

## Antioxidant Properties of Different Types of Torbangun Herbal Tea

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### Abstract

*In this study, Torbangun leaves are processed into three different types varying in their oxidation degree, namely unoxidized, semioxidized and oxidized Torbangun leaves. Each type is then brewed into herbal tea and analyzed in terms of its total phenolic, total flavonoid and antioxidant activity. Unoxidized Torbangun herbal tea shows higher content of total phenolic (44.22 mg GAE/g) and total flavonoid (17.02 mg QE/g) compared to oxidized (24.66 mg GAE/g total phenolic content and 8.61 mg QE/g total flavonoid content) and semioxidized (33.83 mg GAE/g total phenolic content and 10.68 mg QE/g total flavonoid content) Torbangun herbal tea. In terms of the antioxidant activity, processing the Torbangun leaves into herbal tea tremendously decreased the antioxidant activity based on the IC50 value of 1400.89 µg/mL for unoxidized Torbangun herbal tea, 3211.71 µg/mL for semioxidized Torbangun herbal tea and 4504.78 µg/mL for oxidized Torbangun herbal tea. However, steam blanching used in the preparation of unoxidized Torbangun leaves is proven to increase the total phenolic and flavonoid content of raw or unprocessed Torbangun leaves from 39.02 to 44.22 mg GAE/g and an increase of total flavonoid from 10.32 to 17.02 mg QE/g.*

**Keywords:** antioxidant; flavonoid; herbal tea; phenolic; torbangun

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### INTRODUCTION

Tea is considered as the most widely consumed beverage worldwide beside water (McKay and Blumberg, 2002). Traditionally, tea refers to a beverage made by brewing the leaves of *Camelia sinensis* plant. The leaves of *C. Sinensis* are commonly processed in three manners that produce the popular black, green and Oolong teas. In the production of black tea, oxidation of most polyphenols in the leaves is encouraged. On the contrary, green tea processing prevents the oxidation of the leaf polyphenols, while Oolong tea is made through partial oxidation process.

Other than *Camellia sinensis*, various types of leaves, spices and seeds can also extracted using hot water. Such product is generally known as herbal tea,

herbal drink or herbal infusion. *Coleus amboinicus* (L.), synonym *Plectranthus amboinicus*, is a plant indigenous to South Sumatera and locally known as 'Torbangun' or 'Bangun-bangun'. It belongs to the plant family Lamiaceae. Torbangun leaves are commonly consumed in Indonesia by nursing mothers due to their ability to promote quantity and quality of breast milk (Damanik *et al.*, 2017; Iwansyah *et al.*, 2016; El Sakka *et al.*, 2014). This promoting effect may be due to the antioxidant, phenolic and flavonoid present in the raw ingredients. Most of the phenolic and flavonoid compounds have phytoestrogen activity that can trigger the secretion of prolactin and mammary gland, resulting in an increase of casein production and

lactose synthesis (Wirawati *et al.*, 2018; Al-Snafi *et al.*, 2015).

Damanik *et al.* (2006) concluded that Torbangun leaves in form of traditional cuisine such as soup helped in increasing the quantity and quality of breastmilk and can therefore be considered as lactagogue. However, the study only focus in comparing the lactagogue effect of Torbangun when consumed as soup compared to other lactagogue materials. Recently, Torbangun become commercially available in form of dried leaves that is consumed as herbal tea. This dried form of Torbangun leaves is more popular due to longer shelf-life and convenience rather than the traditional form (soup). Antioxidant activity of Torbangun leaves tea was studied by Saragih (2014). However, the antioxidant activity was only studied on the most preferred Torbangun tea because the study focused on observing the acceptability of Torbangun tea. Antioxidant properties data for the other types of Torbangun tea is not available in that study. Previous studies on the antioxidant activity of tea and herbal tea were done with the emphasis on dry leaf extract using organic solvents mainly ethanol (Atoui *et al.*, 2005). This often did not correlate to the reality since such product is consumed using water as its solvent. Therefore, it becomes necessary to gather antioxidant properties data of uncommon herbal tea such as Torbangun in its practical form. Furthermore, the objective of this study is to observe the effect of different processing methods of Torbangun leaves, particularly the oxidation degree, on the antioxidant properties of Torbangun herbal tea. This is may become valuable information in using particular drying technique that provides better antioxidant properties that may result in better lactagogue effect.

## MATERIALS AND METHODS

### Materials and Equipment

The raw materials that is used in this study are drinking water and Torbangun leaves (*Plectranthus amboinicus* (Lour.) Spreng.) from Wonderful Agriculture Indonesia (WAIN) farm in Bogor. The leaves vary in size (5-7 cm) with an average age of 3 months. Reagents that are used include methanol (proanalysis, Smart Lab), DPPH (proanalysis, Sigma-Aldrich), aluminium chloride (proanalysis, Merck), quercetin (proanalysis, Sigma-Aldrich), folin-ciocalteu (proanalysis, Merck), sodium carbonate (proanalysis, Merck), gallic acid (proanalysis, Merck), magnesium sulfate (proanalysis, Merck), magnesium (proanalysis, Merck), amyl alcohol (proanalysis, Merck), hydrochloric acid (proanalysis, Smart Lab) and ferric chloride (proanalysis, Smart Lab). Meanwhile, the main quipment used in this study are cabinet dryer, steamer, glassware, vortex, analytical balance (AR2140, Ohaus Corp) and uv-visible wavelength spectrophotometer (U1800, Hitachi).

### Preparation of Torbangun leaves

In this study, fresh Torbangun leaves were treated in three different manners based on the

production of green tea, black tea and Oolong tea. Each of the resulting Torbangun leaves were brewed and analyzed in terms of antioxidant activity, total phenolic, total flavonoid as well as phytochemical identification of phenolic, flavonoid and tannin. Herbal tea sample was obtained by brewing 6 g of dried Torbangun leaves in 250 ml of 70°C water for 3 minutes.

### Preparation of Unoxidized Torbangun Herbal Leaves

The preparation procedure for unoxidized Torbangun leaves was modified from Panda (2011) and followed the Japanese-style green tea processing by utilizing steam. Once the leaves were sorted from any defects, they are washed with running water and drained. Afterwards, the leaves were steam-blanching in a steam cooker for 90 seconds and manually rolled. Rolled leaves were then reduced in size by cutting into  $\pm 1$  cm in width and dried in a cabinet dryer at 50°C for 18 hours.

### Preparation of Semioxidized Torbangun Leaves

The preparation procedure for semioxidized Torbangun leaves was done according to the processing of Oolong tea by Zhen (2002) with modification. Prior to oxidation process, the leaves were sorted, washed and drained. The oxidation process was done in two stages. The first oxidation was done by exposing the leaves to sunlight for 90 minutes and further oxidation was done at room temperature for 5-8 hours. Indoor oxidation was done by placing the leaves on a flat surface without covering the leaves, subjecting them to open air. Subsequently, the leaves were lightly pressed to bruise the leaves. Before drying, the leaves were manually rolled and cut into  $\pm 1$  cm in width. Drying was then done at 50°C for 18 hours in a cabinet dryer.

### Preparation of Oxidized Torbangun Leaves

The preparation procedure for oxidized Torbangun leaves was done using conventional black tea processing method according to Emdadi *et al.* (2009) with modifications. Similar to the other two preparation steps, Torbangun leaves were sorted, washed and drained. Unlike the semioxidized Torbangun leaves, oxidation was done the longest. The leaves were laid flat and subjected to open air at room temperatura for 15 hours. The leaves were then lightly pressed to bruise the leaves for easier oxidation and manually rolled. Afterwards, the leaves were covered with cloth and left to oxidize at room temperatura for 2 hours. Oxidized leaves were cut into  $\pm 1$  cm in width and dried in a cabinet dryer at 50°C for 18 hours.

### DPPH Radical Scavenging Activity

The antioxidant activity of Torbangun herbal tea was measured on the basis of the DPPH radical scavenging activity assay according to the method described by Sudaryat *et al.* (2015) with slight modifications. Various concentrations of Torbangun herbal tea (0.8 ml) were mixed with 1 ml of 0.2mM DPPH solution. The mixture was shaken vigorously

and stored for 30 minutes in a dark environment. Mixture of 0.8 ml methanol and 1 ml DPPH solution was used as control. The absorbance reading was done at 517 nm. IC<sub>50</sub> (µg/ml) was used to identify the antioxidant activity of the herbal tea to inhibit free radicals as much as 50%.

#### Determination of Total Phenolic Content

The total phenolic content of Torbangun herbal tea was measured by the Folin-ciocalteu method as described by Borkatakya *et al.* (2013) with modifications. 0.3 ml sample was mixed with 1.5 ml 10% Folin-Ciocalteu. The mixture was vortexed and after 5 minutes, 1.2 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> was added to the mixture, vortexed and placed in a dark environment for 30 minutes. Absorbance was measured at 765 nm. Calibration curve of gallic acid was used as a standard. The total phenolic content in the Torbangun herbal tea was expressed in terms of gallic acid equivalent (mg GAE/L).

#### Determination of Total Flavonoid Content

The total flavonoid content of Torbangun herbal tea was measured by aluminium chloride colorimetric assay adapted from Nabavi *et al.* (2008) with slight modifications. 2 ml sample was added with 2 ml of 2% AlCl<sub>3</sub> and homogenized using vortex. Absorbance was measured at 415 nm. Total flavonoid content was expressed as mg quercetin equivalent per litre sample (mg QE/L).

#### Preliminary Phytochemical Screening

The phytochemical screening of Torbangun herbal tea was conducted based on the procedure describe by Mamta and Jyoti (2012) and Rao *et al.* (2016) with modifications. Phytochemical screening in this study was done to qualitatively test the suspected presence of flavonoid, phenolic and tannin. To quantitatively check the presence of flavonoid, 5 ml of Torbangun herbal tea was added 1 ml HCl, 0.2 g Mg, 1 ml amyl alcohol and homogenized. Change in color to red, yellow or orange indicates the presence of flavonoid. For phenolic, 5 ml of Torbangun herbal tea was mixed with 5 drops of 5% FeCl<sub>3</sub> and homogenized. Change in color to blue, green, red or purple indicates positive presence of phenolic. Lastly, 5 ml of Torbangun herbal tea was mixed with a few drops of 0.1% FeCl<sub>3</sub> to qualitatively test the presence of tannin. Color change to greenish-black indicates the presence of tannin in the sample.

#### Moisture Content Determination

Moisture content determination follows the AOAC method. Evaporating dish were placed in the oven and measured until a constant weight is reached. About 5 g of sample was placed in the constant evaporating dish and dried in an oven at 105°C for 6 hours. The dish was then removed and placed in a desiccator for 10 minutes and weighed. The sample was placed back into the oven and reweighed until a

constant weight has been reached. Moisture content of raw materials, tea leaves and tea samples were calculated using the formula as follows:

$$\text{moisture content (\%)} = \frac{(\text{final sample weight} - \text{initial sample weight})}{\text{initial sample weight}} \times 100$$

## RESULTS AND DISCUSSION

### Antioxidant Properties of Raw Torbangun Leaves

Prior to the making of Torbangun herbal tea using different preparation techniques, the antioxidant activity, total phenolic and total flavonoid of the raw Torbangun leaves were analyzed (Table 1). Antioxidant can be classified into four categories according to its activity; very strong activity with IC<sub>50</sub> value between 0 to 50 ppm, while strong activity shows an IC<sub>50</sub> value of 50-100 ppm. IC<sub>50</sub> value of 100-150 ppm indicates moderate activity and weak activity can be seen in IC<sub>50</sub> value between 151-200 ppm (Tobing *et al.*, 2017). Based on the result in Table 1, antioxidant activity of unprocessed Torbangun leaves used in this study, 114.27 µg/mL, can be categorized as moderate activity. Furthermore, the antioxidant activity of Torbangun leaves used in this study is greater than the antioxidant activity found by Tafzi *et al.* (2017), where IC<sub>50</sub> value of methanolic extract of Torbangun leaves is reported at 155.24 µg/mL. However, Tafzi *et al.* (2017) reported noticeable greater value on total phenolic (265.83 mg GAE/g) and total flavonoid (59.32 mg QE/g) content. Study done by Iwansyah *et al.* (2016) also reported greater value on total phenolic (53.88 mg GAE/g) and total flavonoid (14.19 mg QE/g) content of Torbangun leaves. In addition to environmental factors (geography, maturity, and climate), these differences may also be due to different methods of extraction or different types of solvent being used. Iwansyah *et al.* (2016) used multiple-maceration using both ethanol and methanol while Tafzi *et al.* (2017) ground the Torbangun leaves into powder for the analysis.

### Moisture Content of Unoxidized, Semioxidized and Oxidized Torbangun Leaves

Moisture content of the unoxidized, semioxidized and oxidized *Torbangun* leaves was performed to examine whether the moisture content of the leaves have met the SNI (Standar Nasional Indonesia) standard for tea. In order to be considered as 'tea', SNI declares a maximum of 8% moisture content. Table 2 below presents the moisture content of each type of Torbangun leaves in the present study.

Table 1. Total phenolic, total flavonoid and antioxidant activity of raw Torbangun leaves

Parameter	Result
Total phenolic [mg GAE/g]	39.02±0.18
Total flavonoid [mg QE/g]	10.32±0.10
Antioxidant activity (IC <sub>50</sub> ) [µg/mL]	114.27±4.43

Table 2. Moisture content of unoxidized, semioxidized and oxidized Torbangun leaves

Types of Torbangun leaves	Moisture content [%]
Unoxidized	7.64±0.20
Semioxidized	7.18±0.15
Oxidized	7.79±0.16

Prior to the processing of Torbangun leaves, moisture content of the raw Torbangun leaves was analyzed. Moisture content of the raw Torbangun leaves used in this study is 91.06±0.48 %, which is in accordance to the moisture content found by Iwansyah *et al.* (2016). Moisture content of the raw Torbangun leaves is known to be higher than the true tea leaves, *Camellia sinensis*, which has moisture content of 75-78 % (Mujumdar, 2014). Drying process applied in the present study resulted in moisture content that is in accordance with SNI. In addition, moisture content of all Torbangun leaves used in the present study is in accordance to the maximum moisture content of dried medicinal plant leaves that is between 10 to 12 % as reported by Müller and Heindl (2006).

#### Phytochemical Identification of Different Types of Torbangun Herbal Tea

Phytochemical identification is done in order to qualitatively describe the presence of phenolic, flavonoid and tannin compounds in each type of Torbangun herbal tea, namely unoxidized, semioxidized and oxidized. Figure 1 shows the appearance of the Torbangun herbal tea in which the oxidized Torbangun leaves resulted in slightly darker colour herbal tea compared to the semioxidized and unoxidized. All herbal tea samples indicated the presence of phenolic, flavonoid and tannin (Table 3). Slight difference in color changes during phenolic identification test is observed between unoxidized and oxidized herbal tea, where unoxidized herbal tea exhibited darker positive color than oxidized herbal tea. Positive results on flavonoid and tannin are also observed in the Torbangun extract used by Tafzi *et al.* (2017), while positive results on phenolic identification confirmed reports by previous studies on the presence of some phytochemicals, including phenolic, in Torbangun (Arumugam *et al.*, 2016).



Figure 1. Torbangun herbal tea from oxidized leaves (left), semioxidized leaves (middle) and unoxidized leaves (right)

Table 3. Phytochemical identification of different types of Torbangun herbal tea

Types of herbal tea	Phenolic	Flavonoid	Tannin
Unoxidized	+	+	+
Torbangun			
Semioxidized	+	+	+
Torbangun			
Oxidized Torbangun	+	+	+

#### Antioxidant Properties of Different Types of Torbangun Herbal Tea

##### Total Phenolic

The effect of different processing conditions applied to the Torbangun leaves on the total phenolic content of Torbangun herbal tea can be seen in Figure 2. In terms of total phenolic, unoxidized Torbangun herbal tea shows a significantly higher content (44.22 mg GAE/g) compared to oxidized Torbangun herbal tea (24.66 mg GAE/g). Significant difference ( $p < 0.05$ ) is only observed between unoxidized and oxidized Torbangun herbal teas. The higher total phenolic content may be due to the minimal processing applied (no oxidation) and steam blanching process prior to rolling and drying. According to Panda (2016), steam blanching step in green tea processing allows the leaves to inactivate enzymatic activity that might occur, preventing the oxidation process. On the contrary, the occurrence of oxidation is purposely done in the processing of semioxidized and oxidized Torbangun herbal tea in which the latter is oxidized to a greater extent compared to the other types. As described in the research and methodology section, the oxidized Torbangun leaves was exposed to air at a much longer period compared to the semioxidized Torbangun leaves. Therefore, although insignificant, the total phenolic content of the oxidized Torbangun herbal tea is lower than the semioxidized.

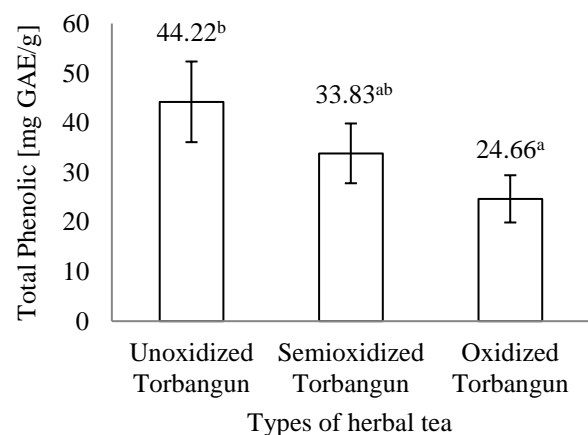


Figure 2. Total phenolic content [mg GAE/g] of different types of Torbangun herbal tea. Different superscript letter indicates significant difference statistically ( $p < 0.05$ )

In this study, correlation between oxidation process of the leaves and the phenolic content of the herbal tea can be seen. In comparison to the raw or unprocessed Torbangun leaves, the oxidation process decreases the total phenolic content of Torbangun from 39.02 mg GAE/g to 33.83 and 24.66 mg GAE/g depending on the oxidation degree. This reduction in total phenolic content may be due to the occurrence of oxidative degradation. However, the processing manner of unoxidized leaves gives an increasing effect on the total phenolic content. In addition to inactivate enzymatic activity that might decrease the total phenolic content, the use of steam-blanching in the preparation of unoxidized Torbangun leaves may release the possibly trapped phenolic compounds as seen in green leafy vegetables (Adefegha and Oboh, 2011), resulting in higher total phenolic content.

Total phenolic content present in this study is lower than the one found by Khattak *et al.* (2013). This difference may be due the different form of sample and solvent used. In the previous study, the sample was obtained from a ground Torbangun that was extracted in methanol, resulting in greater amount of phenolic compound. Similar effect of oxidation on phenolic content of herbal teas is also seen in the study done by Ariffin *et al.* (2011) using *Cantella asiatica*. The study also showed a decreasing pattern in total phenolic content from non-oxidized, partially oxidized and fully-oxidized herbal teas. When compared to true tea from *Camellia sinensis*, Atoui *et al.* (2005) also reported decreasing trend between green tea and black tea that also confirms the deterioration effect of phenolic compounds due to oxidation process involved in the making of black tea.

### Total Flavonoid

Similar to the total phenolic content results, total flavonoid content is highest in unoxidized Torbangun herbal tea at 17.02 mg QE/g, followed by semioxidized and oxidized Torbangun herbal teas at 10.68 mg QE/g and 8.61 mg QE/g, respectively. The flavonoid content of all herbal tea samples observed in this study are in the range of flavonoid content of three different clones of Torbangun reported by Andarwulan *et al.* (2014). Significant difference ( $p < 0.05$ ) is seen between the unoxidized Torbangun and both semioxidized and oxidized Torbangun. However, no significant difference ( $p > 0.05$ ) is existed between the semioxidized and oxidized Torbangun herbal teas. Although insignificant difference is seen between semioxidized and oxidized Torbangun herbal teas, the different oxidation degree may be the leading factor that results in the decrease of flavonoid content.

In the study of total phenolic and flavonoid content of true tea, Izzreen and Fadzelly (2013) also reported that total flavonoid is found at higher level in green tea compared to in black tea. In other herbal tea such as rooibos, decreasing trend in total flavonoid is also seen between unoxidized, semioxidized and oxidized rooibos teas (von Gadow *et al.*, 1997).

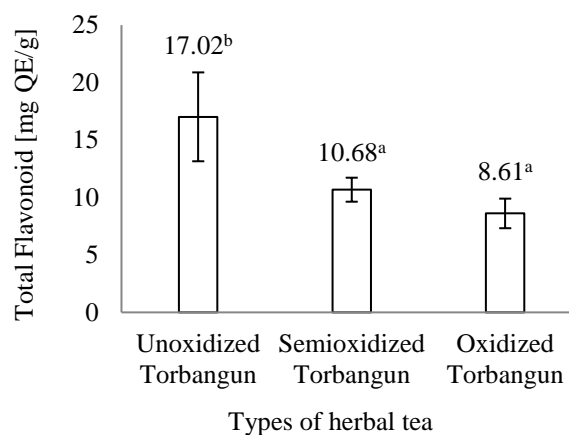


Figure 2. Total flavonoid content [mg QE/g] of different types of Torbangun herbal tea. Different superscript letter indicates significant difference statistically ( $p < 0.05$ )

As the oxidation period increases, the total flavonoid content decreases. The absence of oxidation may be responsible for the greater content of both phenolic and flavonoid content in unoxidized Torbangun herbal tea. Steaming, or subjecting the Torbangun leaves to heat in order to stop enzymatic activity seem to give the same promoting effect as seen in total phenolic content. Similar to the finding of Adefegha and Oboh (2011), Hossain *et al.* (2017) also observed an increasing trend in total flavonoid of green leafy vegetables when subjected to high temperature.

### Antioxidant Activity

Antioxidant activity of the unoxidized, semioxidized and oxidized Torbangun herbal teas is expressed in IC<sub>50</sub> value [ $\mu\text{g/ml}$ ] obtained from DPPH free radical scavenging assay. The ability of the Torbangun herbal teas to inhibit DPPH free radical as much as 50% ranges between 1400 and 4505  $\mu\text{g/ml}$ . Among the three herbal teas, unoxidized Torbangun shows significantly lower IC<sub>50</sub> value at 1400.89  $\mu\text{g/ml}$  compared to the semioxidized Torbangun at 3211.71  $\mu\text{g/ml}$  and oxidized Torbangun at 4504.78  $\mu\text{g/ml}$ . It is generally accepted that lower IC<sub>50</sub> value indicates better scavenging activity or greater antioxidant activity. Significant difference ( $p < 0.05$ ) in IC<sub>50</sub> value is found between the tree types of herbal tea. All of the Torbangun herbal teas shows very weak antioxidant activity ( $> 200$  ppm).

Yang and Liu (2015) studied the antioxidant activity of true tea, namely green tea, black tea and Oolong tea. The study reported that the highest antioxidant is displayed by green tea, followed by black tea and Oolong tea. Although the study mentioned that the difference between black tea and Oolong tea is not significant, the antioxidant activity of the different types of Torbangun herbal tea is found to be consistent with the trend seen in true tea.

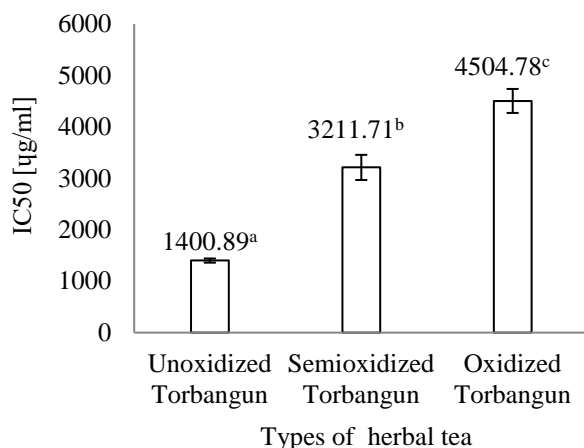


Figure 3. IC50 value [µg/ml] of different types of Torbangun herbal tea. Different superscript letter indicates significant difference statistically ( $p < 0.05$ )

In the study on antioxidant activity of Torbangun plant, Suryowati *et al.* (2015) reported the IC50 value of ethanolic extract of Torbangun at 247.942 µg/mL. The noticeably lower IC50 value may be due to the use of different solvent to extract the Torbangun. This suggests that ethanol is more effective in terms of extracting the antioxidant-contributing compounds, such as phenolics and flavonoids, in Torbangun compared to water. Hendrawan *et al.* (2019) used distilled water (aquadest) as the extraction solvent to study the antioxidant activity of dried Torbangun leaves extract using microwave assisted extraction method. In that study, the lowest IC50 value is found at around 9000 µg/mL using the highest microwave power (300 watts) and the longest extraction time (3 minutes). This proves that the traditional method of tea brewing using hot water continue to give better extraction process in terms of antioxidant activity.

In addition, compared to raw Torbangun leaves, much higher IC50 value is seen in processed (unoxidized, semioxidized and oxidized) leaves. This indicates that processing the Torbangun leaves into herbal tea has tremendous effect on the antioxidant activity. Subjecting the leaves to heat for long period of time (cabinet drying at 50°C for 18 hours) decreases the antioxidant activity of Torbangun leaves. However, heat application in unoxidized Torbangun leaves (steam blanching) resulted in higher total phenolic and flavonoid content compared to the raw or unprocessed Torbangun leaves.

## CONCLUSIONS

In terms of total phenolic, unoxidized Torbangun herbal tea shows a significantly higher content (44.22 mg GAE/g) compared to oxidized Torbangun herbal tea (24.66 mg GAE/g), which may be due to the absence of oxidation and steam blanching process. Similar to the total phenolic content results, total flavonoid content is highest in unoxidized Torbangun herbal tea (17.02 mg QE/g). Although insignificant difference is seen between semioxidized

and oxidized Torbangun herbal teas, the different oxidation degree may be the leading factor that results in the decrease of flavonoid content.

All of the Torbangun herbal teas shows very weak antioxidant activity (1400.89 µg/mL for unoxidized Torbangun herbal tea, 3211.71 µg/mL for semioxidized Torbangun herbal tea and 4504.78 µg/mL for oxidized Torbangun herbal tea). Based on the IC50 value of raw or unprocessed Torbangun leaves (114.27 µg/mL), processing the Torbangun leaves into different types of herbal tea tremendously decreased the antioxidant activity. On the other hand, steam blanching used in the preparation of unoxidized Torbangun leaves resulted in higher total phenolic and flavonoid content compared to the raw or unprocessed Torbangun leaves with an increase of total phenolic from 39.02 to 44.22 mg GAE/g and an increase of total flavonoid from 10.32 to 17.02 mg QE/g.

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