Ultrasound-assisted Extraction of Oil from *Calophyllum inophyllum* Seeds: Statistical Optimisation using Box-Behnken Design

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Abstract: *Ultrasound-assisted extraction (UAE) of oil from Calophyllum inophyllum Linn seeds was studied and the effects of four factors (extraction time, ultrasound power, extraction temperature, and liquid to solid (L/S) ratio) on the oil yield were optimised by using a statistical tool. Specifically, the optimisation was carried out by employing a Box-Behnken statistical experimental design. The experimental data were fitted to a quadratic model using multiple regression analysis giving high determination coefficient value (R^2) of 0.984. The predicted oil yield was optimum (56.2%) when the extraction were conducted for 21 min, 210 W ultrasound power, 42°C extraction temperature and 21 ml/g L/S ratio. Based on the model summary statistics, the experimental values agreed closely with the predicted values, indicating an excellent fit of the model used. The results indicated that Response Surface Methodology (RSM) was effective for optimising the UAE conditions of oil from C. inophyllum seeds.*

Keywords: *Calophyllum inophyllum*, ultrasound, seed oil, Box-Behnken design, optimisation

1. INTRODUCTION

Calophyllum inophyllum Linn is an oilseed ornamental evergreen tree, commonly known as Penaga Laut in Malaysia. It belongs to the Clusiaceae family having average height of 8–20 m with a broad spreading crown of irregular branches. *C. inophyllum* are also known as Alexandrian laurel, Tamanu, Bintangor, Nyamplung and Kamani.[1–3] A number of medicinal and therapeutic properties have been described to various parts of *Calophyllum* multipurpose tree, including the treatment of rheumatism, varicose veins, hemorrhoids and chronic ulcers.[4]

The seeds of *C. inophyllum* have a very high oil content and most of them are unsaturated oleic and linoleic acid.[5] The oil does not only possess anti-inflammatory, antimicrobial and antiaging properties, but it can be used to treat diabetic sores, anal fissures, sunburn, dry skin, blisters and sore throat.[4,6] The pain relieving properties of *C. inophyllum* oil has been used traditionally to relieve neuralgin, rheumatism and sciatica.[6]

Traditional techniques of extraction, such as heating, boiling or refluxing, used for the solvent extraction of natural products are associated with longer extraction times and lower yields, use of large amount of organic solvents, and poor extraction efficiency.[7] Thus, developing an optimised novel extraction technology is necessary in pharmaceutical, food and cosmetic industries.

Recently, ultrasound-assisted extraction (UAE) has been developed as a novel technique to extract oil from plants. UAE is being used widely in extraction of oil from various types of seeds including pomegranate, papaya, tobacco, grape, hemp and black seed.[8–13] This technique has been considered as a desirable method of extraction offering many advantages. Such advantages include less extraction time, low extraction temperature, and high extraction efficiency.[14] In addition, it is inexpensive, environment friendly and simple to operate.[15,16] The mechanism of ultrasonic enhancement is mainly attributed to behaviour of cavitation bubbles upon propagation of acoustic waves. The collapsing of these bubbles can produce chemical, physical and mechanical effects which result in disruption of material matrix, facilitating release of extractable compounds and enhancing mass transfer of solvent into the sample thus increasing the release of target compounds from matrix into the solvent.[17]

Response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of several factors, and obtaining optimum condition of factors for desirable responses. RSM provides the relationship between one or more measured dependent responses and a number of input factors.[18] The optimisation process involves studying the response of the statistically designed combinations, estimating the coefficients by fitting it in a mathematical model that fits best the experimental conditions, predicting the response of the fitted model, and checking the adequacy of the model.[19]

Box-Behnken design (BBD) is one of the most common RSM tools, which has been widely used by researchers for optimisation of experimental trials. It is more efficient to conduct experiments using a BBD over traditional methods because it simplifies the complexity of the experimental trials needed to evaluate multiple variables and their interactions.[20] BBD is not only capable in determining the accurate optimum values of experimental parameters but also provides the

possibility to evaluate the interaction between variables with a reduced number of experiments.[21] BBD does not contain combinations for which all factors are simultaneous at their highest or lowest levels. It is also useful in avoiding experiments performed under extreme conditions, for which unsatisfactory results are often obtained.[22] Its other advantages including the following: less number of experiments involved; suitability for multiple variables, which can reveal possible interactions between variable; relativity search between multiple variables; and finding the most suitable correlation and forecast response.[18]

Even though UAE technique offers many advantages, the feasibility of using UAE for the extraction of *C. inophyllum* seed oil has not yet been explored in the literature. Furthermore, to our best knowledge, reports on optimisation of extraction conditions on *C. inophyllum* oil using RSM are very limited. Hence, the objectives of our study is to investigate and optimise the effect of UAE process variables such as extraction time, ultrasonic power, extraction temperature and liquid to solid (L/S) ratio on the yield of *C. inophyllum* oil using Box-Behnken response surface design. The optimised controlled conditions determined in this study should offer important reference values for any subsequent studies.

2.   EXPERIMENTAL

2.1   Material

C. inophyllum fruits were collected from Taman Kerian, Parit Buntar, Perak, Malaysia. The species was identified by Dr. Rahmad Zakaria (USM Herbarium 11565) from the School of Biological Sciences, Universiti Sains Malaysia. Prior to extraction, the fruits were slightly crushed to obtain the seeds. Then, the cleaned seeds were ground in a laboratory mill and sieved using a 10-mesh (pore size 2 mm) sieve. Analytical grade n-hexane (Merck) was used as extraction solvent.

2.2   Moisture Content of the Seeds

The moisture content of the seeds was determined by oven drying method at 105 ± 1°C for 24 h.[23] The moisture content (wet basis) was calculated as:

$$\text{Moisture content (\%)} = \frac{(m_i - m_d)}{m_i} \times 100 \qquad (1)$$

where m_i and m_d is the initial and final mass of the seed (g), respectively.[24]

2.3   Ultrasound-assisted Extraction

The extraction was performed using an ultrasonic water bath (Transsonic Digital S Model T 840DH), with internal tank dimension 327 mm × 300 mm × 200 mm, volume 18 l, having a power consumption of 1100 W and a fixed operating frequency of 40 kHz. It is equipped with adjustable power output from 70 to 250 W. The ultrasonic bath was filled with water approximately 2/3 of its volume. The seed sample (5 g) and the extracting solvent, n-hexane was placed in an Erlenmayer flask (250 ml) covered with aluminium foil. Then, the flask was immersed into the centre position of the ultrasonic bath and this position was kept constant throughout the experiments. During extraction, the temperature was controlled and maintained at the desired level by water circulating from a water bath. After extraction, the liquid extract was separated from the seed residue by using a centrifuge at 4000 rpm for 20 min. The solvent was then removed by using a rotary evaporator and the oil obtained were dried until a constant weight was reached. The extracted oil was collected in a pre-weighed 50 ml beaker for the yield calculation.

2.4   Determination of *C. inophyllum* Oil Yield

Extraction yield of *C. inophyllum* oil was calculated using Equation (2):

$$Y = \frac{M_1}{M_0} \times 100\%$$
(2)

where Y is the extraction yield of *C. inophyllum* oil (%), M_1 is the mass of *C. inophyllum* oil extracted from the sample (g) and M_0 is the mass of the sample used (g).[25] The mass of *C. inophyllum* oil extracted from the sample, M_1 was calculated by the difference between the mass of beaker containing the oil and the mass of the empty beaker used.

2.5   Experimental Design

A four-factor, three-level BBD was employed to determine the optimal conditions for UAE of *C. inophyllum* oil. In order to evaluate the effect of process variables, 29 experiments including five replicates at the central point were performed randomly. Four independent variables involved in this study were extraction time (X_1), ultrasonic power (X_2), extraction temperature (X_3) and liquid to solid ratio (X_4), while the dependent variable was the yield of *C. inophyllum* oil (Y). The ranges of the independent variables were chosen based on the results of preliminary experiments. All independent variables and their respective levels used in BBD were shown in Table 1.

Table 1: Independent variables and their respective coded levels employed in BBD.

Independent variables	Levels		
	−1	0	+1
Extraction time, X_1 (min)	15	20	25
Ultrasonic power, X_2 (W)	190	210	230
Extraction temperature, X_3 (°C)	35	40	45
Liquid to solid ratio, X_4 (ml/g)	15	20	25

Each of these independent variables were coded at three levels between −1, 0 and +1. The coding of the variables was done according to the following equation:[26]

$$x_i = \frac{x_i - x_z}{\Delta x_i} \quad i = 1, 2, 3, ..., k \tag{3}$$

Where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_Z is the real value of an independent variable at the centre point, and Δx_i is the step change of the real value of the variable i corresponding to a variation of a unit for the dimensionless value of the variable i. The experimental data were analysed by multiple regressions to fit the following quadratic polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i \angle j}^{3} \sum_{j}^{4} \beta_{ij} Xi \, X_j + \sum_{i=1}^{4} \beta_{ij} X_2^{i} \tag{4}$$

Where Y is the predicted response and β_o is an intercept. B_i, β_{ii} and β_{ij} are regression coefficients for linear, quadratic, and interactive terms, respectively. X_i and X_j are the coded independent variables.[27]

2.6   Statistical Method

The statistical analysis was carried out using Design Expert (Version 6.0.6, Stat-Ease Inc., Minneapolis, Minnesota, USA). Modeling of data started with a quadratic model including linear, squared and interaction terms. The adequacy of the model was determined by evaluating the coefficient of determination (R^2), the lack of fit, adequate precision and the F-test value obtained from the analysis of variance (ANOVA) that was generated. The regression coefficients obtained from the model were then used for the statistical calculations to generate response

surface plots.[28] Additional confirmation experiments were subsequently conducted to verify the validity of the statistical model.

3.   RESULTS AND DISCUSSION

3.1   Statistical Analysis and Model Fitting

The *C. inophyllum* seeds used in this study have a moisture content of 9.45%. The extraction parameters involved in the UAE of *C. inophyllum* oil were optimised using the BBD. The experimental design matrices with their respective response of the *C. inophyllum* oil yield are shown in Table 2.

Table 2 : Box-Behnken experimental design and results for extraction yield of *C. inophyllum* oil.

No.	X_1 (min)	X_2 (W)	X_3 (°C)	X_4 (ml/g)	Y, oil yield (%)	
					Experimental	Predicted
1	20	190	35	20	52.79	52.67
2	20	210	45	15	54.84	54.91
3	20	210	40	20	56.24	56.01
4	15	190	40	20	53.45	53.43
5	25	210	35	20	53.87	53.83
6	20	230	40	25	54.88	54.90
7	25	210	40	25	54.56	54.52
8	15	210	40	15	52.93	52.92
9	25	190	40	20	54.12	54.28
10	20	190	40	25	53.82	53.94
11	20	210	35	15	52.08	52.18
12	20	190	40	15	53.67	53.71
13	15	210	40	25	54.52	54.60
14	20	230	35	20	53.25	53.37
15	25	210	45	20	54.89	54.93
16	15	230	40	20	54.03	53.87
17	20	210	35	25	54.16	54.08
18	25	210	40	15	54.77	54.64

Table 3: (*continued*)

No.	X_1 (min)	X_2 (W)	X_3 (°C)	X_4 (ml/g)	Y, oil yield (%)	
					Experimental	Predicted
19	25	230	40	20	54.65	54.66
20	20	230	45	20	54.61	54.69
21	20	210	40	20	55.59	56.01
22	20	210	45	25	54.69	54.58
23	20	190	45	20	54.75	54.58
24	15	210	45	20	54.53	54.62
25	20	210	40	20	56.02	56.01
26	20	210	40	20	55.93	56.01
27	20	210	40	20	56.28	56.01
28	20	230	40	15	53.63	53.56
29	15	210	35	20	52.48	52.50

X_1 = Extraction time, X_2 = Ultrasonic power (W), X_3 = Extraction temperature, X_4 = liquid to solid ratio (ml/g)

The resulting oil yield ranged between 52.08% and 56.28% where the maximum oil yield was obtained under the following extraction conditions: 20 min extraction time, 210 W ultrasonic power, 40°C extraction temperature and 20 ml/g L/S ratio. By applying multiple regression analysis on the experimental data, the predicted response variable and the independent variables were found to correlate by a second-order polynomial equation. The equation was expressed in terms of coded factors, described as follows:

$$Y = 56.01 + 0.41X_1 + 0.20X_2 + 0.81X_3 + 0.39X_4 - 0.90 X_1^2 - 1.05X_2^2 - 1.14X_3^2 - 0.94X_4^2 \quad (5)$$
$$-0.012X_1X_2 - 0.26X_1X_3 - 0.45X_1X_4 - 0.15X_2X_3 + 0.28X_2X_4 - 0.56X_3X_4$$

The coefficients with single factor represent the effect of that particular factor towards the *C. inophyllum* oil yield, while those with second-order terms and two different factors represent the quadratic and interactive effects, respectively. Analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. Table 3 provides the regression coefficient values of equation obtained from the statistical analysis results.

Table 4: ANOVA of the regression quadratic model for the prediction of the *C. inophyllum* oil yield.

Source	Sum of squares	Degrees of freedom	Mean square	*F* value	*p*-value
Model	31.93	14	2.28	61.54	< 0.0001
Residual	0.52	14	0.04		
Lack of fit	0.21	10	0.02	0.27	0.9566
Pure error	0.31	4	0.08		
Cor total	32.45	28			

R^2 = 0.9840; R2= R^2_{Adj} = 0.9680; = R^2_{pred} 0.9478; C.V.% = 0.35; Adeq precision = 27.66

The regression model of *C. inophyllum* oil yield was considered highly significant owing to the values of both *F* value and *p*-value, which were 61.54 and < 0.0001, respectively. Meanwhile, the *p*-value for the lack of fit (0.9566) was higher than 0.05. This shows that it was not significant relative to the pure error and indicates that the fitting model is adequate to describe the experimental data. These two values confirmed the goodness-of-fit and suitability of the regression model. The adequacy of the model was further tested by evaluating the determination coefficient (R^2). The determination coefficient (R^2 = 0.9840) indicates that only 1.6% of the total variation are not explained by the model. The value of adjusted determination coefficient (R^2_{Adj} = 0.9680) also confirmed that the model was highly significant. At the same time, a relatively low value of coefficient of variation (C.V.% = 0.35) clearly proves that the experimental values of regression model were precise and reliable. Adequate precision is a measure of the range in predicted response relative to its associated error and a value greater than four indicate that the model can be used within the region of operation.[29] In this study, an adequate precision value of 27.66 indicated that the model has an adequate signal. In conclusion, the established model is adequate for prediction in the range of experimental variables. The significance of each coefficient measured using *F*-value and *p*-value is listed in Table 4. For each terms in the model, a large *F*-value and a small *P*-value would imply a more significant effect on the respective response variable.[30] All regression coefficients were significant ($P < 0.05$) towards the response variable except for two interactive coefficients, which were the interaction between extraction time and ultrasonic power (X_1X_2) as well as ultrasonic power and extraction temperature (X_2X_3).

Table 5: Estimated coefficients and significance test for linear, quadratic and interactive factors of the regression model.

Source	Coefficient estimate	Standard error	F value	p-value	Significance
X_1	0.41	0.06	54.43	< 0.0001	S
X_2	0.20	0.06	13.50	0.0025	S
X_3	0.81	0.06	210.68	< 0.0001	S
X_4	0.39	0.06	49.88	< 0.0001	S
X_1^2	−0.90	0.08	143.26	< 0.0001	S
X_2^2	−1.05	0.08	192.40	< 0.0001	S
X_3^2	−1.14	0.08	226.35	< 0.0001	S
X_4^2	−0.94	0.08	153.33	< 0.0001	S
X_1X_2	−0.01	0.10	0.02	0.8985	N-S
X_1X_3	−0.26	0.10	7.16	0.0181	S
X_1X_4	−0.45	0.10	21.85	0.0004	S
X_2X_3	−0.15	0.10	2.43	0.1415	N-S
X_2X_4	0.28	0.10	8.16	0.0127	S
X_3X_4	−0.56	0.10	33.54	< 0.0001	S

S = Significant, N-S = Non-significant

3.2   Comparison of Experimental and Predicted *C. inophyllum* Oil Yield

A regression model provides the ability to predict future observations on the response (*C. inophyllum* oil yield) corresponding to particular values of the variables. However, verification of the model is essential to ensure that adequate approximation to the actual values is done. Proceeding without proper analysis and optimisation of the fitted response surface would probably cause disingenuous results.[28] Therefore, diagnostic plots such as the experimental versus predicted values shown in Figure 1 were used to judge the model adequacy and display the correlation between experimental and predicted values. Each of the experimental value is compared to the predicted ones computed from the model. The data points on this plot are positioned close to the straight line and signify that there is sufficient agreement between the actual data and the model data. This result implies that the regression model used in this extraction process were able to predict optimum operating conditions for *C. inophyllum* oil extraction.
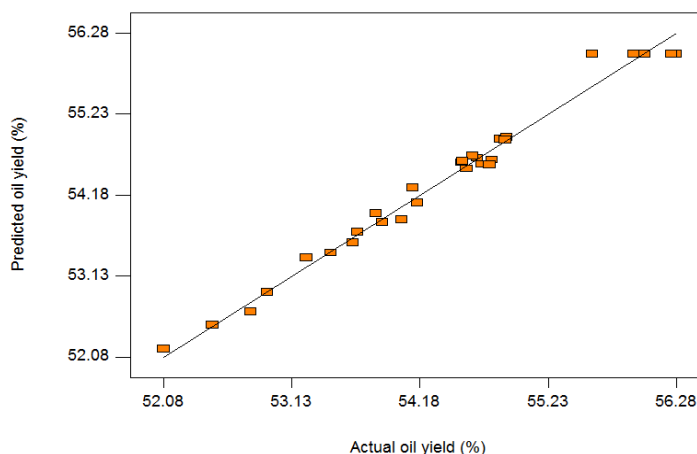
Figure 1: Comparison between predicted and experimental oil yield.

3.3 Response Surface Optimisation of the *C. inophyllum* Oil Extraction Conditions

Three-dimensional response surface and two-dimensional contour plots generated by the Design Expert software version 6.0.6 were used to visualise the relationship between independent and dependent variables and the interactions between two variables. Different shapes of the contour plots indicate whether mutual interactions between the independent variables are significant or not. The circular contour plots indicate the negligible interactions between the corresponding variables, while an elliptical contour plots indicate the significant interactions between the corresponding variables.[31] The three-dimensional representations of the response surfaces generated by the model are shown in Figure 2, 3, 4 and 5. Among these four variables studied, two variables were kept constant at their respective zero level, when the other two variables within the experimental range were depicted in the three-dimensional surface plots.
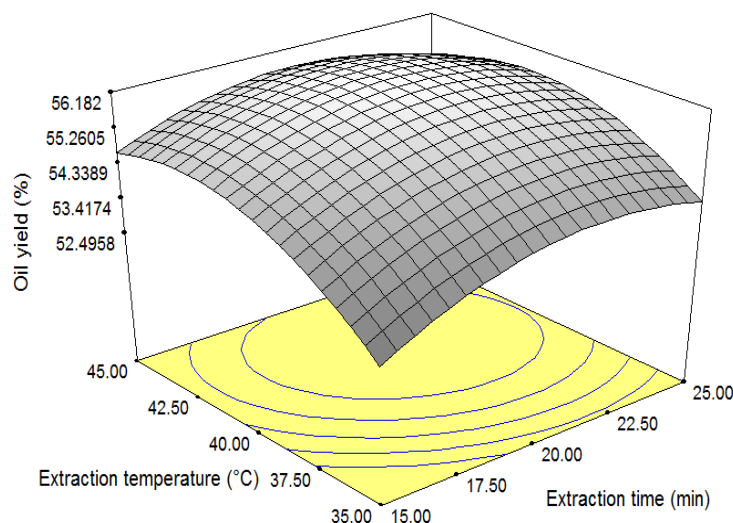
Figure 2: Response surface plot showing the effects of extraction time and extraction temperature on the yield of *C. inophyllum* oil. The ultrasonic power and L/S ratio were at 210 W and 20 ml g^{-1}, respectively.

Figure 2 illustrates the effects of extraction time and extraction temperature on *C. inophyllum* oil yield at an ultrasonic power of 210 W and L/S ratio of 20 ml g^{-1}. Increases in extraction time from 15 min to 20 min and extraction temperature from 35°C to 40°C gradually increased the oil yield and then it began to level off and decrease slightly at elevated temperatures (> 40°C) and longer extraction time (> 20 min). The initial sharp increase in the extraction yield was due to the large oil concentration gradient between the extracting solvent and the seeds and also due to easier extraction of oil from the most outer part of the seeds. As the extraction time proceeded, the concentration gradient decreased; as the mass transfer was increased with continuous exposure to ultrasound, the extraction became difficult due to interior part of the seeds. The continuous increase in the release of the oil resulted in a saturated solvent, leading to a negligible mass transfer and extraction.[32]

According to Jovanovic-Malinovska et al.,[33] this observation can be well explained by Fick's second law of diffusion, which stated that the final equilibrium between the solute concentrations in the solid matrix (seeds) and in the bulk solution (solvent) will be achieved after certain time. Hence, an excessive extraction time did not lead to enhanced oil yield. Therefore, the final equilibrium between the oil concentration within the seeds and in the n-hexane was achieved at approximately 20 min of extraction time. This result was in agreement with the findings reported by Zhang et al.[34] on the UAE of epimedin

A, B, C and icariin from *Herba Epimedii*. The increasing trend of the oil yield along the increasing temperature (35°C to 40°C) is probably due to the improvement of the mass transfer resulting from the increased solubility of *C. inophyllum* oil and the decreased viscosity of the solvent.[35] On the other hand, the reverse trend could be explained by a combination of acoustic cavitation and thermal effect. The temperature displayed a positive effect on vapour pressure. Therefore, high temperature led to the increase in the vapour pressure of solvent molecules within cavitation micro-bubbles, causing the damping of the bubble collapse and decrease in cavitation intensity.[36] Sun et al.[37] reported the same trend in their research on all-*trans*-β-carotene extraction from citrus peels by using ultrasound treatment.
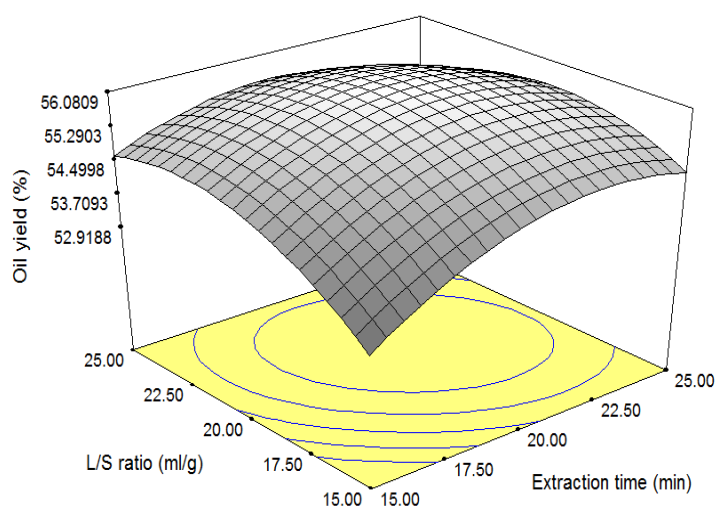

Figure 3: Response surface plot showing the effects of extraction time and L/S ratio on the yield of *C. inophyllum* oil. The ultrasonic power and extraction temperature were at 210 W and 40°C, respectively.

Figure 3 shows the effects of extraction time and L/S ratio on *C. inophyllum* oil yield when the ultrasonic power and extraction temperature were maintained at 210 W and 40°C. The oil yield increased significantly at a lower range of extraction time (15 to 20 min) and L/S ratio (15 to 20 ml g^{-1}). In contrast, when extraction time and L/S ratio were raised to a higher level, the oil yield did not show any remarkable improvement. A high ratio of liquid to solid material implied greater concentration difference between the interior plant cells and the exterior solvent, and the diffusion of oil occurred more quickly. In this case, increasing L/S ratio from 15 to 20 ml g^{-1} created a larger concentration difference between the interior seeds and exterior solvent, thus enhancing the oil yield. The

oil yield did not improve further when L/S ratio was increased higher from 20 to 35 ml g^{-1} due to the prolonged distance of diffusion towards the interior tissues.[38] Fu et al.[39] studied UAE of oleanolic and ursolic acids from pomegranate (*Punica granatum* L.) flowers and they found out that solvent to material ratio of 20 ml g^{-1} was the best condition for the extraction and a larger ratio did not increase the extraction yield.
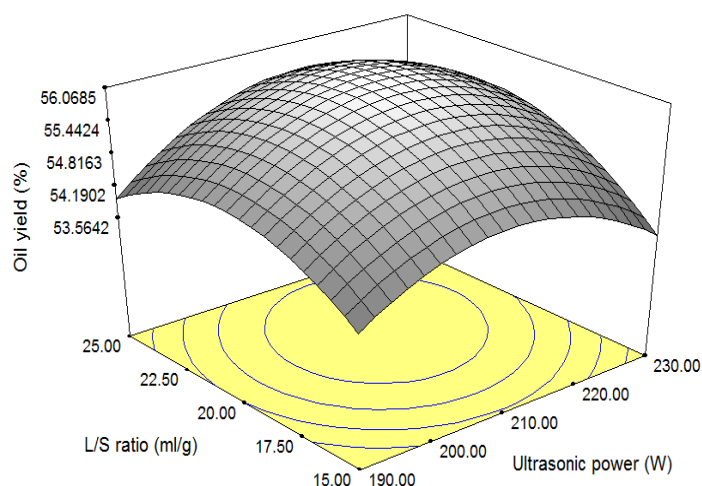

Figure 4:   Response surface plot showing the effects of ultrasonic power and L/S ratio on the yield of *C. inophyllum* oil. The extraction time and extraction temperature were at 20 min and 40°C, respectively.

Figure 4 illustrates the response surface plot for the effects of ultrasonic power and L/S ratio on *C. inophyllum* oil yield when the extraction time and extraction temperature were held constant at 20 min and 40°C respectively. It can be seen that higher oil yield was reached at an ultrasonic power between 190 W and 210 W and L/S ratio between 15 ml g^{-1} and 20 ml g^{-1}. An increase in ultrasound power promotes a more vigorous destruction of the seed's cell walls. The higher the ultrasound power, the more solvent could enter the interior of the cells and the more oil will be released into the solvent, hence improving the extraction efficiency.[40] However, beyond 210 W and 20 ml g^{-1}, the oil yield started to reduce. This observation can be explained by the increase of acoustic intensity with the increasing of ultrasonic power. In this case more bubbles were formed which hampers the propagation of shock waves and the bubbles may coalesce to form bigger ones and implode weakly. Therefore, the extraction efficiency would decrease.[41] Sun et al.[42] in their study on the UAE of five isoflavones from *Iris tectorum* Maxim reported the same trend where the highest extraction yield for

all isoflavones were achieved at an ultrasound power of 150 W. The extraction yield decreased when the power was above 150 W.
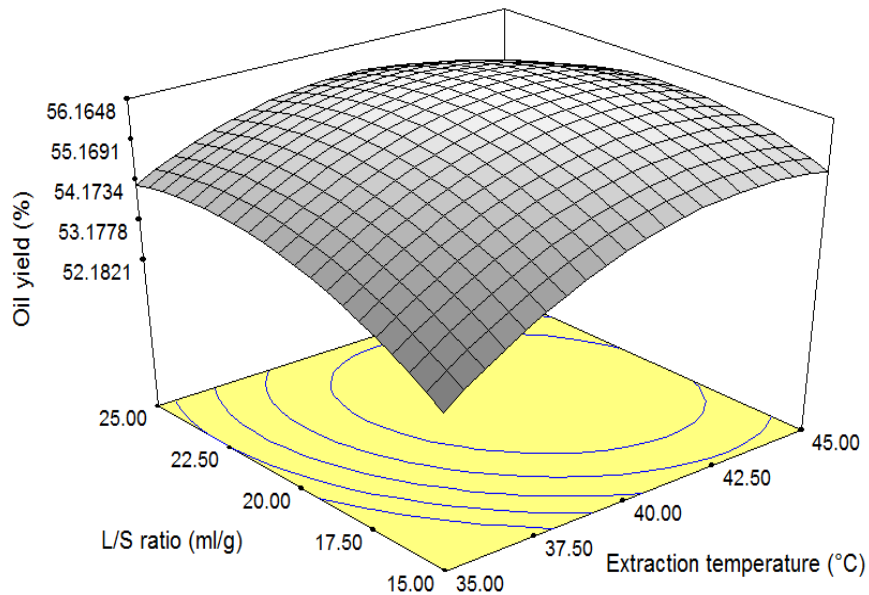

Figure 5:   Response surface plot showing the effects of extraction temperature and L/S ratio on the yield of *C. inophyllum* oil. The extraction time and ultrasonic power were at 20 min and 210 W, respectively.

Figure 5 shows the effects of extraction temperature and L/S ratio on *C. inophyllum* oil yield whereas the extraction time and ultrasonic power were set constant at their respective centre values of 20 min and 210 W. The oil yield increased as the extraction temperature and L/S ratio increases in the range between 35°C to 40°C and 15 to 20 ml g^{-1} respectively. The highest oil yield was obtained at approximately 40°C with L/S ratio of 20 ml g^{-1}. Further variation in the temperature (40°C to 45°C) and L/S ratio (20 to 25 ml g^{-1}) however caused a slight decrease in the oil yield.

3.4   Validation of the Predictive Model

The regression model proposed by BBD predicts optimum conditions which gives the highest *C. inophyllum* oil yield. Optimum conditions identified were as follows: extraction time 20.8 min, ultrasonic power 211.74 W, extraction temperature 41.54°C and L/S ratio of 20.5 ml g^{-1}. The combination of these extraction conditions were expected to obtain maximum oil yield of 56.2%. For

operational convenience, the optimum conditions were 21 min, 210 W, 42°C and 21 ml g^{-1} for extraction time, ultrasonic power, extraction temperature and L/S ratio, respectively. However, validation of the predicted optimum conditions is required to determine the adequacy and reliability of the model equation. Therefore, five sets of confirmatory experiments were conducted at the suggested optimum extraction conditions. As tabulated in Table 5, the experimental oil yield was 56.03% and it was close to the predicted value (56.2%). Additionally, the percentage error differences between the experimental and predicted values were in the range of 0.30–3.75% (< 5%), thus indicating that the predicted conditions and response were verified for optimising UAE of *C. inophyllum* oil. As a result, the model developed by BBD was suitable and could be effectively used to optimise the extraction parameters of *C. inophyllum* oil extraction.

Table 5: Experimental and predicted *C. inophyllum* oil yield under optimum conditions.

	Optimum conditions				Y, Oil yield (%)		Error (%)
Run	X_1 (min)	X_2 (W)	X3 (°C)	X4 (ml g–1)	Experimental	Predicted	
1	21	210	42	21	55.13	56.20	1.90
2	21	210	42	21	56.41	56.20	0.37
3	21	210	42	21	54.09	56.20	3.75
4	21	210	42	21	55.68	56.20	0.93
5	21	210	42	21	56.03	56.20	0.30

X_1 = Extraction time; X_2 = Ultrasonic power; X_3 = Extraction temperature; X_4 = L/S ratio

The efficiency of UAE technique in extracting oil from *C. inophyllum* seeds was compared to a study reported by Jahirul et al.[43] They have conducted the extraction of *C. inophyllum* seed oil by using a conventional solvent (hexane) extraction technique. The highest oil yield obtained was approximately 51% after 8 h of extraction. In addition, they also used mechanical extraction technique (screw press) in order to obtain the oil. However, this technique was less efficient as it took over an hour to process just one sample and the oil yield was low (approximately 25%). In contrast, UAE technique in the present study only required 21 min to give a maximum oil yield of 56%. This shows that the application of ultrasound have successfully reduced the extraction time needed, thus making UAE a more effective and promising technique compared to the conventional extraction technique.

4. CONCLUSION

The Box–Behnken response surface design was successfully employed to optimise the UAE of oil from *C. inophyllum* seeds. Four independent variables

such as extraction time, ultrasound power, extraction temperature and L/S ratio significantly affect the *C. inophyllum* oil yield. The developed model gave a high determination coefficient value (R^2) of 0.984, implying a satisfactory fit to the experimental data. The optimum conditions were found to be as follows: extraction time 21 min, ultrasound power 210 W, extraction temperature of 42°C and L/S ratio 21 ml g^{-1}. Under these optimised conditions, the maximum oil yield observed was 56.03% and it was in good agreement with those predicted by the regression model.

5. ACKNOWLEDGEMENT

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