# Antioxidant Activity of Methanol Extract from Several Indonesian Green Teas

Adelina Simamora[1], Michael Steven[1], Adit Widodo Santoso[2], Flora Rumiati[3], Kris Herawan Timotius[3]

[1]*Department of Biochemistry, Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia*

[2]*Department of Biology, Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia*

[3]*Department of Physiology, Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia*

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*ABSTRACT* *As a common beverage, green tea is supposed to have beneficial health effect, such as antioxidant. At present, there are many green teas available in the market in Jakarta. Their quality, in their antioxidant activity, should be proved. This study aimed to investigate the antioxidant activity of extracts of green tea products commercially available in Jakarta. Four green tea samples from different factories (coded as A, B, C, and D) were selected and macerated using methanol. DPPH radical scavenging activity, reducing power ability and total antioxidant capacity were used to measure the antioxidant activity. The total phenolic content (TPC) was also determined. The studied green teas had varied TPC from 23.80 to 84.03 mG GAE/g extract, ranked as B > D > A > C. All samples exhibited various but strong antioxidant activity by DPPH assay, even better than standards ascorbic acid and butylated hydroxytoluene (BHT). However, all extracts showed similar activities in their reducing power ability and total antioxidant capacity, with activities less than standards. These findings confirm that the quality of the Indonesian commercial green teas were heterogeneous both in TPC or DPPH scavenging capacity. But, there is an indication that they are good as an antioxidant containing beverage.*

## INTRODUCTION

As natural antioxidant, tea beverage is cheap, easy to prepare and available all the time. The quality of any tea can be evaluated based on its antioxidant activity. However, antioxidant quality of tea is affected by environmental factors (Kaur et al., 2014). Due to the processing procedures, green tea which does not undergo fermentation is supposed to have better antioxidant activity compared to black tea. Several epidemiology studies have reported that green tea consumption can reduce the occurrence of various degenerative diseases (Vuong, 2014, Yuan, 2013, Arab et al., 2013). Green tea products are available in the market all around Indonesia. To date, there is little report on the green tea quality, in particular its antioxidant activity. Thus, this study was carried out to compare the total phenolic content (TPC) and antioxidant activities of methanol extracts from several green tea products available in Jakarta market. Three different assays were used to evaluate antioxidant activities of green tea samples, *i.e.* DPPH radical scavenging activity, reducing power ability, and total antioxidant capacity (phosphomolybdenum assay).

## MATERIALS AND METHODS

### Chemicals

All solvents and chemicals used in the experiments were of analytical grade. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3,5-di-tert-butyl-4-hydoxytoluene (BHT), Folin & Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, USA). Gallic acid (GA) was purchased from Santa Cruz Biotechnology (Dallas, USA). Sodium carbonate ($Na_2CO_3$), potassium ferricyanide $K_3Fe(CN)_6$, ferric chloride $FeCl_3$, and ammonium molybdate $(NH_4)_6Mo_7O_{24}.\ 4H_2O$ were purchased from Merck (Darmstadt, Germany). Ascorbic acid was purchased from VWR BDH Prolabo Chemicals (Tingalpa, Australia).

### Methanol extraction

Four commercial green teas (A, B, C, and D) were selected for study based on their availability and popularity in Jakarta market. All tea samples were extracted with methanol. Each tea sample (13 grams) was macerated with methanol (200 ml) for two days. The mixture was filtered and the filtrate was concentrated using rotary evaporator at 50 $^oC$ (Buchi, Switzerland) to get a brown liquid. The filtrate was frozen and freeze-dried at 46 Pa pressure and at – 50 $^oC$ (MRC) to obtain dried extract. All extracts were stored at -20 $^oC$ until used.

### Determination of total phenolic content

Total phenolic content of tea extracts was determined spectrophotometrically by Folin-Ciocalteu (F-C) method described previously (Khatoon et al., 2013). Briefly, an aliquot (0.5 ml) of dilute extract of sample and standard was mixed with 2.5 mL Folin– Ciocalteu reagent (10%) and left to stand for 10 minutes at 25 $^oC$. The reaction mixture was then added with 2.5 ml of sodium carbonate (75 g/L) and incubated for two hours in the dark at 25 $^oC$.

*Correspondence:*
*Adelina Simamora, Department of Biochemistry, Faculty of Medicine, Krida Wacana Christian University, Jakarta, Indonesia*
*Email:adelina.simamora@ukrida.ac.id*

The absorbance was determined at 765 nm using a spectrophotometer (BioChrom Libra S22). Standard curve was prepared using different concentrations of gallic acid (12.5 – 200 µG/mL). Results were expressed as mG gallic acid equivalent (GAE)/G dry weight of extract. All measurements were carried out in triplicate.

**Determination of DPPH radical scavenging activity**

The free radical scavenging activity of the green tea samples was measured based on 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay according to the method (Shen et al., 2010) with minor modification. DPPH solution (0.6 mM in ethanol) was prepared and 1 ml of this solution was added to 3 ml of green tea extracts and reference solutions in various concentration (10 - 200 µG/mL). The mixture was immediately vortexed and incubated for 30 minutes in darkness at room temperature. The decrease in absorbance was measured at 517 nm using spectrophotometer. Ascorbic acid and BHT were used as reference solutions and ethanol was used as a control. The percentage of inhibition activity was determined based on the following equation:

$$\text{Inhibition (\%)} = \frac{Acontrol - Asample}{Acontrol} \text{X100\%}$$

Where A(control): absorbance of control, A(sample): absorbance of the sample. The percentage inhibition was then plotted against concentration and $IC_{50}$ was determined using a linear regression analysis. The values expressed as µG/mL and was compared to the standard solutions. All measurements were repeated three times.

**Determination of reducing power ability**

The reducing power ability was determined using potassium ferricyanide reduction assay described by (Oyaizu, 1986). Different concentrations of green tea extracts and standards (ascorbic acid and BHT) in water (25, 50, and 100 µg/mL) were prepared and 1 mL of each sample solution was added with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1 % w/v). The mixture was incubated for 20 mins at 50 $^oC$. Into this mixture was added 2.5 ml of trichloroacetic acid (10 % w/v) and the reaction was centrifuged for 10 mins at 3000 rpm. The upper layer of the solution (2.5 mL) was added with water (2.5 mL), followed by addition of 0.5 mL of $FeCl_3$ (0.1 % w/v). The absorbance of each sample was read at 700 nm by spectrophotometer and was compared with the standards. All measurements were repeated three times.

**Determination of total antioxidant activity**

Total antioxidant capacity of the green tea extracts was estimated using a phosphomolybdenum method described by (Alam et al., 2013). Reagent solution was prepared containing sulfuric acid (0.6 M), sodium phosphate (28 mM), and ammonium molybdate (4 mM). 3 mL of this solution was added to 0.3 ml extract solution and standards (ascorbic acid and BHT) in water (25, 50, and 100 µG/mL) placed in capped tubes. The reaction mixture was incubated in water bath at 95 $^oC$ for 1.5 hours and the mixture was let to cool at room temperature. Blank solution contained 3 ml of reagent solution and 0.3 ml of water in place of extract, and the

solution was treated under the same condition as samples. The absorbance was measured at 695 using spectrophotometer and was compared with standard. All measurements were repeated three times.

**Statistical analysis**

All data are the average of three measurements and presented as mean ± SD.

## RESULTS

**Extract yields and total polyphenol contents**

The recovery of the methanol extract from various green teas was similar, obtaining 9.60 to 10.90 mG extract/100 gram green tea. All extracts contained various TPC, ranging from 23.80 ± 0.15 to 84.03 ± 0.82 mg of GAE/g of extract (Table 1). TPC of the tea B was the highest among others, ranked as B > D > A > C. The difference in TPC suggests that there are different tea material and tea processing.

Table 1. Total phenolic content (TPC) and DPPH scavenging activity ($IC_{50}$) of green tea extracts and standards. AA: ascorbic acid, BHT: butylated hydroxytoluene. The values were expressed as mean ± SD (n=3).

| Green tea | TPC (mG GAE/G) | $IC_{50}$ (µG/mL) |
|---|---|---|
| A | 42.74 ± 0.13 | 52.89±0.46 |
| B | 84.03 ± 0.82 | 15.83±1.66 |
| C | 23.80 ± 0.15 | 56.80±1.81 |
| D | 56.56 ± 0.19 | 43.21±0.39 |
| AA | | 53.24±0.82 |
| BHT | | 21.36±0.90 |

Three different assays were used to evaluate antioxidant activities of green tea samples, i.e. DPPH radical scavenging activity, reducing power ability, and total antioxidant capacity (phosphomolybdenum assay). Commercial antioxidants such as ascorbic acid and BHT were used for comparison.

**DPPH Radical Scavenging Activity**

Free radical scavenging activity of methanol extract from commercial green tea was measured by DPPH free radical method. The method has been widely used for the screening of antioxidant activity of a wide range of samples including plant extracts (Koleva et al., 2002).

Table 1 shows the $IC_{50}$ of the tea samples and the standards. The $IC_{50}$ of all tea extracts exhibited strong activity of radical scavenging, ranged from 15.83 ± 1.66 to 56.80 ± 1.81 µG/mL, which were stronger or similar to $IC_{50}$ of commercial standards ascorbic acid and BHT obtained under similar reaction conditions.

**Reducing power capacity**

The reducing power ability is a good indicator for potential antioxidant activity (Jayaprakasha et al., 2001, Gulcin et al., 2007). In the present study, a reduction assay using potassium ferricyanide was used to directly measure the reducing capacity of tea extract based on the transfer of an electron to $Fe^{3+}$ to generate the $Fe^{2+}$ form. Figure 1 shows the reducing power for all commercial tea samples and standards (ascorbic acid and BHT) at various concentrations (25, 50, and 100 µG/mL).

All samples exhibited a concentration dependent reducing activity. Similar to radical scavenging activity by DPPH method, the reducing power of all samples increased with increasing concentrations, as observed by an increase in absorbance. However,

there is no difference observed in the antioxidant activity among various tea samples, at each concentration level. Both ascorbic acid and BHT at the same concentration levels showed a larger increase in absorbance, indicating a stronger reducing power capacity.

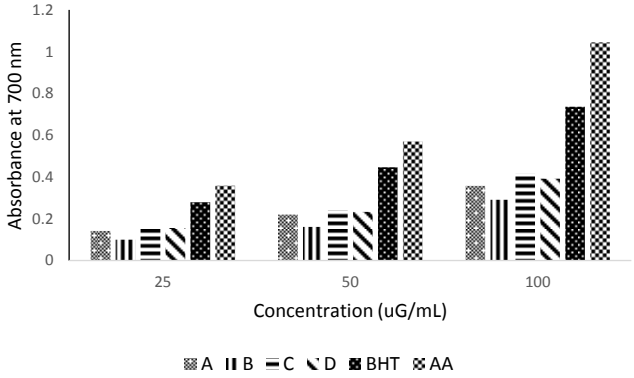


Figure 1. Reducing power of green tea methanol extracts and standards (Values were expressed as mean ± SD (n=3)).

**Total antioxidant capacity**

Total antioxidant capacity of the range of tea samples was estimated by a phosphomolybdenum assay. The assay which is based on the transformation of Mo(VI) to Mo(V) was widely used to determine antioxidant capacity in various samples including plant extracts (Pisoschi et al., 2016, Dhull et al., 2016). As in the reducing power assay, the extracts and standards were tested at concentrations of 25, 50, and 100 μG/mL. The results are shown in Figure 2. It can be seen that tea samples showed considerable amount of antioxidant activity which is comparable to ascorbic acid and BHT. For each tea sample, the absorbance of the Mo(V) complex formed in the reaction increased linearly with increasing concentrations, indicating an effective reduction reaction by the antioxidant compounds present in the tea samples. Similar to the results obtained from reducing power assay using ferricyanide method, there is no difference observed in the antioxidant activity among various tea samples, as can be seen from the absorbance at each concentration level.

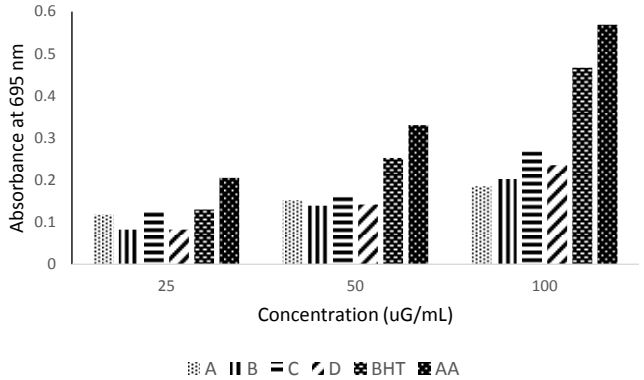


Figure 2.Total antioxidant capacity of commercial green tea extracts and standards (Values were expressed as mean ± SD (n=3)).

## DISCUSSION

Radicals may react with a wide range of molecules found in cells such as amino acids, lipids, sugar, and nucleotides causing oxidative damages in biological systems. This may lead to the induction of many chronic and degenerative diseases. Due to the high reactivity of free radicals, ability of an antioxidant to scavenge these radicals is an important antioxidant activity.

There are a number of ways in which an antioxidant can protect biological system from oxidative damage associated with free radicals. These include mechanisms through hydrogen donating reaction and single electron transfer mechanism (Pisoschi et al., 2016). To account for these mechanism, methanol extracts of green tea at various concentrations were evaluated for their antioxidant activity using different assays: DPPH radical scavenging activity, reducing power ability, and total antioxidant capacity (phosphomolybdenum assay).

An antioxidant ability to scavenge free radical is an essential antioxidant activity. This can be achieved by transferring an electron or a hydrogen to free radical, so that odd electron in the radical which is responsible for its reactivity can be neutralized, forming an unreactive species (Wang et al., 2008). All extracts of green teas exhibited strong scavenging activity. This result indicated that antioxidant compounds in the extracts are significantly able to reduce DPPH radical into its nonradical form DPPH-H. Therefore, our results suggested that tea extracts may be a promising source of antioxidant which may protect against diseases induced by radicals (Patlevič et al., 2016, Spadiene et al., 2014).

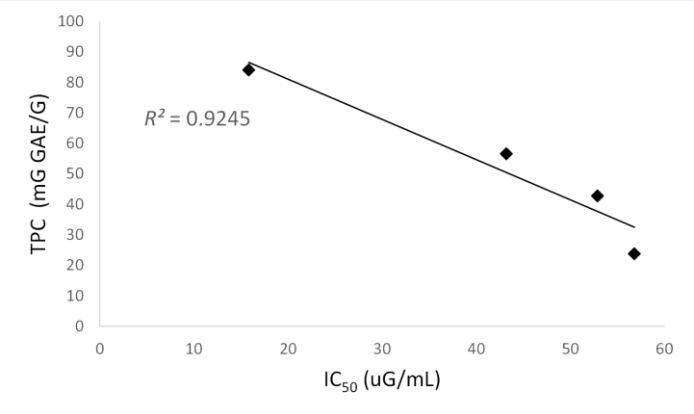


Figure 3. Linear correlation of DPPH radical scavenging activity ($IC_{50}$) as a function of total phenolic content (TPC).

Recent studies have highlighted the importance of polyphenolic compounds due to their importance in antioxidant activities and other biological activities (Makris et al., 2007). In particular, it is also known that phenolic compounds exert antioxidant effect (Adjimani and Asare, 2015), including in plant extracts (Genwali et al., 2013, Paixão et al., 2007). The relationship between total phenolic content and radical scavenging activity was also demonstrated in our study. It was observed that antioxidant activities

($IC_{50}$) and total phenolic compounds content of the studied green tea were highly correlated ($R^2$ = 0.9245, Figure 3). This finding provides evidence that polyphenols in the tea extract are the predominant source of the radical scavenging activities of these tea, which is likely exerted through donation of a hydrogen or electron transfer from the phenolic structure to the radicals (Adjimani and Asare, 2015). Previous studies reported this property comes from polyphenols such as epichatechin, epichatechin galate (Kristanti and Punbusayakul, 2008), and especially epigallochatechin gallate (Wang et al., 2017) which is a catechin derivate possessing eight free hydroxyl groups, thus explaining high antioxidant activity.

It has been reported that antioxidant activity is concomitant with the development of reducing power (Duh, 1998). The reducing activities are considered to be generally associated with the presence of reductones (Jayaprakasha et al., 2001, Gulcin et al., 2007). Previous reports suggested that one of the mechanisms of the antioxidant action of reductones is based on the breaking of free radical chain by donating a hydrogen to neutralize free radical (Jayaprakasha et al., 2001). Therefore, the reducing capacity of tea extracts may serve as a good indicator for potential antioxidant activity. In our study, reducing activities of tea extracts were investigated by ferricyanide and phosphomolybdenum assays. Both assays were widely used determination of antioxidant capacity in various samples including plant extracts (Pisoschi et al., 2016, Dhull et al., 2016). In both assays, at increasing concentrations, all samples exhibited increasing absorbance, indicating reducing power activities. The reductones present in green tea extracts caused the reduction of Fe(III) to Fe(II) and Mo(VI) to Mo(V) for the respective assay, therefore proved the reducing power. Thus, our data indicated that tea extracts may act as electron donors which react with free radicals, converting them to more stable products, thus can terminate radical chain reaction. Previous study reported that plant extract possessing reducing ability can prevent liver injury by inhibiting the formation of lipid peroxides (Khennouf et al., 2010). Therefore, in addition to having potent free radical scavenging activities, tea extracts also have reducing power capacity. These findings suggest that green tea can be used as an economical source of antioxidant.

It should be noted, however, that the three methods used for antioxidant activity showed different results. The DPPH assay was found able to differenciate antioxidant activity among the teas. On the other hand, the other two methods could not differenciate the antioxidant activity among the samples, with results showing better activities for the standards.

## CONCLUSION

The methanol extract of the four selected Indonesian green tea (coded as A,B,C, and D) varied in TPC and antioxidant activities. The best green tea in antioxidant activity was B which was closely related to its highest TPC.

**Conflict of interest:** We declare that we have no conflict of interest.

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