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Optimization of extraction condition of *Gynura procumbens* extract enriched with flavonoid and antioxidant compounds using Response Surface Methodology

Devi Permatasari[1*], Anisyah Is Purwati[1], Hismiaty Bahua[2], and Agus Supriyono[1]

[1]RC for Pharmaceutical Ingredients and Traditional Medicine, Research Organization for Health, National Research and Innovation Agency, South Tangerang, 15314, Indonesia
[2]RC for Sustainable Production System and Life Cycle Assessment, Research Organization for Energy and Manufacture, National Research and Innovation Agency, South Tangerang, 15314, Indonesia

Abstract: *Gynura procumbens* is known as one of the herbal medicinal plants found in Indonesia and has been used from time to time. It is claimed to have various efficacy such as anti-hyperglycemic, anti-hypertension, anti-microbial, anti-cancer, and antioxidant. Other studies mention that G. *procumbens* possessed high antioxidant compounds and had been used as a natural-based medicinal supplement. However, further studies on optimizing the extraction process of *Gynura procumbens* in Indonesia have yet to be reported. Therefore, this study aimed to optimize the extraction condition of *G. procumbens* leaves by maceration with three variables: solvent concentration, extraction time, and the ratio of solid-liquid used. Each of those variables contained three different levels. Determination of total flavonoid and antioxidant activity was measured using aluminium chloride colourimetric assay and 2, 2-diphenyl-1-picryl-hydrazil (DPPH) assay, respectively. In the optimization process, Response Surface Methodology (RSM) was used to explore the main effects and interaction between parameters and their correlations with dependent variables. The results were analyzed using the Box-Behnken method using Minitab software 17. This study shows that the most significant effect of the variable for both flavonoid and antioxidant activity was solvent concentration, with a $P<0.05$. The results showed that the extraction process to obtain *G. procumbens* extract with optimal flavonoid content and antioxidant activity (IC_{50}) was predicted at 70% solvent concentration, 1 h maceration time, and a solid-liquid ratio of 1:9.8 w/v, with results of 17.599 mg QE/g extract and 0.211 mg/mL, respectively. This study was expected to complement other studies and can be used as an additional reference for the development of the extraction process on a larger scale.

Keywords: *Gynura procumbens,* Optimization, Antioxidant activity, Flavonoid content, Response Surface Methodology.

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***Corresponding author. E-mail:** devi.permatasari@brin.go.id.

1. INTRODUCTION

Gynura procumbens or "Sambung Nyawa"-as local people said- is one of the herbal medicines widely known for its efficacy as anti-hypertension, anti-hyperglycemia, anti-cancer, anti-microbial, antioxidant, and also anti-inflammation (1). As the American people said, this longevity spinach possesses high antioxidant activity (2), which can be a source of longer-lasting health quality, like the origin of the name. Antioxidant itself is one of the chemical compounds used to prevent oxidative stress in the biological system within the body that has the potential to cause diseases (3). Antioxidant compounds eliminate free radicals through specific mechanisms and can be obtained from endogenous (enzymes within the body) or exogenous sources (food, nutritional supplements, or pharmaceuticals) (4). The previous study mentioned that Sambung Nyawa leaves contains phenolics compounds such as gallic acid, protocatechuic acid, hydroxybenzoic acids (HBA),

vanillic acid, and syringic acid, while other study reported that it has flavonoid compounds like rutin, quercetin, kaempferol, myricetin, and apigenin that can be served as an antioxidant and an anti-photoaging agents (5,6).

This study focused on the optimization process which was conducted by using Response Surface Methodology (RSM), a technique to optimize the response from the experiment as influenced by its variables. This tool is helpful for modelling and analysis using mathematical and statistical approaches (7), selecting and constructing the variables needed to produce reliable results in the response optimizer. RSM has been used in the optimization process to reduce experimental trials. Thus, the study can be less expensive and less time-consuming (8–10). The most common design used in RSM is Box-Behnken Design, which requires three central points in the experiments (James Regun Karmoker). However, the final results generated from one experiment to another might have slight differences because of the variance in setting each variable within a specific range (11). The extraction process is commonly influenced by multiple variables. Therefore, the effects should be evaluated simultaneously to obtain accurate results.

In this study, the extraction process was conducted using a traditional maceration technique and assisted with stirring to increase the effectiveness of the process (12). Maceration was a method to get plant extracts easily and effectively. This process can be easily upgraded on a lab, pilot, or larger scale, like for a manufacturer. Despite the extraction technique, the extract's quality can be affected by many other factors, such as time of extraction, temperature, type and concentration of the solvent, and the solid-liquid ratio between the dry sample and solvent (13).

Based on previous studies, many studies of the antioxidant activity of this plant have been carried out, including the influence of the part of the plant used (14) and the effect of different solvents used in the extraction process (2,15). Another study also showed that this plant could be cultivated and produced in large quantities as a natural and affordable health supplement, especially in tropical countries (16). Hence, this study aims to equip previous studies by finding the maximum operating conditions in the extraction process using Response Surface Methodology to produce an extract with high total flavonoid content (TFC) and antioxidant activity. Besides, this study also aimed to find a contribution between each independent variable with the result within the extraction process.

2. EXPERIMENTAL SECTION

2.1. Chemical and reagents

Quercetin ≥95% (HPLC), 2, 2-diphenyl-1-picryl-hydrazil (DPPH), and Aluminum chloride were purchased from Sigma Chemical Company (St. Louis MO, USA). Potassium acetate was purchased from BDH Chemicals Ltd (Poole, England). HPLC-grade methanol was obtained from Merck, 96% food-grade ethanol was obtained from a local supplier, and all other solvents were analytical or HPLC grade.

2.2. Plant collection

Sambung nyawa or *Gynura procumbens leaves* were harvested from cultivation by BRIN in Sulusuban, Lampung. Fresh leaves of *G. procumbens* were dried using an oven with a temperature of up to 60 °C, then ground into a semi-powder using a milling machine before use.

2.3. Extraction

30 g of powdered leaves *of G. procumbens* were extracted in glassware with a magnetic stirrer using food-grade ethanol with various levels of solvent concentration, time of extraction, and solid-liquid ratio (w/v). Each variable contains 3 levels, as stated in the experiment design in Minitab below. The stirring process was conducted at 500 rpm using an IKA magnetic stirrer, and then the filtrate was filtered using filter paper and concentrated using a Buchi rotary vacuum evaporator.

Table 1: The design of the variable used in the experiment.

Independent Variables	Levels of Variables			Dependent Variables
	-1	0	1	
Ethanol Concentration (X_1, %)	30	50	70	Yield (Y_1, %)
Time for Maceration (X_2, h)	1	2	3	TFC (Y_2, mg QE / g extract)
Solid-Liquid Ratio (X_3, w/v)	1:8	1:10	1:12	IC 50 (Y_3, mg/mL)

The yield extract of *G. procumbens* obtained from the experiment was then calculated using the equation below:

$$Yield(\%) = \frac{Mass\ of\ extract\ (g)}{Mass\ of\ G.\ procumbens\ leaves} x\ 100 \quad (1)$$

2.4. Determination of total flavonoid assay

The total flavonoid content (TFC) of the extracts was determined by the aluminium chloride colourimetric method by Dyah (17) with a slight modification. Quercetin solution in methanol with various concentrations from 15-50 ppm was used as a standard. A standard solution and a viscous extract of *G. procumbens* leaves in methanol with an amount of 0.5 mL were put into the test tube containing 1.5 mL of methanol. Then 0.1 ml of $AlCl_3$ (10%) and 1 M CH_3COOK were added into the test tube, respectively. The solution was then homogenized and incubated for 30 minutes. Then, using a UV–Vis spectrophotometer (Genesys 10 UV, Thermo Electron Corporation, USA), the samples were measured in wavelength 433 nm (Optimum for Quercetin). The absorbance of the standard series of concentrations shows y= 0.0151x – 0.0055 with the value of R^2 = 0.9992. Then, the total flavonoid content from *G. procumbens* extracts was obtained using the formula below:

$$TFC\left(mg\,QE/g\,extract\right)=\frac{C\times V}{M} \quad (2)$$

$$Total\,Flav.\,Cont.\,(\%)=\frac{C\times V\times fp\times 10^{(-3)}}{Wu}\,x\,100$$

With :
C : TFC from the standard equation (mg/mL)
V : Volume extract (mL)
M : Mass extract (g)

2.5. Determination of antioxidant activity

Free radical scavenging activity from *G. procumbens* extract was determined by using 0.5 mM DPPH (2, 2-diphenyl-1-picryl-hydrazil) according to Pant (18), with some modifications. A series of concentrations from each sample extract *G. procumbens* were made to determine IC_{50}. Briefly, 240 µL extract of *G. procumbens* and 60 µL were added to 96-well plates, respectively. Then the samples were homogenized and incubated for 30 minutes in a dark place. Using an Elisa Thermo Multiskan Ascent 354 microplate reader, the absorbance of samples was measured at wavelength 520 nm. The free radical scavenging activity (or inhibition percentage**)** and IC_{50} value were obtained using the formula below:

$$Inh(\%)=\frac{\mathrm{Abs}\,of\,Blank-\mathrm{Abs}\,of\,Sample}{\mathrm{Abs}\,of\,Blank}\,x\,100 \quad (3)$$

$$lC\,50=\frac{50-a}{b} \quad (4)$$

with a = intercept and b = slope from linear regression.

2.6. Statistical Analysis

Data processing and the design of the experiment were conducted using Minitab 17 with response surface methodology (RSM). Box-Behnken was chosen as a design planner, using three variables with three levels in each variable. It was more efficient than the 2^k level factorial (19) because the number of runs can be deducted. The relationship between independent variables and the response will be provided by a second-order polynomial equation. Thus, the total number of runs with two-times replication was 30.

3. RESULTS

3.1. Effect of extraction condition and its correlation in each variable response

The results of experimental data obtained from Box-Behnken are shown in Table 2. The experiment result showed that a more significant yield was obtained at the lowest concentration of ethanol. On the contrary, the higher TFC and IC_{50} values were obtained at the highest concentration of the solvent.

The maximum yield resulting from the experiment was obtained at a configuration of 30% solvent concentration, 3 hours maceration time, and a 1:10 ratio of solid-liquid with a value of 54.78%. Meanwhile, the extract with the highest flavonoid content (TFC), 18.89 mg QE/g extract, can be obtained at a setting of 70% solvent concentration, 2 hours maceration, and a 1:12 sol:liq ratio, whereas the same solvent concentration, 3 hours maceration time, and a 1:10 ratio of sol:liq can produce the lowest IC_{50} values at 0.28 mg/mL. The higher amount of extract and flavonoid content showed a higher value of yield (Y_1) and TFC (Y_2), while the lower value of IC_{50} (Y_3) showed the more prominent free radicals scavenging (antioxidant) activity.

Table 2: Result of each dependent variable; Yield, TFC, and IC_{50} from the experiment and predicted models from RSM.

	Independent Variables			**Dependent Variables**			**Predicted Dependent Variables**		
Run order	**Ethanol Concentration (X_1, %)**	**Time for Maceration (X_2, h)**	**Solid-Liquid ratio (X_3, w/v)**	**Yield (Y_1, %)**	**TFC (Y_2, mg QE / g Extract)**	**IC 50 (Y_3, mg/mL)**	**Yield (Y_1, %)**	**TFC (Y_2, mg QE / g Extract)**	**IC 50 (Y_3, mg/mL)**
1	30	1	10	35.60 ± 6.60	0.32 ± 0.01	1.66 ± 0.38	38.65	0.09	1.79
2	70	1	10	14.43 ± 1.37	17.62 ± 0.23	0.32 ± 0.05	16.57	17.65	0.22
3	30	3	10	54.78 ± 13.60	0.33 ± 0.06	1.89 ± 0.18	52.64	0.29	1.99
4	70	3	10	14.75 ± 1.01	16.16 ± 3.14	0.28 ± 0.07	11.70	16.39	0.14
5	30	2	8	43.44 ± 3.50	0.25 ± 0.01	2.64± 0.32	43.21	0.85	2.40
6	70	2	8	12.86 ± 1.32	15.81 ± 1.57	0.28 ± 0.07	13.54	16.15	0.28
7	30	2	12	49.54 ± 0.95	0.25 ± 0.03	1.82 ± 0.04	48.87	-0.08	1.82
8	70	2	12	15.30 ± 0.75	18.89 ± 2.19	0.29 ± 0.07	15.53	18.28	0.53
9	50	1	8	15.26 ± 0.99	3.29 ± 1.32	0.29 ± 0.08	12.45	2.92	0.39
10	50	3	8	14.11 ± 0.44	2.09 ± 0.14	0.35 ± 0.07	16.49	1.52	0.49
11	50	1	12	18.12 ± 0.64	2.10 ± 0.01	0.40 ± 0.11	15.75	2.66	0.26
12	50	3	12	18.01 ± 1.26	2.61 ± 0.75	0.37 ± 0.12	20.83	2.99	0.28
13	50	2	10	17.77 ± 0.90	2.33 ± 0.30	0.42 ± 0.16	17.19	2.11	0.40
14	50	2	10	16.83 ± 0.75	1.86 ± 0.13	0.42 ± 0.01	17.19	2.11	0.40
15	50	2	10	16.98 ± 1.53	2.13 ± 0.27	0.35 ± 0.03	17.19	2.11	0.40

*(Data expresses as mean ± SD, n = 2)

The interaction between independent and dependent variables was processed with ANOVA. The variable that held a significant effect in the experiment expressed with a P-value <0.05, indicates that the model's prediction was significant at 5% (20). Table 3 below showed that solvent concentration (X_1) had the most significant effect on all dependent variables (Y_1, Y_2, Y_3) with P<0.001. In addition, for the dependent variable yield, extraction time (X_2) and its correlation with solvent concentration (X_1X_2) showed a significant impact in the experiment with P=0.046 and P=0.005 successively. Furthermore, the correlation between solvent concentration and sol:liq ratio (X_1X_3) proved to be significant with P<0.05 for Antioxidant/IC_{50} values (Y_3).

Table 3: Correlation of yield, TFC, and antioxidant activity to each independent variable in the experiment.

Factor	Yield (Y_1, %)		TFC (Y_2, mg QE/g extract)		IC_{50} (Y_3, mg/mL)	
	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Regression	34.16	< 0.001	134.98	< 0.001	45.51	< 0.001
X_1	216.67	< 0.001*	952.21	< 0.001*	283.52	< 0.001*
X_2	4.54	0.046*	0.96	0.339	0.29	0.595
X_3	3.19	0.089	1.21	0.283	2.73	0.114
X_1X_2	9.71	0.005*	0.9	0.353	0.93	0.345
X_1X_3	0.37	0.552	3.96	0.06	8.38	0.009*
X_2X_3	0.03	0.865	1.25	0.277	0.08	0.777
X_1^2	71.31	< 0.001*	253.21	< 0.001*	106.75	< 0.001*
X_2^2	0.15	0.704	0.08	0.787	3.08	0.095
X_3^2	0.02	0.895	0.58	0.457	1.42	0.247
Lack of fit	2.49	0.096**	1.13	0.367**	6.77	0.003***

*Significant for P <0.05; **Significant for lack of fit P > 0.05; ***Non-significant lack of fit P > 0.05

3.2. Fitting the RSM model

Several conditions must be fulfilled to prove the suitability of the experiment with the RSM model, which was the P-value of the model <0.05 (significant) and the P-value for the lack of fit being >0.05 (non-significant), and coefficient determination (R^2) (21). The lack of fit from Table 3 showed that it was non-significant (P>0.05) for Yield (Y_1) and TFC (Y_2); therefore, the RSM model fitted well with the prediction from the experiment (20). Although the condition of lack of fit in IC_{50} was significant (P<0.05) and did not meet the requirement, the model can still be used (21). Normality and residual plot were also considered as additional information to ensure the suitability of the predicted RSM model with the experiment (19). The experiment's results were considered good, showing homogeneous data distribution around the linear line in the normality plot and heterogeneous data distribution in the residual plot. Each dependent variable in this experiment shows that the experimental data were normally distributed (data not shown). Thus, mathematical models from the experimental data were expressed as equations 5-7 below:

$$Y_1 = 17.194 - 15.754X_1 + 2.281X_2 + 1.911X_3 - 4.717X_1X_2 - 0.916X_1X_3 + 0.261X_2X_3 + 13.303X_1^2 - 0.608X_2^2 - 0.210X_3^2 \quad (5)$$

$$Y_2 = 2.108 + 8.415X_1 - 0.267X_2 + 0.301X_3 - 0.367X_1X_2 + 0.768X_1X_3 + 0.431X_2X_3 + 6.388X_1^2 + 0.110X_2^2 + 0.305X_3^2 \quad (6)$$

$$Y_3 = 0.396 - 0.854X_1 + 0.027X_2 - 0.084X_3 - 0.069X_1X_2 + 0.208X_1X_3 - 0.021X_2X_3 + 0.772X_1^2 - 0.131X_2^2 + 0.089X_3^2 \quad (7)$$

Table 4: Differences in coefficient of equation between experimental and predictional study.

Factor	Yield (%)		TFC (mg QE/g Extract)		IC_{50} (mg/mL)	
	Exp. Coef	***Pred. Coef***	***Exp. Coef***	***Pred. Coef***	***Exp. Coef***	***Pred. Coef***
Constant	17.194	85.502	2.108	40.151	0.396	11.466
X_1	-15.754	-3.413	8.415	-1.331	-0.854	-0.281
X_2	2.281	15.199	-0.267	-1.946	0.027	0.828
X_3	1.911	2.889	0.301	-2.764	-0.084	-0.726
X_1X_2	-4.717	-0.236	-0.367	-0.018	-0.069	-0.003
X_1X_3	-0.916	-0.023	0.768	0.019	0.208	0.005
X_2X_3	0.261	0.131	0.431	0.216	-0.021	-0.010
X_1^2	13.303	0.033	6.388	0.016	0.772	0.002
X_2^2	-0.608	-0.608	0.110	0.110	-0.131	-0.131
X_3^2	-0.210	-0.053	0.305	0.076	0.089	0.022
	R^2	**Adjusted R^2**	**R^2**	**Adjusted R^2**	**R^2**	**Adjusted R^2**
	93.89%	91.14%	98.38%	97.65%	95.34%	93.25%

The experiments also resulted in model predictions, as shown in Table 4 above. Compared with the experimental data, the coefficients of the predicted models were drastically different. However, the predicted model was still considered valid because of R^2 and Adj. R^2 was still around 0.9 for all dependent variables, meaning 90% of the total variation can be explained by the models. R^2 was used to evaluate the accuracy between predicted and experimental values (22). The closer value of R^2 to 1 indicates the fitness of the models and the experimental data (23). The difference value between R^2 and Adj. R^2 means the percentage of total variation not explained by the models (24). Values of the dependent variable from the predicted RSM models are shown in Table 4.

3.3. Optimization of extraction condition

Contour plot graphs below visualized the correlation between X_1, X_2, X_3, and Y_1, Y_2, and Y_3 more easily. From Figure 1, the maximum TFC value (dark green area) was obtained in the range of higher solvent concentrations (X_1) and sol:liq ratios (X_3). Meanwhile, extraction time (X_2) did not significantly affect the response. Thus, the maximum TFC value can be obtained using a 70% solvent concentration with a 2-hour extraction time and a 1:12 sol:liq ratio.

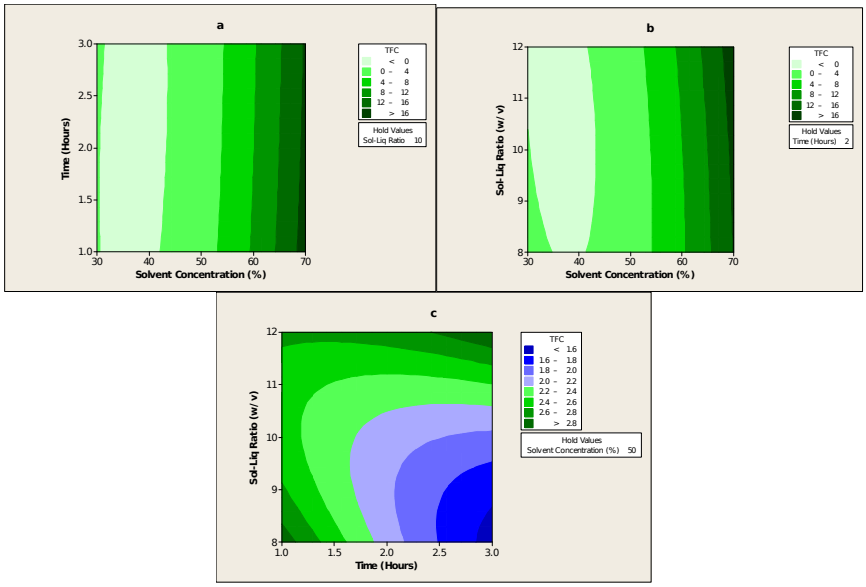

Figure 1: Contour plot on TFC in correlation with; a) time vs solvent concentration, b) sol:liq ratio vs solvent concentration, and c) sol:liq ratio vs time.

In contrast with Figure 2, the highest antioxidant activity/the lowest IC_{50} value obtained was marked with a light green colour. Higher levels of X_1 increase the antioxidant activity proportionally, while X_2 and X_3 have almost no effect. For the sol:liq ratio variable (X_3), the antioxidant activity content did not show any alteration as the ratio increased. The details of the plots can be seen in Figure 2. To sum up, determining exact values in each parameter to produce the lowest IC_{50} values was more challenging in this case than in the TFC, except for the solvent concentration. Therefore, a response optimizer was used to gain more reliable setting parameters by combining calculations from experimental and predicted models.

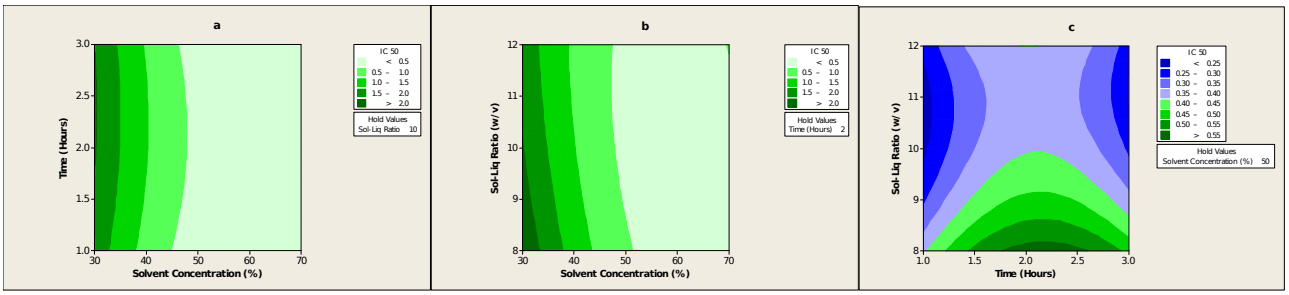

Figure 2: Contour plot on IC_{50} in correlation with; a) time vs solvent concentration, b) sol:liq ratio vs solvent concentration, and c) sol:liq ratio vs time.

Optimization was carried out to produce an extract with maximum TFC and antioxidant activity. Response optimizers were used by adding dependent variables like TFC and IC_{50} at the maximum setting. Response optimizer from Figure 3 showed that to obtain the maximum content of flavonoid and antioxidant activity, the extraction condition should be set at 70% solvent concentration, 1 hour extraction time, and almost a 1:10 ratio in the sol:liq ratio. This condition was likely to have a similar result as predicted since the composite desirability was considered high (>0.9). However, the result will not produce a high-yield extract since it was excluded from the optimizer, and the correlation of yield with the desired parameters was the opposite.

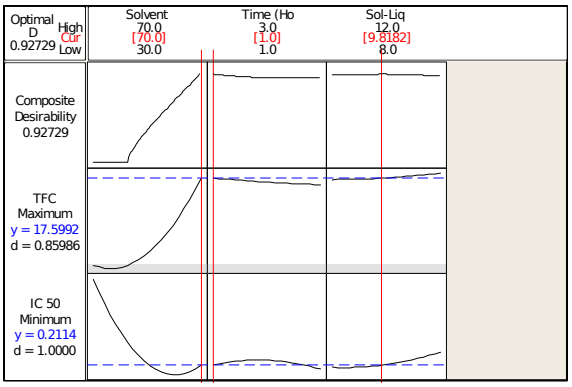

Figure 3: Optimization plot of operation process for extraction of *G. procumbens* for high TFC and IC_{50}.

4. DISCUSSION

The bioactivities of the plant medicines, especially antioxidant activity, were strongly influenced by the solvent concentration, while other variables did not show a significant effect (8). Solvent concentration plays a major role in the amount of flavonoid, antioxidant, and other bioactive compounds (23). The extraction of *G. procumbens* with methanol has more TPC and antioxidant activity than extraction with ethanol or water (25). However, the extract with ethanol still contained the high antioxidant quality of extract (8,15). A solvent with more water composition was likely to draw the polysaccharides inside the sample out, resulting in a higher yield obtained and lower activity. Meanwhile, the extraction time only affected the yield produced and did not affect the antioxidant activity (26). Therefore, this variable did not appear to be significant (24).

Sol:liq variable in this study did not appear to hold any significance in antioxidant and total flavonoid content. Meanwhile, in correlation with solvent concentration, it did contain a significant role in determining antioxidant activity (20,24,27–29) as others because of the maximum amount of solvent that penetrated the sample. A solvent with a higher ethanol composition has a lower dielectric constant that can disrupt the plant matrices easier, as the extraction process involves the mass transfer between solid and liquid. Mass transfer depends on driving forces and resistance within the process. Different concentrations, as a result of the sol:liq ratio, lead to a higher driving force and diffusion rate (27,29).

Long exposures in variables such as sol:liq ratio, extraction temperature, and time resulted in the extract with the highest yield. Nevertheless, as the temperature increased, the antioxidant activity decreased (20). Extraction temperature is likely to have more influence on maximizing the yield produced while affecting the antioxidant activity content within it (22,30). TFC and radical scavenging activity decreased as the temperature rose due to the degradation of bioactive compounds, which are commonly sensitive to heat (23,30).

Many compounds can be classified as having antioxidant activity, including flavonoid compounds. It was known to have potent scavengers of free radicals and was potentially used as medicine for oxidative damage and degenerative diseases such as cancer (31). Previous studies stated that the ethyl acetate fraction of *G. procumbens* extracts exhibits antioxidant activity with IC_{50} values of 0.05 mg/mL (2) and 0.2 mg/mL (5). This ethyl acetate fraction contains some flavonoid compounds such as myricetin, quercetin, rutin, apigenin, and kaempferol that are responsible for antioxidant activity within the fraction. Another experiment showed crude methanol extract contains a TFC value of 10.33 mg QE/g DW and an IC_{50} 0.47 mg/mL, proving that both variables have some correlation (5). However, the result can not be compared with this experiment due to the differences in the solvent used. Medicinal plants were known to have very high antioxidant activity with IC_{50} less than 0.05 mg/mL and high with IC_{50} around 0.05-0.1 mg/mL, while the IC_{50} around 0.1-0.15 mg/mL and 0.15-0.2 mg/mL were mentioned as medium and low antioxidant capacity (32). The value was still higher than IC_{50} obtained in this experiment (0.28 mg/mL). However, judging from previous studies, further purification of this extract allows for an increase in the antioxidant activity of this plant.

5. CONCLUSION

To be concluded, the established RSM models were adequate for determining extraction conditions for certain goals. This experiment shows that for obtaining an extract with both maximum TFC and antioxidant activity, extraction conditions must be held at 70% solvent concentration, 1 hour extraction time, and a 9.8 sol:liq ratio with an estimated value of 17.599 mg QE/g extract and 0.211 mg/ml, respectively. Further, this experiment still needs some validation of the result suggested by the optimizer. Besides, this method can also be used for other traditional medicines. It can be developed as an optimization tool for the purified extract to elevate the effectiveness of the traditional medicines used.

6. CONFLICT OF INTEREST

The authors state no conflict of interest.

7. ACKNOWLEDGMENTS

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8. REFERENCES

1. Tan H, Chan K, Pusparajah P, Lee L, Goh B, Lee L. Gynura procumbens : An Overview of the Biological Activities. Front Pharmacol. 2016;7(52):1–14. Available from: <DOI>

2. Yam MF, Sadikun A, Asmawi MZ, Rosidah. Antioxidant potential of Gynura procumbens. Pharm Biol. 2008 Sep;46(9):616–25. Available from: <DOI>

3. Werdhasari A. Peran Antioksidan Bagi Kesehatan. J Biotek Medisiana Indones. 2014;3(2):59–68. <DOI>

4. Francenia Santos-Sánchez N, Salas-Coronado R, Villanueva-Cañongo C, Hernández-Carlos B. Antioxidant Compounds and Their Antioxidant Mechanism. IntechOpen. 2019;1–28. Available from: <URL>

5. Kaewseejan N, Sutthikhum V, Siriamornpun S. Potential of Gynura procumbens leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. J Funct Foods [Internet]. 2015;12:120–8. Available from: <DOI>

6. Kim J, Lee C, Kyung E, Lee S, Park N, Kim H, et al. Inhibition effect of Gynura procumbens extract on UV-B-induced matrix-metalloproteinase expression in human dermal fibroblasts. J Ethnopharmacol. 2011;137(1):427–33. Available from: <DOI>

7. Montgomery DC. Design and Analysis of Experiments Eighth Edition. Arizona State University. Vol. 2009, Copyright. 2013. 2001 p. ISBN: 978-1-118-14692-7. Available from: <URL>

8. Şahin S, Şamli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason Sonochem. 2013;20(1):595–602.Available from: <DOI>

9. Khuri AI, Mukhopadhyay S. Response surface methodology. Wiley Interdisciplinary Reviews: Computational Statistics. 2010;2(2):128–49. Available from: <DOI>

10. Vuong Q V., Nguyen VT, Thanh DT, Bhuyan DJ, Goldsmith CD, Sadeqzadeh E, et al. Optimization of ultrasound-assisted extraction conditions for euphol from the medicinal plant, Euphorbia tirucalli, using response surface methodology. Ind Crops Prod. 2015;63:197–202. Available from: <DOI>

11. Khuri AI. Response Surface Methodology and Its Applications In Agricultural and Food Sciences. Biometrics Biostat Int J. 2017;5(5):155–63. Available from: <DOI>

12. Hairon H, Sabtu R, Talib NA, Awang MA, Aziz R, Suan CL, et al. Extraction of Gynura procumbens Leaves (Sambung Nyawa) with Different Parameters Using Maceration Process. In: 6th International Conference on Biotechnology for the Wellness Industry (ICBWI). Malaka, Malaysia: Institute of Bioproduct Development, Faculty of

Chemical and Energy Engineering, Universiti Teknologi Malaysia; 2016. p. 131–3.

13. Rosidah I, Bahua H, Mufidah R, Pongtuluran OB. Pengaruh Kondisi Proses Ekstraksi Batang Brotowali (Tinospora crispa (L) Hook.f & Thomson) Terhadap Aktivitas Hambatan Enzim Alfa Glukosidase. Vol. 25, Media Penelitian dan Pengembangan Kesehatan. 2015;25(4):203-210. Available from: <URL>

14. Krishnan V, Ahmad S, Mahmood M. Antioxidant Potential in Different Parts and Callus of Gynura procumbens and Different Parts of Gynura bicolor. Biomed Res Int. 2015;2015. Available from: <DOI>

15. S.S. Maw, M.M. Mon, Z.K. Oo. Study on Antioxidant and Antitumor Activities of Some Herbal Extracts. World Acad Sci Eng Technol. 2011;51:450–5. Available from: <URL>

16. Tristantini D, Setiawan H, Santoso LL. Feasibility assessment of an encapsulated longevity spinach (Gynura procumbens l.) extract plant in Indonesia. Appl Sci. 2021 May 1;11(9). Available from: <DOI>

17. Dyah NA, Endang K, Fahrauk F. Penetapan Kadar Flavonoid Metode AlCl3 Pada Ektsrak MEtanol Kulit Buah KAKAO (Theobroma cacao L.). Kartika J Ilm Farm. 2014;2(2):45–9. Available from: <DOI>

18. Pant G, Simaria C, Varsi RAH, Bhan P, Sibi G. In vitro Anti-Cholesterol and Antioxidant Activity of Methanolic Extracts from Flax Seeds (Linum usitatissimum L .). Res J Med Plant. 2015;9(6):300–6. Available from: <DOI>

19. Faulina R, Andari S, Anggraeni D. Response surface methodology (RSM) dan aplikasinya. Magister Stat Its. 2011;152–75. Available from: <URL>

20. Chuyen H V., Roach PD, Golding JB, Parks SE, Nguyen MH. Optimisation of extraction conditions for recovering carotenoids and antioxidant capacity from Gac peel using response surface methodology. Int J Food Sci Technol. 2017;52(4):972–80. Available from: <DOI>

21. Karmoker JR, Hasan I, Ahmed N, Saifuddin M, Reza MS. Development and Optimization of Acyclovir Loaded Mucoadhesive Microspheres by Box – Behnken Design. Dhaka Univ J Pharm Sci. 2019;18(1):1–12. Available from: <DOI>

22. Aydar AY, Bagdatlioglu N, Köseoglu O. Effect of ultrasound on olive oil extraction and optimization of ultrasound-assisted extraction of extra virgin olive oil by response surface methodology (RSM). Grasas y Aceites. 2017;68(2). Available from: <DOI>

23. Shirzad H, Niknam V, Taheri M, Ebrahimzadeh H. Ultrasound-assisted extraction process of phenolic antioxidants from Olive leaves: a nutraceutical study using RSM and LC–ESI–DAD–MS. J Food Sci Technol. 2017;54(8):2361–71. Available from: <DOI>

24. Elksibi I, Haddar W, Ben Ticha M, Gharbi R, Mhenni MF. Development and optimisation of a non conventional extraction process of natural dye from olive solid waste using response surface methodology (RSM). Food Chem. 2014;161:345–52. Available from: <DOI>

25. A. Akowuah G, Ahmad M, M. Fei Y. Effects of Gynura procumbens Leaf Extracts on Plasma Lipid Peroxidation and Total Antioxidant Status in CCl4-Treated Rats. Nat Prod Journale. 2013;2(4):247–51. Available from: <DOI>

26. Tian Y, Xu Z, Zheng B, Martin Lo Y. Optimization of ultrasonic-assisted extraction of pomegranate (Punica granatum L.) seed oil. Ultrason Sonochem. 2013;20(1):202–8. Available from: <DOI>

27. Yan F, Fan K, He J, Gao M. Ultrasonic-Assisted Solvent Extraction of Carotenoids From Rapeseed Meal: Optimization Using Response Surface Methodology. J Food Qual. 2015;38(6):377–86. Available from: <DOI>

28. Raza A, Li F, Xu X, Tang J. Optimization of ultrasonic-assisted extraction of antioxidant polysaccharides from the stem of Trapa quadrispinosa using response surface methodology. Int J Biol Macromol. 2017;94:335–44. Available from: <DOI>

29. Samaram S, Mirhosseini H, Tan CP, Ghazali HM, Bordbar S, Serjouie A. Optimisation of ultrasound-assisted extraction of oil from papaya seed by response surface methodology: Oil recovery, radical scavenging antioxidant activity, and oxidation stability. Food Chem. 2015;172:7–17. Available from: <DOI>

30. Akowuah GA, Mariam A, Chin JH. The effect of extraction temperature on total phenols and antioxidant activity of Gynura procumbens leaf. Pharmacogn Mag. 2009;4(17):81–5. Available from: <URL>

31. Zainol MK, Abd-Hamid A, Yusof S, Muse R. Antioxidative activity and total phenolic compounds of leaf, root and petiole of four accessions of Centella asiatica (L.) Urban. Food Chem. 2003;81(4):575–81. Available from: <DOI>

32. Tristantini D, Ismawati A, Tegar Pradana B, Gabriel Jonathan J. Pengujian Aktivitas Antioksidan Menggunakan Metode DPPH pada Daun Tanjung (Mimusops elengi L). Semin Nas Tek Kim Kejuangan. Prosiding Seminar Nasional Teknik Kimia “Kejuangan” 17 Mar 2016;ISSN 1693-4393:1–7. Available from: <URL>