



## Application of microwave assisted extraction in extracting Torbangun leaves (*Coleus ambonicus*, L.) and its effects on polyphenol and flavonoids content

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### KEYWORDS

Flavonoids  
Microwave Assisted  
Extraction  
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### ABSTRACT

Torbangun leaves (*Coleus ambonicus*, L.) contain polyphenol compounds, flavonoids and antioxidant compounds that can be obtained by extraction methods. However, with the conventional extraction method it has the disadvantage of long extraction time and requires a lot of solvents. Therefore, this study discusses the use of microwave assisted extraction (MAE) method to extract the leaves of Torbangun. This study uses two treatment factors on MAE i.e. power variations (100, 180 and 300 Watts) and extraction time (1, 2 and 3 minutes). This study aims to analyze the effect of MAE on the content of polyphenol compounds and flavonoids in the extraction process of Torbangun leaf. The results showed that the highest total phenol (4196.59 mg GAE/g extract) was found in the treatment of 300 watt of power with extraction time of 3-minutes with IC50 value of 9.89 mg/ml. The highest total flavonoid value was 300 watt of power with 1-minute extraction time which was 4.54 mg QE/g DW.

### Introduction

Indonesia is one of the countries that has the highest drug diversity in the world. Indonesia's tropical forest areas have the second highest biodiversity in the world after Brazil. As many as 400,000 species of flora in the world, there are 30,000 species found in Indonesia and 940 of them are known to be efficacious as medicines and have been used in traditional medicine for generations by various ethnic groups in Indonesia (Elfahmi et al., 2014; Sukara, 2014). According to the tradition of the Batak people in North Sumatra, Indonesia, Torbangun (*Coleus ambonicus*, L.) leaves are believed to be efficacious as laktogogum, increasing the quality and quantity of breast milk. Aside from being a laktogogum, the Batak people also believe in the efficacy of the Torbangun leaves as a uterine cleansing agent, tonicum, painkillers, antimicrobial / antibacterial agents and drugs to cure diseases such as canker sores and coughing (Silalahi et al., 2015). The phytochemical database (Guldiken et al., 2018) reports that this leaf contains vitamin C, vitamin B1,

vitamin B12, beta-carotene, niacin, carvacrol, calcium, fatty acids oxalic acid, and fiber. These compounds have the potential for a variety of biological activities, such as antioxidants, diuretics, analgesics, cancer prevention, anti-tumor, antivertigo, immunostimulants, anti-inflammatory, anti-infertility, hypocholesterolemic, hypotensive, and other properties that need further investigation. As one of the sources of medicinal plants in Indonesia, the benefits of Torbangun leaves need to be continuously explored and developed. Therefore, phenolic compounds and antioxidant compounds can be obtained by using the extraction process (Casagrande et al., 2018; Trinh et al., 2018).

Previously, Tobing et al. (2017) extracting Torbangun leaves to extract flavonoid compounds using maceration method with 150 ml hexan solvent and macerated for 48 hours. In addition, Suryowati et al. (2015) also carried out the extraction process of Torbangun leaves to extract phenolic compounds using solvent maceration method 600 ml ethyl alcohol 96% and macerated for 3 hours. Based on

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the two studies, the extraction process of the phenolic compounds from the Torbangun leaves has a weakness i.e. the long extraction time, large amount of solvents, and the yield produced is small. Therefore, the extraction process of phenolic compounds can be modified by other methods such as the microwave assisted extraction (MAE) method (Alara et al., 2018; Moral et al., 2018). The MAE radiation technique is an extraction technique that utilizes microwave radiation to heat solvents quickly and efficiently. The advantages of MAE extraction are short extraction times, low solvent requirements, and simple process (Jain et al., 2009; Ekezia et al., 2017). In addition, MAE extraction is very suitable for extracting compounds that are not heat resistant (Angiolillo et al., 2015). The MAE method can also help to increase the amount of crude extract in the extraction time and the lower amount of solvent compared to conventional extraction methods (Vinatoru et al., 2017; Yuan et al., 2018; Zhong et al., 2018). In the MAE method, the microwave generated can increase the temperature of the solvent in the material which can cause the cell wall to break and the substances contained in the cell out towards the solvent, so that the yield produced increases (Chemat and Giancarlo, 2013). The purpose of this study was to obtain extracts of polyphenols and flavonoids contained in Torbangun leaves produced from the MAE method effectively and efficiently so that the extraction process can be shortened.

### Research Methods

This research was conducted at the Laboratory of Agrochemical Technology and Bioindustry Laboratory, Department of Agricultural Industrial Technology, Universitas Brawijaya, Indonesia.

### Materials

The materials used in this study include: the dried Torbangun leaf as the main ingredient to be extracted (Jatiasih, Bekasi, West Java, Indonesia); aquades as extraction solvents; methanol and 1,1-diphenyl-2-2-picrylhydrazyl (DPPH) as test materials for antioxidant activity. Other materials include quercetin; aluminum chloride; sodium nitrite; and sodium hydroxide were used as a total flavonoid test material. Some materials like gallic acid; sodium carbonate solution; folin ciocalteau solution were used as a total phenol test material.

The tools used in this study include: Microwave (Samsung type MG23H3185); UV-Vis spectrophotometer (Libra S12) to measure the color of polyphenol compounds at 760 nm wavelength, at 350 nm for flavonoids, and at 517 nm for antioxidant activity; rotary vacuum evaporator;

digital balance sheet; vortex; oven; thermometer; and stopwatch.

### Methods

Torbangun leaves that have been dried in an oven for 48 hours at a temperature of 50°C and are reduced in size, weighed 3 g. Then put in a 500 ml beaker glass. After homogenization, the initial temperature measurement is carried out using a thermometer. Then extraction is done using MAE. This process is carried out with three time variations i.e. 1 minute, 2 minutes, and 3 minutes with three variations of power i.e. 100 Watts, 180 Watts and 300 Watts. After the extraction process was completed, temperature measurements were taken again and maceration was carried out for one hour in each sample. After that, the filtering process is done twice using fine filter paper. Furthermore, purification of the extract from the solvent was carried out by evaporation. At this stage a rotary evaporator was used with a temperature of 50°C and a rotating speed of 60 rpm for 23 minutes 15 seconds. The purpose of evaporation is to get a more concentrated and pure extract. After the extraction process was completed, then the extract from the evaporation was tested for total phenol using the Folin-Ciocalteau method (Rover and Brown, 2013), and measured the total flavonoid content by the aluminum chloride calorimetry method and antioxidant activity using the DPPH method (Baranowska and Bajkacz, 2018). The length of time and the power used is based on the preliminary research. Preliminary research is a study conducted to obtain information about the research that will be conducted. The selection of time and power variations is based on preliminary research where at a greater power with a longer time, temperatures can reach 70°C which can cause damage to antioxidant compounds. In addition, at this stage it also determines the right time in the sample evaporation process. In each treatment, the greater the power and the longer time used for extraction, the higher the temperature. This increase in temperature is caused by microwave contact with materials which then cause a heat effect that affects the extraction process.

The total phenol test is a test used to determine the total phenol content of the samples by estimating the value of the phenolic compound as a whole. For the determination of the total phenol value, Eq. (1) is used.

$$TP = \frac{A-b}{a} \times df \times \frac{V}{M} \quad (1)$$

Where,

- TP : total phenol (mg Gallic Acid Equivalent (GAE)/g extract)  
 A : measured absorbance value  
 a : values that have standard curve equations  
 $y = ax + b$   
 b : values that have standard curve equations  
 $y = ax + b$   
 df : dilution factor  
 V : extract volume (ml)  
 M : mass extract (g)

After measuring all the absorbance values of the concentration of gallic acid solution using a UV VIS spectrophotometer with a wavelength of 760 nm, then the graph of the relationship between the concentration of solution is then made to obtain a linear regression equation:  $y = ax + b$ . The 0.5 ml sample prepared will be reacted with 10% Folin-Ciocalteu reagent and 7.5% Sodium Carbonate reagent. In this reaction it will show a change in color to blue which indicates containing phenol compounds.

The total value of flavonoids was measured by the method of aluminum chloride ( $\text{AlCl}_3$  calorimetry) and presented in units of mg QE (Quercetin Equivalent)/g DW. The aluminum chloride method is a method of calculating total flavonoids using a comparison. This study used quercetin as a comparison. Quercetin is the largest compound of the flavonol group, quercetin and its glycosides are in the amount of about 60-75% of flavonoids (Nile et al., 2017). The sample of 2.5 ml was taken, then weighed the mass. The next step, a 0.5 ml of distilled water is added, 0.3 ml of  $\text{AlCl}_3$  10% 0.8 M solution, 4 ml of 1 M NaOH and again added 6.4 ml of distilled water, then absorbance at 350 nm was measured and a linear equation standard curve was made  $y = ax + b$ .

In this study the determination of the value of antioxidant activity expressed with  $\text{IC}_{50}$  value aims to determine the magnitude of antioxidant activity of Torbangun extract which is extracted using the MAE method with a variation of 100 Watts, 180 Watts and 300 Watts of power as well as variations of extraction time i.e. 1 minute, 2 minutes and 3 minutes. The antioxidant activity testing method uses DPPH (2,2-Diphenyl-1-Picrylhydrazil), which utilizes DPPH free radical compounds in polar solvents which in this study used methanol to test an antioxidant compound in reducing free radicals. DPPH solution was weighed 0.0039 g and dissolved with 50 ml methanol. Then the extract was weighed 0.250 g and dissolved with methanol 25 ml, then obtained a concentration of 10 mg/ml. After that, varying the concentration of samples 2.5, 5, and 7.5

mg/ml by diluting a solution of 10 mg/ml. As a control, a solution of 0 mg/ml was made without sample solution. The next step, at each concentration 0.5 ml of sample was taken into the test tube. Then it was added 3.5 ml methanol and 1 ml DPPH 0.1 nM solution to each test tube. After that, it was vortexed and left for 30 minutes in the dark room. Then absorbance measurements were carried out with a wavelength of 517 nm.

The scavenger activity of free radicals is calculated as a percentage of DPPH color reduction by using Eq. (2).

$$\% \text{inhibition} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100\% \quad (2)$$

To determine the  $\text{IC}_{50}$  value, a linear regression equation is needed  $y = cx + d$ . This equation is obtained from the graph of the relationship between sample concentration and percentage of DPPH inhibition.  $\text{IC}_{50}$  values are calculated by Eq. (3).

$$\text{IC}_{50} = \frac{50-d}{c} \quad (3)$$

$\text{IC}_{50}$  : Inhibitor concentration 50% (mg/ml)  
 50 : Setting value for  $\text{IC}_{50}$  determination

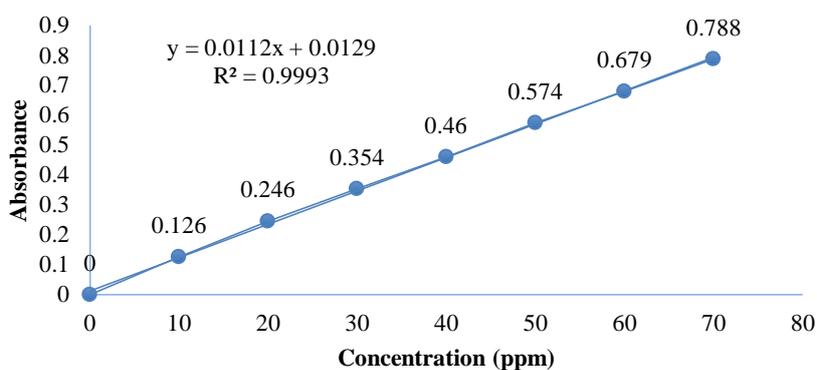
## Results and Discussion

The total phenol value uses the ratio of the gallic acid curve, the results can be seen as shown in Fig. 1. Based on the total test results of total phenol extract, the total phenol values ranged from 218.23 to 4196.59 mg GAE/g. The average graph of the total phenol value of Torbangun leaf extract due to the treatment of MAE power variations and extraction time can be seen in Fig. 2.

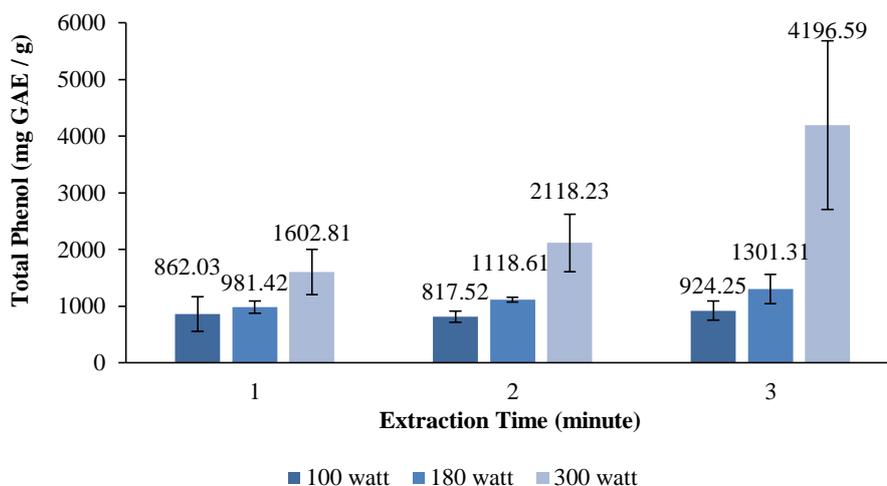
Based on the results of the study, Fig. 2 shows that the total phenol value of Torbangun leaf extract with the treatment of MAE power variation and extraction time obtained the highest value at 300-watt of power with extraction time of 3 minutes i.e. 4196.59 mg GAE/g extract and the lowest value at power variation of 100 Watts with extraction time of 2 minutes which is equal to 817.52 mg GAE/g extract. Besides that, Fig. 2 also shows that the higher the MAE power and the longer the extraction time is used, the greater the total phenol value produced. However, there was a decrease in power of 100 Watts with an extraction time of 2 minutes. This can occur because during the evaporation process, there are still solvents left behind which reduce the purity of the extract material and can affect the results of the total phenol value. Microwave generators and long extraction times are

two factors that influence each other. The combination of low power and long extraction time is a good choice considering the combination can avoid the thermal degradation of the product (Mandal et al., 2009). According to (Shu and Ko, 2003; Razzaghi et al., 2019), extraction efficiency increases with increasing microwave power. However, according to (Gao et al., 2006) increased

efficiency at low power is achieved by extraction with a short duration. However, at higher power, power variations do not have a significant effect on extraction yields. The results of ANOVA analysis showed that the variation of MAE power and extraction time had a significant effect on  $\alpha = 0.05$  on the total phenol value contained in Torbangun leaf extract.



**Figure 1.** Gallic acid standard curve



**Figure 2.** The mean graph of total phenol of Torbangun leaf extract with treatment of variations in MAE power and extraction time

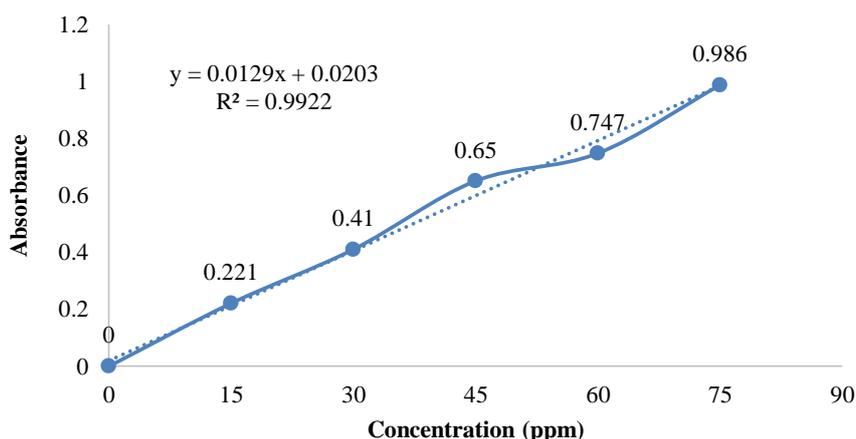
Antioxidant activity is indicated by 50% (IC50) Inhibitory Concentration, which means the sample concentration can reduce DPPH radicals by 50%. The higher IC50 value indicates that the antioxidant activity is low, and vice versa if the IC50 value is low then the antioxidant activity is high. The total value of flavonoids is calculated using a comparison of the standard quercetin curve which the result is shown in Fig. 3. Based on the results of the study it was found that the average flavonoid content was 3.63-4.54 mg QE/g DW. The total graph of the flavonoid of Torbangun leaf extract is presented in

Fig. 4. Fig. 4 shows that the extraction time of 1 minute, 2 minutes and 3 minutes indicated a fluctuating value. This is due to the difference in MAE power used and affects the temperature produced during the extraction process. From the fluctuating data, it shows that the phenomenon that occurs is not in the form of a linear trend, where the more the treatment value is raised, the result will increase or decrease. But from the data obtained it can be seen that there is an optimal point of power and length of extraction time. The highest flavonoid content at 1 minute is at power 300 of 4.54 mg QE/g

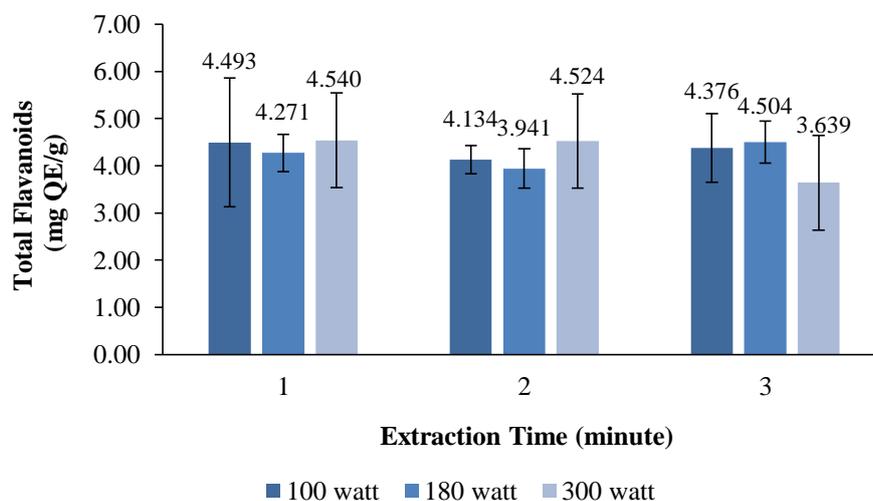
DW. The highest content at 2 minutes is at 300 Watts i.e. 4.52 mg QE/g DW. The highest content at 3 minutes is at 180 Watts i.e. 4.50 mg QE/g DW. The mean flavonoid content at 1 minute with the power of 100 Watts, 180 Watts and 300 Watts is 4.27-4.54 mg QE/g DW. The mean flavonoid content at 2 minutes with the power of 100 Watts, 180 Watts and 300 Watts is 3.94-4.52 mg QE/g DW. While at 3 minutes with the power of 100 Watts, 180 Watts and 300 Watts, the average content of flavonoids is 3.63-4.50 mg QE/g DW.

The test results were measured using an UV-VIS spectrophotometer to determine the wavelength absorbed. The greater the decrease in DPPH absorption, the stronger the antioxidant activity (Zamani et al., 2018). The reduction in color intensity that occurs is related to the number of DPPH electrons that capture hydrogen atoms. Reducing color intensity indicates an increase in the ability of antioxidants to capture free radicals. In

other words, antioxidant activity is obtained by calculating the amount of DPPH purple color intensity reduction which is proportional to the reduction in the concentration of DPPH solution through the measurement of the absorbance of the test solution (Abdel-Aty et al., 2019). After that, percentage of antioxidants were calculated to be able to get IC50 values. Percentage of antioxidants show the ability of compounds to reduce free radicals. According to Takaidza et al., (2018), the value of 0% means that the sample does not have antioxidant activity, while the value of 100% means that testing of antioxidant activity needs to be continued with sample dilution to determine the limits of concentration of activity. An ingredient can be said to be active as an antioxidant if the presentation of antioxidant activity is more or equal to 50%. The smaller the IC50 of a test compound, the more effective the compound as an antidote to free radicals.



**Figure 3.** Standard quercetin curve

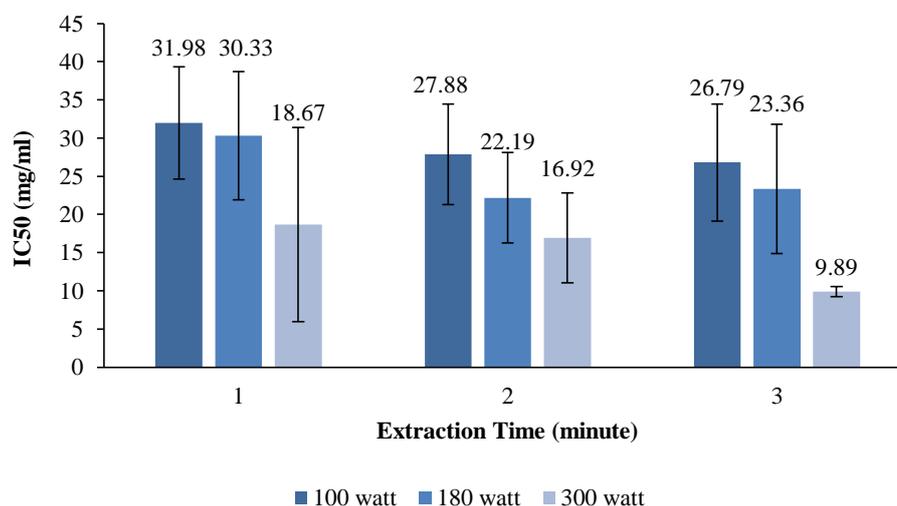


**Figure 4.** The graph of total flavonoid extract of the Torbangun leaf with the treatment of variations in MAE power and extraction time

Based on the antioxidant activity test obtained, antioxidant activity expressed with IC<sub>50</sub> values ranged from 9.89 to 31.98 mg/ml. Fig. 5 shows a graph of the mean IC<sub>50</sub> value of Torbangun leaf extract due to the treatment of power variations and extraction time.

Fig. 5 shows that IC<sub>50</sub> value of Torbangun leaf extract with the treatment of MAE power variations and extraction time obtained the best / the lowest value in the treatment with 300 Watts of power with extraction time of 3 minutes at 9.89 mg/ml. The worst IC<sub>50</sub> value / the highest value (31.98 mg/ml) reached in the treatment with a variation of 100 Watts of power with the extraction time of 1 minute.

In addition, from Fig. 5, it can also be seen that in each treatment the variation in extraction time of the IC<sub>50</sub> value will tend to show a decrease in value along with the increase in MAE power used i.e. 100 Watts, 180 Watts and 300 Watts. The greater the power used and the longer the extraction time used, the higher the antioxidant activity. In this study the results of ANOVA analysis show that MAE power variation and extraction time have a significant effect on  $\alpha = 0.05$  on IC<sub>50</sub> values.



**Figure 5.** IC<sub>50</sub> graph of Torbangun leaf extract with treatment of MAE power variation and extraction time

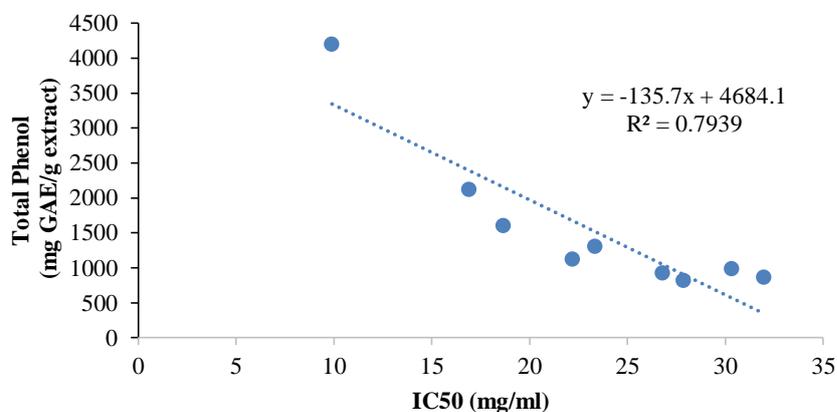
The correlation between the value of total phenol and the value of antioxidant activity represented in IC<sub>50</sub> values in this study was positively correlated. In this study it can be seen that the greater the total phenol value will make the IC<sub>50</sub> value smaller. This means that fewer extracts are needed to inhibit free radicals by 50%. In this study, the longer the extraction time and the greater the power used, the greater the phenol value as well as the high antioxidant activity. The correlation between the phenol compounds and the antioxidant activity of the Torbangun leaf extract is shown in Fig. 6. In general, bioactive compounds in agricultural products are phenolic compounds. According to Caillet and Lacroic (2006), phenolic compounds have strong antioxidant properties resulting in a correlation between the two. From the results of testing, the relationship between

antioxidant activity and total flavonoids is presented in Fig. 7.

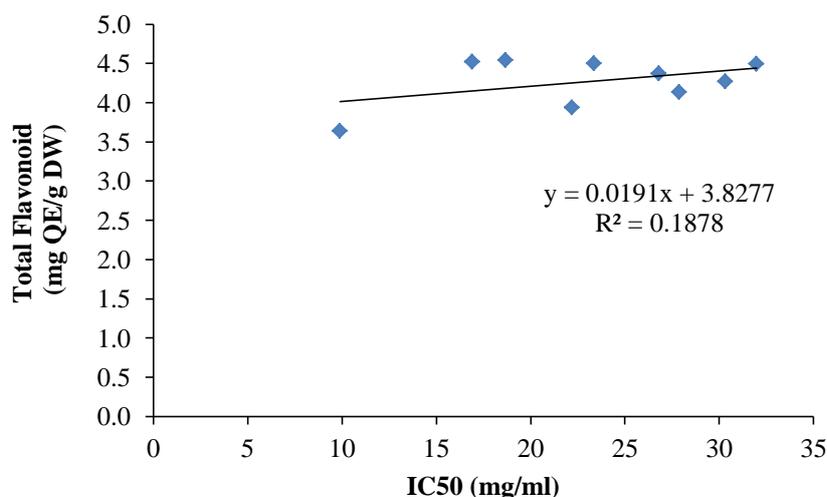
Fig. 7 shows that the lower the total flavonoids, the IC<sub>50</sub> obtained is also low. The minimum value obtained from the test on the total value of flavonoids was 3.63 mg QE/g DW and IC<sub>50</sub> of 9.89 mg/ml. Fluctuations in the graph can be caused by certain minimum points that affect the extraction process. It can be concluded that the amount of power used and the extraction time that is too long can cause flavonoids and antioxidant activity to decrease. This can be caused by the presence of antioxidant compounds other than flavonoids. Based on previous research on the composition of the chemical content of Torbangun leaves (Hullatti and Bhattacharjee, 2011), it was found that in this leaf contains essential oils of 0.043% in fresh leaves or 0.2% in dried leaves. The results of the

chromatogram analysis from Torbangun leaves were carried out by Suryowati et al. (2015) showed the levels of chemical compounds n. Hexadecanoic acid ( $C_{16}H_{32}O_2$ ) was reported to have biological activity as an antioxidant. Torbangun leaves contain vitamin

C, which also functions as an antioxidant that is hydrophilic. The results of this study also show that phenolic compounds as a component of antioxidants have high value.



**Figure 6.** Graph of correlation between total phenol and IC50



**Figure 7.** Graph of correlation between antioxidant activity (IC50) with total flavonoid content

Comparison of extraction results can be seen in Table 1 where the best phenol value in the study using MAE was 300 Watts with an extraction time of 3 minutes, i.e. 4196.59 mg GAE/g extract. The lowest result is at 100 Watts and 2 minutes which is 817.52 mg GAE/g extract. While the phenol value of maceration extraction, which is for 1 hour at room temperature of 27°C, is smaller at 771.30 mg GAE/g extract. The flavonoid test as presented in Table 2 shows that extraction using the MAE method can increase the total value of flavonoids. The highest total flavonoid value was found in the treatment with

300 Watts of power and 1-minute extraction time of 4.54 mg/QE DW. The lowest flavonoid value in the MAE treatment is in the treatment of 300 Watts of power with an extraction time of 3 minutes which is 3.63 mg/QE DW. Whereas the macerated control has a value of 1.84 mg/QE DW flavonoids. Table 3 in the antioxidant activity test shows that the MAE treatment has a higher antioxidant activity which is indicated by a lower value than without MAE treatment. The best results were found in the extraction treatment using MAE with a variation of 300 Watts of power with an extraction time of 3

minutes i.e. 9.89 mg/ml. The lowest results in the MAE extraction treatment were found in the 100-Watts treatment of 2 minutes extraction time which was 27.88 mg/ml. Whereas the control without MAE treatment was 35.70 mg/ml. This can prove the hypothesis that there was an increase in the extraction of polyphenols and flavonoids from the

extraction of Torbangun leaves using the MAE method compared to the conventional method wherein this study was macerated for one hour with room temperature of 27 °C with the same solvent (aquadest).

**Table 1.** Comparison of the results of the phenol value of the extraction of the MAE method with the control

Methods	Total Phenol (mg GAE/g extract)
MAE 300 watt 3 minutes	4196.59
MAE 100 watt 2 minutes	817.52
Maceration	771.30

**Table 2.** Comparison of the results of the flavonoid extraction method of MAE with control

Methods	Total Flavonoid (mg QE/g DW)
MAE 300 watt 3 minutes	4.54
MAE 100 watt 2 minutes	3.63
Maceration	1.84

**Table 3.** Comparison of the results of the antioxidant extraction activity method MAE with control

Methods	Antioxidant Activity IC50 (mg/ml extract)
MAE 300 watt 3 minutes	9.89
MAE 100 watt 2 minutes	27.88
Maceration	35.70

## Conclusions

This research used two treatments of microwave assisted extraction (MAE) i.e. power and extraction time to extract Torbangun leaves. The selection of extraction time and power variations is important. The greater the power and the longer time used for extraction, the higher the temperature. This increase in temperature is caused by microwave contact with materials which then cause a heat effect that affects the extraction process. For this reason, optimal power and extraction time are needed. The best results for total phenols were shown by 300 Watts of power and 3 minutes of extraction time which obtained phenol yields of 4196.59 mg GAE/g extract and IC50 of 9.89 mg/ml. The best treatment of total flavanoid with 4.54 mg QE/g DW was obtained by using 300 Watts in 1-minute extraction time. In this study it can be concluded that there was an increase in the extraction results of polyphenol compounds and flavonoids from Torbangun leaf extract using

the MAE method compared to the conventional methods.

## Conflict of interest

The authors declare that there is no conflict of interest in this publication.

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