Response Surface Optimization of Yield of Agarwood (Aquilaria Malaccensis) Leaf Extract using Soxhlet Extraction

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Abstract ***Extracts from plant materials are continuously being tested using various techniques in the quest to find new therapeutic agents including from agarwood species. To date, the current existing method and parameters of extracting A. malaccensis leaves are still indefinite. Hence, this study was carried out to redefine the standard method of Soxhlet extraction by optimizing the parameters in order to maximize the yield of A. malaccensis leaves. The yields of Aquilaria malaccensis leaves extract (ALEX) were statistically optimized using Response Surface Methodology (Central Composite Design) with two factors: (A) extraction time (12, 15 and 18 hours) and (B) solid to solvent ratio (1:50, 1:60 and 1:70). The optimization of ALEX yield revealed that Run 5 had the highest yield of 184.482 ± 5.849 mg/g (18.45% wt/wt) with A: 18 hours and B: 1:70 while the lowest yield was at Run 12, 160.173 ± 15.342 mg/g (16.02% wt/wt) with A: 12 hours and B: 1:70. Subsequently, the analysis of variance (ANOVA) revealed that optimization study was well explained by a quadratic polynomial model (R2=0.7964 and Adj. R2=0.6510) implying the acceptable accuracy and general availability of the polynomial model. The data presented that only the effect of A was highly significant (P-value = 0.0123) towards the yield of ALEX although the interaction between variables A and B were significant as indicated by a small P-value=0.0220 (<0.05). Subsequently, the model validation showed that the experimental value accorded considerably well with the predicted value and ultimately the yield of ALEX was successfully optimized.***

Keywords: Agarwood, Aquilaria malaccensis, Extraction, Optimization, Soxhlet.

I. INTRODUCTION

The armamentarium of medicinal plants has often maintained their popularity for historical and cultural reasons. According to Mushtaq et al. (2018), World Health Organization (WHO) stated that 80% of the world's population are reliant to plant-based drug template for primary health care [1]. Thus, extracts from plant materials including from agarwood species are continuously being tested using various techniques in the quest to find new therapeutic agents. To date, *Aquilaria* species which is very well-known for its aromatic resin-containing heartwood (agarwood) has been reported to be one of the most valuable wood with high demand and price due to its myriad of applications particularly in medicine, perfume and incense [2]. Agarwood is known by various names based on cultures around the world such as gaharu or karas (Malay), oud (Arab), agar (India), jin-koh (Japan) and chenxiang (Chinese) [3, 4].

Aquilaria malaccensis is in genus *Aquilaria*, family *Thymeleaceae* and class *Magnoliopsida*; and a primary producer of the resin-impregnated agarwood. It is a fast-growing large evergreen forest tree, which can grow up over 15 to 30 m tall with 1.5 to 2.5 m in diameter [3]. *A. malaccensis* is widely distributed in South and South-East Asia and can be easily found in Malaysia. Leaves of *A. malaccensis* generally has a length of 5 to 11 cm and diameter of 2 to 4 cm with an elliptical blades shape [3, 5, 6]. The leaves have become recent interest in research due to its abundant supply and phytochemicals with functional groups relating to various pharmacological properties such as anticancer, antioxidant and anti-inflammatory [4,6,7].

Consequently, numerous studies involving the extraction of Aquilaria leaves employing different methods have been explored with the aims to improve the extraction efficiency and overall yield while preserving the desired compounds for biological assessment [3, 4, 7-10]. Nonetheless, there is still limited report on *A. malaccensis* leaves whilst its extract obtained from the current existing method is still indefinite to fulfil the market demand. The selection of the ideal extraction method frequently depends on several considerations in which the method should preferably produce high yield of the desired compound(s) with high purity, usage of non-toxic and safe solvents, environmentally friendly, rapid and low energy consumption as well as economically profitable and technologically feasible [11, 12]. The classical Soxhlet apparatus was designed by Franz Ritter Von Soxhlet, a German chemist in 1879 for the extraction of lipid. Later, the Soxhlet extractor was extensively used to extract various bioactive compounds from different natural sources. Soxhlet extraction is a standard model technique which is used as a reference extraction method to evaluate the performance of any new solid-liquid extraction approaches even for the most advanced methods due to its simplicity, capability to produce high yield,

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low cost per sample as well as inexpensive and robust extraction apparatus [11, 13]. Soxhlet method is used as a reference method in the US EPA official methods such as EPA 3540B and in the AOAC and British Standards; as well as being the recommended method for several analytical determinations [13]. Nevertheless, Soxhlet extraction is not suitable for extracting the thermolabile compounds since prolonged heating may expose the compound towards thermal degradation as the extraction only occurs at the boiling point of the solvent [12, 13]. It is therefore, the interest of this present study to redefine the Soxhlet method for extracting *A. malaccensis* leaves extract (ALEX) by optimizing the parameters in order to maximize the yield of which may facilitate in the scale-up of the extraction process

II. MATERIALS

A. Raw materials and sample preparation

Fresh leaves of non-inoculated *Aquilaria malaccensis* (agarwood plant which was not being induced or injected with microbial concoction for development of resin) were freshly harvested from local agarwood farm in Bangi, Selangor, Malaysia in February 2018. The leaves were identified according to their morphology and voucher specimen deposited at KAED Herbarium at the International Islamic University Malaysia. The leaves were thoroughly washed with running water and left to dry prior to drying 50^{o}C for 24 hours. The dried leaves were then mechanically ground into fine powder with a particle size of 0.2 mm using laboratory grinder. The powdered leaves were stored in Schott bottle wrapped with aluminum foil at the room temperature and protected from light until further extraction processes [14].

III. METHODS

A. Preliminary screening of Soxhlet extraction parameters

A preliminary screening was first conducted in order to determine the best set of parameters and value range for the ethanolic Soxhlet extraction method prior to optimization study. The extraction parameters chosen for screening were solid to solvent ratios of 1:30, 1:40, 1:50, 1:60 and 1:70 and extraction times of 3, 6, 9, 12, 15 and 18 hours at the constant sample weight of 6 g and particle size of 0.2 mm. The extract and excess solvent were evaporated under reduced pressure at 40^{o}C using a rotary evaporator (Heidolph-instruments, Rotavapor, Germany) to give concentrated crude ethanolic extracts. The weight of the extracts was measured after solvent evaporation. The extracts were then kept in the petri dish sealed with aluminum foil for further analysis [9, 15, 16]. The yields of ALEX were calculated by using the following Eq. 1 [11]:

$$y = \frac{w2}{w1} \qquad (1)$$

Where y: yield of extract (mg/g)
w1: weight of sample (g)
w2: weight of agarwood leaf extract (mg)

B. Optimization study of Soxhlet extraction parameters

Based on results from the preliminary screening, response surface central composite design (CCD) was generated using Design Expert version 7.0.0 software to optimize the Soxhlet extraction parameters. The experimental design involved two parameters: (1) extraction time (12, 15 and 18 hours) and (2) solid to solvent ratio (1:50, 1:60 and 1:70) using absolute ethanol as solvent with sample weight of 6 g and particle size of 0.2 mm. Meanwhile, yield of ALEX (mg/g) was the main response of the optimization study.

IV. RESULT AND DISCUSSION

A. Preliminary screening of Soxhlet extraction parameters

a. The effect of extraction time on the yield of ALEX

According to Rostagno and Prado (2013), extraction time is a parameter that is directly related to temperature. It is indicated that the increase of extraction time may increase the yield. However, the prolonged exposure of the solid material to high temperatures may cause the degradation of targeted compound(s) [11]. The best range set of Soxhlet extraction time against the yield of ALEX was assessed at a minimum of 3 hours and further prolonged up to 18 hours with 15 minutes per cycle which gave four cycles per hour for a constant solid to solvent ratio of 1:50. From Fig. 1, it can be observed that, the yield of ALEX gradually increased with increasing extraction times of 3 to 12 hours, 83.368 to 112.875 mg/g respectively. Interestingly, from 12 to 15 hours, the yield sharply increased from 112.875 mg/g to 166.096 mg/g. The yield started to slightly decrease from 166.096 mg/g to 160.105 mg/g after 15 to 18 hours of extraction.

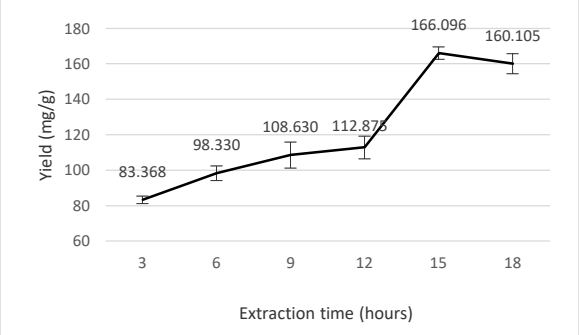


Fig. 1. The effect of extraction time (hours) on the yield (mg/g) of ALEX at the constant sample weight of 6 g, particle size of 0.2 mm and solid to solvent ratio of 1:50. The highest yield of ALEX was 166.096 mg/g (±s.d.), obtained at 15 hours of extraction. Each points represent the mean of ALEX yield, n=3, ±s.d.

This phenomena could be explained by the Fick's second law of diffusion, in which the final equilibrium between the solute concentrations in the solid matrix and in the bulk solvent might be reached after a certain time [17, 18], in this case after 15 hours of extraction resulting in deceleration of ALEX yield.

Furthermore, the declination of yield might be due to the decomposition and oxidation of certain bioactive compounds after long exposure towards the unfavourable environmental factors such as light, temperature and oxygen [17]. Therefore, extraction time of 15 hours was selected as the optimization point since the maximum yield of ALEX was obtained at this particular hour with a total of 60 cycles. Consequently, the extraction time range of 12, 15 and 18 hours were selected for further optimization studies of ALEX yield.

b. The effect of solid to solvent ratio on the yield of ALEX

The ideal solid to solvent ratio suggested by European Pharmacopeia is 1:10 g/ml in which the sample is expected to be completely covered by the solvent while sufficiently protecting the compounds from degradation [10]. Nevertheless, in the case of 500 ml Soxhlet chamber used in this present study, ratio of 1:10 and 1:20 were found to be insufficient for the extraction solvent to reach the overflow level in order to be rinsed back by siphon into the distillation flask which resulted the sample in the flask to be burnt. Therefore, the starting solid to solvent ratio used was 1:30 and the screening of solid to solvent ratios was carried out until the increment in yields of ALEX was no longer observed. For the screening of solid to solvent ratio, the extraction time was kept constant for 6 hours since it was observed that the colour of solvent in the thimble turned into colourless, its original colour indicated that maximum yield was extracted [9, 19].

Fig. 2 depicts the yield of ALEX extracted using 180, 240, 300, 360 and 420 ml of absolute ethanol for 6 hours with constant sample weight of 6 g which gave the solid to solvent ratio of 1:30, 1:40, 1:50, 1:60 and 1:70 respectively. It can be observed that the yields of ALEX were slightly and abruptly decreased when using ratios of 1:40 and 1:50 respectively. Interestingly, the maximum yield of 152.772 mg/g, was obtained at ratio of 1:60 and the screening was stopped after declination of the yield was observed when using ratio 1:70. The decrease of yield at ratio 1:70 (420 ml) could be explained due to the fact that heat was wasted on heating the solvent rather than the sample within that 6 hours. Moreover, when larger amount of solvent is used, the time taken for the solvent to reach its boiling point and condensed into the Soxhlet chamber becomes longer, thus reduces the number of solvent cycles as well as the extraction efficiency.

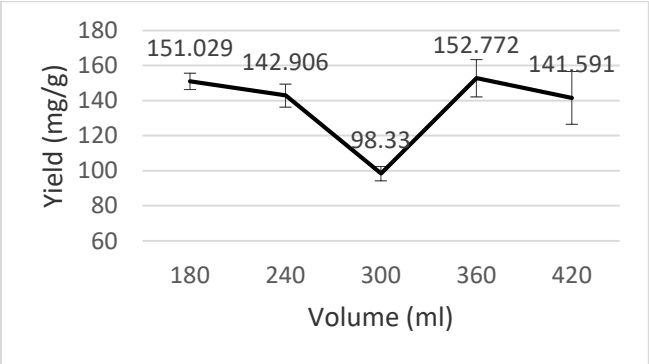


Fig. 2. The effect of solid to solvent ratio on the yield (mg/g) of ALEX at the constant sample weight of 6 g, particle size of 0.2 mm and extraction time of 6 hours. The highest yield of ALEX was 152.772 mg/g (±s.d.) obtained at 1:60 solid to solvent ratio. Each point represent the mean of ALEX yield, n=3, ±s.d.

Hazwan et al. (2013) stated that the ratio of solid to solvent is an important parameter in extraction process that need to be considered in order to achieve maximum extractability which can facilitate the scaling up process [20]. It is notable that the usage of large solvent volume would cause the extraction procedure to be more complex and complicated particularly causing difficulties for solvent removal. Conversely, smaller solvent ratio might lead to incomplete extraction of the bioactive compounds while also exposing the compounds to overheating [10, 20]. Thus, it is important for the extraction process to reach equilibrium in which the solute concentration in both phases, solid and solvent are equal. When the solid to solvent ratio is sufficient to cater the solubility of the solute, solution adhering to the solids will have the same concentration as liquid or solvent phase. Hence, within the contact or extraction time investigated, adequate amount of solid to solvent ratio should be determined prior to the extraction process. By increasing the solid to solvent ratio up to a specific limit, the yields of ALEX were observed to be increased. This could be explained by the greater concentration gradient between the solid and liquid phase which enhance the mass transfer rate [20].

B. Optimisation of Soxhlet extraction parameters

a. Statistical analysis of Soxhlet optimization

The optimization study attempted to determine the best factors for maximizing the yield of ALEX. The independent screening studies showed that extraction of ALEX using Soxhlet method with solid to solvent ratio (g/ml) of 1:60 and extraction time of 15 hours produced the highest yield and thus, were selected as the optimization point. Subsequently, the optimization study of Soxhlet extraction of ALEX was performed using response surface methodology (RSM), central composite design (CCD) with two factors, three levels and five centre points, generated using Design Expert software version 7.0.0 which gave a total of 13 runs. The parameters optimized were solid to solvent ratio (range of 1:50 to 1:70) and extraction time (range of 12 to 18 hours) at constant sample weight of 6 g, particle size of 0.2 mm with the studied response of ALEX yield (mg/g). Table 1 shows the actual and coded values for the optimization process in which each factor was coded at three levels; the low, medium and high levels of the independent variables denoted by -1, 0 and +1 respectively.

Table 1: The actual and coded independent variables at three levels, for the central composite design of the optimization Soxhlet extraction process.

Factor	Factor name (Units)	Factor coded levels		
		-1 (Low)	0 (Mean)	1 (High)
A	Extraction time (h)	12	15	18
***B**	Solid to solvent ratio (g/ml)	1:50	1:60	1:70

*Solid to solvent ratios of 1:50, 1:60 and 1:70 represent the volume of 300, 360 and 420 ml of absolute ethanol respectively for a constant sample weight of 6 g.

The optimization of ALEX yield using Soxhlet method (Table 2) showed that Run 5 had the highest yield of 184.482 ± 5.849 mg/g (18.45% wt/wt) obtained after 18 hours of extraction with solid to solvent ratio of 1:70 while the lowest yield was at Run 12, 160.173 ± 15.342 mg/g (16.02% wt/wt) obtained after 12 hours of extraction with ratio of 1:70. The adequacy and significance of the generated model and its terms were evaluated using analysis of variance (ANOVA). Several statistical analysis parameters were used such as F-value, P-value, coefficient of determination (R^2) and adjusted determination of coefficient (Adj. R^2). Meanwhile, the value of adequate precision was used as the indicator of the signal to noise ratio. For this present study, ANOVA revealed computed F-value of 5.48 (> 4.0) and P-value of 0.0228; which can be interpreted as a marginally significant outcome since this outcome translated to 97.72% confidence [21]. Further, the insignificant lack-of-fit of 0.8187 (> 0.05) relative to the pure error attained for the model, justified its suitability to predict the experiment.

Table 2: Response surface methodology design arrangement; response (yield) in actual and predicted values for Soxhlet extraction of ALEX. The yield (mg/g) of ALEX for 13 runs were based on two factors; Extraction time (h) and solid to solvent ratio (g/ml) at the constant sample weight of 6 g and particle size of 0.2 mm Results are presented as mean ±s.d. (n=3).

Std. order	Run number	Time	Solid to solvent ratio	Actual value	Predicted value
				Yield of ALEX [mg/g] ± SD	Yield of ALEX [mg/g]
6	1	15	0.0833333	171.662 ± 16.779	177.12
11	2	15	0.0833333	179.549 ± 19.732	177.12
5	3	15	0.0833333	170.816 ± 20.979	177.12
8	4	15	0.0833333	180.648 ± 10.229	177.12
9	5	18	0.0902778	184.482 ± 5.849	185.76
1	6	15	0.0902778	176.146 ± 10.338	172.87
12	7	12	1:50	170.663 ± 8.658	170.57
3	8	18	0.0833333	184.372 ± 5.580	183.91
2	9	12	0.0833333	174.437 ± 10.123	172.53
7	10	15	0.0833333	180.542 ± 6.611	177.12
4	11	18	1:50	170.564 ± 13.562	169.75
13	12	12	0.0902778	160.173 ± 15.342	162.17
10	13	15	1:50	168.164 ± 24.059	169.07

Table 3 showed that A: extraction time, AB: interaction terms between extraction time to solid ratio; and B^2: quadratic term for solid to solvent ratio were significant at $p<0.05$.The value of adequate precision which measures the ratio of signal to noise was 8.340, higher than 4 (Adeq precision > 4), indicating the sufficient signals were achieved and the model gave reasonable performance in prediction [22]. The positive sign in front of the terms describes the synergistic effects while the negative sign implies an antagonistic effect which influenced the independent variables of a response. Hence, the positive terms for the variables of; A: extraction time versus B: solid to solvent ratio, as presented in Table 3 (b) for both actual and coded final equations, +6.10*A*B and +0.034*A*B respectively showed their synergistic effects that would improve the yield of ALEX.

Accordingly, the response (yield of ALEX) of this optimization study was well explained by a quadratic polynomial model in which the value of R^2 and Adj. R^2 were 0.7964 and 0.6510 respectively; in which the proportion of variation explained by the model relative to the mean (overall average of the response) is acceptable, implying the strong correlation of the polynomial model [21, 23]. The positive sign of the coefficient indicated that the independent variables were directly proportional to the response variable and vice versa. Moreover, the coefficient for the yield of ALEX showed an acceptable agreement between the predicted and actual responses [23]. Fig. 3 (a) shows the normal plot of residuals in which the points on the graph represent the normal percentage probability of the percentage yields with respect to the residuals. It can be observed from the graph that, the distribution of points for the 13 experimental runs are approximately linear showing that the plot of residuals fit the expected pattern, implying normal distribution of the residuals [22]. Meanwhile, Fig. 3 (b) presents the relationship between the predicted and experimental (actual) values of the response which displayed an acceptable level of agreement implying that the model had a good equation fit.

Table 3: (a) ANOVA for Response Surface Quadratic model and (b) Final equations and processed factors for the yield of ALEX.

Note: *Significant at P < 0.05

(a) Analysis of variance (ANOVA)

Source	Sum of squares	Degree of freedom	Mean square	F-value	p-value (Prob>F)
Model	474.49	5	94.9	5.48	0.0228*
A: Time	194.31	1	194.31	11.21	0.0123*
B: Solid to solvent ratio	21.7	1	21.7	1.25	0.3001
AB	148.94	1	148.94	8.59	0.0220*
A^2	3.33	1	3.33	0.19	0.6744
B^2	104.52	1	104.52	6.03	0.0437*
Residual	121.3	7	17.33	-	-
Lack of fit	22.85	3	7.62	0.31	0.8187
Pure error	98.45	4	24.61	-	-
Corr total	595.79	12	-	-	-

(b) Response: Yield of ALEX

Final equation	Coded factors	Yield = +177.12 + 5.69*A + 1.9*B + 6.10*A*B + 1.10*A^2 – 6.15*B^2
	Actual factors	Yield = +126.293 – 13.966*A + 0.754*B + 0.034*A*B +0.122*A^2 - 0.002*B^2
R^2	0.7964	
Adj R^2 value	0.6510	
Pred R^2 value	0.4144	
Adequate precision	8.340	

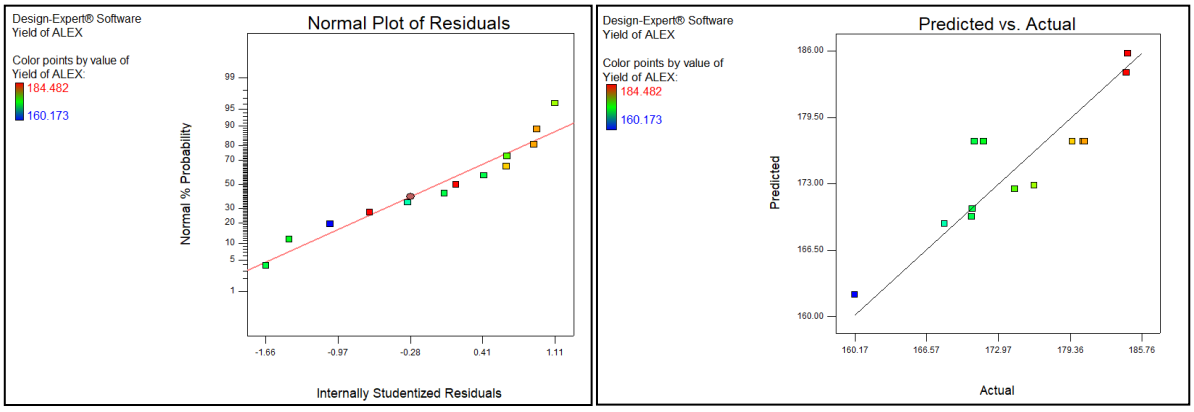


Fig. 3. (a) Normal plot of residuals presenting the summary of fit plot for experimental design (b) The relationship between the predicted and experimental (actual) values of the studied response.

On the other hand, Fig. 4 depicts the plot of externally studentized residuals which is also known as Outlier T. This plot presents the outliers (points that are distributed outside the control limit ranges (red lines)). This indicates that the points are not well fitted by the current model either due to the incorrectness of the value or the model. Thus, for this study, the outlier T detection showed that the data points of the experimental runs were located within the Design-Expert control limit ranges of the outlier T plot of +4.56 and –4.56 of standard deviation for the yield of ALEX. Consequently, no outlier requires to be removed in this model since the normal plot implies the model was normal and acceptable. In addition, the randomly distributed points which showed no pattern, indicated that the model was suitable to the data and satisfied the independent normally distributed residuals which are usually assumed [21, 22].

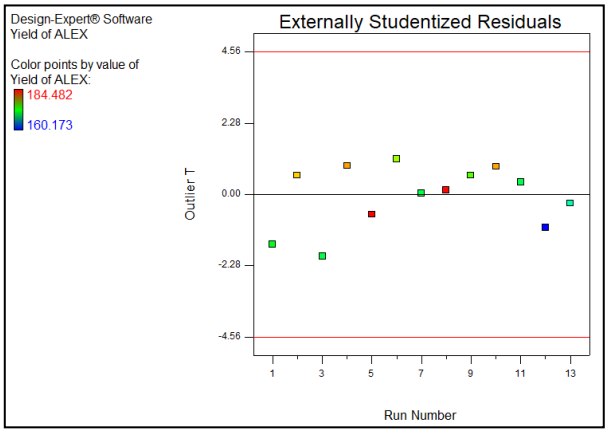


Fig. 4. Externally studentized residuals plot presenting that all 13 experimental data points of the studied response were located within Design-Expert control limit ranges of the outlier T plot of + 4.56 and – 4.56 of standard deviation for the yield of ALEX.

b. Effect of Soxhlet extraction parameters of the yield of ALEX

The effects of the Soxhlet process variables in this optimization study; A: extraction time and B: solid to solvent ratio on the studied response; on the yield of ALEX are presented through the perturbation plot, response surface and contour plot. The perturbation plot helps to compare the effect of all variables at a particular point in the design space. This plot is like "one factor at a time" experimentation which does not show the effects of interactions. The coded units which shows the position relative to the coded (-1 to +1) scale at x-axis of the plot reflect the distance of the experimental value from the reference. Based on the plots in Fig. 5, the data clearly demonstrate that the yield of ALEX increased linearly with extraction time. Conversely, the yield of ALEX improved when the solid to solvent ratio was increased up to 0.5 before a decline was observed. Hence, both of the steep slope and curvature of variables A and B respectively indicate that the response was sensitive to the process variables.

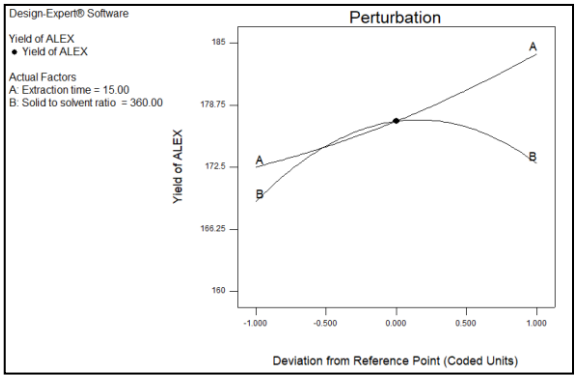
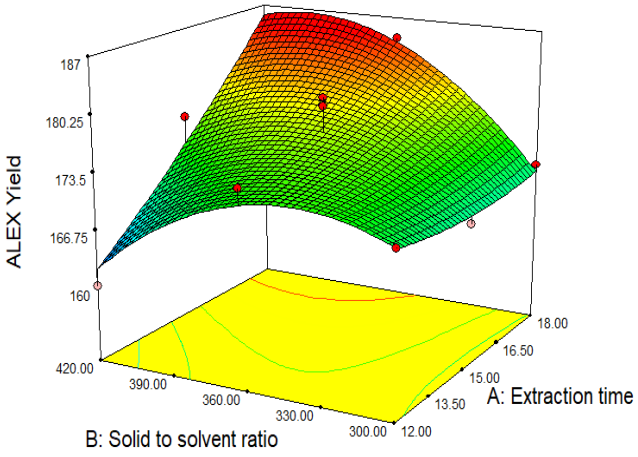


Fig. 5. The deviation of the reference point for the yield of ALEX value for the effect of A: extraction time and B: solid to solvent ratio.

Similarly, the surface and contour plots in Fig. 6 (a) and (b) respectively show that the yield of ALEX is directly proportional with extraction time. The yield of ALEX was further increased with increasing solid to solvent ratio. This might be due to the longer time of extraction resulting in more cycles of fresh solvent available to extract the bioactive compounds from solid materials which led to a higher yield. Table 3 (a) revealed that the interaction between variables A and B were significant as indicated by a small P-value of 0.0220 (< 0.05). The data also presented that only the effect of extraction time was highly significant with P-value of 0.0123 while solid to solvent ratio was not significant, towards the yield of ALEX. This is also supported by the fact that the first and second highest yields of ALEX, Run 5 (184.482 mg/g) and Run 8 (184.372 mg/g) had the difference by only 0.110 mg/g regardless of being extracted at different solid to solvent ratios of 1:70 and 1:60 respectively for similar extraction time of 18 hours. Furthermore, the present study also noted that Run 12 carried out at shortest extraction time of 12 hours with the highest solid to solvent ratio of 1:70 produced lowest yield of ALEX (160.173 mg/g). Therefore, the data evidently indicates that extraction time plays crucial role in affecting the yield of ALEX in which a maximum yield of ALEX was achieved at longest extraction time irrespective of solid to solvent ratio. Nevertheless, the required extraction time varies depending on the type and part of plant material as well as extraction equipment [10].

The contour plot in Fig. 6 (b) shows that the direction of optimization point was towards the highest point of extraction time and solid to solvent ratio. The plot also describes that extraction process conducted between 17 to 18 hours and solid to solvent ratio (g/ml) between 1:60 (360 ml) and 1:70 (420 ml) yielded the highest ALEX (mg/g). Yield of ALEX was increased with increasing extraction time due to a higher accessibility of solvent molecules and more compounds which able to permeate into the ethanol within that sufficient time while lowest yield attained when extracting for 12 hours might be due to insufficient time for the solvent to solubilize and extract all the bioactive compounds out of the cells to the fluid medium [10]. Moreover, increasing the extraction time, might enhance the diffusion rate thus, reducing the viscosity and surface tension of the solvent [11, 18]. The swelling of plant cell wall for a longer time might increase the penetration of the solvent into the plant material, yielding a higher leaves extract [9].



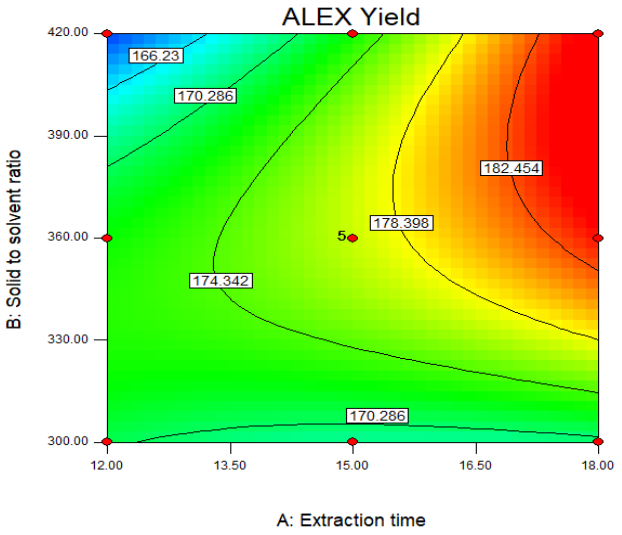


Fig. 6. Response (a) surface plot and (b) contour plot describing the effects of A: extraction time and B: solid to solvent ratio towards the main study response, yield of ALEX.

Nevertheless, longer or prolonged extraction time might interfere the stability of bioactive compounds. This is because, Soxhlet is operated at the boiling point of the extraction solvent used. Henceforth, the compounds would continuously be in contact with the solvent at high temperature. Consequently, the compounds with lower boiling point or thermolabile might be exposed to the chemical and thermal degradation. In addition, prolonged extraction time would also increase the decomposition of bioactive compounds due to the long exposure towards light and oxygen. Combination of such changes would greatly reduce the mass transfer during extraction. These circumstances would usually reduce the efficiency of the Soxhlet method and the quality of the extracted compounds [11].

Meanwhile, the plot also indicates that the lowest yield was obtained at extraction time between 12 to 13 hours with solid to solvent ratio (g/ml) between 1:65 (390 ml) and 1:70 (420 ml). Therefore, it suggests that 18 hours is the ideal time to extract maximum bioactive compounds from 6 g of powdered *A. malaccensis* leaves when using 420 ml ethanol. However, it is noteworthy that prolonged extraction time may increase the yield of ALEX but may result a low quality of bioactive compounds. Therefore, in the case of extracting the target compounds which is thermosensitive, longer extraction time is not advisable. Other than that, increase of the solid to solvent ratio also may be irrelevant for Soxhlet method since the process allows the recycle of fresh extraction solvent.

Likewise, previous studies revealed that the yields of Soxhlet extract were highly influenced by the extraction times regardless of the solvent types and solid to solvent ratios [18, 24, 25]. Sulaiman et al. (2015) observed that agarwood oil extracted using Soxhlet for 6, 9, 12 and 16 hours with n-hexane as solvent yielded 0.60%, 1.20%, 1.60% and 1.67% respectively [24]. Meanwhile, significant difference of percentage yields, 3.60%, 8.26% and 19.19% were obtained after 4, 6 and 8 hours of extraction using 95% ethanol [18]. Thus, a longer extraction time led to a higher percentage of extract due to the longer time of contact between the solute and solvent. The longer contact time facilitated the process to have greater mass transfer. Nevertheless, excessive extraction time would be unnecessary as the solvent and sample would be in the final equilibrium after certain duration based on Fick's second law of diffusion. After certain time, the extraction rate of the compounds would decelerate [17, 18].

c. Validation of experimental design

Table 4 compared the yield of *A. malaccensis* leaf extract using different extraction method. It can be concluded that in terms of yield, the ethanolic ALEX in this present study was successfully optimised by two-fold higher than the yields reported in the previous studies.

Table 4 Comparison of *A. malaccensis* leaf extract yield using different extraction methods

Extraction methods	Solvent	Percentage yield (wt/wt %)	Reference
Soxhlet	Methanol	9.20	[26]
	Ethanol	10.00	[9]
		18.45	[present work]
Maceration	Water	7.28	[27]
		52.12	[3]
		2.10	[9]
Hydro distillation		0.05	[10]
Ultrasound-assisted		3.83	[28]

Validation of the model equation to predict the optimum response value was performed using the optimization function in Design Expert® 7.0.0 software. The optimized condition was established with the aim of minimizing the extraction time and solid to solvent ratio while maximizing the yield of ALEX. Thus, the model was validated at extraction time of 12 hours and solid to solvent ratio of 1:50 to obtain the predicted yield of 173.295 mg/g. The average experimental value for the triplicate yield of ALEX obtained as presented in Table 5 was 162.116 mg/g which accorded quite well with the predicted value with standard deviation and percentage error of 7.905 and 6.451% (<10%) respectively implying that optimization by the CCD model can be considered accurate and relevant.

Table 5: Standard deviation and percentage error of the experimental (actual) yield from predicted yield of ALEX.

Time [h]	Solid to solvent ratio	Pred yield [mg/g]	Sample	Yield (mg/g)		Design (actual) ± SD	Percentage error [%]
				Triplicate	Average; ± SD		
12	1:50	173.295 (17.33%)	R1	159.674	162.116 ± 3.401 (16.21%)	7.905	6.451
			R2	166.001			
			R3	160.673			

V. CONCLUSION

In conclusion, the findings from this present study revealed that the extraction time of Soxhlet process showed signifcant effect towards the yield of *Aquilaria malaccensis* leaves extract (ALEX). Conversely, the solid to solvent ratio did not show significant effect on ALEX although the interaction between variables were significant. The optimization study was well explained by a quadratic polynomial model with a value of R^2 of 0.7964 implies that the model was accurate. Subsequently, the model validation showed that the experimental value accorded considerably well with the predicted value. Ultimately the Soxhlet extraction process of *A. malaccensis* leaves and its yield were succesfully redefined and optimized.

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