy beverage for people. Thus, we aimed to evaluate phenol and flavonoid content as well as antioxidant activity of green tea leaves from PPTK Gambung Ciwidey West Java.

2. Materials and Method

2.1. Green tea extraction

Oolong tea (*C. sinensis*) was obtained from a tea plantation in East Java. Oolong tea was extracted with 96% methanol (1:4) using a maceration technique. The filtrate was filtered with Na_2SO_4 and collected every 24 h until the filtrate became colorless. The filtrate was evaporated at 40^0C in an evaporator until a dried pellet was obtained. The ethanol extracted pellet was stored at 4^0C [24].

2.2. Fractination (Thin-layer Chromatography)

Thin-layer chromatography is an early identification of flavonoid. Extract was dissolved in three solvents: water, ethyl acetate, and n-hexane. Solution was filtered, and then evaporated. End product was spotted onto KLT plate, and eluted with mixture of water:ethyl acetate:n-hexane. Dots were observed and Rf was measured [24].

2.3. Total phenolic content

Total phenolic content was determined by Folin Ciocalteu method. Folin Ciocalteu's reagent (10%, 75 µl) was added to the samples (15 µl). Sodium carbonate solution (7,5 %, 60 µl) was then added to the samples, whilts DMSO (135 µl) was added into blank. Plates were incubated for 10 min in the dark at 50°C. The absorbance was measured at 760 nm wavelength. The total phenolic content was expressed as gallic acid equivalents (GAE) mg/g of tea leaves.

$$y = ax + b$$

$$Total\ phenolic\ content = \frac{Sample\ absorbance - b}{a}$$

2.4. Measurement of flavonoid

Green tea leaves extract and quercetin (75 µl) was added into samples and blank well. Alumunium chloride (2%, 75 µl) was added into samples, whilst DSMO (75 µl) was added into blank well. Absorbance was recorded at 415 nm wavelength. Flavonoid total was measured according to linier regression of quercetin.

$$y = ax + b$$

$$Total\ flavonoid = \frac{Sample\ absorbance - b}{a}$$

2.5. DPPH (2,2- diphenyl-1 picrylhydrazyl) scavenging activity

Briefly, 50 µl extracts and eugenol (Sigma-Aldrich) were added to a microplate followed by 200 µl DPPH (Sigma-Aldrich) solution (0.077 mmol/l in methanol). The mixtures was shaken vigorously and kept in the dark for 30 min at room temperature; DPPH scavenging activity was determined with a microplate reader at 517 nm. Negative control was 50 µL DMSO and 200 µL DPPH, and blank was 50 µL sample and 200 µL DMSO 10% [25]. The radical scavenging activity of each sample was measured according to following formula:

$$DPPH - scavenging\ activity = 1 - \frac{Samples\ absorbance}{Negative\ control\ absorbance} \times 100$$

3. Results

Phenolic content was measured with Folin-Ciocalteu colorymetry method in which polyphenol of plants reacts to redox specific reagent to form blue complex [26]. As shown in Table 3, phenolic total of green tea leaves extract was 36,64 ± 0,39 mg GAE/mg extract.

Table 1: Analysis of phenolic content of tea leaves extract.

Sample	Phenolic content (mg GAE/mg extract)	Flavonoid content (1 mg QE/1 mg sample)
Green tea leaves extract	36,64±0,39	8,43±0,39

Flavonoid content was measured with complementary colorimetry of yellow complex formed by reaction between $AlCl_3$ and flavonols [27]. Total flavonoid of green tea leaves extract obtained in this study (Table 3) was 8,43 ± 0,39 mg QE/mg extract.

Table 2: DPPH-scavenging activity of gree tea leaves extract.

Green tea leaves extract (ug/ml)	DPPH-scavenging activity (%)	IC_{50} (µg/mL)
25,0	$94,55 \pm 0,78^{e}$	0,54 ± 0,08
12,5	$75,96 \pm 0,40^{d}$	
6,25	$64,72 \pm 0,17^{c}$	
3,13	$54,08 \pm 0,74^{b}$	
1,56	$48,04 \pm 0,16^{a}$	

Data are presented in Mean ± Standard Deviation. Superscript letters $^{a-e}$ in each column indicates signicant difference ($p < 0,05$) (Tukey HSD post hoc test).

DPPH-scavenging activity and IC_{50} value of green tea leaves extract are presented in Table 1. Green tea leaves extract showed highest DPPH-scavenging activity (94,55 ± 0,78%). The IC_{50} of the extract was 0,54 ± 0,08 µg/mL.

4. Discussion

In the present study, green tea leave extract exhibited antioxidant properties as shown in high DPPH-scavenging activity. The test with the DPPH radical aims at labeling the ability of its neutralization via the antioxidants included in the solution [28]. The result of presents study showed highest DPPH-scavenging activity of green tea leaves extract, was at concentration of 25 ug/ml (DPPH = 94,55%;IC_{50} = 0,54 µg/mL). This was higher than previous study done by Nazliniwaty, et al (2021) in which C. sinensis extract exhibits lower antioxidant activity (IC50 = 11.83 ± 0.005 µg/ml) [29].

Antioxidant properties are associated with presence of polyphenols, flavanoids and epigallocatechin gallate in tea leaves [30]. Polyphenols are secondary metabolites of plants generally involved in defense against ultraviolet radiation, aggression by pathogens [31], and stress [32]. In the present study, total phenolic content and flavonoid of green tea leaves extract were 36,64 and 8,43 mg QE/mg extract respectively Previous study on green tea from Argentina shows total polyphenol was ranged from 21.02 +/- 1.54 to 14.32 +/- 0.45% of gallic acid equivalents (GAE) [33]. In addition, Almajano et al. (2008) revealed that total phenolic content in green tea was higher than black tea [34].

Different polyphenol content among tea varieties might be infuenced by several factors such as degree of ripeness, environmental factors, processing and storage [35]. Maturation of tea leaves influence polyphenols content and antioxidant activities due to the morphological changes and chemical compounds transportation within the plant [36]. Levels of EGCC and ECG was found higher in young tea leaves than mature leaves [37]. In contrast, study carried by Lin *et al*. (2003) shows that EGCG, EGC, EC and catechin are richer in old leaves than in young leaves [18]. Different solvents also plays role in antioxidant activities. Solvent that has higher polarity (aqueous-methanol), is more efficient to scavenge free radicals than less polar solvent (methanol and hot water) [38].Ethanol is frequently considered as an efficient solvent to extract polyphenols [39].

Although there are many studies regarding the promising medicinal properties of green tea in Asia, we believe that this study is new addition from Indonesian plants, especially from West Java region, which can be further compared to other tea variants. We plan to optimize and to explore more activities from green tea in the near future.

5. Conclusion

Green tea leave extract exhibited antioxidant activity which is associated with its phenolic content. Further in vitro and in vivo studies to observe the efficacy of green tea leave extract, are encouraged.

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