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Optimization of Total Phenolic Content from *Gynura Procumbens* Leaves Under Oven and Microwave Drying

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ABSTRACT

The optimization of the process variable; temperature, power, and drying time, under oven and microwave drying were studied for total phenolic content of *Gynura procumbens* leaves. The optimization conducted using response surface methodology central composite design of experiment. The optimum drying condition was obtained at 65.61°C at 10 hours with 201.78 mgGAE/g of TPC yield for oven drying while, for microwave drying was 436.19 Watt at 8.37 minutes with 12.24 mgGAE/g of TPC yield. The response surface methodology used in this study was able to predict the optimum drying condition for total phenolic content via linear and interaction mathematical model with higher adjusted coefficient determination $\left(\overline{R^2}\right)$, for both oven and microwave drying, 0.9383 and 0.7785, respectively. Hence, the models gave good prediction of the total phenolic content obtained from *Gynura procumbens* leaves under the experimental conditions used in this study. In addition, these outcomes are also expected to contribute to the production of shelf stable *Gynura procumbens* leaves which can be used for pharmacological purposes.

Keywords: Gynura procumbens, drying, oven, microwave, optimization

INTRODUCTION

Gynura procumbens, a member of the Asteraceae family, is commonly known as longevity spinach and grows widely in India, Bangladesh, and Southeast Asia, particularly in Indonesia, Malaysia, and Thailand (Tahsin et al., 2022). In the Malay language, *G. procumbens* leaves are referred to as 'Sambung Nyawa', which translates to "prolongation of life." On the other hand, the Chinese refer it as Bai Bing Cao, which means "100 diseases" (Rahman & Asad, 2013). This is because it has a history of being applied topically as well as internally in the practice of traditional medicine, to treat a wide variety of illnesses and diseases (Krishnan et al., 2015). The leaves are believed to alleviate kidney discomfort, reduce the risk of heart disease, lowering cholesterol and blood sugar levels (Kanzil Mowla Mou & Pristesh Ranjan Dash, 2016; Khuzaidatul Azidah et al., 2019). This is because the *G. procumbens* is high in antioxidants that are used for the treatment of various ailments and has a wide range of claims in pharmacological properties such as anti-inflammatory, anticancer, anti-diabetic, anti-herpes simplex virus, anti-ulcerogenic, vasorelaxant and toxicity (Mou & Dash, 2016; Rosidah et al., 2008; Tan et al., 2016; Tan et al., 2020). According to Roleira et al. (2018), the extracts of *G. procumbens* contain the major groups of phenolic plant metabolites, with flavonoids being one of the most abundant. Other than that, a study by Tristantini et al. (2021) stated that the main phytochemical compound that can be found in the *G. procumbens* leaves is p-hydroxycinnamic acid which belongs to the phenolic compound.

However, the usage of *G. procumbens* leaves in pharmacological industry can be difficult if the *G. procumbens* leaves experience interrupted supply due to a shorter lifespan. Fresh leaves of *G. procumbens* have a high moisture content and are chill-sensitive and classified as highly perishable (Ghasemi et al., 2017). To ensure that the leaves could be supplied throughout the year for their medicinal uses and facilitates their storage period prior extraction process, a pre-treatment to prolong the life span of fresh *G. procumbens* leaves should be carried out. The application of drying as a pre-treatment process could indirectly reduce processing costs thus lowering energy consumption of the process.

The drying process involves the complete removal of water from the product, thus reducing microbial deterioration as well as physical and chemical changes that happen during storage (Yan Lim et al., 2013). Traditional drying methods usually rely on sun-drying in the open air as products are being subjected to external factors such as weather, consumption by other animals or insects, and contamination from contact with animals such as rodents (Pirasteh et al., 2014). Although sun-drying is easily accessible and cheap, the process takes a long time and this process is not suitable to dry herbs as it will result in low product quality degradation (Thamkaew et al., 2021). Therefore, modern drying techniques could minimize these influences by drying in conditions that can be more controllable. An advance drying technology with shorter drying time requiring lower energy consumption and minimum processing cost is highly needed, hence, oven and microwave drying are opted in. Oven drying has been practiced for a long time and considered to be one of the most common drying methods that is also cost-effective. While microwave drying provide rapid drying, reducing drying time by almost four times (Elhussein & Şahin, 2018).

The objective of this study is to optimize the drying condition of *G. Procumbens* leaves using response surface methodology (RSM) through oven and microwave drying. Optimization by using response surface methodology (RSM) can simultaneously determine many responses. This optimization method will be able to analyse the impact of several factors and their interactions on one or more response variables. Therefore, optimization by using response surface methodology (RSM) can assist in establishing the best conditions for drying of *G. procumbens* leaves. The number of experiments needed to determine a particular number of factors and their levels is reduced by using the response surface methodology, which is frequently employed in experiment design. The term optimization has been widely used in analytical chemistry to describe the process of determining the best conditions under which to apply a procedure to produce the best possible response (Bezerra et al., 2008). Since phenolics are highly sensitive to heat, an appropriate condition by considering the temperature or power and drying time under oven and microwave drying are required to maintain the high amount of phenolic compounds. Thus, this study can help to extend the shelf life of *G. procumbens* leaves by determining the best conditions for preserving the leaves, which can be used for pharmacological purposes in the future.

MATERIALS AND METHODS

Sample Preparations

The fresh leaves of *G. procumbens* were collected from Alor Setar, Kedah, Malaysia. The dirt on the leaves was cleaned thoroughly and the leaves were separated from its stem. Then, only healthy, and fully expanded leaves without any defect were selected and stored in a zip-lock polyethylene bag at a temperature of 2 – 4 °C in the chiller (Berjaya 2D/DCF-SM) to maintain its freshness until the drying process as proposed by Elhussein & Şahin, (2018). Before drying, the leaves were separated from its midrib and petiole and then ground by using a food chopper for 10 seconds until uniform particles were achieved. The initial moisture level of the leaves was determined by using a moisture analyser (A&D MX-50) and the moisture ranged from 82% to 85%.

Drying Process

A forced air convection oven (Memmert UNB 300) and a microwave (Fujison FS-MO300) were used to dry the *G. procumbens* leaves. Each drying runs for oven were performed at different temperatures (40°C – 70°C) and drying time (5 – 15 hours) while each drying runs for microwave were performed at different powers (100-500 W) and drying time (3– 10 minutes) according to the central composite design experimental design given in Table 1. Then, 20 g of samples were placed on a steel petri dish with the same size and thickness. The samples were duplicated for both oven and microwave drying. After that, the moisture loss of leaves was determined by weight loss using an analytical balance (A&D Galaxy HR-100AZ) with a precision of 0.01g for 20 minutes and 30 seconds time interval for oven and microwave respectively.

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Optimization Process

Optimization tool used in this study is the response surface methodology (Minitab 20). For oven drying, two independent factors which were temperature (N_1) and drying time (N_2) in a range of 40 °C – 70 °C and 5 – 15 hours respectively were selected based on a preliminary screening process. Next, for microwave drying, two independent factors which were power (N_3) and drying time (N_4) in a range of 100 – 500 W and 3 – 10 minutes respectively were selected. The central composite design was composed of 13 treatments including 2^2 factorial points, four axial point (α=1.41421) and five repetition at the centre points. The response that was measured was the total phenolic content. The design of experiments and dependent variable values were as shown in Table 1. The effects of the temperature or power and the drying time were measured via multiple regressions as described in Equation 1 for oven drying and Equation 2 for microwave drying.

$$Y = \beta_0 + \beta_1 N_1 + \beta_2 N_2 + \beta_3 N_1^2 + \beta_4 N_2^2 + \beta_5 N_1 N_2 \quad \textbf{(1)}$$

$$Y = \beta_0 + \beta_1 N_3 + \beta_2 N_4 + \beta_3 N_3^2 + \beta_4 N_4^2 + \beta_5 N_3 N_4 \quad \textbf{(2)}$$

$\beta_0, \beta_1, \beta_2$ = linear terms

$\beta_0, \beta_1, \beta_2, \beta_5$ = linear and interaction terms

$\beta_0, \beta_1, \beta_2, \beta_3, \beta_4$ = linear and square terms

$\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5$ = full quadratic terms

Table 1 Design of experiments and dependent values

Run	Oven Optimization				Microwave Optimization			
	Factor		**Response**		**Factor**		**Response**	
	Temperature (°C)	**Time (hour)**	**TPC Yield**	**Predicted**	**Power (Watt)**	**Time (min)**	**TPC Yield**	**Predicted**
1	47.50	7.50	172.56	172.812	200.00	4.75	0.192	0.169
2	62.50	7.50	173.89	177.595	400.00	4.75	2.894	5.383
3	47.50	12.50	162.06	160.448	200.00	8.25	5.553	5.551
4	62.50	12.50	177.75	179.590	400.00	8.25	9.729	12.239
5	44.39	10.00	183.47	184.865	158.58	6.50	1.604	2.137
6	65.61	10.00	205.27	201.782	441.42	6.50	13.572	10.552
7	55.00	6.46	157.93	155.565	300.00	4.03	2.229	1.000
8	55.00	13.54	147.96	148.232	300.00	8.97	10.912	9.654
9	55.00	10.00	173.23	174.638	300.00	6.50	9.410	9.117
10	55.00	10.00	180.27	174.638	300.00	6.50	8.878	9.117
11	55.00	10.00	173.23	174.638	300.00	6.50	8.080	9.117
12	55.00	10.00	173.23	174.638	300.00	6.50	9.410	9.117
13	55.00	10.00	173.23	174.638	300.00	6.50	9.809	9.117

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Extraction

Based on method proposed by Roslan et al., (2020). 5 g of dried *G. procumbens* powdered sample that has been crushed using mortar was weighed and transferred into a conical flask. Then, 50 ml of 80% ethanol was added, and the mixture was placed on a 30°C incubator shaker (Innova 4080) for 5 hours with 400 rpm. Next, the mixture was centrifuged at 3500 rpm for 15 minutes before filtered using ashless filter paper into bottle and kept at - 20 °C.

Standard Solution

Referring the method developed by Shirazi et al., (2014), 1 g of gallic acid was dissolved in 100 ml of methanol to get 1% solution of Gallic acid (10 mg/ml). A standard gallic acid curve was constructed by preparing the dilutions of (1.0, 2.0, 3.0, 4.0, 5.0 mg/ml).

Total Phenolic Content Determination

The total phenolic content of the *G. procumbens* leaves for both oven and microwave drying were determined by the folin-ciocalteu method based on the method by Shirazi et al., (2014) with few modifications. 100 µl of each of the gallic acid dilutions were mixed with 500 µl of water and then with 100 µl of folin-ciocalteu reagent. Then, it was left to stand for 6 minutes at room temperature in the dark. Next, 1 ml of 7% sodium carbonate and 500 µl of distilled water was added to the reaction mixture. The absorbance was recorded after 90 minutes at 765 nm. The total phenolic content of the leaves was calculated as gallic acid equivalents (mgGAE/g). All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Optimum Drying Condition

Figure 1 displays the outcomes of the response optimizer for oven drying under ideal conditions for target, maximum and minimum. Each ideal condition has two points which was lower and upper that needs to be determined according to the highest response value from two different groups which were the experimental (TPC) and predicted data (FITS). Table 2 represents the comparison of values of target for oven drying for different goals and values for predicted responses, as well as the feasibility of the goals. As shown in Table 2, the target goal was found to be feasible. This can be seen from Figure 1 as the optimum point of the target goal was right above the shaded region which indicating the feasibility of the goal. Thus, the target was ready to be validated. Meanwhile, both maximum and minimum goals were removed from being selected as the optimum condition since the optimal point were at the shaded region, making them not feasible. Therefore, the optimum oven drying condition for the *Gynura procumbens* leaves was found to be at the target goal which was 65.61 °C drying temperature and 10 hours drying time with 201.78 mgGAE/g TPC yield.

Table 2 Comparison values of target for different goals and values for oven drying predicted response

Goal		Lower	Target	Upper	Optimum Condition		Predicted Response	Feasible
					Temp (°C)	Time (hour)		
Target	TPC	147.96	201.78	205.27	65.61	10.00	201.78	Feasible
	FITS	148.23	205.26	225.79				
Max	TPC	147.96	205.27	205.27	65.61	10.25	201.92	Not Feasible
	FITS	148.23	201.78	201.78				
Min	TPC	147.96	147.96	205.27	50.61	13.54	144.96	Not Feasible
	FITS	148.23	148.23	201.78				

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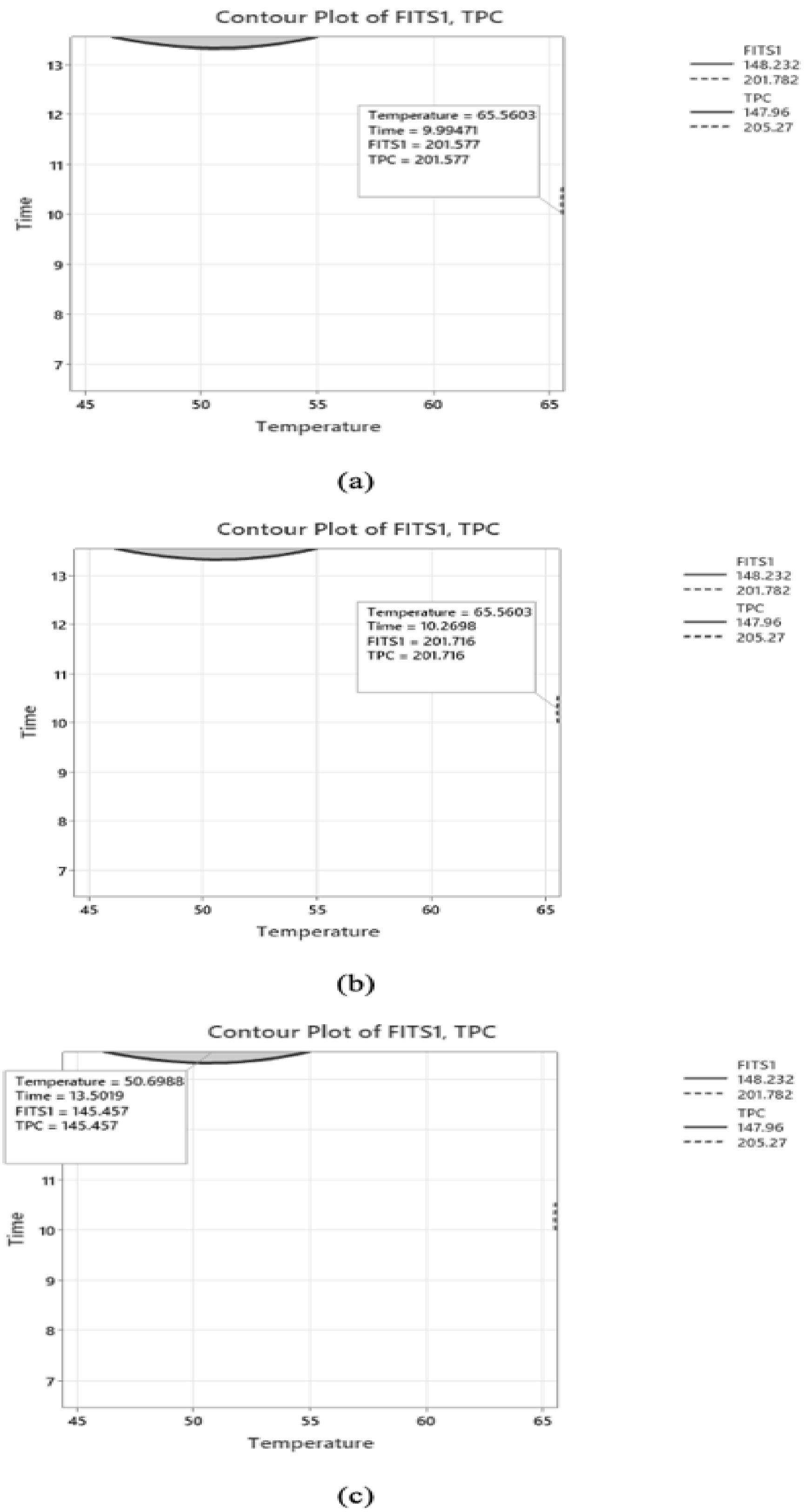

Figure 1 Overlaid contour plot for oven optimization process of Gynura procumbens leaves (a) Target (b) Maximum (c) Minimum

Table 3 referring to the optimum conditions of microwave drying for the three goals, namely, target, maximum and minimum. The target goal was considered as feasible. As shown in Figure 2, the optimal point of the target goal was at the unshaded region while maximum and minimum were at the shaded region. Target goal was validated further while maximum and minimum goal were removed from the selection of optimum condition. Therefore, the optimum microwave drying condition for *Gynura procumbens* leaves was revealed at 436.19 Watt and 8.37 minutes drying time with 12.24 mgGAE/g TPC.

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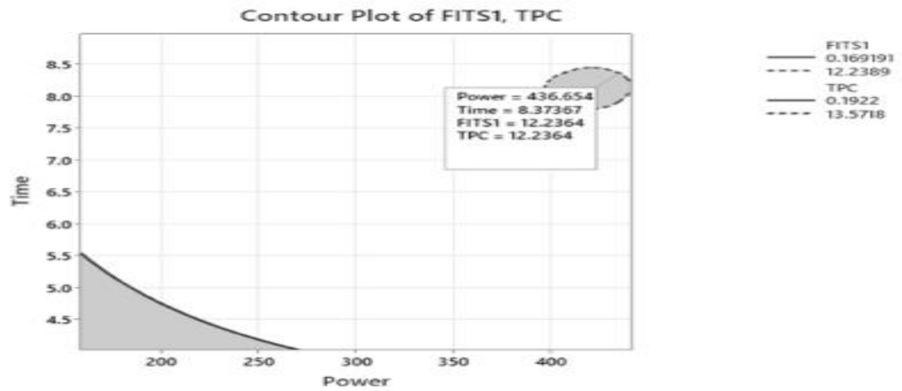
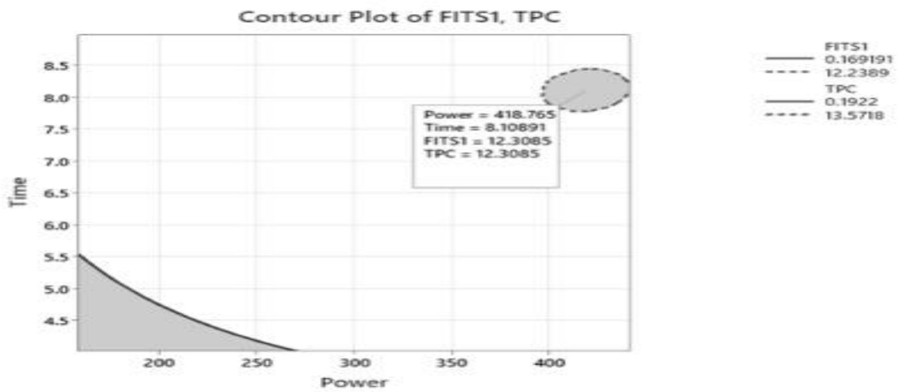
Table 3 Comparison values of target for different goals and values for microwave drying predicted response.

Goal		Lower	Target	Upper	Optimum Condition		Predicted Response	Feasible
					Power	Time		
Target	TPC	0.19	13.57	13.57	436.19`	8.37	12.24	Feasible
	FITS	0.17	12.24	12.24				
Max	TPC	0.19	13.57	13.57	418.57	8.12	12.31	Not Feasible
	FITS	0.17	12.24	12.24				
Min	TPC	0.19	0.19	13.57	158.58	4.03	-5.24	Not Feasible
	FITS	0.17	0.17	12.24				



(a)



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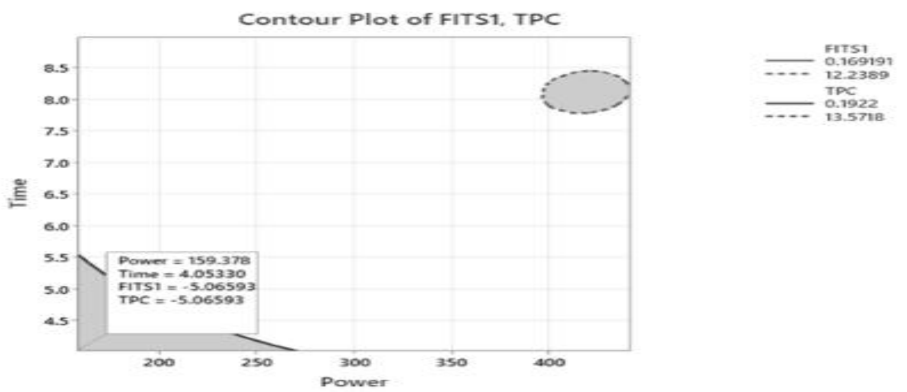

(c)

Figure 2 Overlaid contour plot for microwave optimization process of Gynura procumbens leaves (a) Target (b) Maximum (c) Minimum

Model Fitting

The experimental data were analysed using multiple linear regression, which resulted in the production of second-order polynomial models for predicting total phenolic content models as shown in Table 4. Response surface and contour plots were constructed for each of the fitted models as a function of two independent variables to visualize the combined effects of two factors on response (Johnson, 2022). This was done while maintaining the variable's central value throughout the process. The analysis of these plots allowed for discussion of the effects of the variables on the response's models. The percentage of the total variation in the response that can be accounted for by the model was indicated by R^2 error sum of squares to the total sum of squares. R^2 is the metric that is used to evaluate how well the model fits the data (Alexander et al., 2015). The greater the R^2 number, the more closely the model matches the data as it represents smaller differences between the observed data and fitted values (Frost, 2018). The R^2 value for oven and microwave drying reached 96.40% and 87.08% respectively. This indicated that 96.40% and 87.08% of the regression model is a good fit for the responses data for both oven and microwave. A similar trend can also be found in a study of dried moringa leaves by Alim et al. (2023), which highlighted that for a good fit model, the R^2 value should be more than 80% to indicate that the models were highly compatible. However, since R^2 increases with the number of terms, making the higher value of R^2 to be obtained easily by simply adding the number of terms that are tested in one relationship, therefore, use of R^2 as a coefficient of determination does not fully demonstrate the perfect relationship between factors and response. As a result, adjusted determination coefficient (adj R^2) is utilised in assessing the correlation between factors and responses to enhance precision and reduce errors in research as the coefficient determination provided was based on a correlation between additional terms upon existing terms. Hence, it is more suitable to utilise (adj R^2) as an indicator in selecting the optimal design to develop the best model to depict the correlation between factors and responses. Thus, based on the adj R^2, the best model to describe the relationship between temperature or power and drying time for oven and microwave drying on total phenolic yield via linear and interaction model with given adjusted R^2 were 93.83% and 77.85% respectively. Next, the equation for both oven and microwave drying are clearly illustrated in Table 4 where it gives a higher number of significant terms (6 terms) as equal to full quadratic model and has significant value for the regression with reading P value < 0.05. Therefore, the best model which represents the relationship between temperature and drying time for oven and power and drying time for microwave are clearly represented by the equations in Table 4.

Table 4 Model analysis for oven and microwave drying.

Dryer	Equation	R^2	R^2 (adj)
Oven	$Y = 567.0 - 19.39N_1 + 24.82N_2 + 0.1661N_1^2 - 1.819N_2^2 + 0.1915N_1N_2$	96.40%	93.83%
Microwave	$Y = -45.7 + 0.0993N_3 + 9.16N_4 - 0.000139N_3^2 + 0.619N_4^2 + 0.00211N_3N_4$	87.08%	77.85%

Effect of Variables on Total Phenolic Content Yield

Table 4 demonstrated the equation for oven and microwave drying obtained from mathematical model. For oven drying, the equation clearly showed that temperature (N_1) gave a relatively larger impact compared to drying time (N_2) because temperature (24.82) has a higher coefficient than the drying time (-19.39). It indicated that total phenolic content raised with increase of temperature but with decreasing of drying time. Meanwhile, for microwave drying, the power (N_4) gave a relatively stronger effect than the drying time (N_3), represented by a higher coefficient for the power (9.16) in comparison to the drying time (0.0993), indicating that total phenolic content raised with increasing microwave power but with the decreasing drying time.

3-D response surface plots have been proposed and recognised as extremely helpful and explicit for interpreting the interaction behaviour of system variables (Omorogie et al., 2017). A 3-D surface is represented graphically using a contour plot, which plots contours—constant z slices—on a 2-D format. For connecting the (x, y)

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coordinates where the z value occurs, Z lines were drawn. It represents the regression equation that shows how two factors behave while their values are fixed. Figure 3 (a) indicate the surface plot for oven drying. Oven drying reveals that the goal target was at the saddle point where the point is an inflexion point between a relative maximum and relative minimum (Bezerra et al., 2008). Other than that, as indicated in Figure 4 (a) is the surface plot for microwave drying has a concave shape. Both figures showed that there is an interaction between temperature or power with time. In general, both factors which are temperature and time for oven, and power and time for microwave have a positive effect on total phenolic yield.

Figure 3 (b) displays the 3-D response contour plot of the goal target for oven drying where saddle was at a low point between two areas of higher ground, resulting to an hourglass shape. As shown in Figure 4 (b), microwave drying has a circular distribution in the contour plot, moving from lower to higher total phenolic yield, the circles were growing in parallel with the increase in drying time and power. Both figures portray the positive effects of the variables on the total phenolic yield.

Accuracy, repeatable, reproducible, and robust upon predicted response total phenolic yield was determined by at five repetitions of proposed optimum condition as shown in Table 5. Validation was carried out at the proposed optimum drying point for both oven and microwave. Then, the results showed that no significant differences were detected between the repeated and predicted TPC yield by given P value > 0.05 (one - sample T-test, P value 0.281 and 0.103) as depicted in Table 6. The optimize models have sufficient control in process conditions with little variation which creates an ideal situation for model verification and accuracy. Thus, the proposed optimum drying point obtained in this study for both oven and microwave were accurate, reliable, and repeatable**.**

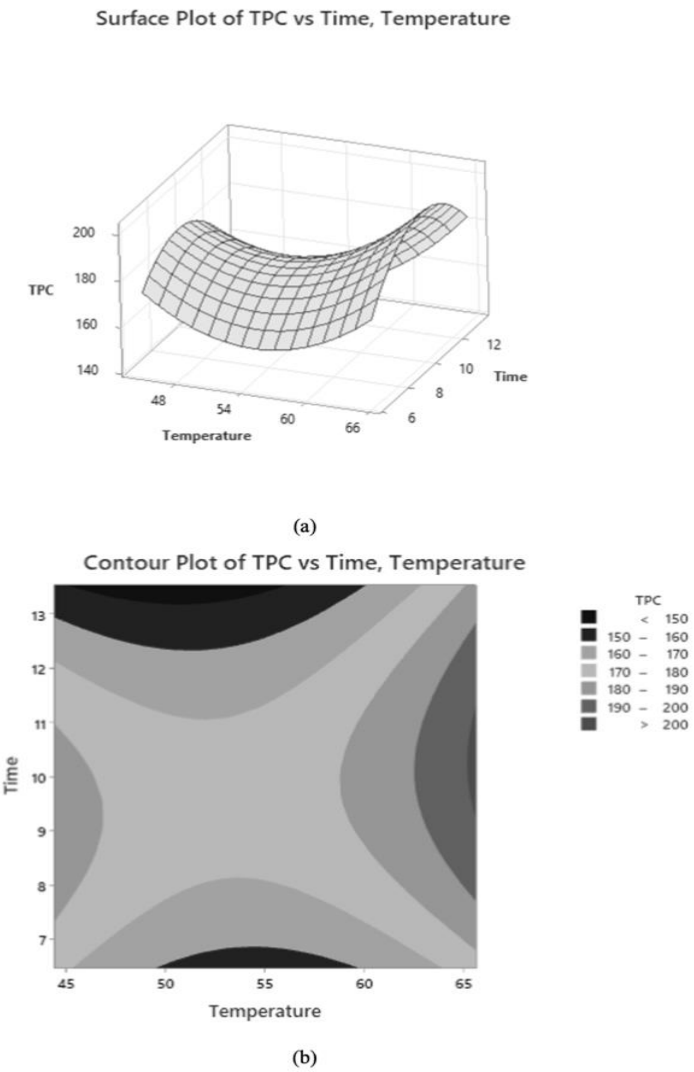

Figure 3 Contour and surface plot for target phenolic content in oven optimization process (a) contour plot (b) surface plot

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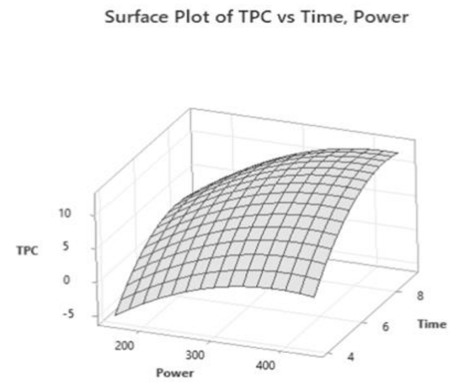

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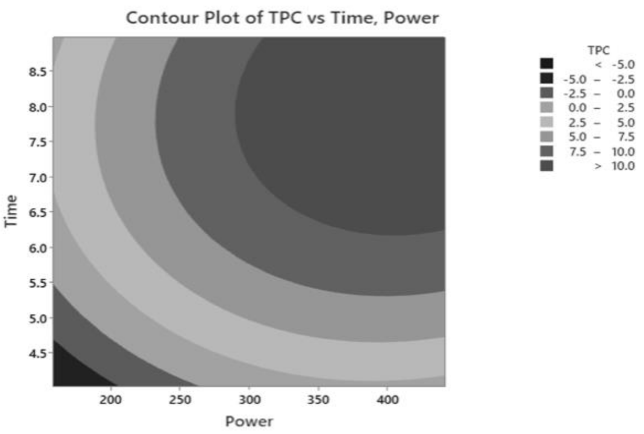

(b)

Figure 4 Contour and surface plot for target phenolic content in microwave optimization process (a) contour plot (b) surface plot

Table 5 Optimum point for oven and microwave optimization

Dryer	**Process Variables**		**TPC Yield (mgGAE/g)**
	Temperature (°C) /Power (W)	**Drying Time (hour/minute)**	
Oven	65.61 °C	10.00 hours	201.78
Microwave	436.19 W	8.37 minutes	12.24

Table 6 Validation for oven and microwave drying

Dryer	**P-Value**	**P-value > 0.05**	**Result**
Oven	0.281	0.281 > 0.05	No significant difference
Microwave	0.103	0.103 > 0.05	No significant difference

CONCLUSION

In conclusion, the optimum drying condition for oven is at 65.61 °C and 10 hours drying time by generated model predicted a 201.78 mgGAE/g TPC yield. Meanwhile, for microwave was found to be at 436.19 W and 8.37 minutes drying time by generated model predicted a 12.24 mgGAE/g TPC yield. From this study, it shows that although microwave drying provides a shorter drying time, oven drying of *G. procumbens* leaves can provide a higher TPC yield. The linear and interaction mathematical model for both oven and microwave gave good prediction of TPC yield obtained from *G. procumbens* leaves under experimental conditions used in this study where the adjusted coefficient determination $(\overline{R^2})$, are 0.9383 and 0.7851, respectively. Also, this study managed to identify that the variables, temperature or power and drying time, made a significant difference to the total phenolic content yield of the leaves. Therefore, the correlation between temperature and time for oven drying and power and time for microwave drying, can help to provide predictions of the impact of process variable on the TPC yield of *G. procumbens* leaves. In addition, the outcomes from this study are expected to contribute the production of a shelf stable *G. procumbens* leaves which can be used for pharmacological purposes.

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