Antioxidant Capacity of White Tea (*Camelia Sinensis*) Extract: Compared to Green, Oolong and Black Tea

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Abstract. Tea is the most popular natural beverage ingredients in Indonesian community. It was four types for commercial tea consumption, i.e. white tea, green tea, Oolong and black tea. Nowadays public opinions that the white and green tea processed without or slightly fermentation had stronger antioxidant capacity than Oolong and black tea processed in full fermentation. The aim of the research was to compare the antioxidant capacity of the white tea (WTE), green tea (GTE), oolong tea (OTE) and black tea aqueous extract (BTE). Approximately 50 g dried tea was extracted using aquadest (1:10 (w/v), macerated in waterbath shaker (100 rpm/60±2°C/10 minutes) and filtrated using Whatman paper. The solution was concentrated with rotary vacuum evaporator till obtained a viscous liquid then it was freeze-dried for solvent removal and kept in the refrigerator for further analysis. Each extract was to determinate of total phenolic compounds, flavonoid content and DPPH radical scavenging activity. The results showed that the antioxidant capacity of extract as the total phenolic compound expressed as gram gallic acid equivalent (g-GAE/100g-extract) were BTE<WTE<GTE<OTE respectively. Meanwhile, the total flavonoid of extract expressed as gram quercetin equivalent (g-QE/100g-extract) were BTE < WTE < OTE < GTE respectively. The antioxidant capacities of extract (1000-1500 µg/ml) against the DPPH radical were BTE<GTE<OTE<WTE, respectively. There were significant differences of radical scavenging activity-DPPH among extracts in all concentrations (p<0.05). As conclusion WTE has the strongest antioxidant capacities, so it has great potential as a natural beverage and also to be applied as natural antioxidant in food system.

Keywords: white tea; oolong tea; green tea; black tea; antioxidant capacities
1. Introduction

Tea is the most popular natural beverage ingredients in Indonesian community. It is made from the top buds and leaves of varieties of tea plant species *Camellia sinensis* (Linn.) Kuntz and produced by acceptable processes for making tea in order to consumption as a beverages. Tea is consumed for daily drinking, normally at the morning and evenings. Most people make a beverage tea with the manner of brewing tea on their own, while others purchase a drinking tea in pack. Base on the differences in raw material and processing method, tea products are classified into four main class, i.e.: white tea, green tea (both unfermented tea), Oolong tea (semi-fermented tea) and black tea (completely-fermented tea)[1,2]. Meanwhile there is another class which classified into six classes including white tea, Jasmine tea, black tea, green tea, oolong tea, and herbal tea.

The area tea plantation and tea production during the last ten years increased slightly. Data the area tea plantations in Indonesia in 2016 was 117,268 hectare which consists of smallholders 53,141 ha (46%), belonging to the government was 35,655 hectare (31%) and private plantation was 28,472 hectare (24%)[3]. The total tea production in same time recorded was 144,015 ton, derived from smallholders plantation 49,364 ton, government plantation 55,976 ton and private sector was 38,675 ton. However, tea consumption per capita has decreased significantly from 0.69 kg per capita (2006) to 0.61 kg per capita (2014)[4]. It is probably caused lack of information to the community that is tea has great health impact. On the other hand demand of the tea drink in pack (ready to drink) increased in two digits during the last ten years [5].

Tea has a bioactive compound such as polyphenolic which related an attractive aroma, good taste and health-promoting effect [1, 2]. Because of its high polyphenolic compound such as catechin family i.e. (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin 3-gallate (EGCG), (-)-epigallocatechin (EGC), and (-)-epicatechin 3-gallate (ECG) [1,6]. Some of these related health effects are as antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, anti-cancer and anti-mutagenic [1,2,6,7]. White and green tea contains more polyphenolic compound than black and oolong tea [1,2]. Previous research related to antioxidant activity of aqueous WTE was successfully detected but it was not as strong as grape seed extract and BHA [8]. The aim of the research was to compare the antioxidant capacity of the white tea (WTE), green tea (GTE), oolong tea (OTE) and black tea aqueous extract (BTE).

2. Materials and Method

The white tea, green tea, oolong tea, and black tea commercial grade were directly purchased from PT. Perkebunan Nusantara IX, Semarang Central Java, a gallic acid (Sigma Chemical Co. St. Louis USA), quercetine (Waco Pure Chemical Industry-Osaka Japan), hydrochloric acid (HCl), ferrous chloride (FeCl2), ferric chloride (FeCl3), ammonium thiocyanate, K3Fe(CN)6, trichloroacetic acid (TCA), tungstophosphoric acid, 1,1-diphenyl-2-picryl hydroxyl radical (DPPH) (Aldrich Chemical Co.), Folin Ciocalteu reagent, paper filter Whatman No.4 (Whatman International, Ltd. England). The pieces of equipment were analytical weight Shimadzu AUW 120 (Shimadzu, Kyoto Japan), a rotary vacuum evaporator (YHChem, Shanghai), freeze dryer (Zhengzhou Nanbei, China), water-bath shaker (Julabo SW 22), UV-Visible spectrophotometer (UV-1601 Shimadzu, Japan).

2.1 Tea extraction

Approximately 50 g dried tea was extracted using aquades at ratio of material to solvent = 1:10 (w/v), then macerated in 100 rpm water bath shaker (60±2°C/10 minutes) and filtrated using Whatman paper according to [9]. Then the solution was concentrated with rotary vacuum evaporator till obtained a viscous
liquid. Then a viscous liquid extract was freeze-dried for solvent removal and kept in the refrigerator for further analysis. This process was repeated 3 times.

2.2 Determination of total phenolic compounds and flavonoid content

Total phenolic content of tea extract was measured using Folin-Ciocalteu reagent (FCR) colorimetric method using spectrophotometer at $\lambda$ 765 nm according to the procedure applied by Ebrahimzadeh et al., [11], with gallic acid as standard (g GAE/100 g extract). Total flavonoid content was measured using spectrophotometer at $\lambda$ 415 nm using protocols based on previous researches with quercetine as standard (g QE/100 g extract) [9,10].

2.3 DPPH radical scavenging activity assay

Measurement of radical scavenging activity -DPPH of aqueous tea extract referred to procedure applied by Vasi and Austin [10]. Approximately 0.5 mL tea extract at various concentrations (1000-1500 μg/ml) in 50% ethanol added with 0.5 mL 2,2-diphenyl-1-picryl hydroxyl radical (DPPH)-100 μM then incubated in the dark at room temperature (37±2°C) for 30 minutes, in triplicate for all samples. Its absorbance was read at $\lambda$ 517 nm using UV-Vis spectrophotometer (UV-1601 Shimadzu). DPPH radical scavenging activity can be calculated as follows refereed [11].

$$RSA-DPPH \text{ (%) } = \left[ 1 - \frac{Abs \text{ sample}}{Abs \text{ kontrol}} \right] \times 100\% \quad \text{……………….1}$$

2.4 Statistical analysis

Experimental results are expressed as mean ±SD. All measurement were conducted in triplicate, analyzed using variance analysis ($p<0.05$). A significant difference was measured using Duncan’s Multiple range tests.

3. Results and Discussion

3.1 Chemical content

The results of the chemical analysis of four commercial teas i.e a green tea (GT), black tea (BT), Oolong tea (OT) and a white tea (WT) are presented at Table 1. The water content for all samples are at the range 7.43±0.04 - 9.80±0.17 % (wb). On behalf of Indonesian National Standar SNI 3836:2013 about the tea product that the maximum water content was 8% [12]. Thus of the four teas, the only a WT as the sample meets the standar. Previous research cited that the moisture content of white tea as 6.9% and 7.44 ±0.064 [7,8]. The ash content for all sample was at the range 5.39±0.08-6.37±0.14%, so for all sample appropriate the standar the maximum ash content was 8%. The protein content for all sample was at the range 14.48±0.15 - 28.62±0.10%, lipid 0.19±0.06 - 0.34±0.05% and the carbohydrate content (by difference) 46.11±0.06 - 53.97±0.17 %. During production on the GT, BT and OT undergone drying process, rolling and shaping, but not on the WT. So that these three tea types are more porous and hygroscopic. Moreover, moisture absorption on the three teas above is higher than the WT.
Table 1. Chemical content of four commercial tea product

| Sample | Water content (%) | Ash (%) | Protein (%) | Lipids (%) | Carbohydrate (%) |
|--------|------------------|---------|-------------|------------|------------------|
| GT     | 9.29±0.14        | 6.37±0.14 | 14.48±0.15  | 0.34±0.05  | 52.06±0.29       |
| BT     | 9.80±0.17        | 5.49±0.35 | 18.84±0.16  | 0.19±0.06  | 49.09±0.29       |
| OT     | 8.60±0.16        | 5.67±0.30 | 23.57±0.16  | 0.28±0.06  | 53.97±0.17       |
| WT     | 7.43±0.04        | 5.39±0.08 | 28.62±0.10  | 0.32±0.01  | 46.11±0.06       |

Note: Different small letter within the same chemicals denote the significant differences (p<0.05), n=3

3.2 The extract yield

Different tea types namely GT, BT, OT, and WT were extracted using hot water (60±2°C/10 minutes) by maceration methods. The yield extract of four commercial tea listed in Table 2. The highest yield which was 14.80 % (g-extract/100 g-dried tea) obtained at BT extraction. While the lowest yield was 2.24% obtained at WT extraction. Characteristic of the BT in which of more porous and bulky than others due to cutting, tearing and curling (CTC) processed of it alleged effected on yield. Other factors were the solvents, time of extraction, the ratio of solvent-to-plant materials, temperature and methods [1,2,7,12]. Previous research said that the extract yield of white tea as 14.4% (wb) used by hot water [8].

Table 2. Yield extraction of four tea extract

| Samples | Yield (%-db) |
|---------|--------------|
| GTE     | 6.72 ± 0.04  |
| BTE     | 14.80 ± 0.03 |
| OTE     | 5.64 ± 0.03  |
| WTE     | 2.24 ± 0.04  |

Note: Different small letter within the same column denote the significant differences (p<0.05), n=3

3.3 Total of Phenolic compound

The total of phenolic compound of four commercial tea listed in Table 3. Total phenolic compound is one of quality attribute of commercial dried tea. The Indonesian National Standard- SNI 3836:2013 required the total phenolic content was more than 5.2 % used by ethanol solvent [11]. Different tea types resulted in difference of the total phenolic value. From Table 3 it could be declared that all of the samples fulfill criterium as commercial dried tea.
Table 3. Total of phenolic of four commercial tea extract

| Samples | Total of phenolic content (g-GAE/100 g-) |
|---------|------------------------------------------|
| GTE     | 30.89 ± 0.01c                           |
| BTE     | 25.67 ± 0.01a                           |
| OTE     | 31.93 ± 0.49d                           |
| WTE     | 29.93 ± 0.04b                           |

Note: Different small letter within the same column denote the significant differences (p<0.05), n=3

The lowest total phenolics was 25.67 ± 0.01 which obtained on black tea. That was possible because the conversion of tea phenolics occurred in the manufacture of black tea within fermentation processes[1]. This conversion was accomplished by endogenous enzymes the polyphenol oxidase. Previous research the white tea was extracted three times using hot water (60±2°C/10 minutes) obtained the total phenolics 18.56±0.25 (g-GAE/100) [8], others using methanol 70% average 21.54 %, 19.18%, and 16.5% of white tea, green tea, and black tea respectively [1], at the range 21 - 25 % it used ethanol 96% solvent [2]. Extraction of total phenolic content of white tea with various solvent including n-hexane, ethyl-acetate, and ethanol were 22.04%, 57.54% and 59.32% respectively [7].

3.4 Total of flavonoid

The total flavonoid of four commercial tea listed on Table 4. The total flavonoid of four samples was at the range 15.60-17.52% (g-QE/g), in which the highest was 17.52% obtained from green tea and the lowest one was 15.60 % at the white teas. That was significant difference between the total flavonoid of the samples (p<0.05). Green tea was relatively rich in proanthocyanidins (0.85%) and lower in bisflavanols (0.05%), meanwhile the black tea had higher amounts of bisflavanols (0.65%) and lower in proanthocyanidins (0.5%) [1]. The galloylated proanthocyanidins were degraded during fermentation [13,1]. Wellknown the tea’s polyphenols including flavanols, flavandios, flavonoids, and phenolic acids [1] then flavonols consisted quercetin, kaempferol, and myricetin are present in form of their O-glycosides [1,14]. As already mentioned tea flavonoids are claimed to be responsible for most of the positive health effects of tea. The flavonols family group is catechins, especially epigallocatechin gallate (EGCG), theaflavins and flavonol-glycosides are thought to be responsible for antioxidative properties in tea.

Table 4. Total flavonoid content of four commercial teas extract

| Samples | Total Flavonoid (g-QE/100 g-) |
|---------|-------------------------------|
| GTE     | 17.52 ± 0.42d                |
| BTE     | 14.73 ± 0.36a                |
| OTE     | 16.44 ± 0.53e                |
| WTE     | 15.60 ± 0.58b                |

Note: Different small letter within the same column denote the significant differences (p<0.05), n=3
The total catechins of green tea, black tea, and white tea were analyzed with HPLC reportedly are 12.95% (g/100 g), 4.2 and 13.22 % respectively. While the flavonol glycosides content of green tea was 2.27%, white tea 1.25% [1]. Catechins and their gallates are the main phenolic compounds in unfermented fresh tea leaves [2]. Using the quercetin as reference to determined total flavonoid content on tea extract may not suitable, catechins is better and relevant.

3.5 Radicals scavenging capacity

The antioxidant capacity as radical scavenging DPPH of tea extract (1000-1500 μg/ml) listed in Table 5. There were significant differences in the radical scavenging DPPH among samples (p<0.05). The highest radical scavenging was 93.61% obtained on the white tea extract (1500 μg/ml ), then the lowest was 72.07 % on the black tea extract. It has similarly with result work done by Hilal and Engelhardt that the white tea has radical scavenging capacity strongest than others [1]. Other literature revealed that the DPPH radical scavenging activity (IC₅₀(μg/ml) of hot water white tea extract was 99.9 ± 4.9 % [6]. Nevertheless, all of the tea extracts was able to reduce the stable radical DPPH within monitoring due to changes of purple to the yellow colored diphenylpicrylhydrazine. The scavenging effect increased with increasing concentration of the extract [1,2,6,8,9].

Table 5. Antioxidant capacity radicals scavenging DPPH of four tea extract

| Samples concentration (μg/ml) | GTE | BTE | OTE | WTE |
|------------------------------|-----|-----|-----|-----|
| 1000                         | 86.32 ± 0.11ᵇ | 55.48±0.69ᵃ | 87.20 ± 0.22ᶜ | 92.91 ± 0.08ᵈ |
| 1250                         | 89.22 ± 0.09ᵇ | 71.90±0.30ᵃ | 92.19 ± 0.29ᶜ | 93.27 ± 0.19ᵈ |
| 1500                         | 93.40 ± 0.20ᶜ | 72.07±0.31ᵃ | 92.86 ± 0.02ᵇ | 93.61 ± 0.09ᶜ |

Note: Different small letters within the same raw denote the significant differences (p<0.05), n=3.

The catechins, especially epigallocatechin gallate (EGCG), theaflavins and flavonolglycosides are thought to be responsible for antioxidative properties in tea [1].

4. Conclusions

The hot water white tea extract showed that it has antioxidant capacity as radicals scavenging DPPH with the green tea extract which has similarity, but not than others. The radical scavenging DPPH of the oolong and black tea extract is more weakness than green and white tea extract. The use of the quercetin as reference to determine the total flavonoid on tea extract may not suitable.

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