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Extraction of *Phenolic* Compounds from *Coleus amboinicus* Leaves by Microwave-assisted Extraction: Optimization of the Operating Condition

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***Abstract*— Torbangun *(Coleus amboinicus L)* leaf is one of the herbs from Indonesia that has been reported to have many bioactive compounds for human health. These *phytochemicals* are predominantly incorporated into *phenolics*, such as *flavonoids*. An extraction process is needed to obtain all the valuable compounds, and conventional solvent extraction, such as heating and maceration, could be easily applied because of its simplicity. However, this method requires high energy, a longer extraction time, and a large amount of solvent. To overcome these limitations, in this study, microwave-assisted extraction (MAE) was utilized to extract phenolic compounds, including *flavonoids* from *torbangun* leaves. Three factors (solvent to feed ratio, microwave power, and extraction time) were evaluated to affect the three responses (extract volume, TPC, and TFC). The extraction process optimization was carried out to get a higher response. The Box-Behnken experiment design and Response Surface Methodology (RSM) determined the optimal operating condition. The result shows 30 ml/g of solvent to feed ratio, 300 W of microwave power, and 4.6 minutes of extraction time as the optimum condition. Applying those conditions, the extract is expected to reach 10.13 ml of extract volume, 9.17 mg GAE/ ml extract of total phenolic, and 3.62 mg QE/ ml extract of total flavonoid. In sum, MAE was useful for extracting *phenolic* compounds from *torbangun* leaves and could be an alternative method due to its high efficiency.**

***Keywords*— *Coleus amboinicus;* Torbangun; MAE; TPC; TFC; RSM.**

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I. INTRODUCTION

Torbangun (*Coleus amboinicus* L.) leaves are one Indonesian's plant which has many phytochemical compounds incorporated into phenolic compounds: flavonoids, isoflavones, tannins, and saponins [1], [2]. Those components have abundant benefits to human health, for example, antioxidant, anti-inflammatory, anti-dandruff, anti-virus, and antibacterial [2]. The ancient people believed that this leaf could increase post-partum mothers' breast milk [3], [4]. It is supported by the previous study that was reported galactagogue compounds containing in this leaf: *quercetin*, *kaempferol*, and *diosgenin* [4]–[6]. The *phytochemical* components of *torbangun* leaves are potentially useful for antioxidants. The evaluation of antioxidant activity resulted in higher contents of *phenolic* and *flavonoids* from the leaf than those in the stem [7]. Based on some studies, the higher the phenolic and flavonoid compounds, the higher antioxidant activity [8]–[10].

To gain all the beneficial components, the extraction process is necessary to get those bioactive compounds from the plant. The choice of extraction method is considered based on the extracted compound properties, economic value, and efficiency of the process [11], [12]. The most conventional methods were maceration and heat extraction. These methods are very simple and easy to be applied [13]. However, the conventional extraction requires heat and takes a longer extraction time which causes high consumption of energy [14]. Also, heat extraction at a certain temperature was reported to decrease the extract yield because some phenolic compounds are degraded by the high temperature [15], [16]. To reduce the energy consumption that affected by heat, some novel extraction methods such as electric-assisted extraction [17]–[19], ultrasound-assisted extraction (UAE) [20], [21], and microwave-assisted extraction (MAE) were applied [22]–[25].

Based on the recent studies, MAE was known as a novel extraction method that could reduce solvent usage and extraction time as well as increase the extraction yield [26]. The evaporation of raw material residual water is increased by the microwave radiation, then eventually destroys the plant cells wall to stimulate the extraction via internal diffusion. Besides, the energy loss to the surrounding in MAE is

pressingly less because the heating ensues specifically in the targeted material [27]. Considering these advantages, MAE was expected to decrease extraction time and reduce energy consumption. In addition, the short extraction time could prohibit heat accumulation; thus, thermo-sensitive compounds such as phenolic could be maintained.

Commonly, the success of MAE was influenced by many factors, such as the power of the microwave, frequency, extraction time, solvent to feed ratio, and solvent properties. As a result, the optimization of the extraction condition is necessary to obtain a high bioactive compound. RSM is a well-known effective method for evaluating the influence of the variables and their interactions on the response parameters. This method combines mathematical and statistical appliances to simultaneously analyze the dataset, resulting in the recommendation for optimum operating conditions. Many researchers have utilized RSM to optimize phytochemical compounds extraction, including antioxidant compounds [28], [29]. Nonetheless, there is no reference to optimizing antioxidant compounds extraction from torbangun leaves to the best of our knowledge.

The objective of the present study is to determine the optimum operating condition in the phenolic-MAE from *torbangun* leaves (*Coleus amboinicus* L.) by using RSM. The effect of some extraction variables (solvent to feed ratio, microwave power, and extraction time) on the observed responses (extract volume, total phenolic content (TPC), and total flavonoid content (TFC)) was also evaluated.

II. Materials and Methods

A. Material and Equipment

Fresh Torbangun leaves were obtained from Jatiasih-Bekasi, Indonesia. Pure analysis chemical material was used: distilled water, gallic acid, *sodium bicarbonate*, *Folin-ciocalteau* reagent, *Quercetin*, *aluminum chloride*, and *acetic* acid.

To conduct the extraction, microwave oven (Samsung MG23H3185), blender (Philips HR 2106), rotary vacuum evaporator (Heidolph), and UV-Vis spectrophotometer (Thermo Scientific Spectronic Genesys 10 S) were used. The RSM optimization was conducted using Design Expert 9.0.3 *(trial version)*.

B. Methods

1) Extraction of Torbangun Leaves. The fresh and clean Torbangun leaves were dried using a microwave at 450 W and 4 minutes drying. The dried leaves were milled and sieved (60 mesh) before being stored in a refrigerator. In the extraction process, the powdered leaves were blended with distilled water and extracted using a microwave with a certain operating condition in Table 1. After extraction, the mixture was filtered using Whatman filter paper (No. 40) and then evaporated using a rotary vacuum evaporator at 50 °C for 2 hours. The extract was stored in the amber bottle at 4 °C before being analyzed.

2) Determination of Total Phenolic Content (TPC). The TPC of Torbangun extract was evaluated based on *Folin-ciocalteau* methods using Gallic acid as a standard. The standard solution of Gallic acid was prepared with concentrations of 0, 10, 20, 30, 40, 50, 60, and 70 mg/L to make a calibration curve. For each concentration, the standard solution of 0.5 ml was added to 2.5 ml of 10% Folin-Ciocalteau solution, then vortexed and allowed to stand for 5 minutes. The 2 ml of 7.5% Natrium bicarbonate solution was then added to the solution. The solution was then vortexed and let stand for 15 minutes. Furthermore, the absorbance was measured with a UV-Vis spectrophotometer at 765 nm (maximum wavelength). To determine the TPC, 0.5 ml of the sample was reacted with 2.5 ml of 10% Folin-Ciocalteau solution, then it was vortexed and allowed to stand for 5 minutes. Next, the mixture was added with 2 ml of 7.5% Na_2CO_3 solution, then vortexed and allowed to stand for 15 minutes. Furthermore, the absorbance is measured at a wavelength of 765 nm. The result of absorbance of the sample obtained was converted to concentration using a standard curve of gallic acid. The TPC was expressed as milligram *gallic acid equivalent* (GAE) per milliliter extract (mg GAE/ ml extract).

3) Determination of Total Flavonoid Content (TFC). The TFC of Torbangun extract was analyzed based on the aluminum chloride colorimetric method using *quercetin* as a standard. To determine the TFC of the samples, 2.5 ml sample was added by 0.5 ml of distilled water and 0.3 ml of 20% $NaNO_2$ solution. The mixture was then allowed to stand for 6 minutes. The 0.3 ml of 10% $AlCl_3$ was added to the mixture and allowed to stand for 6 minutes. Hereinafter, 4 ml of 1 M NaOH and 2.4 ml distilled water were added to the mixture. The mixture was then vortexed and incubated for 30 minutes at room temperature in dark conditions. Centrifugation was applied for 20 minutes with 7500 rpm of angular velocity to separate the impurities. Furthermore, the absorbance was measured with a UV-Vis spectrophotometer at 350 nm of wavelength (maximum wavelength). The result of the sample absorbance was then converted to concentration using a calibration curve of *Quercetin*. The calibration curve was prepared with the range of 0-75 mg/L of quercetin concentration. The TFC was expressed as milligram *quercetin equivalent* (QE) per milliliter extract (mg QE/ ml extract).

4) Optimization using Response Surface Methodology (RSM). The optimization of Torbangun extraction was proceeded using RSM, a common method to simultaneously calculate the experiment data both mathematically and statistically. Also, this method was used to make a model for the experiment that has two or more factors and to optimize the desired responses. The observed factors in this research were solvent to feed ratio, microwave power, and extraction time; each factor has three levels (low, center, high) that were provided in Table 1.

TABLE I
FACTORS AND LEVEL

Factor	Unit	Low level (-1)	Center (0)	High level (1)
Solvent to feed ratio	ml/g	30	45	60
Microwave Power	W	300	450	600
Extraction Time	min	1	3	5

Because the experiment using three factors, 13 runs will be experimented with based on Box-Behnken design. Besides, the replication of the center point (0,0,0) was necessary a minimum of 3 times. To make the experiment more precise, 5 replications were applied, thus the number of experiment samples was 17. Each factor consists of 3 levels that have a low level (-1), center (0), and high level (1) as Box-Behnken design. The experiment was conducted according to the Box-Behnken design, as shown in Table 2.

III. Results and Discussion

Particularly, in this study, the extraction could become an optimum process if all responses are maximum. However, the maximum value of each response cannot be reached under the same condition. Therefore, the use of *Response Surface Methodology (RSM)* to obtain the optimal result was necessary. In addition, to reduce the number of experiments, the Box-Behnken design was applied. The response values from extraction are given in Table 2. The table consists of extraction variables (solvent to feed ratio, microwave power, and extraction time) and the responses (extract volume, TPC, and TFC). RSM analysis included ANOVA (*Analysis of Variance*) was applied to find the appropriate model that can connect each response with all factors. Besides, supported by Design-Expert software, the relationship of all factors and responses can be provided on a colorfully 2-dimensional graph (contour) and 3-dimensional graph (response surface). The color blue, green, yellow, and red indicates the condition of the response; for example, the red area is the maximum value of the response, and the blue area is vice versa.

TABLE II
Extraction factors and responses using Box-Behnken design

Run	Solvent to Feed Ratio (ml/g)	Microwave Power (W)	Extraction time (min)	Extract Volume (ml)	TPC (mg GAE/ ml extract)	TFC (mg QE/ ml extract)
1	30	300	3	30	4.13	1.58
2	60	300	3	30	2.32	0.80
3	30	600	3	10	5.47	2.07
4	60	600	3	17	2.93	1.39
5	30	450	1	39	3.70	1.39
6	60	450	1	43	1.86	0.87
7	30	450	5	2.4	12.05	4.97
8	60	450	5	5	5.45	2.08
9	45	300	1	42	2.44	0.93
10	45	600	1	35	2.38	0.85
11	45	300	5	5.7	5.28	2.30
12	45	600	5	3.6	6.43	3.28
13	45	450	3	29	2.83	1.44
14	45	450	3	26	2.74	1.47
15	45	450	3	30	2.78	1.46
16	45	450	3	30	2.78	1.48
17	45	450	3	30	2.72	1.54

A. RSM Analysis on Extract Volume

RSM analysis of the extract volume results that all existed factors influence the extract volume, and the relationship is represented by the quadratic model (Eq.1), where Y_1 is the extract volume. All combinations of experiments in Table 2 were processed in the DX software, including fitting data and reducing factors, resulting in a prediction result. The relationship between actual experiment results and predicted results was plotted in **Fig. 1**. The coefficient correlation (R^2 = 0.9687) represents that the model can represent 96.87% of the predicted data.

$$Y_1 = 29.00 + 1.70A - 5.26B - 17.79C + 1{,}75AB - 3.24A^2 - 4.01B^2 - 3.41C^2 \quad (1)$$

In addition, the model resulted in a contour and response surface of extract volume, as provided in **Fig 2.** The figure shows that the maximum extract volume was reached under these operation conditions: solvent to feed ratio of 45 ml/g, microwave power of 300-450 W, and extraction time of 1 minute (minimum extraction time).

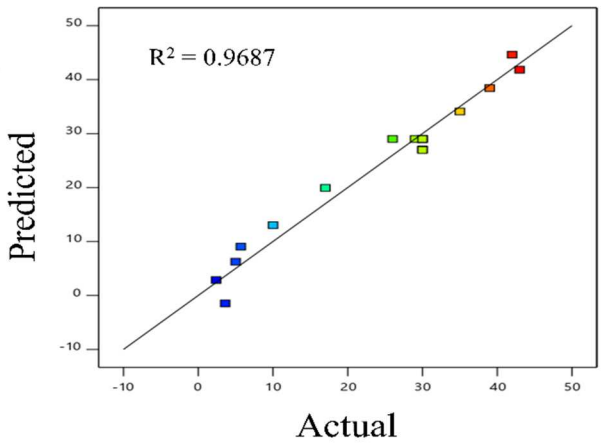

Fig. 1 Plotted graph of model predictions and actual results of extract volume

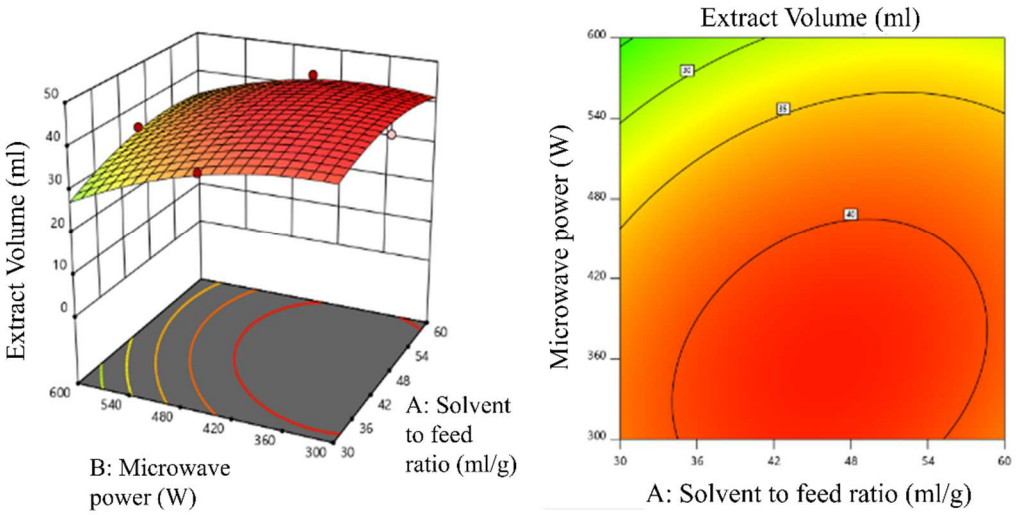

Fig. 2 Response surface of Torbangun leaves extract volume

B. RSM Analysis of Total Phenolic Content (TPC)

The RSM analysis of TPC resulted that TPC was influenced by all existed factors and represented as a quadratic model **(Eq. 2)**, where Y_2 is TPC. This model is similar to the previous model (Eq. 1). In the model, some variables did not exist (AB, BC, and B^2) because this model has been evaluated. Therefore, the coefficient which has minimum effect has been eliminated. This evaluation process was based on the p-value as the indicator of whether the variables are significant or insignificant.

$$Y_2 = 2.63 - 1.6A + 0.3804B + 2.36C - 1.19AC + 1.27A^2 + 1.69C^2 \quad (2)$$

Similarly, with that on the extract volume response, the suitability of the predicted data from the model with the actual experimental result was provided by the plotted graph (Fig. 3). Briefly, the coefficient correlation of the graph (R^2= 0.9349) indicates that the model can represent 93.49% of data. To explain the complex relationship among variables, the contour and response surfaces have been provided (Fig. 4). Expressed by the red area, the contour and response surface shows that maximum TPC was reached at a minimum solvent to feed ratio, 30 ml/g, maximum microwave power of 600 W, and maximum extraction time of 5 minutes.

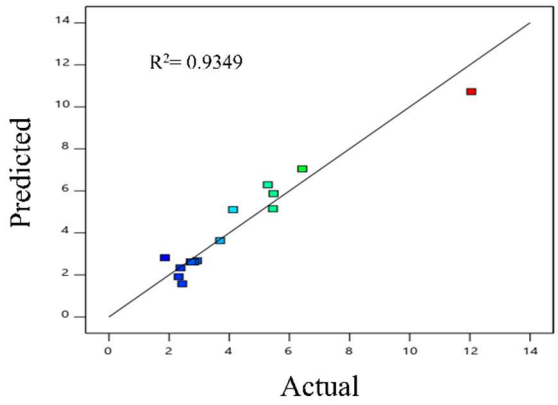

Fig. 3 Plotted graph of model predictions and actual results of TPC

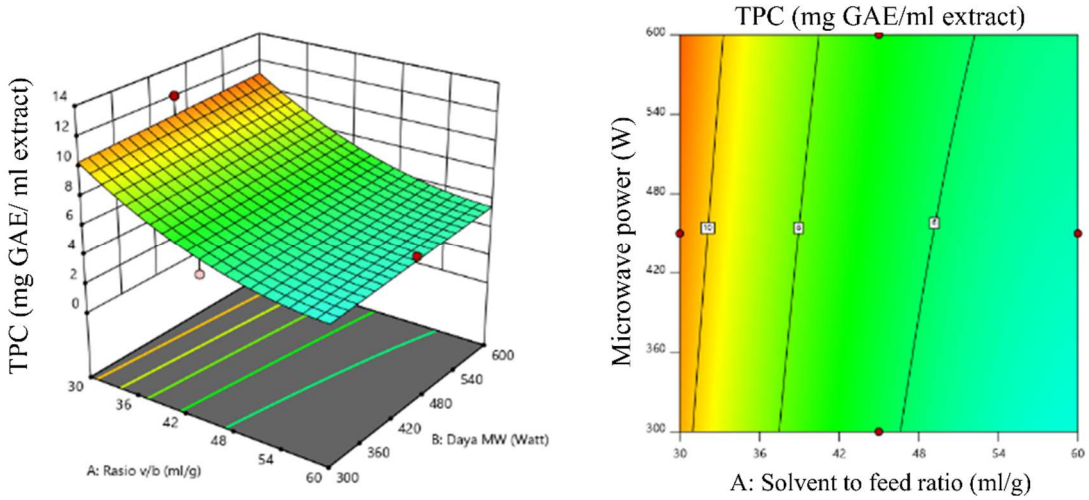

Fig. 4 Response surface of TPC of Torbangun leaves extract

C. RSM analysis of Total Flavonoid Content (TFC)

The RSM analysis of TFC resulted that TPC being also influenced by all factors and represented as a quadratic model (Eq. 3), where Y3 is TFC. This model is similar to the previous (Eq. 1 and 2). Elimination of the insignificant variables is also applied. The relationship between the predicted TFC from the model with actual data is provided in Fig. 5.

$$Y_3 = 1.47 - 0.6096A + 0.24774B + 1.07C - 0.5932AC + 0.6135C^2 \quad (3)$$

The coefficient correlation of the graph is 0.9198, which indicates that the model can represent 91.98% of data. To explain the complex relationship among parameters, the contour and the response surfaces have been provided (**Fig. 6)**. The red area of the contour (maximum TFC) is reached with a minimum solvent to feed ratio of 30 ml/g, maximum microwave power of 600 W, and maximum extraction time of ~5 minutes.

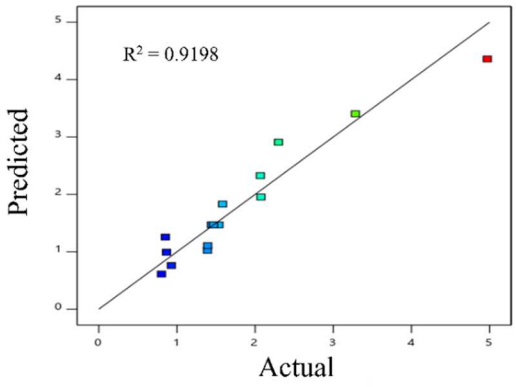

Fig. 5 Plotted graph of model predictions and actual results of TFC

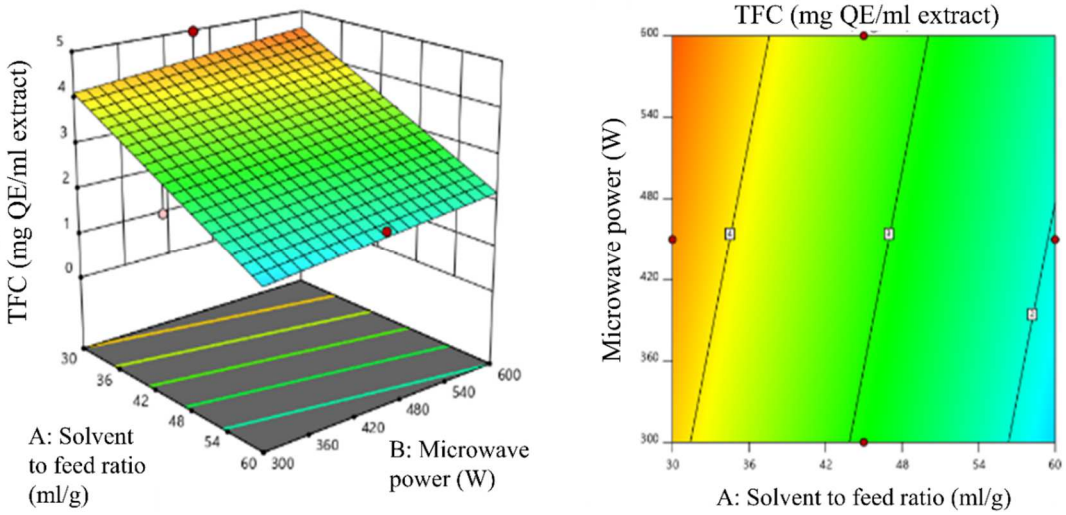

Fig. 6 Response surface of TFC of Torbangun leaves extract

D. Selection Process and Optimization

All responses should reach the maximum condition to obtain an optimum extraction product. However, the optimum operation conditions for each response are different; therefore, an optimization process is necessary. RSM was used to simultaneously proceed with the data to achieve an optimum operating condition. Firstly, the response priority should be selected. In this study, TPC and TFC were arranged as the most priority among responses, while extract volume was placed as the second priority. Then, the software DX 9.0.3 proceeds the information and results in several operations combinations, which produce the optimum extraction responses. The optimum operation condition could be achieved by selecting the highest desirability value from the solution.

TABLE III
SOLUTIONS OF OPTIMAL EXTRACTION PARAMETERS

No	Solvent to Feed Ratio	Microwave Power (W)	Extraction Time (min)	Extract Volume (ml)	TPC (mg GAE/ ml extract)	TFC (mg QE/ ml extract)	Desirability	
1	30.000	300.001	4.644	10.130	9.166	3.618	0.620	Selected
2	30.000	300.002	4.661	9.931	9.220	3.641	0.620	
3	30.000	300.002	4.614	10.483	9.071	3.578	0.620	
4	30.000	301.622	4.642	10.172	9.162	3.617	0.620	
5	30.000	300.003	4.577	10.915	8.955	3.528	0.620	
6	30.000	301.807	4.661	9.944	9.224	3.644	0.619	
7	30.115	300.001	4.620	10.460	9.051	3.577	0.619	
8	30.000	302.959	4.681	9.718	9.290	3.672	0.619	
9	30.000	303.618	4.620	10.437	9.098	3.591	0.619	
10	30.000	300.001	4.749	8.898	9.501	3.759	0.618	

The factor of microwave power (W) was adjusted to the closest exact value of the first solution (300 W) due to the limitation of the microwave equipment. The final solutions from the software are provided in Table 3. Finally, a solvent to feed ratio of 30 ml/g, 300 W microwave power, and 4.64 min extraction time is suggested as optimum operating conditions for extraction. Under those conditions, the extraction process is predicted to produce an extracted volume of 5.86 ml, TPC of 10.34 mg GAE/ ml extract, and TFC of 4.11 mg GAE/ ml extract.

E. Discussion

Based on the optimization result, 30 ml/g of solvent to feed ratio was expected to produce optimum extraction responses, which means more Torbangun leaves could increase the evaluated parameters as the number of extracted compounds is still lower than its solubility in ethanol. On the other hand, a long time with minimum microwave power was also predicted as the optimum operating condition. A high-power of the microwave was reported to increase the extracted temperature simultaneously. In more detail, most of the absorbed power is converted into heat within the material; therefore, the heating rate ($\frac{\Delta T}{At}$) of a particular component is related to the power that is absorbed per unit volume (Pd). The relationship of each factor is described in Eqs 4 and 5.

$$\frac{\Delta T}{At} = \frac{Pd}{\rho C_p} \tag{4}$$

$$Pd = 2\pi f \varepsilon_0 \varepsilon''_{eff} |E|^2 \tag{5}$$

which ρ and C_p in Eq. 4 are the material density and specific heat capacity. While f, ε_0, ε''_{eff}, and $|E|^2$ in Eq. 5 are microwave frequency, the permittivity of free space (8.85 x 10^{-12} F/m), relative dielectric loss factor, and magnitude of the electric field, respectively [30]. The high temperature of the extract could cause the degradation of phenolic compounds, as reported by the previous study [15], hence decreasing the TPC and TFC.

A summarized comparison of the present extraction to other studies is provided in Table 4. Compared to other Torbangun extraction studies, TPC from the suggested optimum condition (34.79 mg GAE/ g dw) was much higher than those were resulted by maceration (TPC = 8.8 ± 0.01 mg GAE/ g dw) or a combination of lyophilization-maceration (TPC = 18.87 ± 16.65 mg GAE/g dw); while TFC (13.73 mg QE/ g dw) was higher than that was extracted by water shaking (6.66 ± 0.004 mg QE/ g dw) and slightly lower than that was extracted by maceration (14.19 ± 0.09 mg QE/ g dw). On the other hand, a combination with maceration seems necessary as the results are still lower than those were extracted by four hours maceration (TPC = 39.08 GAE/ g dw; TFC = 31.37 mg QE/ g dw) and PEF-maceration (TPC = 60.16 mg GAE/ g dw; TFC = 34.94 mg QE/ g dw). In brief, even though the combination with other extraction methods and the observation of other extraction parameters are needed, this study represents the benefits of MAE for phenolic extraction to shorten the extraction time.

Considering the efficiency of the extraction method, MAE has the shortest extraction time compared to the other studies in Table 4. By MAE, 5 minutes extraction time could produce higher TPC and TFC than those were extracted by a conventional method. A shorter extraction time might be a solution for thermal sensitive compounds, such as phenolic compounds, as it could minimize the heat accumulation of the extract. Supported by the other reported studies [31]–[33], MAE could be promoted as a green and efficient extraction technology.

TABLE IV
COMPARISON OF STUDIES IN *TORBANGUN* EXTRACTION

Author	Extraction methods	Operating condition	TPC	TFC
Dewi *et al*) [9]	Solvent: water PEF & maceration with microwave drying methods	MAE drying at 450 W for 4 min); PEF at 2.5 kV/cm 20s & maceration 4h	60.16 mg GAE/ g dw	34.94 mg QE/ g dw
	Solvent: water Maceration with microwave drying method	MAE drying at 450 W for 4 min); Maceration 4h	39.08 mg GAE/g dw	31.37 mg QE/ g dw
Iwansyah [34]	Solvent: Ethanol Maceration	24 hours (repeated)	8.8 ± 0.01 mg GAE/g dw	14.19 ± 0.09 mg QE/g dw
Andarwulan *et al.* (2014) [35]	Lyophilization and conventional extraction with shaking and water	-	18.87 ± 16.65 mg GAE/g dw	6.66 ± 0.004 mg QE/ g dw
This study (suggested by RSM)	**MAE**	**5 min; 450 W; 30 ml/gr**	**9.17 mg GAE/ ml extract = 34.79 mg GAE /g dw** *converted unit)*	**3.62 mg GAE/ ml extract= 13.73 mg GAE/g dw** *(converted unit)*

IV. CONCLUSION

The extraction of phenolic compounds of Torbangun leaves was successfully conducted by Microwave-assisted extraction. The extraction operating condition was optimized using RSM. The optimum extraction parameters were predicted to be obtained by these operating conditions: 30 ml/g of solvent to feed ratio, 300 W of microwave power, and 4.64 minutes of extraction time; while the extract was predicted to have 10.13 ml of extract volume, 9.17 mg GAE/ ml extract of TPC, and 3.62 mg GAE/ ml extract of TFC. In brief, the lower solvent to feed ratio and microwave power was necessary to gain optimum responses. The best extraction result from this study was comparable with other Torbangun extraction studies. Assuredly, MAE was applicable for the extraction of phenolic and flavonoid compounds from Torbangun leaves and could be a favored alternative method due to its high efficiency.

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