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Total polyphenols and antioxidant activities of green tea powder from GMB 7 and GMB 9 tea clones

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Abstract. People's interest in green tea powder is increasing currently. The addition of green tea powder to food products could increase antioxidant activity and other health benefits. GMB 7 and GMB 9 are local tea clones of the Assam variety widely planted in Indonesia's tea plantation. This study aimed to determine green tea powder's total polyphenols and antioxidant activity from local tea clones (GMB 7 and GMB 9). Total polyphenol and antioxidant activity were determined by Folin-Ciocalteu and free radical of DPPH methods, respectively. Our results showed that the total polyphenols of green tea powder were 27.61% and 27.31% for the GMB 7 and GMB 9 clones, respectively. Furthermore, the antioxidant activity of green tea powder with IC₅₀ was 15.41 mg/L and 17.32 mg/L for clones GMB 7 and GMB 9, respectively. The results indicate the potential for the development and utilization of local clones to fulfill domestic green tea powder production. Further research is needed to determine the health benefits of green tea powder from clones of The Assam varieties.

1. Introduction

People's interest in green tea powder is increasing nowadays. Many research and development have been carried out on food products enriched with green tea powder, including sponge cake [1], biscuits [2], bread [3], dried noodle [4], and ice cream products [5]. The addition of green tea powder to food products can increase antioxidant activity and significantly reduce peroxide production during product storage [3]. Green tea powder contains high catechin, lutein, and vitamin K, which has protective effects against cognitive decline in community-dwelling older women if consumed daily [6]. The chemical composition of green tea powder contains the chemical composition to promote health benefits such as: anticarcinogenic, anti-inflammatory, cardioprotective, antiviral, and regulating carbohydrate metabolism [7]. In addition, from a health perspective, the addition of green tea powder to bakery products has a low glycemic index [8].

Green tea powder contains high nutrition that has benefits for health. Green tea powder contains ash content, and crude fiber varied between 5.97-7.49% and 9.23–10.71, respectively [9]. Other results



reported that green tea powder's ash content and crude fiber ranged between 5.2-8.7% and 10.7-17.5%, respectively [10]. Green tea powder also contains a high vitamin C. The study reported that green tea powder from various vitamin C products ranged from 1.63 to 3.98 mg/g [10]. According to Jakubczyk et al., infusion of green tea powder contains vitamin C between 32.12-44.80 mg/L depending on the type of matcha and water temperature for preparing green tea powder [11]. The study reported by Koláčková et al. green tea powder contains caffeine in a board range of 14.4 to 34.1 mg/g, estimated about 1.10-4.32 mg/g and 0.46-2.73 mg/g of chlorophyll-a and chlorophyll b, respectively [10].

The Indonesian National Standard (SNI) 01-4453-1998 defines green tea powder as a dry powder produced from processing the shoots and young leaves of the *Camellia sinensis* plant without going through a fermentation process [12]. Green tea powder is produced by various process technologies such as jet milling, ball milling, stone milling, spray drying, and microencapsulation [13]. Green tea powder produced in Indonesia is mostly from tea clones of the Assam variety, while green tea powder from Japan is mostly from tea clones of the Sinensis variety [13,14].

GMB 7 and GMB 9 are tea clones from the Assam variety with different morphological and genetic characteristics [15,16]. Indonesia Minister of Agriculture officially released both clones in 1990 and then widely planted them in Indonesian tea plantations. GMB 7 and GMB 9 have high productivity, with an average production of 5.8 and 4.7 tons. ha⁻¹ per year and well growth in different altitudes [17]. A previous study by Martono et al. only reported the total polyphenols and antioxidant activity of tea leaves extract from GMB 7 at different altitudes [18]. Studies reporting total polyphenols and antioxidant activity of green tea powder from local clones of tea Assam variety are still rare. This study aimed to determine green tea powder's total polyphenols and antioxidant activity from local tea clones (GMB 7 and GMB 9). The result of this study might be helpful guideline production green tea powder from Assam variety.

2. Materials and methods

2.1. Plant materials

Tea leaves specification used for green tea production in this research was a bud and two young leaves from GMB 7 dan GMB 9 tea clones (*Camellia sinensis* var. Assamica). The research was carried out in Indonesia Research Institute for Tea and Cinchona (IRITC) Gambung tea plantation (Latitude: 7° 08' 37"N, longitude: 107° 30' 54" R, altitude 1200 m) in West Java. The fresh leaves were brought to mini plant tea processing for further processing of green tea powder.

2.2. Manufacturing green tea powder

Ten kilograms of tea leaves that have been picked are then processed in the green tea mini-processing laboratory, IRITC. Green tea processing stages include inactivation of enzymes with hot steam (2.5 minutes; 100°C), cooling with blower fan for 10 minutes, and drying using a rack drier (CNC, Sri Lanka) at 90°C for 60 minutes to obtain final moisture content of 2.5% green tea products. Before storage, dried tea leaves were crushed then stored in a closed plastic container until it was used as raw material for the manufacture of green tea powder.

As described in our previous study, green tea powder was produced using a millstone grinder machine developed by the IRITC Gambung [13]. Green tea powder was then packaged and stored in a room with ≤60% humidity before further analysis.

2.3. Extract preparation

Green tea powder was extracted using methanol 70% (Merck, pro analysis) according to our previous study [19]. About 500 mg of green tea powder was added with 20 ml boiled methanol 70%, then extracted for ten minutes in hot plate and maceration in the dry oven (Gallenkamp) With a temperature of 70°C for 120 minutes. After that, the extract was sonicated (Bransonic-220) for 10 minutes and filtered using Whatman filter paper No. 41 to obtain a clear filtrate. Added green tea powder extract with 70% methanol to obtain a final volume of 25 ml and stored at 4°C for further analyze total polyphenols and antioxidant activity.

2.4. Determination of total polyphenols

Total polyphenols were determined using the Folin-Ciocalteu method refers to ISO 14502-1:2005 [20]. Tea extract (0.5 ml) was diluted with distilled water up to 50 ml. Then, 1 ml was taken and put into a test tube. Add 5 ml of Folin-Ciocalteu (Sigma-Aldrich) 10% (diluted using distilled water) followed by vortex for 5 minutes, then add 4 ml of 7.5% sodium carbonate (Merck) solution. The sample was then stored in a dark room for 1 hour. The absorbance of the sample at a wavelength of 740 nm was measured using a UV-vis Spectrophotometer (Varian Carry win UV). Total polyphenols were determined from the standard curve equation for gallic acid (Sigma-Aldrich) with a 10-100 mg/L concentration range.

2.5. Determination of antioxidant activity

Antioxidant activity was determined using the free radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH). A total of 2 ml of sample extract with concentrations of 10, 15, 20, and 25 µg/ml was added with 3 ml of 0.1 mM DPPH (Sigma-Aldrich). Samples were incubated for 30 minutes in a dark room and continued to measure absorbances at 515 nm using a UV-vis Spectrophotometer (Varian Carry win UV). Methanol 70% was used for the blank sample. The scavenging activity of DPPH as antioxidant activity is calculated using the formula:

$$\text{Scavenging activity of DPPH (\%)} = \frac{A_{\text{Blank}} - A_{\text{Sample}}}{A_{\text{Blank}}} \quad (1)$$

Where: A_{blank} : blank absorbance; A_{sample} : sample absorbance. The IC₅₀ value is defined as the sample concentration required to inhibit 50% free radicals (y), indicating antioxidant capacity. The IC₅₀ value is calculated from the linear regression equation $Y = a + bx$.

2.6. Analysis data

Data collection for each analysis was carried out at least three times. Data was presented based on the mean value with standard deviation. All data obtained were statistically analyzed using the t-test with a significant P-value <0.05. All data was performed using GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com).

3. Results and discussion

3.1. Total polyphenols of green tea powder

Total tea polyphenols are around 30% of the total chemical components, of which most are flavanols (catechins) [21]. Figure 1 shows the total polyphenols of green tea powder from GMB 7 and GMB 9 clones. Total polyphenols (TP) content of green tea powders is 27.6% (w/w) and 27.3% (w/w) for GMB 7 and GMB 9, respectively. Even though GMB 7 has higher total polyphenol than GMB 9, t-

test analysis showed that the total polyphenol content of GMB 7 and GMB 9 did not significantly differ.

A previous study reported that tea from the Assam cultivar has total polyphenols content was significantly higher than the Chinese cultivar [22]. A research study reported the total polyphenol content of green tea powder or matcha from three countries extracted using 80% methanol ranges from 169-273 mg GAE/g [10]. Our results were similar to those mentioned above, 27.6 and 27.3 % for GMB 7 and GMB 9, respectively. Other results reported that the total polyphenol content of matcha green tea is 19,84% [23].

Our result shows that GMB 7 and GMB 9 have higher total polyphenol content than green tea powder produced from Fener and Derepazari 17 clones with 22.73 and 22.0 g GAE/100 g, respectively [9]. However, Peng et al. reported that the total polyphenols content of green tea powder was lower than green tea even the result was not significantly different [24]. The total polyphenol content in green tea powder was influenced by several factors such as shading level, shooting periods, and clones [9]; powdering process [25,26]; and different particle sizes of green tea powder [27].

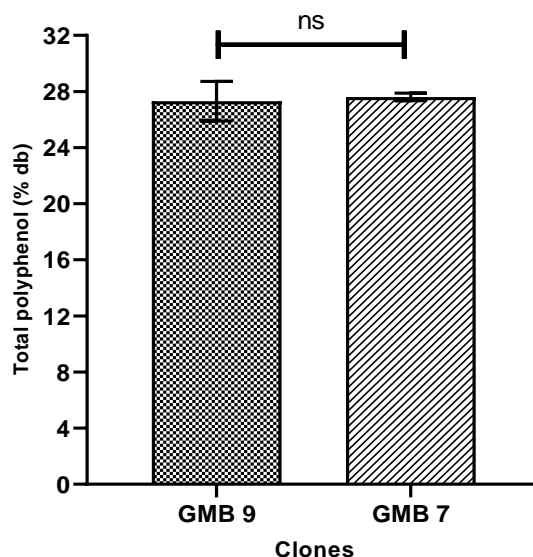


Figure 1. Total polyphenol content (% db) of green tea powder from GMB 7 and GMB 9 clones.

The content of polyphenols in tea is always associated with good health benefits. Dietary supplementation of matcha for six weeks improved metabolic capacity and liver function also prevented elevated blood glucose and excessive visceral and hepatic lipid [23]. Green tea powder containing higher polyphenol has potential application in food, cosmetics, and pharmaceutical industries [28].

3.2. Antioxidant activity of green tea powder

In the present study, we assessed the antioxidant activity of green tea powder from GMB 7 and GMB 9 clones. Figure 2A showed the antioxidant activity of green tea powder from GMB 7 and GMB 9 clones. DPPH scavenging activity of GMB 7 clones is higher than GMB 9 with 74.55% and 66.87% at 25 $\mu\text{g/ml}$ concentration, respectively. A study reported that the antioxidant activity of superfine green tea powder was 59.2%; therefore, it is not significantly different from the antioxidant activity of green tea [24]. DPPH scavenging activity of green tea powder is also dependent on the increasing

concentration of green tea powder extract, serving temperature, powdering process, and particle size of green tea powder [11,25,27].

Figure 2B shows IC 50 value (Inhibition concentration of 50%) for green tea powder. GMB 7 clone has IC 50 value lower than GMB 9 with 15.41 $\mu\text{g/ml}$ and 17.32 $\mu\text{g/ml}$, respectively. Statistically, there were significantly different IC 50 values between GMB 7 and GMB 9 on t-test analysis (p -value <0.05). A lower IC 50 value means the higher antioxidant activity of green tea powder. The total polyphenol content also influences the antioxidant activity of tea. It is related to our results of total polyphenols (Figure 1) which green tea powder from GMB 7 clone has higher total polyphenol content and DPPH scavenging capacity.

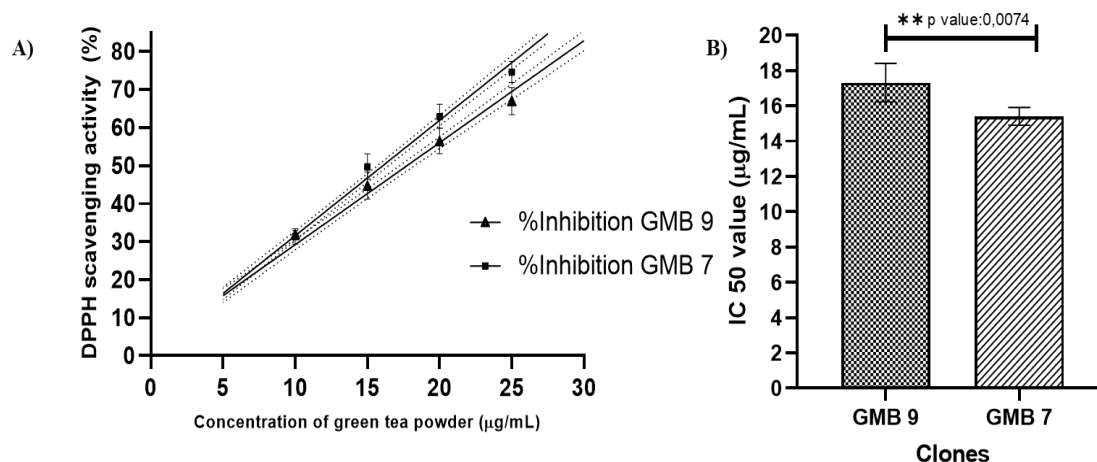


Figure 2. DPPH scavenging activity (A) and IC50 value (B) of green tea powder from GMB 7 and GMB 9 clones.

The Assam variety produced tea antioxidant capacities higher than the Sinensis variety [22]. A research study by Koláčková et al. reported that radical scavenging activity of 12 matcha ranges 288-346 mg Trolox equivalent/g [10]. Other results also reported that the IC 50 value of Turkish green tea powder ranges from 0.31-0.36 mg/mg DPPH [9]. Otherwise, the IC 50 value from two various matcha brands is 29.93 $\mu\text{g/ml}$ and 27.55 $\mu\text{g/ml}$ [29]. Comparing the IC50 value of green tea powder from various works of literature is very difficult because the basic units used are different.

Green tea powder is the source of natural antioxidants. Polyphenols in tea have intense antioxidant activity compared to vitamin C and vitamin E [30]. The phenolic compound in green tea powder containing a hydroxyl group is attached to an aromatic ring contributing to antioxidant activity [22]. EGCG ((-)-epigallocatechin-3-gallate) is one of the dominant compounds in catechins that play a role in the antioxidant activity of green tea powder [23,25,27,31]. Mechanism antioxidant activity of tea polyphenols can counteract and scavenge free radicals such as reactive oxygen species (ROS) and reactive carbonyl species (RCS) through hydrogen/electron donation, so interrupt propagation lipid peroxidation or blocking tissue damage [32].

4. Conclusion

The green tea powder from local clones (GMB 7 and GMB 9) possessed higher potential total polyphenol and antioxidant activity than other green tea powder products discussed above. The results also indicate potentiality in developing and utilizing local clones to produce green tea powder. Further

research regarding the health benefits of green tea powder from Indonesia's clones is needed to be determined.

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