Modeling and optimization of pilot-scale subcritical water extraction on *Zingiber zerumbet* by central composite design

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Abstract. *Zingiber zerumbet* (*Z. zerumbet*) or locally called '*lempoyang*' is one of the ginger species extensively cultivated and utilized in the Southeast Asia region. In order to extract valuable ingredients from *Z. zerumbet*, a green and non-toxic extraction process is implemented namely subcritical water extraction (SWE). Modeling and optimization of SWE of *Z. zerumbet* are performed using the central composite design (CCD) by 20 runs with 6 repetitions at the center point. The independent variables investigated in this research are temperature (130-170°C), time of extraction (20-40 minutes) and solid to solvent ratio (20-40 ml/g) that focused to identify the optimized process parameters for zerumbone concentration, antioxidant activity, and yield of extracts. All independent variables researched were analyzed to be significant as the p-value for zerumbone concentration, antioxidant activity and yield are 0.0001, which are less than 0.05 for a model to be significant. The optimum process parameters for all responses of *Z. zerumbet* extract are at 170°C, 20 minutes and 20 ml/g. This optimum condition was validated and the correlation between predicted and experimental values was within 95% which indicated the range of variables selected was valid. Thus, the outcome from this research may be beneficial on subcritical water extraction of ginger species especially *Z. zerumbet.*

Keywords: *Zingiber zerumbet*, subcritical water extraction, central composite design, zerumbone, antioxidant activity

1.0 Introduction

Z. zerumbet is one of the largely grown and used ginger species in the region of Southeast Asia [1]. It is known as shampoo ginger or locally called '*lempoyang*' in Malaysia. *Z. zerumbet* has been used extensively as food and traditional medicine for centuries. Many researchers have investigated *Z. zerumbet* with reports showing abundant benefits and possessing the potential to fight disease. A number of studies have identified that the major constituent of *Z. zerumbet* is zerumbone as mentioned in a review by *Singh et.al.* [2,3]. The other research on *Z. zerumbet* also proved that zerumbone is the major bioactive compound extracted as zerumbone compositions made up 79.79% of the extracts [4].

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In order to get important benefits of *Z. zerumbet*, an extraction process is vital. It is important to establish a green, non-toxic and safe extraction process to ensure that the products do not harm users. The common techniques used to extract bioactive compounds are conventional liquid-liquid and solid-liquid extraction, and more advanced techniques are subcritical extraction of water, microwave-assisted extraction and ultrasonic extraction [5,6]. However, standard extraction processes depend on alcohol solvents such as ethanol and methanol to extract bioactive compounds compared to advanced extraction processes with minimal use of solvents, which were the primary concern to maximize extracted quality [7].

One of the modern methods of extraction is subcritical water extraction (SWE). It was created to decrease the use of organic solvents and enhance the extraction process quality [8]. Subcritical water extraction or superheated water extraction employs a great technique to keep the water in a liquid state. In this technique, the design procedure for temperature is set between 100°C and 374°C. Meanwhile, the pressure in the subcritical water extraction system is high and adequately introduced to guarantee that it retains its liquid state. By utilizing subcritical water extraction, time of extraction could be greatly minimized to less than an hour compared to traditional extraction methods which are can take up until eight hours for Soxhlet extraction and several days for maceration [9,10].

The Surface Response Methodology (RSM) is used to evaluate the reaction of several factors in order to determine the optimum factors that contribute to the maximum or minimum reaction values [11,12]. Face central composite design (FCCD) in RSM is suggested by Olivera *et. al.* (2018) for the optimization of liquid-solid extraction process [13]. The aim of this study is therefore to use FCCD in RSM to explore the impacts of major parameters such as temperature, extraction time, and solvent to solid ratio in reaction to zerumbone concentration, antioxidant activity, and yield in pilot-scale subcritical water extraction of *Z. zerumbet.*

2.0 Methodology

2.1 Chemicals and reagents

HPLC grade chemicals, methanol, and acetonitrile were purchased from Friendemann Schmidt Pty Ltd, Washington, US. The 2,2-Diphenyl-1-picryl hydrazil (DPPH) was procured from Sigma-Aldrich, US, and ethanol from J-Kollin, UK.

2.2 Plant materials and sample preparation

Z. zerumbet rhizome was acquired from a farm at Kuala Krau, Pahang, Malaysia. The samples were washed to remove soils. The cleaned samples were sliced to 1 mm thick using an industrial slicer and dried for 3 days in the oven at 50°C until the final moisture content was below 10%. Moisture content in dried *Z. zerumbet* was analyzed using OHAUS Moisture Analyzer (MB25), USA. Then, in order to get smaller size, the samples were ground and sieved.

2.3 Subcritical water extraction

Pilot-scale subcritical water extraction with 5.0 L capacity has been used in this research which comprises both 5.0 L extraction and collection vessels. The required volume of dried *Z. zerumbet* was placed in the extractor. Operating variables such as temperature and time were manipulated during the experiments while the operating pressure was fixed at 2.0 MPa.

In the pilot-scale subcritical water extraction, the desired amount of dried *Z. zerumbet* was placed in a sample holder made from stainless steel with 1.0 mm diameter mesh. Afterward, the sample holder was placed in the extraction vessel and distilled water was filled into the vessel, followed by a fitted cover. Nitrogen gas was then loaded into the high pressure vessels from the nitrogen tank until the operating pressure was reached. Before maintaining the pressure on the extraction vessel, the nitrogen gas was purged for one minute to release oxygenated oxygen available in the solution. The extraction began once the desired temperature was reached. The speed of the stirrer was maintained at 100 rpm throughout the experiment. The extracts were streamed into the cooling vessel after it reached the desired extraction time, then the extract was collected for analysis.

2.4 Experimental design

An experiment was performed to investigate the impact of three factors (variables) namely temperature, time of extraction and solid to solvent ratio on subcritical water extraction of *Z. zerumbet*. Three responses were evaluated from the experiments, namely zerumbone concentration, antioxidant activity and yield of the extracts.

Response surface methodology (RSM) was selected to identify the combination of three factors on the three responses that optimize the extraction process. Central composite design (CCD) was employed to perform this experiment and twenty runs were required to disguise all possible combinations of factor levels. The independent variables used in the CCD and the respective levels are shown in Table 1.

Table 1 Factors and levels of independent variables in CCD

Independent variables	Levels			References
	-1	0	1	
X_1 - Temperature (ºC)	130	150	170	[9,14,15]
X_2 - Time (min)	20	30	40	
X_3 – Solvent to solid ratio (ml/g)	20	30	40	

Table 2 CCD for three factors in the experiment

Run	Temperature (°C)	Time (minutes)	Solid to solvent ratio (ml/g)	Zerumbone concentration (mg/g)	Antioxidant activity (%)	Yield (%)
1	150	30	30	10.28	36.52	15.05
2	130	40	40	8.03	32.66	14.62
3	150	40	30	8.71	35.26	17.95
4	150	20	30	9.66	34.01	16.03
5	130	20	20	10.20	37.32	16.14
6	150	30	30	9.28	33.27	16.66
7	170	40	40	12.80	39.46	19.10
8	150	30	30	10.12	33.09	15.82
9	150	30	30	10.19	35.20	16.50
10	150	30	40	9.48	32.41	15.98
11	170	30	30	15.47	47.27	18.66
12	130	40	20	8.69	32.05	18.91
13	150	30	20	15.23	44.91	18.62
14	130	20	40	7.64	28.34	14.51
15	130	30	30	7.83	31.46	15.06
16	150	30	30	10.63	33.73	15.12
17	170	40	20	18.27	50.28	21.38
18	170	20	40	15.49	41.48	17.94
19	170	20	20	19.86	64.09	20.26
20	150	30	30	10.72	35.88	16.94

2.5 Validation of optimized solution

The optimum process parameters for subcritical water extraction of *Z. zerumbet* (temperature, time of extraction and solid to solvent ratio) were derived from the CCD in Design Expert 7.1.6. In order to confirm the validity of the model, experiments were conducted for the predicted optimum condition to compare with the predicted values.

2.6 High-performance Liquid chromatography

The concentration of bioactive compounds extracted was tested using High-Performance Liquid Chromatography, HPLC (Waters 600-MS, USA) with Photodiode Array Detector (PDA) (Water, USA) and Lichrocart 250-4, 6 Purospher Star RP-8E (5 Mym) column (Merck, Germany). The method was modified from [16]. The mobile phase utilized in this research is 100% methanol (solvent A) and 100% acetonitrile (solvent B). The separation was run in isocratic elution with 35% (solvent A) and 65% (solvent B). The isocratic elution was monitored by PDA detector at a wavelength of 254nm and flow rate of 1 ml/min.

Standard dilutions of zerumbone were prepared in six different concentrations which were 5, 10, 20, 50, 100 and 500 ppm. The standard solution was diluted with methanol. The calibration curve, as in Figure 1, was established by an absorbance plotted against concentration in order to obtain an equation of a straight line.

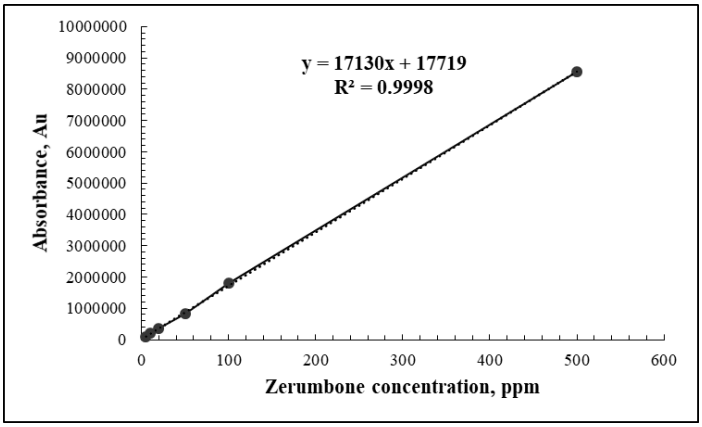

Figure 1 Zerumbone absorbance versus zerumbone concentration

2.7 Uv-Vis Spectrometer

Antioxidant activity of the *Z. zerumbet* extracts was analyzed based on the radical scavenging activity against stable DPPH (1,1-diphenyl-2-picrylhydrazyl hydrate, Sigma- Aldrich, Germany). The preparation of 0.2mM ethanolic DPPH solution was prepared by dissolving DPPH in ethanol which was kept in an amber glass bottle with a screw cap. The absorbance of ethanolic DPPH as a control solution was taken at 517 nm using a spectrophotometer (UV-1800, Shidmazu, Japan) equipped with a quartz cell (optical path length, 1 cm).

The liquid *Z. zerumbet* extracts were prepared by diluting in ethanol at a ratio of 1:4. These solutions were shaken vigorously for 1 minute using a vortex (Heidolph, Germany) and incubated in a dark environment at room temperature. After 1 hour, the absorbance of these solutions was measured at the same wavelength of the control solution.

3.0 Results and Discussion

The variables selected were analyzed by Design Expert 7.1.6 and CCD. These were used in this study to identify the functional relationship between temperature, time of extraction and solid to solvent ratio in the subcritical water extraction, with zerumbone being the targeted bioactive compound to be extracted. Table 2 display the experimental data on zerumbone concentration, antioxidant activity and yield of *Z. zerumbet* extract respectively. What is striking about the figures in this table is that both of the highest zerumbone concentration and antioxidant activity came at a temperature of 170°C and 20 minutes of elapsed time. The higher values of R^2 and adjusted R^2 as stated in Table 3 confirming the response surface quadratic model chosen.

Table 3 Values of R^2 and adjusted R for each response

Response	R^2	Adjusted R^2
Y_1	0.9791	0.9602
Y_2	0.9787	0.9595
Y_3	0.9300	0.8670

*Y_1 – Zerumbone concentration (mg/g); Y_2 – Antioxidant activity (%); Y_3 – Yield (%).

3.1 Influence of extraction variables on zerumbone concentration

Table 4 displays the analysis of variance (ANOVA) for zerumbone concentration and it was found that the model was significant as the P-values was <0.0001. For a model to be significant, the P-value must be less than 0.05. The p-value for each factor was found to be significant, however, the interaction between AB (temperature and time) and BC (time and solid to solvent ratio) were found to be insignificant. Equation 1 shows the model equation for zerumbone concentration in coded factors.

Table 4 Analysis of variance for response surface quadratic equation for zerumbone concentration and regression coefficients of the final reduced models

Source	Coefficient estimate	Mean square	F value	p-value
Model	10.23	26.07	52.00	<0.0001
A	3.95	156.13	311.43	<0.0001
B	-0.63	4.03	8.04	0.0177
C	-1.88	35.35	70.52	<0.0001
AB	-0.40	1.26	2.50	0.1446
AC	-0.83	5.49	10.94	0.0079
BC	0.099	0.079	0.16	0.6994
A^2	1.39	5.30	10.57	0.0087
B^2	-1.08	3.18	6.34	0.0305
C^2	2.09	12.04	24.02	0.0006
Residual	-	0.50	-	-
Lack of Fit	-	0.74	2.83	0.1394
Pure Error	-	0.26	-	-

$$Concentration\ (Y_1) = 10.23 + 3.95 * A - 0.63 * B - 1.88 * C - 0.40 * A * B - 0.83 * A * C + 0.099 * B * C + 1.39 * A^2 - 1.08 * B^2 + 2.09 * C^2 \quad (1)$$

3.2 Influence of extraction variables on antioxidant activity

The ANOVA for antioxidant activity in *Z. zerumbet* extract showed in Table 5. The model for antioxidant activity was found to be significant, as the variables for temperature and solid to solvent ratio were found to be very significant with a p-value of <0.0001. The interaction of B^2 was found to be insignificant with the p-value of 0.3580, meanwhile, the other interactions were found to be significant. Equation 2 shows the model equation for antioxidant activity in coded factors.

Table 5 Analysis of variance for response surface quadratic equation for antioxidant activity and regression coefficients of the final reduced models

Source	Coefficient estimate	Mean square	F value	p-value
Model	35.01	142.87	51.04	<0.0001
A	8.08	652.21	233.00	<0.0001
B	-1.55	24.13	8.62	0.0149
C	-5.43	294.73	105.29	<0.0001
AB	-1.86	27.71	9.90	0.0104

AC	-3.13	78.51	28.05	0.0003
BC	2.67	57.16	20.42	0.0011
A^2	3.76	38.91	13.90	0.0039
B^2	-0.97	2.60	0.93	0.3580
C^2	3.06	25.70	9.18	0.0127
Residual	-	2.80	-	-
Lack of Fit	-	3.50	1.67	0.2933
Pure Error	-	2.10	-	-

$$\textit{Antioxidant activity } (Y_2) = 35.01 + 8.08 * A - 1.55 * B - 5.43 * C - 1.86 * A * B - 3.13 * A * C + 2.67 * B * C + 3.76 * A^2 - 0.97 * B^2 + 3.06 * C^2 \quad (2)$$

3.3 Influence of extraction variables on yield

From the ANOVA listed in Table 6, it is apparent from this table that very few significant variables resulted in the yield of extraction. Only the variables were significant as the p-value for temperature, time of extraction and solid to solvent ratio were <0.0001, 0.0104 and 0.0002 respectively. All interactions were found to be insignificant. Even though all interaction were found to be insignificant, the yield in this investigation was higher compared to those other previous studies [9]. Equation 3 shows the model equation for the yield of the extracts in coded factors.

Table 6 Analysis of variance for response surface quadratic equation for yield and regression coefficients of the final reduced models

Source	Coefficient estimate	Mean square	F value	p-value
Model	16.20	7.48	14.76	0.0001
A	1.81	32.75	64.62	<0.0001
B	0.71	5.02	9.91	0.0104
C	-1.31	17.28	34.09	0.0002
AB	-0.076	0.046	0.091	0.7689
AC	0.16	0.22	0.42	0.5293
BC	-0.33	0.85	1.68	0.2243
A^2	0.39	0.41	0.81	0.3901
B^2	0.52	0.74	1.45	0.2556
C^2	0.82	1.87	3.69	0.0836
Residual	-	0.51	-	-
Lack of Fit	-	0.36	0.54	0.7408
Pure Error	-	0.66	-	-

$$\textit{Yield } (Y_3) = 16.20 + 1.81 * A + 0.71 * B - 1.31 * C - 0.076 * A * B + 0.16 * A * C - 0.33 * B * C + 0.39 * A^2 + 0.52 * B^2 + 0.82 * C^2 \quad (3)$$

3.4 Analysis of the effects of temperature, time of extraction and solvent to solid ratio on the responses.

The findings of this research are consistent with those of prior research, since the temperature was discovered to be the most important variable in subcritical water extraction [17,18]. Temperature plays a crucial part in subcritical water extraction, as properties of water depend on it. Subcritical water condition is unique because the dielectric constant of water is close to organic solvents such as methanol and ethanol which has been widely used in conventional methods in the extraction process. As the temperature of the water is higher, the magnitude of water is three times higher than in ambient water, resulting from a higher concentration of hydrogen and hydroxyl ions. Thus, it can recover or dissolve both polar and non-polar substances from agricultural products [19]. As can be seen in Table 2 and illustrated in a 3D surface graph in Figure 2(a), at a higher temperature of 170°C (20 minutes), the concentration of zerumbone is the highest. At 170°C (40 minutes), the concentration of zerumbone

turned out to be the second-highest because the decreased on the concentration could be attributed to the carbon formation on the extraction vessel resulting from the experiments. This finding proved a statement made by Plaza and Turner (2015), about the further increase of temperature causing a Maillard reaction, caramelization, and hydrolysis [20]. This condition will lead to erroneous results as at 170°C (40 minutes), the zerumbone concentration might be affected by the carbon formation on the surface of extraction vessels and it may be degenerated by the long exposure of sample in the solvent at high temperature. The antioxidant activity and yield of the extracts also increased as the temperature increased as clearly illustrated in Figure 2(b) and 2(c) respectively.

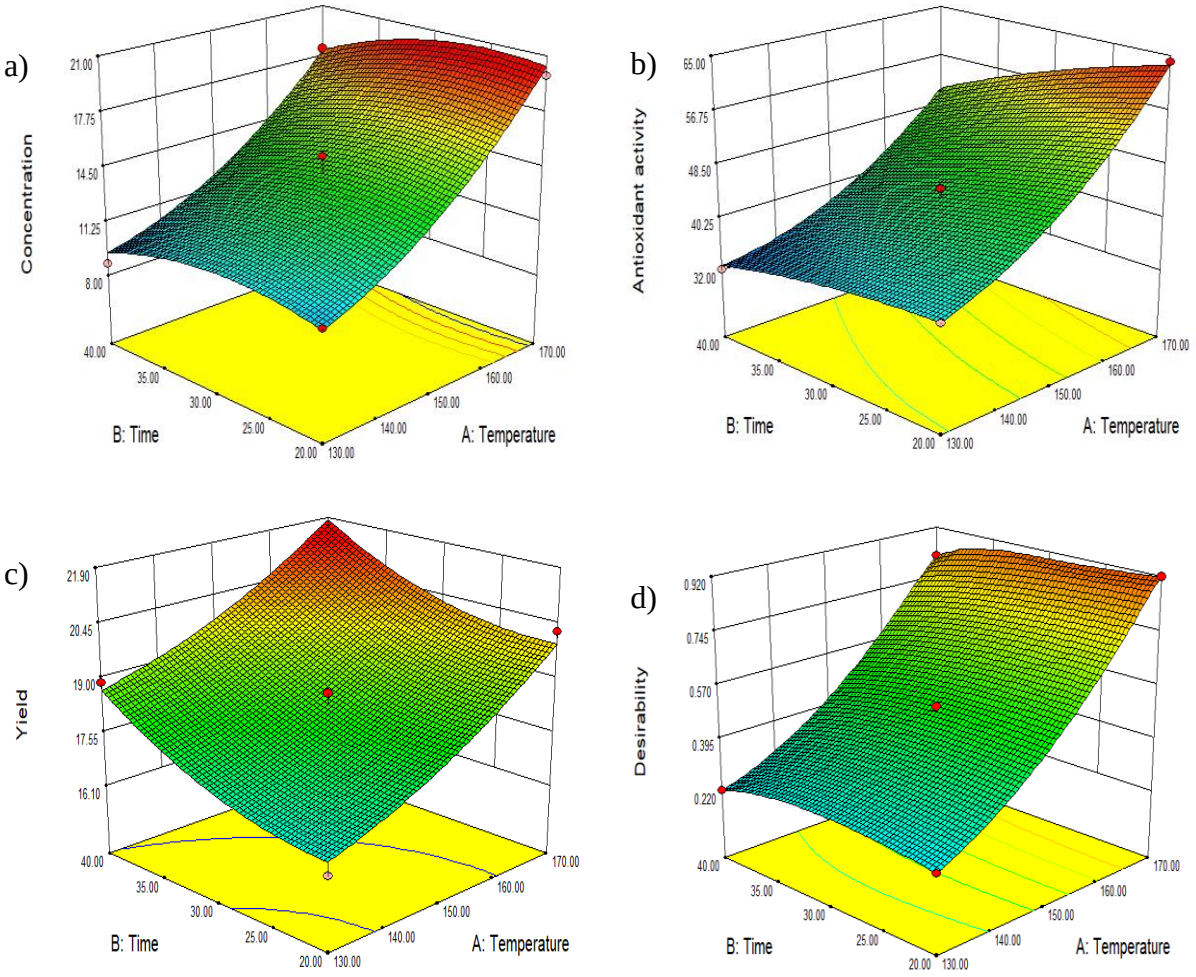

Figure 2 Interaction between temperature and time in the 3D surface graph on a) zerumbone concentration, b) antioxidant activity, c) yield and d) desirability.

Solvent to solid ratio is one of the important variables after the temperature in the process of obtaining the responses in this study. Previous research findings into solid to solvent ratio have been stated that large solvent to solid ratio will increase the extraction efficiency, however, further increased the ratio will cause the extracts to be diluted rather than concentrated [21]. It can be seen in Table 2, as a solvent to solid ratio increased to 40 ml/g, the concentration, antioxidant activity and yield of the extract decreased. However, the responses reached its maximum values by decreasing the solvent to solid ratio to 20 ml/g. Comparison of the findings with those of other studies confirms that by implementing ratio solvent to solid of 20ml/g in subcritical water extraction of *Z. zerumbet*, the higher zerumbone concentration is achieved [9].

Time of extraction is found to be the less significant factor in this research based on the data analyzed by Design Expert 7.1.6 compared to temperature and solid to solvent ratio. The optimum time needed for both concentration and antioxidant activity is 20 minutes. In this study, 20 minutes is

needed to maximize the exposure of the reactive site in order to obtain a compelling extraction process. However, inadequate or shorter extraction time will lead to a less effective extraction process; meanwhile, longer extraction time will lead to degradation of the bioactive compounds to be extracted [22]. From a review by Herrero et. al. (2006), it was stated that by utilizing subcritical water extraction, the time of extraction is shorter compared to other traditional methods [23]. Thus, time is one of the significant factors, as proved by research conducted by Mottahedin et al (2016), who found that the time needed for extraction to be completed is very short [24]. Compared to zerumbone concentration and antioxidant activity, the yield of the extract yielded the highest at time of 40 minutes (170°C).

3.5 Validation of optimized solution in subcritical water extraction of Zingiber zerumbet

In subcritical water extraction of *Z. zerumbet*, the suggested solution from the results analyzed by CCD in Design Expert 7.1.6 is at temperature, time of extraction and solvent to solid ratio of 170°C, 20 minutes and 20 ml/g respectively with the desirability of 0.920. The interaction between temperature and time in the 3D surface graph of desirability was illustrated in Figure 2(d).

Table 7 Validation at optimum condition

Responses	Optimized data (predicted)	Experimental data[a]
Zerumbone concentration (mg/g)	20.42	20.82 ± 0.42
Antioxidant activity (%)	63.58	63.26 ± 0.79
Yield (%)	19.93	20.70 ± 0.67

[a]Mean ± standard deviation of triplicate experimental data.

An experiment was conducted in triplicate and the results were shown in Table 7. The predicted and experimental values suggested a good correlation since the experimental values were within 95% of the predicted values [25].

4.0 Conclusion

Pilot-scale subcritical water extraction of *Z. zerumbet* showed the optimum process parameters for zerumbone concentration and antioxidant activity are at a temperature of 170ºC, solvent to solid ratio of 20 ml/g and time duration of 20 minutes, thus proving that all parameters are highly significant process parameters in subcritical water extraction of *Z. zerumbet*. The validation of the optimum condition showed a good correlation between optimized and experimental data, thus confirming the quadratic model was valid within the range of variables selected.

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Acknowledgments
This work was supported by a PRGS (4L676) grant, Ministry of Education (MOE). The authors fully acknowledge SHIZEN *i-Kohza*, Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia and AM Zaideen Ventures Sdn. Bhd., a spin-off company of UTM (HALEA Skin Care).