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Ultrasound-Assisted Extraction (UAE) of Phytochemicals with Response Surface Methodology (RSM) in *Curcuma Xanthorrhiza*

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Abstract

The genus Curcuma from the Zingiberaceae family consist of 80 species is widely recognized for its culinary and pharmaceutical application and has a significant effect on medicine, food, cosmetic industries, and economic. Considering an eco-friendly extraction method that provides the efficiency of the extraction, it is great of interest to explore the best optimum condition of parameters used in the extraction. This research is carried out to optimize the ultrasound-assisted extraction (UAE) of the extract from C. xanthorrhiza via Response Surface Methodology (RSM). Box-Behnken design (BBD) is used with three variables: extraction time (5-20 min), temperature (30-50°C) and liquid-solid ratio (6-10 mL/g). In the extracts, xanthorrhizol and curcumin are quantified using reversed-phase high-performance liquid chromatography combined with a diode array detector. The optimum condition of yield and concentration of xanthorrhizol are found at the extraction temperature of 50°C, time 20 minutes, and liquid-solid ratio8 mL/g. However, the optimum condition of curcumin is found at the extraction temperature 30°C, time 12.50 minutes, and 10 mL/g liquid-solid ratio. This research recommended that the ultrasound-assisted extraction method under specific parameters has favorable potential to be used in the extraction process which is useful for advanced research.

Keywords:*Curcuma xanthorrhiza; Ultrasound-Assisted extraction; Xanthorrhizol; Curcumin; RSM*

1. Introduction

*Curcuma xanthorrhiza*Roxb belongs to the Zingiberaceae family (ginger family) and originated plants of Indonesia. It is cultivated in Sri Lanka, Malaysia, the Philippines, and Thailand. It is widely used for the traditional treatment of many illnesses in South East Asian countries, including migraines, constipation, liver problems, and inflammatory conditions(Erpina et al., 2017). It has been reported that *C. xanthorrhiza* has utility for hepatitis, rheumatism, cancer, hypertension liver problems, diabetes, and heart disorders (Erpina et al., 2017). *C. xanthorrhiza* has a marketable interest in research and medicinal concerns in the production of novel medications for the treatment of numerous ailments (Anjusha & Gangaprasad, 2014). Meanwhile, *C. xanthorrhiza* known as Javanese turmeric has a long history of Indonesian medicinal use (AzizA et al., 2018) which has also displayed diuretic, anti-cancer, anti-inflammatory, antispasmodic, anti-leucorrhoea, anti-bacterial, anti-oxidant, anti-hypertensive, anti-rheumatic, anti-hepatotoxic, anti-dysmenorrheal, and antifungal effects. This decreases cholesterol, prevents migraines, constipation, and enhances the flow of milk during breastfeeding. *C. xanthorrhiza's* conventional benefits are further confirmed by isolating and recognizing many active chemical compounds comprising xanthorrhizol, curcumin, and a few volatile constituents (Erpina et al., 2017). It is confirmed that curcuminoids and xanthorrhizol, derived from turmeric rhizomes cause the therapeutic effect (AzizA et al., 2018).

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Phytochemical screening of medicinal plants is very useful in discovering new sources of compounds that are of therapeutic and industrial significance. Medicinal plants are important nowadays for the global economy. Many plants are important sources of useful secondary metabolites used in the pharmaceutical, agrochemical, aromatic, and flavor industries. Several secondary plant metabolites are of commercial significance and are used in a variety of pharmaceutical compounds (Anjusha & Gangaprasad, 2014). Besides, the optimization of bioactive compounds and extraction methods are of great interest in the food and medicinal industries for further research and development. Over the years, medicinal shrubs in their innate and administered from have been commonly used in old-style medicine, due to the various biologically active molecules found within them. Extraction is the most critical step in making full use of the bioactive molecules found in medicinal florae (Tušek et al., 2018). The available *C. xanthorrhiza* extraction techniques are versatile and include Soxhlet, microwave-assisted extraction, supercritical extraction of carbon dioxide, and ultrasonic-assisted extraction (Başpınar et al., 2017).

UAE is one of the significant techniques for extricating valuable composites from plant materials and is quite adjustable on a small or large scale (i.e. on a laboratory or industrial scale). Conventionally, an ultrasound device is inexpensive and much simpler to operate (H.-F. Zhang et al., 2009). Additionally, extraction performance can be greatly improved through the analysis of the effect of the extraction parameters. The methods are used for statistical and mathematical analysis (Subuki et al., 2018) to identify optimal conditions for extraction (Hasham-Hisam et al., 2011).According to the literature evidence arising in recent years, the significance of mathematical modeling tools to optimize the extraction process (Tušek et al., 2018) become one of the most widely used methods (Rajha et al., 2014). The purpose of this approach is to detect the most significant variables that affect the response of interest by using RSM (Aydar, 2018). This research was aimed to find the optimum condition of phytochemicals extraction from *C. xanthorrhiza* using RSM by Box-Behnken (BBD) design and verified using ANOVA analysis. The monitored parameters were temperature, reaction time, and liquid-solid ratio. The quantification of xanthorrhizol and curcumin on extracts was carried out by RP-HPLC with a Photo Diode Array detector (PDA). The optimization of UAE from *C. xanthorrhiza* were responses on the percentage of yield, quantification of xanthorrhizol, and curcumin.

2. Literature Review

Traditionally, for hundreds of years, *C. xanthorrhiza* rhizome was used for medicinal purposes through simple preparation (Taher & Sarmidi, 2015). Locally, the biological characteristics of *C. xanthorrhiza*, a well-known traditional medicinal plant used in Malaysia and Indonesia, have been noticed which including anti-inflammatory activity and anti-cancer activity, protective effect on hepatic damage, and neurodegenerative disorder prevention (Zhang et al., 2014). Meanwhile, it is widely used as a condiment, spices, flavoring agents, and dyes sources. It is also important ingredients in traditionally prepared tonics locally known as "Jamu" which is available commercially in Malaysia (Alafiatayo Akinola et al., 2014). Therefore, drug formulations such as syrup and tablets comprise of temulawak rhizome designed to boost appetite. Several bioactivities were recognized in *C. xanthorrhiza* to cure hypertension, hepatitis, cancer, rheumatism, antioxidant, diuretic, liver disease, diabetes, and hepatoprotective effects. The major component present in *C. xanthorriza* rhizome, curcuminoids (mainly curcumin and desmethoxycurcumin), and xanthorrhizol(Ab Halim et al., 2012) is thought to be accountable for these biological activities (Oon et al., 2015). It is therefore essential to identify the class of the components or group of compounds accountable for biological activity (Prabaningdya et al., 2017). Pharmacologically, active compounds are typically in the small amount included in herbal plants and various effective and selective extraction procedures have been introduced to excerpt those compounds from the raw material (Salea et al., 2014).

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C. xanthorrhiza consists of nonvolatile curcuminoids and volatile essential oil (Darmawan & Pramono, 2016). Many probable pharmacological functions of *C. xanthorrhiza* is thought to be possessed to numerous bioactive compounds and phytochemicals, including xanthorrhizol (1.48-1.63%), curcuminoids like curcumin and demethoxycurcumin (1-2%), phellandrene, camphor, tumerol, sineol, borneol, flavonoids, and sesquiterpenes (Theresia et al., 2019).Temulawak plant is used widely as a single drug or in combination with other drugs. There are over 50 traditional medicinal drugs which contain temulawak(Rosidi et al., 2016).Xanthorrhizol, the major component of *C. xanthorrhiza's* essential oil, is a sesquiterpenoid-type bisabolane (Oon et al., 2015). This compound accounts for almost 46.3% of the whole essential oil component via hydro-distillation techniques. (Devaraj et al., 2013). Also, other major compounds that have been discovered from *C. xanthorrhiza* are curcuminoids (AzizA et al., 2018). Curcuminoids are known as Diarylheptanoids and are represented by curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) in *C. xanthorrhiza* (Rajkumari et al., 2017). The presence of curcuminoids induced the yellowish-orange color of the *C. xanthorrhiza* rhizome (Erpina et al., 2017). Moreover, curcuminoids are phenolic antioxidants. The vital roles of antioxidants are utilized to attempt to decrease the fat and oil oxidation cycle (Spigno et al., 2007). It reduces food damage, extends the food industry life, improves food fat stability, and avoids sensory and quality of nutrition losses(Anggarani & Maulana, 2018).

On the other hand, approach and processing conditions used to extract chemical compounds from herbal raw substituents, therefore, perform a key role in evaluating a standardized intermediate phytopharmaceutical's cost-efficiency and overall effectiveness. Based on these considerations, it is of great interest to conduct studies to explore the relationship between extraction parameters and extraction properties to the production of turmeric phytomedicines (Mary et al., 2012). Recently, UAE was commonly used among all of the extraction techniques to excerpt bioactive composites from plant resources due to the high mining efficiencies that can be attained at comparatively low temperatures (Le Pham Tan et al., 2019).

3. Methodology

3.1. Chemicals and Materials

Rhizomes of *C. xanthorrhiza* were purchased from one of the local herbal shops of Larkin markets located in Johor Bahru in November 2019. The rhizomes were chopped in small pieces and dried at room temperature for two weeks. Methanol was used for the extraction of *C. xanthorrhiz*a. Methanol and methanol HPLC grade were purchased from QRec. Xanthorrhizol and curcumin were isolated from the methanol crude extract.

3.2.Experimental Procedure

Ultrasonic cleaning bath with a incidence of 60 kHz and a power of 750 W, armed with time and temperature regulator was used. The extraction of *C. xanthorrhiza* was performed by 17 runs. Powder of *C. xanthorrhiza* rhizome (1 g) has been weighed into a conical flask containing various liquid-solid ratio which was 6 mL/g, 8 mL/g, and 10 mL/g respectively. The conical flask was put in a medium frequency ultrasonic bath at 30° C to 50°C for duration of 5 to 20 minutes' extraction time. The flask containing extract was filtered and concentrated with vacuum rotary evaporator to achieve a waxy crude extract of *C. xanthorrhiza*. The extracts were kept in a refrigerator until further use. All trials were carried out intriplicate.

3.3. HPLC Analysis of Phytochemicals

For further examination, an RP-HPLC Agilent Series 20 equipped with a Photo Detector Array (PDA) with the autosampler was used. This instrument was used to analyze and identify the presence of the phytochemical compounds. Separation of phytochemical compounds was

carried out on a reversed-phase HPLC, Japan Analytical Industry, Tokyo, Japan model:(Shimadzu – NexeraLC-20 ADXR) instrument which armed with Luna® column (description: Luna® 5µm C18(2) 100 Å), (Size: LC Column 150 × 4.6 mm) and UV flash as a detector. The elution solvents were methanol as solvent A and water as solvent B with the flow rate at 1 mL/min. The injection volume was about 10 µl. The compounds were described by comparing the observed retention times at 270 nm (0 – 7 min) and 270 nm (7 – 15 min) to those of the reference standards.

4. Results and Discussion

The effect of independent variables (extraction time, temperature, and liquid-solid ratio) on the response variables of percentage yield, quantification of xanthorrhizol, and curcumin will be discussed. Reversed-phase high-performance liquid chromatography joined with a diode array detector has been used for quantification of xanthorrhizol and curcumin**.** Box-Behnken design (BBD) evaluated the association between the dependent variables and the independent variables.

4.1 Optimization on the UAE of Phytochemical Compounds from *Curcuma Xanthorrhiza* with Box-Behnken design (BBD)

4.1.1. Experimental Design: The experimental design of 17 experimental runs included in this study as shown in Table 4.1

Table 4.1: The variable levels in the Box-Behnken design (BBD) and the response values

	Variable Levels			Response Values		
Run	Time (min)	Temperature (°C)	Liquid-Solid Ratio (mL/g)	Yield (%)	Xanthorrhizol (% w/w)	Curcumin (% w/w)
1	5	30	8	66.30	80.45	29.85
2	20	30	8	65.84	78.94	35.01
3	5	50	8	65.75	78.23	34.96
4	20	50	8	72.20	85.68	34.50
5	5	40	6	66.70	74.00	17.05
6	20	40	6	68.46	74.12	28.50
7	5	40	10	66.21	74.07	36.07
8	20	40	10	70.50	79.11	29.01
9	12.50	30	6	65.22	72.05	22.11
10	12.50	50	6	66.78	72.00	31.78
11	12.50	30	10	64.70	72.05	39.73
12	12.50	50	10	69.00	77.02	33.90
13	12.50	40	8	72.00	82.25	36.99
14	12.50	40	8	71.66	83.00	37.60
15	12.50	40	8	71.74	82.58	38.00
16	12.50	40	8	72.15	83.07	37.43
17	12.50	40	8	71.23	82.54	37.63

4.1.2 Analysis Variance (ANOVA): The assessment of experimental data for statistical analysis was achieved by analysis variance (ANOVA), which is statistically applicable to the mathematical model's representability (Madadi et al., 2020). ANOVA used to determine the significance of the model. The Design-Expert software proposed from the data findings as tabled in Table 4.2 that the quadratic model was the best model for all the responses.

Table 4.2: ANOVA for the response surface model of responses on extraction yield, quantification of xanthorrhizol and curcumin

Response	Model	Standard Deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	Remarks
Yield	Linear	2.64	0.2863	0.1216	-0.1163	142.16	
	2FI	2.75	0.4074	0.0518	-0.4753	187.87	
	Quadratic	0.27	0.9960	0.9909	0.9934	0.84	Suggested
	Cubic	0.35	0.9961	0.9843		+	Aliased
Xanthorrhizol	Linear	4.73	0.1188	-0.0845	-0.5624	515.92	
	2FI	5.08	0.2170	-0.2528	-1.8501	941.14	
	Quadratic	0.28	0.9983	0.9961	0.9931	2.27	Suggested
	Cubic	0.34	0.9986	0.9943		+	Aliased
Curcumin	Linear	5.39	0.3587	0.2107	-0.1702	689.37	
	2FI	4.73	0.6206	0.3930	-0.2330	726.37	
	Quadratic	0.29	0.9990	0.9976	0.9967	1.96	Suggested
	Cubic	0.37	0.9991	0.9964		+	Aliased

PRESS: Predicted Residual Sum of Squares
+ Case(s) with leverage of 1.000: PRESS statistic not defined

The determination coefficient for the proportion of variation in the expected and actual values was denoted as R2 value. The values of R2 in this analysis displeyed in Table 4.3. The summary of ANOVA for each response variable was further described in Table 4.4, 4.5, and Table 4.6.

Table 4.3: Analysis of variance for determination of model fitting

Source of Variation	Percentage Yield	Percentage Xanthorrhizol	Percentage Curcumin
Lack of fit (p-value)	0.01	0.27	0.17
R^2	0.9960	0.9983	0.9990
Standard Deviation	0.27	0.28	0.29
Adjusted R^2	0.9909	0.9961	0.9976
Adeq precision	36.553	62.820	100.194
PRESS	0.84	2.27	1.96
F ratio of Model	9.516	0.27	0.17
P of Model > F	0.9985	0.8422	0.9091

Table 4.4: Summary of ANOVA for extraction yield

Source	Sum ofSquares	Degreeof Freedom	MeanSquare	F-value	Prob > F	Significance
Model	126.84	9	14.09	195.55	< 0.0001	Significant
A-time	18.12	1	18.12	251.42	< 0.0001	
B-temperature	17.02	1	17.02	236.21	< 0.0001	
C-liquid-solid ratio	1.32	1	1.32	18.32	0.0037	
AB	11.94	1	11.94	165.63	< 0.0001	
AC	1.60	1	1.60	22.20	0.0022	
BC	1.88	1	1.88	26.04	0.0014	
A^2	7.62	1	7.62	105.77	< 0.0001	
B^2	35.12	1	35.12	487.27	< 0.0001	
C^2	25.13	1	25.13	348.68	< 0.0001	
Residual	0.50	7	0.072			

Lack of fit	3.575	3	1.192	9.516	0.9985	Notsignificant
Pure Error	0.50	4	0.13			

Table 4.5: Summary of ANOVA for quantification of xanthorrhizol

Source	Sum ofSquares	Degreeof Freedom	Mean Square	F-value	Prob > F	Significance
Model	329.64	9	36.63	453.84	< 0.0001	Significant
A-time	15.40	1	15.40	190.84	< 0.0001	
B-temperature	11.14	1	11.14	138.02	< 0.0001	
C-liquid-solid ratio	12.70	1	12.70	157.37	< 0.0001	
AB	20.07	1	20.07	248.69	< 0.0001	
AC	6.05	1	6.05	74.98	< 0.0001	
BC	6.30	1	6.30	78.06	< 0.0001	
A^2	0.035	1	0.035	0.43	0.5320	
B^2	16.08	1	16.08	199.20	< 0.0001	
C^2	233.95	1	233.95	2898.8	< 0.0001	
Residual	0.56	7	0.081			
Lack of fit	0.096	3	0.032	0.27	0.8422	Notsignificant
Pure Error	0.47	4	0.12			

Table 4.6: Summary of ANOVA for quantification of curcumin

Source	Sum ofSquares	Degreeof Freedom	Mean Square	F-value	Prob > F	Significance
Model	588.52	9	65.39	753.70	< 0.0001	Significant
A-time	10.33	1	10.33	119.05	< 0.0001	
B-temperature	9.10	1	9.10	104.83	< 0.0001	
C-liquid-solid ratio	191.88	1	191.88	2211.65	< 0.0001	
AB	7.90	1	7.90	91.01	< 0.0001	
AC	85.66	1	85.66	987.26	< 0.0001	
BC	60.76	1	60.76	700.34	< 0.0001	
A^2	70.69	1	70.69	814.80	< 0.0001	
B^2	0.092	1	0.092	1.06	0.3384	
C^2	140.42	1	140.42	1618.52	< 0.0001	
Residual	0.61	7	0.087			
Lack of fit	0.070	3	0.023	0.17	0.9091	Notsignificant
Pure Error	0.54	4	0.13			

4.1.3. Analysis of Percentage Yield, Quantification of Xanthorrhizol and Curcumin by RSM: The predicted second-order polynomial regression equations were displayed below:

$$Yield = +71.71 + 1.51\,A + 1.46\,B + 0.41C + 1.73\,AB + 0.63\,AC + 0.68\,BC - 1.35\,A2 - 2.89\,B2 - 2.44\,C2 \quad (4.1)$$

$$Xanthorrhizol = +82.69 + 1.39\,A + 1.18\,B + 1.26\,C + 2.24\,AB + 1.23\,AC + 1.25\,BC + 0.091\,A2 - 1.95\,B2 - 7.45\,C2 \quad (4.2)$$

$$Curcumin = +37.53 + 1.14\,A + 1.07\,B + 4.90\,C - 1.41\,AB - 4.63\,AC - 3.90\,BC - 4.10\,A2 + 0.15\,B2 - 5.78\,C2 \quad (4.3)$$

4.1.4. Diagnostic Analysis: To calculate the model fitting to the quadratic model, the diagnostic plots were plotted. The quadratic models have been plotted four graphs represented as predicted values versus actual values, normal plot, outlier plot, and residual versus run number plot. Figures 4.1, 4.2, and 4.3 showed (a) predicted values versus actual values, (b) the normal plot, (c) outer plot, and (d) residual versus run number plot.

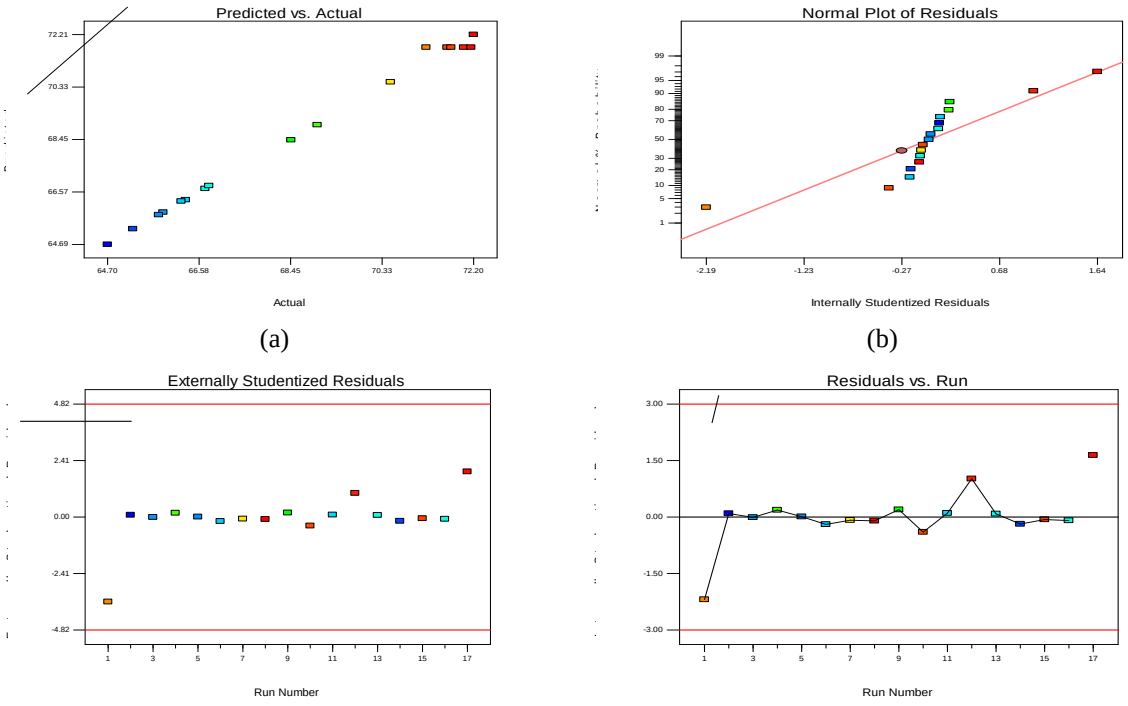
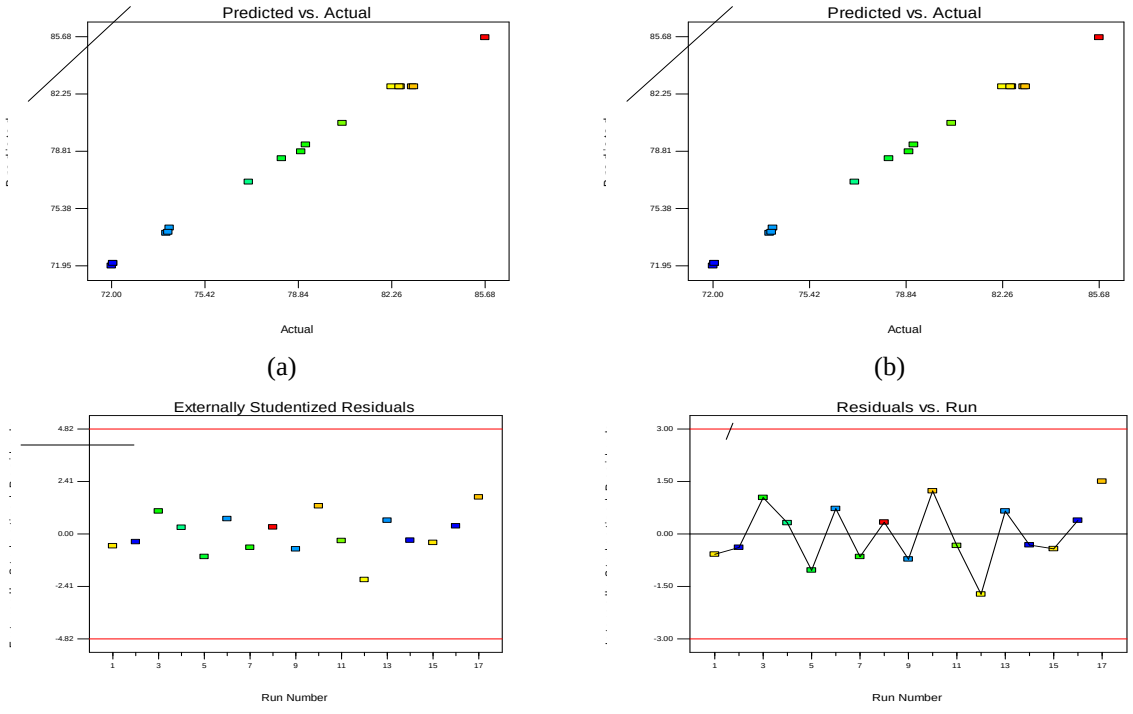
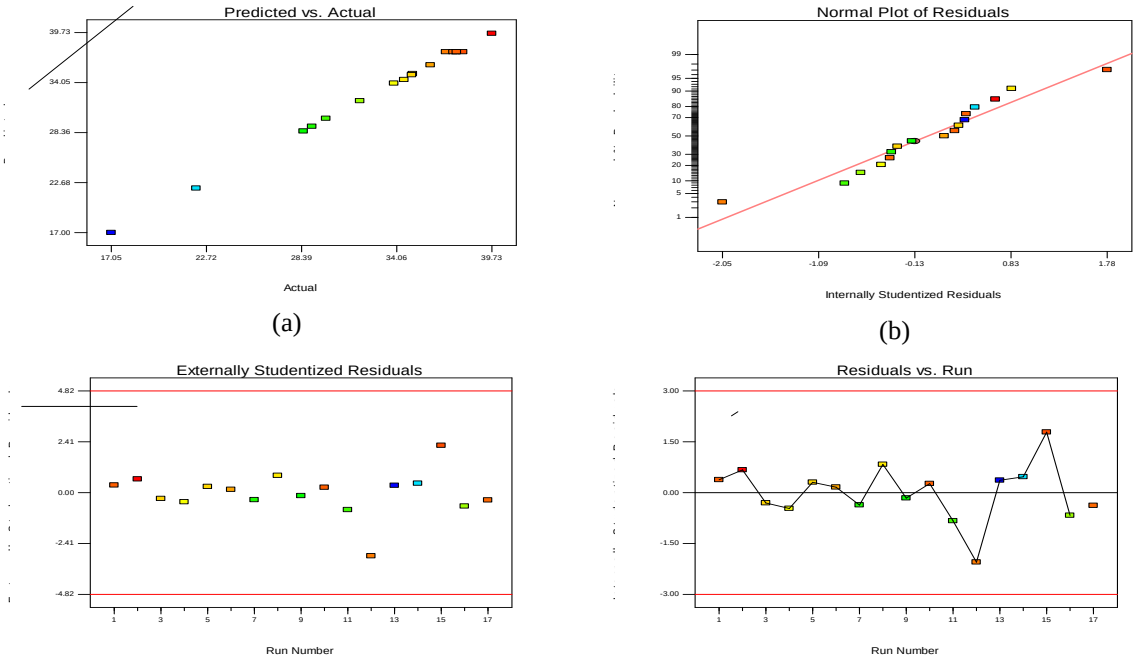


Figure 4.1: Diagnostic plots for percentage yield (a) predicted values versus actual values, (b) normal plot, (c) outlier plot, and (d) residual versus run number plot



(c) (d)

Figure 4.2: Diagnostic plots for quantification of xanthorrhizol (a) predicted values versus actual values, (b) normal plot, (c) outlier plot, and (d) residual versus run number plot



(a) (b)

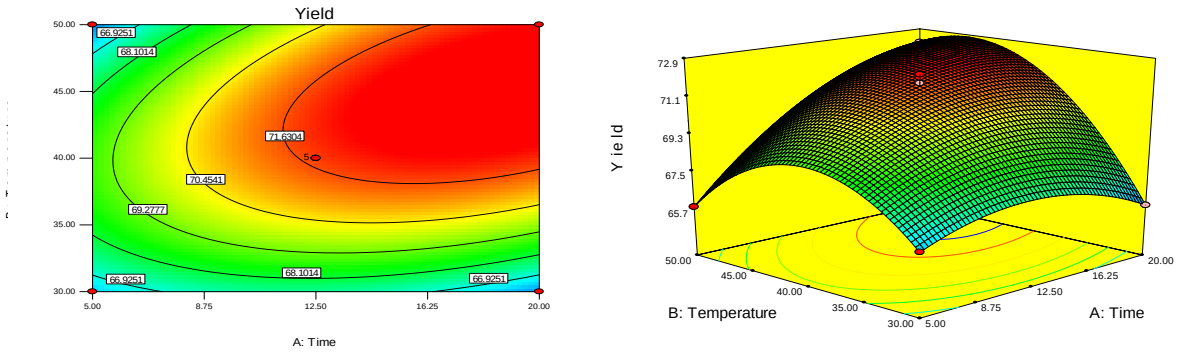
(c) (d)

Figure 4.3: Diagnostic plots for quantification of curcumin (a) predicted values versus actual values, (b) normal plot, (c) outlier plot, and (d) residual versus run number plot

4.1.5. Response Surface Analysis

4.1.5.1. Effect of Time, Temperature, and LS Ratio on Percentage Yield: Temperature, time, and LS ratio impact of extraction on percentage yield of C. Xanthorrhiza were explored in this report. The percentage yields were measured from 64.7 % to 72.2 % for all 17 extracts. The coefficient estimate for time (A) was + 1.51 which represented the highest significant outcome on percentage yield as associated to other terms of the model. Figure 4.4 highlighted (a) response surface and (b) the contour plot showing the outcome of extraction time and temperature on the percentage yield at constant liquid-solid ratio(8mL/g). The interactive effect of Time (A) with Temperature (B) on extract yield from C. xanthorrhiza were < 0.0001 (p-value).The extraction time (F-value= 251.42) revealed the great outstanding value compared to the extraction temperature (F-value= 236.21).

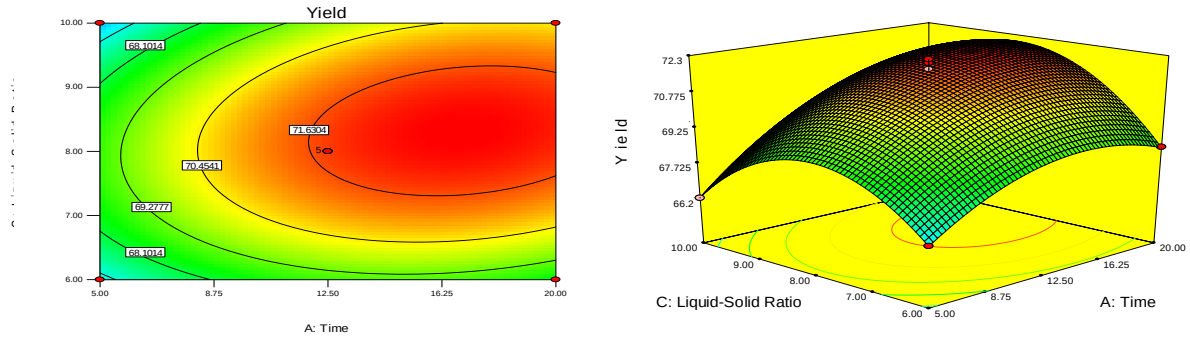
As presented in Figure 4.4, when increasing the extraction temperature and time, the yield can be seen to be increased.High percentage yield as temperatures ranged from 40 ° C to 50 ° C, and the extraction time from 10 min and 20 min. By using the temperature (50 ° C) and time (20 min) at constant liquid-solid ratio (8mL/g) 72.2 % yield was obtained. The results presented in this research are in agreement with the findings by Ayala-soto*et al.* (2016). The journal was reported to have increased sonication time with an increase in temperature resulting in increased corn fiber arabinoxylans yield. (Ayala-Soto et al., 2016). The high temperature speeds up the kinetics of phytochemical compounds in extracts and effectively increases the mass transfer into the solvent (Soquetta et al., 2018). The yield for extraction also raised with an increase in ultrasonic time, similar to the finding of Wang et al.



(a) (b)

Figure 4.4: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of extraction time and temperature on the percentage yield at constant liquid-solid ratio (8mL/g)

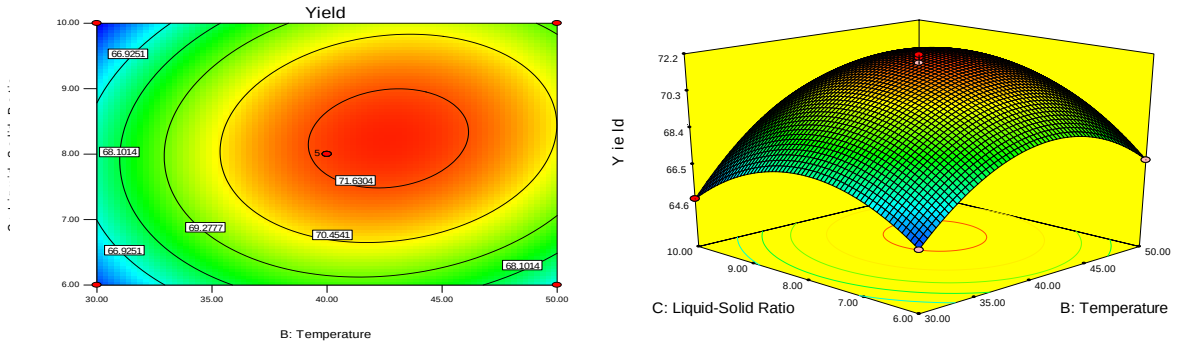
The percentage yield of C. xanthorrhiza was affected via ET (A) and LS ratio (C), as exhibited in Figure 4.5. The interaction of ET and liquid-solid ratio with a p-value of 0.0022 was significant. When comparing the F-value, the effect of ET on percentage yield was greater than the LS ratio. Table 4.4 displayed the F-value of extraction time and LS ratio were 251.42 and 18.32 respectively. The optimum produce was attained at 72.2 % in a constant LS ratio of 8 mL / g. The solubility of phytochemical compounds has been strongly affected by the polarity of the solvent used. Polar compounds may be more easily solved with the polar solvent, and vice versa (Ali et al., 2018). The yield of extraction from C. xanthorrhiza extract was found to be growing with a high liquid-solid ratio and a longer duration of sonication. Meanwhile, AC has been displayed positive value for the coefficient estimate (+ 0.63). This means an increase in the interaction between ET and methanol solvent (LS ratio) would increase the extraction yield.



(a) (b)

Figure 4.5: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of ET and LS ratio on the percentage yield at continuous temperature (40°C)

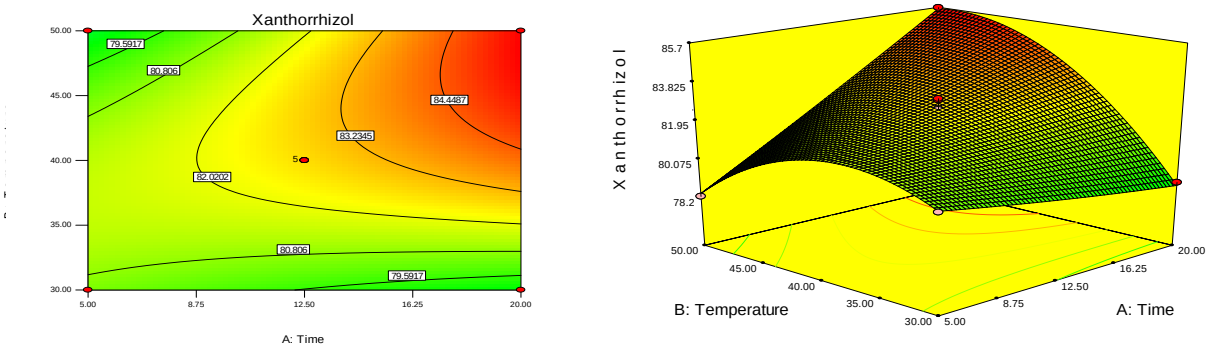
Figure 4.6 demonstrated the 3D surface and a outline plot for the mutual temperature relations (B) with a liquid-solid ratio (C). The interactive effect on the percentage yield of temperature and LS ratio was significantly contributed. For that reason, the p-value (0.0014) for the interactive effect of AC was less than 0.1000 indicate the model terms were significant. Furthermore, the temperature F-value (236.21) was exceptionally excellent compared with the F-value (18.32) of the LS ratio. When comparing the F-value, the effect of extraction temperature on percentage yield was greater than the liquid-solid ratio. As a result, yield extract enhancement may occur when applying the highest temperature and the lowest LS ratio. Besides that, the coefficient calculation (+ 0.68) showed positive value at (BC) interaction.



(a) (b)

Figure 4.6: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of extraction temperature and LS ratio on the percentage yield at the constant time (12.50)

4.1.5.2. Effect of Time, Temperature, and LS Ratio on Quantification of Xanthorrhizol: Quantification of xanthorrhizol for all 17 extracts was carried out in the range from 72 % to 85.68 % in %w/w. Figures 4.7, 4.8, and 4.9 were displayed 3D (response surface plot) of two variables interaction (AB, AC, and BC) on quantification of xanthorrhizol respectively. The coefficient estimate for time (A) was + 1.39 which showed the highest significant effect on xanthorrhizol quantification as compared to other terms of the model. Figure 4.7 demonstrated the response surface showing the effect of extraction time and temperature on xanthorrhizol at a constant LS ratio (8mL/g). The interactive effect of ET (A) with temperature (B) on xanthorrhizol of extracts from C. xanthorrhiza was determined < 0.0001 (p-value). This means that the interactive effect of AB was significant model terms. Temperature of the extraction (F-value= 138.02) was shown to be less than the extraction time (F-value= 190.84). Increasing the extraction temperature with increased time, therefore, showed enhancement in xanthorrhizol quantification. A similar finding was recorded via Haldar et al., (2016). They studied phenolic compounds optimization from Curcuma longa at high temperatures (Haldar et al., 2016).

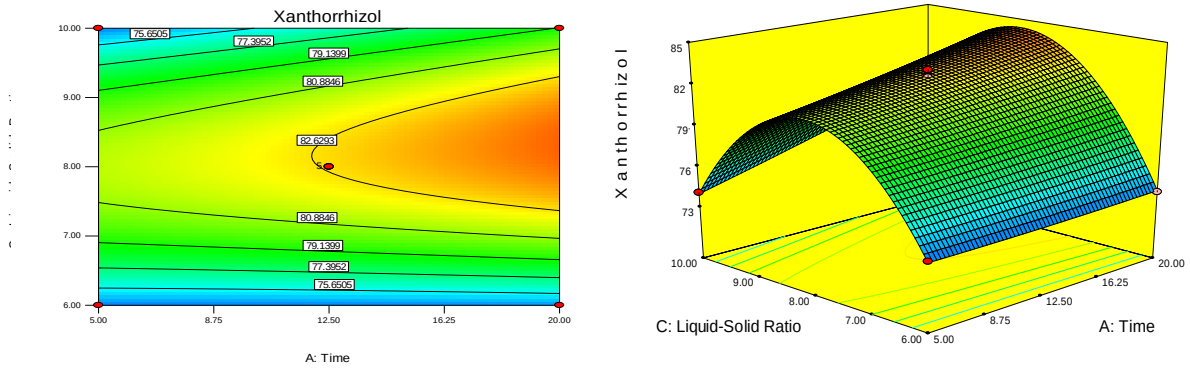


(a) (b)

Figure 4.7: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of ET and temperature on quantification of xanthorrhizol at constant LS ratio (8mL/g)

Figure 4.8 illustrated (a) contour plot and (b) response surface presenting the effect of ET (A) and LS ratio (C) on quantification of xanthorrhizol at constant temperature (40°C). The interaction of ET and LS ratio with a p-value of < 0.0001 was significant. By comparing the F-value, the impact of extraction time on xanthorrhizol quantification was significant than the LS

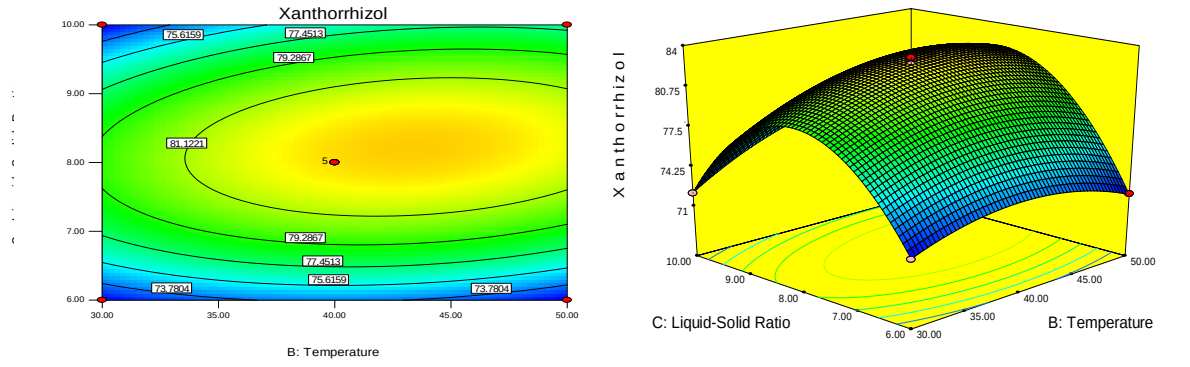
ratio. Table 4.5 reported the F-value of extraction time and the LS ratio was 190.84 and 157.37 respectively. This result is similar to finding by (Nur FauwizahAzahar et al, 2017). Accordingly, the surface plots showed that when performed at specified extraction time, the huge amount of total phenolic compounds can be obtained at an increased ethanol concentration. Such overall findings of phenolic constituents suggest the same pattern as found in Xu et al. phenolic constituent of tea (camellia Sinensis L.) fruit peel where the total phenolic content augmented with an increase in the processing time and independent variables of ethanol concentration till a maximum quantity of phenolic has been obtained. (Azahar et al., 2017).



(a) (b)

Figure 4.8: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of ET and LS ratio on the quantification of xanthorrhizolat constant temperature (40°C)

Figure 4.9 illustrated the 3D surface and a contour plot for the interactive effect of temperature (B) with a LS ratio (C). The interactive influence of temperature and the LS ratio was significantly contributed to xanthorrhizol quantification. For this reason, the p-value (< 0.0001) for the interactive impact of BC was less than 0.1000 indicate the model terms were significant. Moreover, the temperature F-value (138.02) was small as compared to the F-value LS ratio (157.37). The less difference in F-value was presented with the interactive impact of temperature and the LS ratio is being significant. As a result, there may be a drop in the volume of xanthorrhizol when applying the highest temperature and the lowest LS ratio. Meanwhile, AC was showed a positive coefficient estimate (+1.23). The surface plot displayed the highest xanthorrhizol could be attained at a low LS ratio in comparison with a high LS ratio at the constant time (12.50 min) and temperature (40°C).

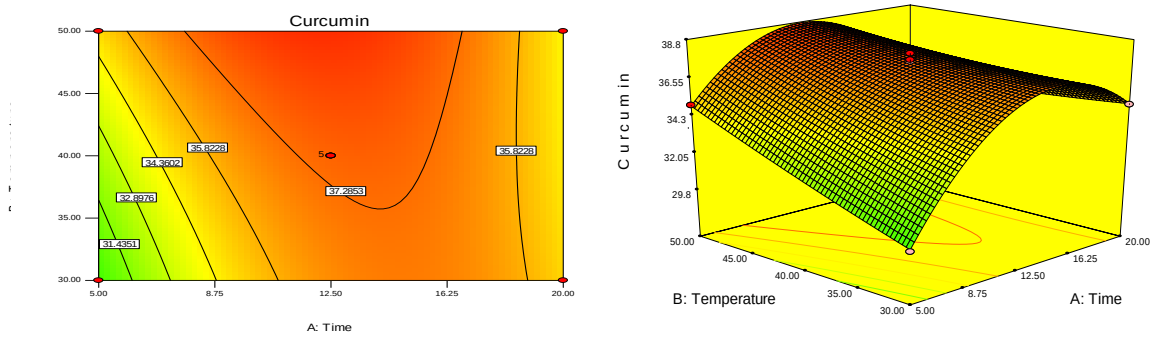


(a) (b)

Figure 4.9: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of extraction temperature and LS ratio on quantification of xanthorrhizol at the constant time (12.50)

4.1.5.3. Effect of Time, Temperature, and LS Ratio on Quantification of Curcumin: Figures 4.10, 4.11, and 4.12 displayed 3D (response surface plot) of two interaction variables (AB, AC, and BC) on the quantification of curcumin. The experimental data that presents quantification of curcumin from C. xanthorrhiza at various extraction conditions were ranged from 17.05 to 39.73 in %w/w of the extract. ANOVA analysis presented with probability the model F-value of 119.05 ($p < 0.0001$), which indicates the experiment was significant. Besides, there was only a 0.64% chance that a model F-value large may happen due to noise. Quantification of curcumin was significantly affected by interaction parameters (AB, AC, and BC), linear (A, B and C), and quadratic parameters (A2 and C2) with probability ($p < 0.05$). ANOVA analysis revealed that the most significant with $p < 0.0001$ on the quantification of curcumin was the liquid-solid ratio.

As shown in Figure 4.10, the 3D (response surface plot) presented the response surface plot as a function of time against temperature at a constant LS ratio (8 mL/g). The response surface plot reported which extraction time and temperature displayed a stronger impact on the quantification of curcumin. From this result, it has been shown that the increase in UAE extraction temperature and duration time leads to an increase in curcumin content. However, the findings of this analysis were different for the extraction time and temperature compared with other studies (Rodríguez-Pérez et al., 2015; Rocchetti et al., 2019, 2015; Wang et al., 2017). The difference may be attributed to the variation in the tree planting styles from different countries. This because C. xanthorrhiza might present the different quantities of phytochemical compounds and also the different range of parameters used for optimization extraction in this study. It may also be attributed to the extraction condition, different composition of Curcuma (various sources), an analytical technique used in the quantification of curcumin (Paulucci et al., 2013).

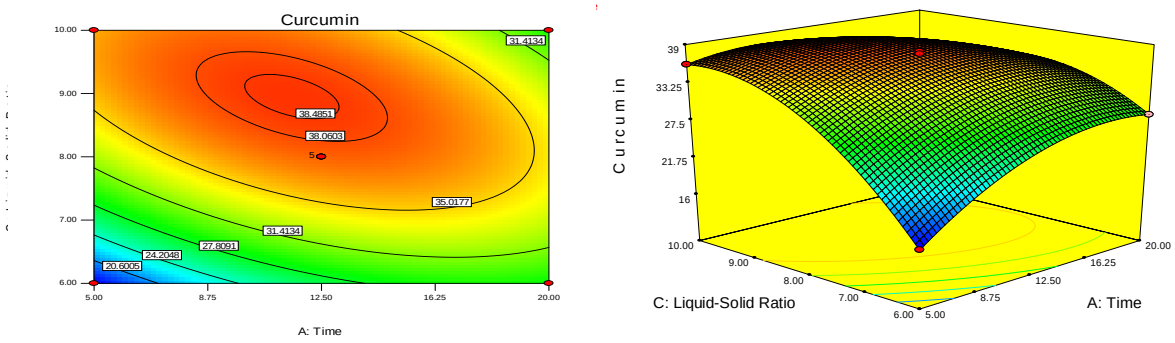


(a) (b)

Figure 4.10: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of ET and temperature on quantification of curcumin at constant LS ratio (8mL/g)

Figure 4.11 shows the plot of the surface reaction between the extraction time and the LS ratio at a constant extraction temperature (40 ° C). The interactive effect of extraction time and the LS ratio was greatly contributed to the quantification of curcumin. Because of this, the p-value (0.0001) for the interactive effect of AC was less than 0.0500 suggested that the terms of the model were significant. However, a negative value (- 4.63) was seen for the AC coefficient estimate. The response surface plots illustrated that the amount of curcumin included in C. xanthorrhiza generally was influenced by the LS ratio. In this study, the highest amount of curcumin can be obtained with the highest LS ratio (10 mL/g) as compared with run 17 with the same extraction time and 8 mL/g LS ratio. This result showed a similar result which has been

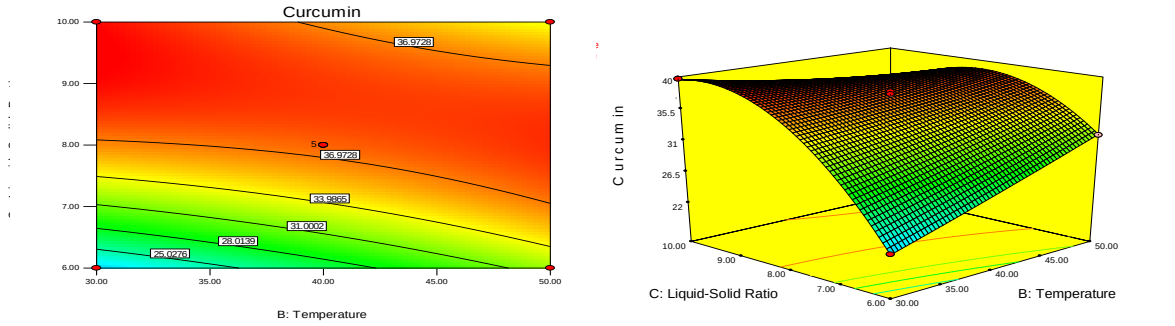
obtained by Wang et al, 2017. According to Wang and coworkers, when the concentration of ethanol was at a certain amount, the extraction content raised dramatically, with the LS ratio increasing from 4 mL/g to 7 mL/g. The finding is in agreement with the theory of mass transfer, guided via the difference of concentration in the liquid between the solid and the bulk. In the mass transfer process, it is observed that at a higher LS ratio the driving force increased (Wang et al, 2017).



(a) (b)

Figure 4.11: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of extraction time and LS ratio on the quantification of curcumin at constant temperature (40°C)

The 3D response surface plot in Figure 4.12 shows the relationship at a constant time (12.50 minutes) between the extraction temperature (B) and the LS ratio (C). This can be confirmed by the rise in curcumin by increasing the LS ratio from 6-10 mL/g (Altemimi et al., 2017). Contrary to this, the highest extraction temperature with the highest LS ratio enhanced in the amount of curcumin. It can be explained by greater particle collision leading to a breakup of the plant tissue. The findings obtained for quercetin are consistent with Jang et al., (2013)'s the previous study on solid onion wastes. The journal reported that the quercetin amounts increased when the extraction temperature goes up to 50 ° C and showed a decreasing trend quercetin above the optimum temperature point (Jang et al., 2013).



(a) (b)

Figure 4.12: Diagram demonstrating (a) contour plot and (b) response surface presenting the effect of extraction temperature and LS ratio on quantification of curcumin at the constant time (12.50)

4.1.6. Optimization and Verification of Model: Table 4.7 showed optimization of the variables analyzed to maximize the three responses simultaneously (percentage yield, quantification of xanthorrhizol, and curcumin.

Table 4.7: Optimal condition of independent variables for all responses

Response	Yield (%)	Xanthorrhizol (% w/w)	Curcumin (% w/w)
Experimental Optimal Condition	20 min	20 min	12.50 min
	50 °C	50 °C	30 °C
	8 mL/g	8 mL/g	10 mL/g
Actual Value	72.20	85.68	39.73
Suggested Optimal Condition	16.75 min	16.75 min	16.75 min
	46.65 °C	46.65 °C	46.65 °C
	8.23 mL/g	8.23 mL/g	8.23 mL/g
Predicted Value	72.63	84.49	36.99

4.2. HPLC Analysis of *Curcuma Xanthorrhiza*Extracts

Standard stock solutions for xanthorrhizol and curcumin were prepared and calibrations were obtained at 5 different concentrations (concentration ranges of 50, 100, 150, 200, and 250 ppm). The linearity of the calibration curve was demonstrated by R2 = 0.9934 for xanthorrhizol and R2 = 0.9986 for curcumin. Furthermore, the limit of detection (LOD) for xanthorrhizol was 71.41831 µg/ml whereas the limit of quantification (LOQ) was 216.41912 µg/ml and compound retention time was 12.063 min. Next, the curcumin limit of detection (LOD) was 6.81339 µg/ml whereas the limit of quantification (LOQ) was 20.64662 µg/ml and compound retention time was 5.840 min.

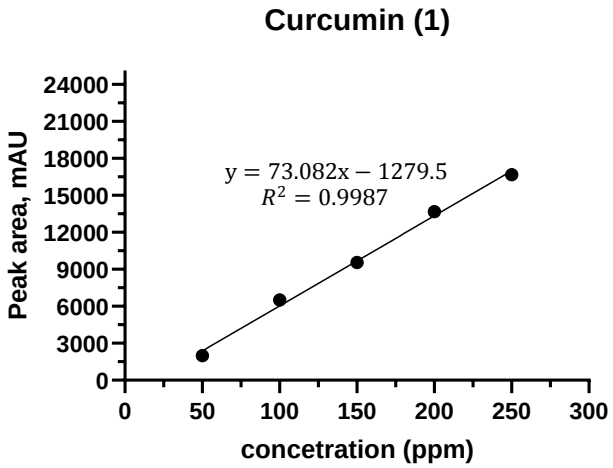


Figure 4.13: Calibration curve of standard curcumin

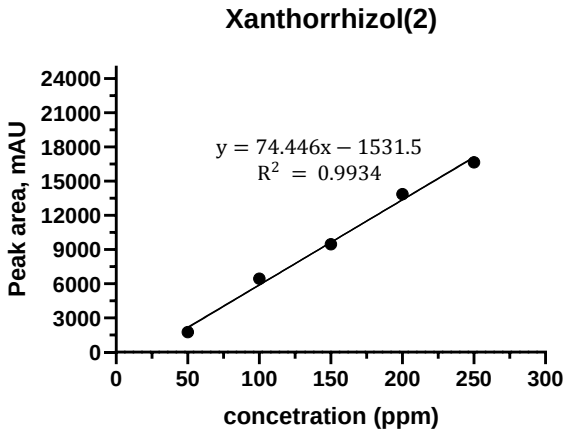


Figure 4.14: Calibration curve of standard xanthorrhizol

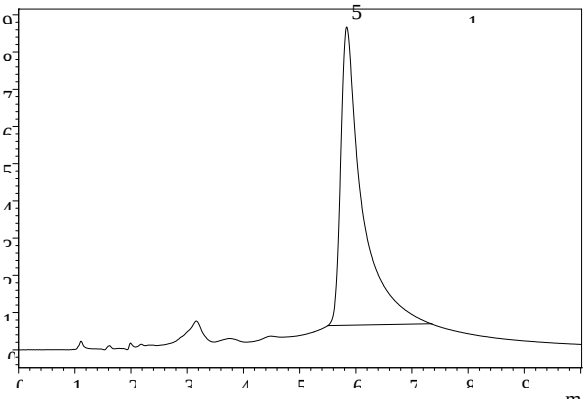


Figure 3.15: HPLC chromatogramof standard curcumin (1)

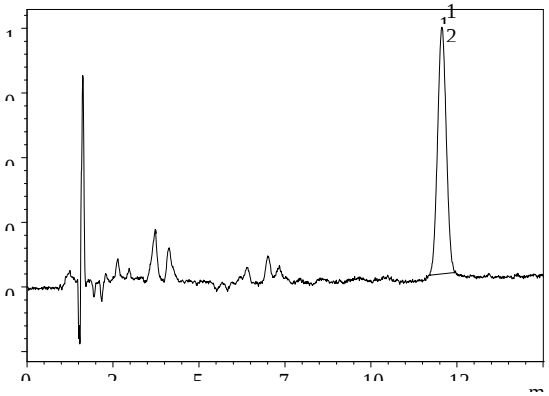


Figure 3.16: HPLC chromatogramof standard xanthorrhizol(2)

4.2.1. HPLC Quantification of *Curcuma Xanthorrhiza*Extracts: Targeted xanthorrhizol and curcumin have been detected by RP-HPLC Agilent Series 20 equipped with a Diode Array Detector (DAD). As can be seen from Figure 4.17, two peaks were observed at 270 nm (from 0-7 min) and 270 nm (from 7-12 min). The first peak was identified as curcumin (peak 1). The next peak was identified as xanthorrhizol (peak 2).

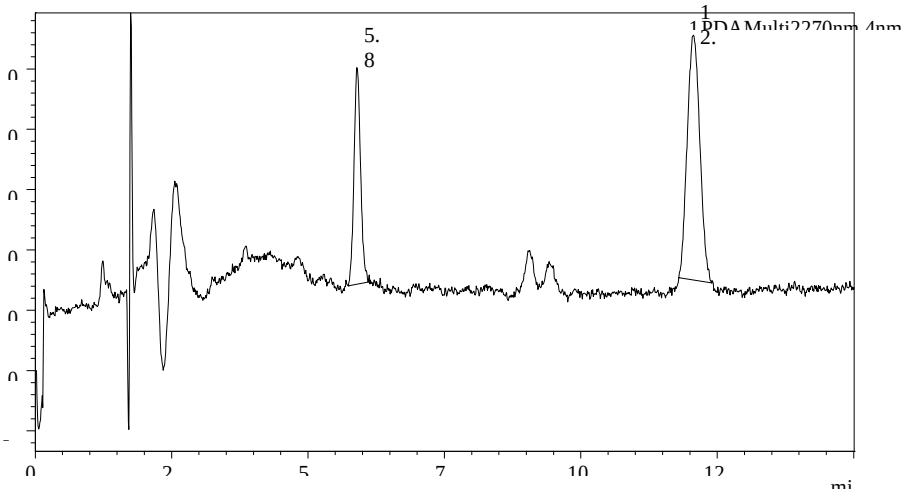
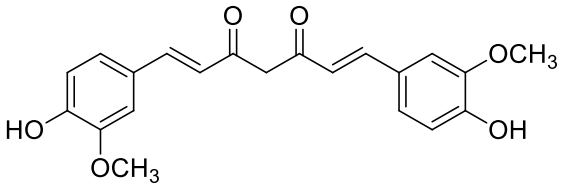


Figure 4.17: A chromatogram in *C. xanthorrhiza*extracts (Run 6

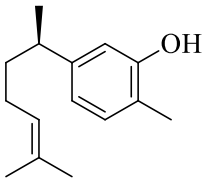
The highest amount of xanthorrhizol was detected under the following conditions extraction temperature 50 ° C, time of 20 minutes, and LS ratio 8 mL/g. The amount of xanthorrhizol ranged from 72 to 85.68 % w / w where the time and temperature had the maximum effect on xanthorrhizol which clearly shows that increasing the time and temperature increases quantification xanthorrhizol.

A small amount of curcumin in all C. xanthorrhiza extracts (between 17.05 and 39.73 in (%w/w) could be clarified by the degradation of curcumin under the ultrasound treatment. The ultrasound treatment demonstrated that curcumin decreases during the UAE. Because of chemical structures, curcumin was very thermolabile (Azahar et al., 2017) The highest curcumin content in this study obtained from rhizome extract under the condition of extraction temperature 30°C, time of 12.50 minutes, and 10 mL/g of LS ratio. It is confirmed that applied ultrasound conditions of temperature, time, and LS ratio did not highly impact the curcumin content. Figure 4.18: shows the chemical structures of curcumin (1) and xanthorrhizol (2). The presence of curcumin (1) and xanthorrhizol (2) and in the sample, as shown in Table 4.8 and

Table 4.9, was analyzed in terms of percentage in extract (w/w %) and percentage in the dried rhizome (w/w %).



(1)



(2)

Figure 4.18: chemical structures of curcumin (1) and xanthorrhizol (2)

Table 4.8: Quantification of curcumin (1) in extracts

Run	Percentage in extract (% w/w)	Percentage in the dried rhizome (%)
1	29.85 ± 0.425	2.985 ± 0.425
2	35.01 ± 0.119	3.501 ± 0.119
3	34.96 ± 0.073	3.496 ± 0.073
4	34.50 ± 0.071	3.450 ± 0.071
5	17.05 ± 0.107	1.705 ± 0.107
6	28.50 ± 0.275	2.850 ± 0.275
7	36.07 ± 0.071	3.607 ± 0.071
8	29.01 ± 4.399	2.901 ± 4.399
9	22.11 ± 5.324	2.211 ± 5.324
10	31.87 ± 0.168	3.187 ± 0.168
11	39.73 ± 0.030	3.973 ± 0.030
12	33.90 ± 0.030	3.390 ± 0.030
13	36.99 ± 0.089	3.699 ± 0.089
14	37.60 ± 0.140	3.760 ± 0.140
15	38.00 ± 0.139	3.800 ± 0.139
16	37.43 ± 0.141	3.743 ± 0.141
17	37.63 ± 0.060	3.763 ± 0.060

Table4.9: Quantification of xanthorrhizol(2) in extracts

Run	Percentage in extract (% w/w)	Percentage in the dried rhizome (%)
1	80.45 ± 0.252	8.045 ± 0.252
2	78.94 ± 0.215	7.894 ± 0.215
3	78.23 ± 0.219	7.823 ± 0.219
4	85.68 ± 0.122	8.568 ± 0.122
5	74.00 ± 0.086	7.400 ± 0.086
6	74.12 ± 0.172	7.412 ± 0.172
7	74.07 ± 0.058	7.407 ± 0.058
8	79.11 ± 6.574	7.911 ± 6.574
9	72.05 ± 3.791	7.205 ± 3.791
10	72.00 ± 0.149	7.200 ± 0.149
11	72.05 ± 0.209	7.205 ± 0.209
12	77.02 ± 0.022	7.702 ± 0.022
13	82.25 ± 0.086	8.225 ± 0.086
14	83.00 ± 0.208	8.300 ± 0.208
15	82.58 ± 0.183	8.258 ± 0.183
16	83.07 ± 0.159	8.307 ± 0.159
17	82.54 ± 0.077	8.254 ± 0.077

4.2.2. Correlation of Yield, Quantification of Xanthorrhizol and Curcumin: The top three ranks based on extract yield, quantification of curcumin (1) and xanthorrhizol (2) were summarized respectively in Figure 4.19, 4.20, and, 4.21.

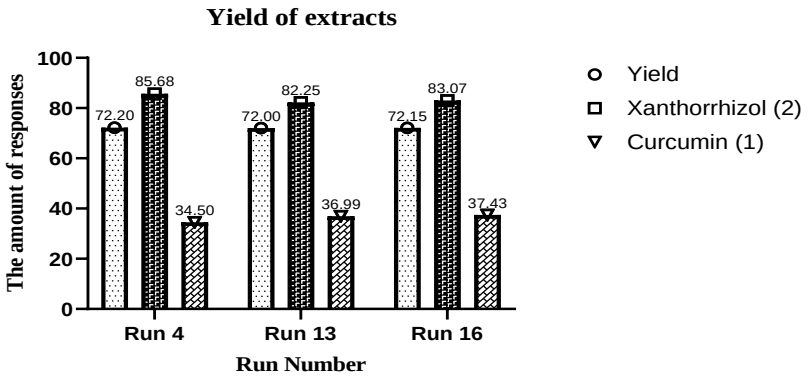


Figure 3.19: Rank of top three extracts based on yields

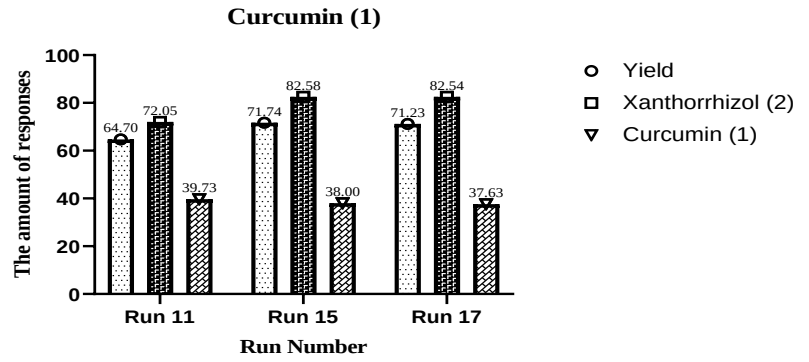


Figure 3.20: Rank of top three extracts based on concentration curcumin (1)

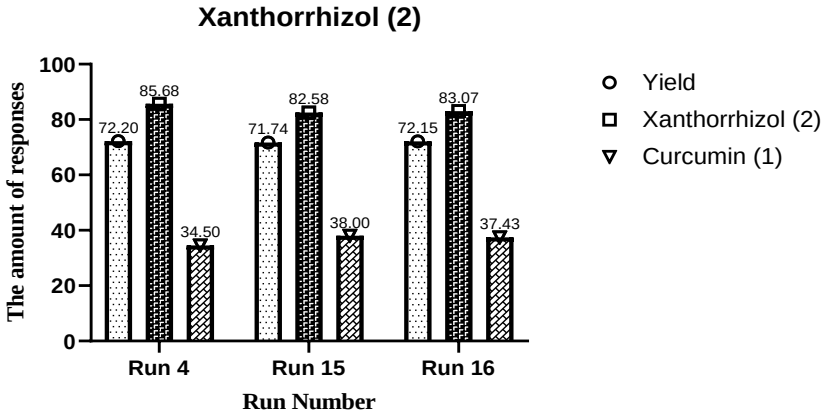


Figure 3.21: Rank of top three extracts based on concentration xanthorrhizol (2)

5. Conclusion

This research carried out on the ultrasound-assisted extraction (UAE) optimization procedure for the phytochemical compounds from C. xanthorrhiza via the Response Surface Methodology

(RSM). In the best of my knowledge in this study, the desirability of the impact of parameters on the extraction yield responses, the quantification of phenolic and sesquiterpenoid compounds, were established. Consequently, the highest percentage of yield (72.20 %) and the concentration of xanthorrhizol (85.68 % in %w/w) were found at the extraction temperature of 50°C, time of 20 minutes and 8 mL/g LS ratio. Nevertheless, a large difference in curcumin concentration (39.73 % in % w / w) with extraction temperature of 30 ° C, 12.50 minute and 10 mL/g LS ratio has been observed. The experimental values have been displayed closed values with the predicted values. The determination coefficient (R2) of extraction yield, xanthorrhizol, curcumin was 0.9990, 0.9986, and 0.9993 respectively. The analysis of variance (ANOVA) indicated a significant statistical and model fitting of the quadratic model. From the numerical data, the result suggested the RSM method's effectiveness in optimizing phytochemical compounds of C. xanthorrhiza. This research has been provided that the ultrasound-assisted extraction approach under specific parameters has favorable potential to be utilized in the extraction process. This extraction method can also enhance the efficacy impact on the yield and quantification of phytochemical compounds.

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