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Extraction of essential oil from kaffir lime (*Citrus hystrix*) leaves using microwave-assisted extraction

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Abstract

Citrus hystrix DC or normally known as kaffir lime is a citrus species from the Rutaceae family with valuable properties that have attracted the interest of the public. Not only that but the leaves and fruits of the kaffir lime also have a diverse range of essential oils and are therefore used for essential oils extraction. The potential for the commercialisation of kaffir lime essential oil on a large scale in the industry remains a challenge due to the low yield of essential oils produced by conventional methods. In this study, microwave-assisted extraction (MAE) has been chosen as an excellent alternative for essential oil extraction to replace conventional industrial methods. The objectives of this study were to identify the optimum particle size, extraction time, and percentage of co-solvent used for obtaining high extraction oils yield and analyse the volatile compounds of extracted kaffir lime essential oils by using gas chromatography-mass spectrum (GC-MS) analysis. Throughout the extraction, the mass of the sample and power in the MAE process remains constant at 6.0 ± 0.003 g and 600 W, respectively. Therefore, the optimisation of the particle size (400–600 µm), percentage of ethanol (10–20%), and extraction time (5–15 min) were made in this study by using Response Surface Methodology (RSM) to achieve the high quality of essential oils. The mathematical models proposed for the study are the quadratic model for both extraction yield and mass of fatty acid recovered and the two-factor interaction for the mass of ester recovered. The optimum essential oil yield was determined as the particle size is 400 µm, extraction time of 14.95 min, and percentage of ethanol is 14.07% resulting in a yield of 23.54%. The GC-MS analysis resulted in the identification of almost 94 compounds for each of the samples. The identified major compounds from kaffir lime leaf oils were esters and followed by fatty acids.

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1.0 Introduction

According to Grand View Research (2020), the global essential oils market is forecast to rise from 2020 to 2028. One of the major forces influencing the global essential oils market is rising demand from major end-use industries such as aromatherapy, food and beverage, cosmetics, and pharmaceuticals. On the other hand, an increase in consumer awareness of natural and organic personal care products has led manufacturers to focus on natural products rather than synthetic ones (Mohammad & Baharun, 2017). This highlights the significance of the essential oils industry in achieving market demand. Aromatic plants have been utilised and recognised as valuable sources of essential oils for many years. In Malaysia, *Citrus hystrix* DC, also known as kaffir lime, is an aromatic plant that is widely used for research and commercialisation purposes (Quyen et al., 2020). These species are used for extraction sources because their fruits, leaves, and seeds contain a diverse range of essential oils with distinct flavours and biologically active compounds (Md Othman et al., 2016). As the essential oils can be extracted from various parts of the

plant, kaffir lime leaves are being used as the source to extract essential oils in this study.

In recent years, there have been numerous methods for extracting essential oils, ranging from conventional to modern methods. The use of conventional methods, such as steam distillation and hydrodistillation for the extraction of essential oils is common because they are simple and easy to implement. Despite that, the microwave-assisted extraction method is preferred in this study because it can produce high quality essential oils (Suresh et al., 2020). Furthermore, the extraction method and co-solvent used to extract the essential oils are also important factors in determining the quantity and quality of essential oils produced. Apart from that, extraction time and particle size used also play an important role in determining the optimum parameter for obtaining the highest essential oils yield through MAE. Thus, this study aims to identify the optimum particle size and extraction time in obtaining a high extraction yield through this method.

The co-solvent is an organic solvent that is added to the extraction process to enhance extraction efficiency. Hence, it was necessary to determine the optimum percentage of co-solvent to obtain high purity of essential oils. The co-solvent used in this study is ethanol. Therefore, the optimisation of the particle size, percentage of ethanol, and extraction time used were done by using Response Surface Methodology (RSM).

RSM is a set of mathematical and statical processes for studying the relationship between several variables (independent variables) and one or more responses (dependent variables) (Kusuma, et al., 2015). RSM is a valuable technique for optimising complex processes in which several factors and interactions influence the responses (Loh et al., 2005). Besides, RSM enables the user to determine the optimum parameters for a selected response while reducing the number of experiments required.

In general, pure essential oils can be categorised into two distinct groups of chemical constituents which are hydrocarbons and oxygenated compounds. Monoterpenes, sesquiterpenes, and diterpenes make up the majority of the hydrocarbons. Meanwhile, oxygenated compounds mostly consist of esters, aldehydes, ketones, alcohols, phenols, and oxides. According to some studies, the major volatiles compounds found in kaffir lime leaves belong to the monoterpene group (Zielińska-Błajet & Feder-Kubis, 2020). Monoterpene groups are the most abundant class of plant secondary metabolites, and they have been shown to exhibit antimicrobial activity and antioxidant biological activity.

The major compounds of the extracted essential oils that are being studied are fatty acids and esters. Servi et al. (2018) stated that the fatty acid composition is rich in polyunsaturated fatty acids such as (Z, Z)-9,12- octadecadienoic acid methyl ester (40.4%), (Z)-9-octadecenoic acid methyl ester (35.0%), and hexadecanoic acid methyl ester (13.0%). Apart from that, the quality of the extracted essential oils can be analysed by using gas chromatography-mass spectrum (GC-MS) analysis. Therefore, the objective of this study is to analyze the major compounds of extracted kaffir lime essential oils.

2.0 Methodology

2.1 Material

Fresh kaffir lime leaves were purchased from a local market in Shah Alam, Selangor. The chemicals used in this study were 10%, 15%, and 20% of ethanol, methanol (CAS no: 67-56-1, ≥ 99.8% purity, Merck), and *n*-hexane (CAS no: 110-54-3, ≥ 99.7%, Merck).

2.2 Sample preparation of kaffir lime leaves

First, the fresh kaffir lime was separated and washed several times with tap water. It was then dried at room temperature for three days until it reached constant weight. The dried leaves samples were then ground in a commercial blender before sieving to an average particle size of 400–600 μm using a digital sieve shaker (Endecotts Octagon 2000). Following that, the sample was packed into airtight packaging and stored in a chiller before proceeding to the extraction process.

2.3 Microwave-assisted extraction (MAE)

The kaffir lime leaves were extracted using a modified microwave oven (NN-D5592B, Panasonic). Fig. 1 illustrates the arrangement of the experimental apparatus. Approximately 6.0 ± 0.003 g of ground samples (X_1) were weighed using an analytical balance. The samples were placed in a tea bag before being transferred to a 500 mL round bottom flask. The solvent mixture of distilled water and ethanol was then added to the flask following the specified ratio (X_3). The flask containing the sample and the solvent was placed in the microwave oven. The microwave oven was switched on and the required time (X_2) and power conditions were set to enable the heating of the sample and the vapors to be produced.

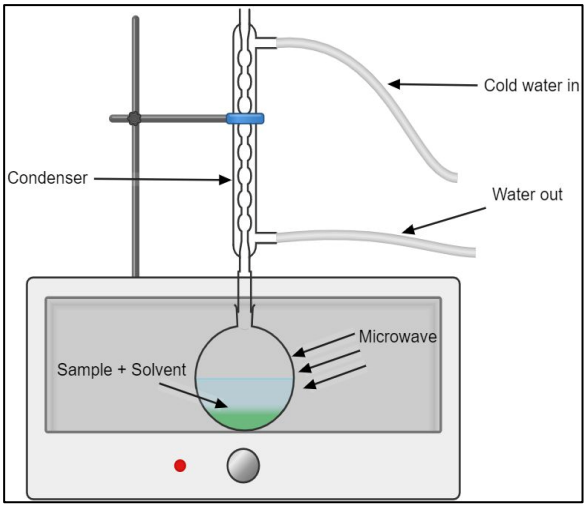

Fig. 1: The experimental setup of MAE

The vapor ascended through the neck of the flask until it reached the condenser, where the vapors were cooled, and the extracted liquid was collected in a trap. After the extraction time, a collection sample of extracted essential oil was filtered with filter paper. The solvent and oil were then separated using a rotary evaporator (Laborota 4000 efficient, Heidolph). Thus, the yield of essential oils extracted was calculated by using Eq. (1):

$$\text{Percentage yield} = \frac{\text{Weight of EO}}{\text{Weight of sample}} \times 100\% \quad (1)$$

where the weight of essential oils and the weight of the sample are in grams.

2.4 GC-MS analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyse the volatile compound of the extracted essential oils. The essential oils were analysed using GC-MS (450-GC coupled with 240-MS, Varian). The column used for this study was HP-5ms Capillary Columns (Agilent). First, the extracted essential oils were mixed with 4 mL of *n*-hexane and 4 mL of methanol. The head column pressure was set to 9.3 psi and the carrier gas was helium with a constant flowrate of 1.0 mL/min. Injector temperature was set at 250 °C with a volume of 1 µL of methanolic extract at a split mode ratio of 100:1. The MS temperature was set at 280 °C with mass range recorded band of 45–450 mass ratios with electron energy of 70 eV. The temperature was kept constant at 60 °C for 3 min and gradually increased to 180 °C at a rate of 5 °C/min and finally dropped to 50 °C. The volatile compounds in the extracted essential oils were analysed by comparing their mass spectra with database libraries.

2.5 Design of experiment

To optimise the experimental parameters for the extraction of essential oils from kaffir lime leaves, a three-factor and three-level Box-Behnken design (BBD) was applied in this study using Design Expert software (Version 13). The design was selected to reduce the number of experimental runs to save time, cost and resources as compared to a full-factorial design. The independent variables were particle size (X_1), extraction time (X_2), and percentage of ethanol (X_3). This design was used to study the effect of three different parameters on the response, including the extraction yield (Y_1), the contents of fatty acids (Y_2), and esters (Y_3). A total of 17 runs were conducted according to the design layout.

3.0 Results and discussion

This section represents the findings that were obtained from the analysis and the optimisation of the three parameters that affect the MAE process using response surface methodology (RSM). The BBD design of experiments and the responses are presented in Table 1.

3.1 Effect of particle size

The yield of essential oils in different particle sizes at constant power and weight of the sample was presented in Fig. 2. The effects of particle size against the yield of essential oils were measured by manipulating the extraction time from 5, 10, and 15 minutes. The microwave power and weight of the sample were kept constant at 600 W and 6 g, respectively for each run.

Fig. 2 shows the effect of particle size on the yield of essential oils from kaffir lime leaves. As the particle size increased from 400 µm to 600 µm, the yield of the extraction of the essential oil drastically decreased by 32.08% from 23.52% to 15.97%. This is due to the increase in surface area when the leaves were ground into smaller size, which allows for greater interaction between the plant matrix and the solvent. It will increase the effective heat transfer area and eventually reduce the mass transfer resistance to the oil solvent mixture from the oil sac to the solution. Under the influence of microwave radiation, these phenomena will rapidly push the essential oils out resulting in higher performance (Tran et al., 2018). Therefore, the yield of the essential oil increased primarily due to the sample size reduction.

Table 1: Experimental results

Run	Particle size (μm), X_1	Extraction time (min), X_2	Percentage of ethanol (%), X_3	Yield (%), Y_1	Mass of fatty acids (μg fatty acids/g sample), Y_2	Mass of esters (μg esters/g sample), Y_3
1	500	5	10	18.61	386.35	826.45
2	600	5	15	16.95	482.55	1154.53
3	400	5	15	13.56	860.87	2109.96
4	500	5	20	10.00	816.38	2457.70
5	600	10	10	11.69	852.14	1532.27
6	400	10	10	17.85	708.68	1486.56
7	500	10	15	20.12	356.55	1543.77
8	500	10	15	13.64	414.82	1011.91
9	500	10	15	14.04	211.95	1697.03
10	500	10	15	13.58	537.31	1276.02
11	500	10	15	20.45	440.70	1953.22
12	400	10	20	10.67	722.14	1390.96
13	600	10	20	10.01	949.67	1745.53
14	500	15	10	15.00	502.25	1638.86
15	600	15	15	15.97	677.58	1704.55
16	400	15	15	23.52	316.11	816.244
17	500	15	20	19.73	692.07	1541.53

However, when the extraction time was increased from 5 min to 15 min, the yield of the essential oils increased gradually from 13.56% to 23.50%. This occurs because of the increased thermal accumulation of microwave energy within the solvent as the extraction time increases. Tran et al. (2018) studied the optimisation of MAE of essential oil from Vietnamese basil using RSM and reported that the yield of the essential oil increases from 0.2% to 0.5% as the basil leaves size changes from whole to ground leaves.

3.2 Effect of extraction time

The extraction time was varied between 5 min, 10 min, and 15 min while maintaining the solvent volume and microwave oven power constant at 150 mL and 600 W, respectively. The effect of extraction time on the yield of essential oils was shown in Fig. 3.

The yield of extracted oils slightly dropped as the extraction time increased from 5 min to 15 min from 18.61% to 15.00%. This is because prolonged exposure of the sample to the intense microwave radiation resulted in the degradation of thermolabile components in the oils (Llompart et al., 2019). In addition, overexposure to microwave radiation causes the extraction system to overheat (Dejkajorn et al., 2021) and causes the chemical structure of the active compounds to degrade.

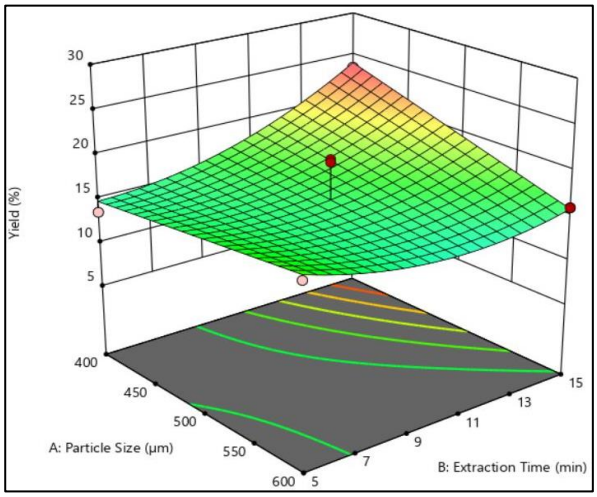

Fig. 2: Response surface for the effect of particle size on the yield of essential oils

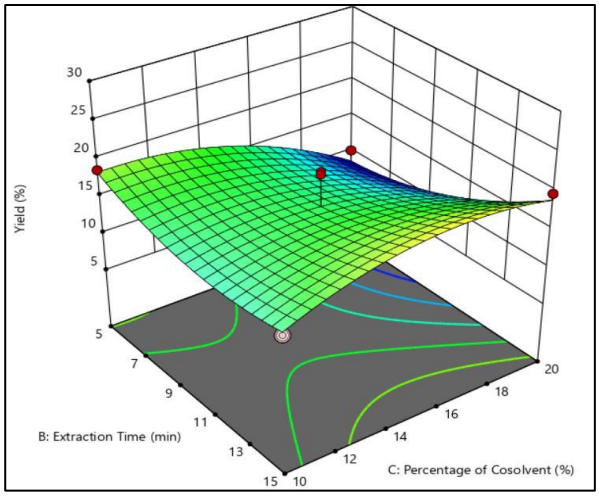

Fig. 3: Response surface for the effect of extraction time on the yield of essential oils

Consequently, a shorter time is required to produce a greater yield of the essential oils. Therefore, the greater yield of the essential oils extracted from the samples with increasing solvent concentration over the same extraction time. Results of the essential oil yield from kaffir lime leaves are similar to those reported by Tran et al. (2018) for the extraction of essential oil from basil leaves, in which the yield of extracted essential oil gradually increased from 0.2% to 0.7% at 90 min but decreased to 0.4% at 105 min.

3.3 Effect of percentage of solvent

In this study, a range of percentages of the solvent of 10%, 15%, and 20% were used, while microwave oven power was fixed at 600 W. The effect of the percentage of solvent on the yield of essential oils was illustrated in Fig. 4.

Fig. 4 shows the yield of the essential oils increases as the percentage of ethanol are increased from 10% to 15%, and then gradually decreases from 23.52% to 19.73% as the percentage of ethanol increase from 15% to 20%. This is due to the concentration gradient during mass transfer within a solid increase as the percentage of solvent increases (Che Radzi et al., 2018), resulting in higher diffusion of essential oil into the solvent. This leads to an increase in oil yield until it reaches its maximum point at 15% of solvent.

Therefore, after reaching its maximum point, the yield of essential oil was found to decrease from 15% to 20%. This could be because the concentration gradient had reached a point of equilibrium, causing the solvent to refuse to take up any more solutes from the samples. Thus, mass transfer and diffusion rates may decrease leading to a reduction in oil yield. Consequently, the yield of the essential oil increased until the maximum point and decreased gradually as the percentage of solvent increased. Furthermore, the yield of the essential oils decreased as the particle size increased. Research by Tran et al., (2018) on the optimisation of MAE of essential oil from Vietnamese basil also found the same trend as in this study where the extract yield increased from 0.2% to 0.5% when the ratio was increased from 1:1 to 3:1. However, the yield of the essential oil decrease from 0.5% to 0.4%. when the ratio was increased from 3:1 to 4:1.

3.4 GC-MS analysis

Fig. 5 illustrates the GC-MS chromatogram obtained for the highest yield of extracted oils from kaffir lime leaves. The highest yield obtained was 23.54% with an extraction time of 14.95 min, 14.07% percentage of ethanol, and 400 μm of sample size. Besides, the results of the GC-MS analysis indicating the composition of essential oil at the detected peak with a retention time between 20 to 42 min are listed in Table 2. It indicates that the kaffir lime leaves were abundant in esters and followed by fatty acids.

3.4.1 Fatty acids and esters

The effect of particle size, and extraction time on compositions of fatty acids and esters obtained in extracted oils were illustrated in Fig. 6(a) and (b), respectively. Fig. 6(a) shows that the mass of fatty acids found in extracted oils steadily increases from 316.11 to 677.58 μg fatty acids/g sample as the particle sizes increases. The same pattern for the effect of particle size on the mass of esters was observed in Fig. 6b, whereby the mass of esters increases from 816.24 to 1704.55 μg esters/g sample when the particle size increases from 400 to 600 μm.

Table 2: GC-MS analysis for extracted oils from kaffir lime leaves

No	Retention Time (min)	Amount (%)	Compound Name
1	22.67	0.0185	2-isobutyl-4-methyl-(1,3,2)dioxaborinane
2	28.60	0.0192	2,2-dimethyl-1-oxa-spiro(2.4)heptane
3	29.54	0.0243	Benzeneethanamine,2-fluoro-á,3,4-trihydroxy-N-isopropyl
4	35.25	0.0182	Hydrazine, 1,1-dimethyl-
5	41.13	0.0327	Nonanedioic acid, bis(2-methylpropyl) ester

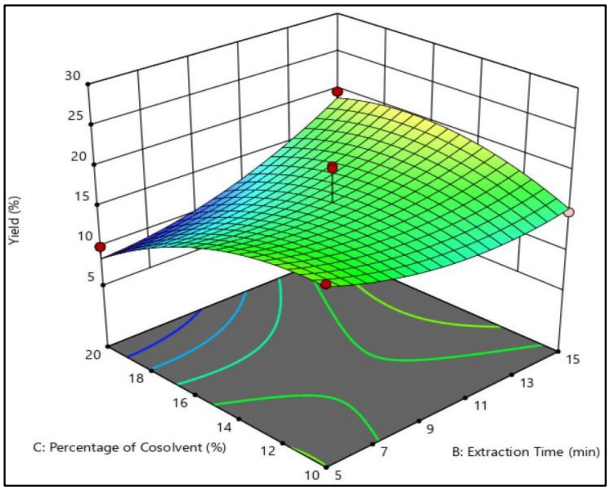

Fig. 4: Response surface for the effect of percentage of solvent on the yield of essential oils

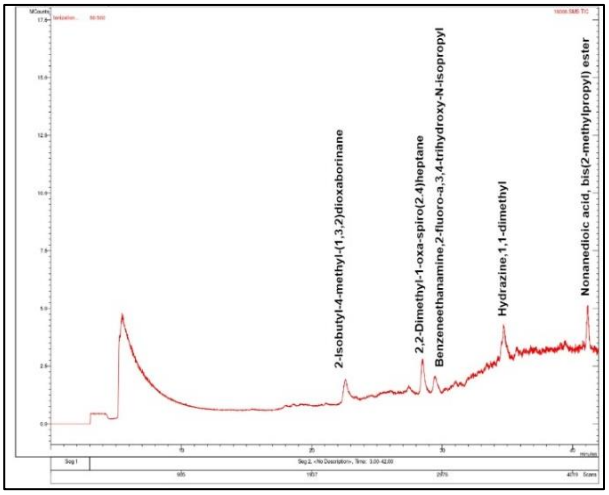

Fig. 5: Chromatogram plot for the highest yield of extracted oil

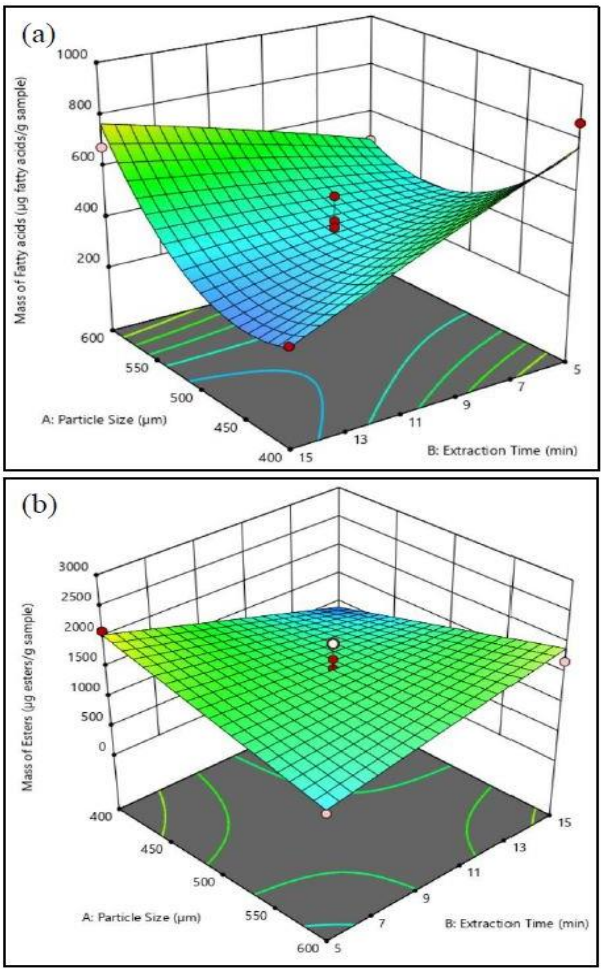

Fig. 6: Response surface for the effect of particle size and extraction time on the (a) mass of fatty acids and (b) mass of esters

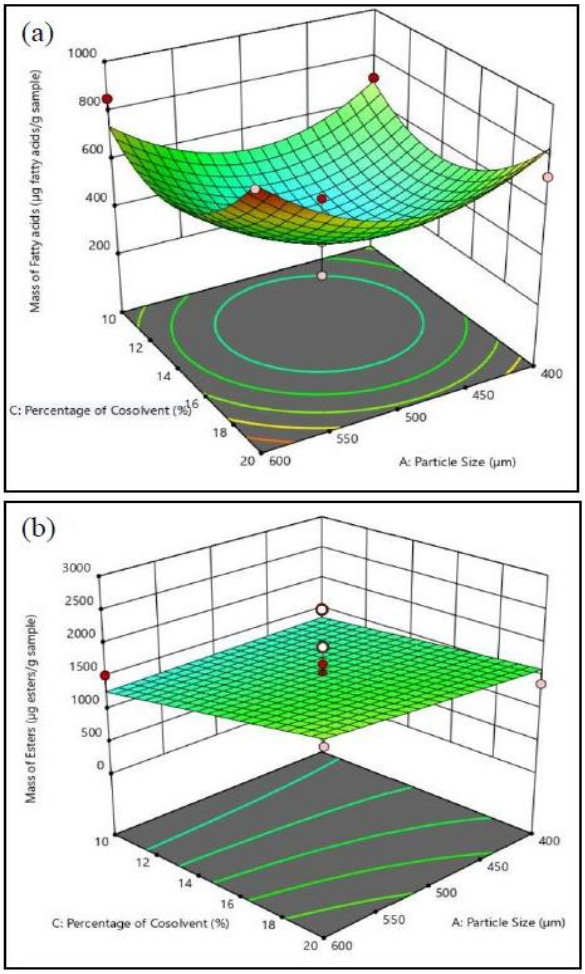

Fig. 7: Response surface for the effect of percentage of ethanol on (a) mass of fatty acids and (b) mass of esters

This is because the solvent has a greater ability to diffuse into the plant particle cell, which in turn increases the extraction rate of fatty acids and esters from the plant particle cells.

Besides, it was observed in Fig. 6(a) that the mass of fatty acids obtained decreased significantly by 63.28% from 860.87 to 316.11 μg fatty acids/g sample as the extraction time was further increased from 5 to 15 min. Fig. 6(b) also shows that the mass of esters decreases as the extraction time increases.

This change occurred because prolonged extraction contributes to the breakdown of fatty acids and esters compounds in the samples, which causes the sample to deteriorate. As a result of this breakdown, the samples are no longer useful and identifiable as fatty acids. Aside from that, a longer extraction time increases the oxidation of the components of essential oils due to prolonged exposure to unfavorable factors such as temperature, light, and oxygen. According to Marr & Ingraham (1962), when the growth temperature at which the compounds can survive increases, there is a gradual decrease in the percentage of the most abundant unsaturated fatty acids such as octadecenoic acid.

Next, the effect of the percentage of ethanol on compositions of fatty acids and esters obtained in extracted oils were illustrated in Fig. 7(a) and (b), respectively.

Fig. 7(a) shows the effects of the percentage of ethanol on the mass of fatty acids obtained from extracted essential oils. The mass of fatty acids decreased from 708.68 to 537.31 μg fatty acids/g sample when the percentage of ethanol increases from 10% to 15% and it was found to be increased to 852.14 μg fatty acids/g sample as the percentage of ethanol further increased from 15% to 20%. Meanwhile, Fig. 7(b) shows a different trend for the mass of esters which the mass of esters decreases slightly from 1486.56 to 1390.96 μg esters/g sample when the percentage of ethanol increases.

This is because the rate of diffusion between the solvent and the plant particles increases when the percentage of the solvent and the particle size are increased, which disrupt the cell walls of plant particles and aid in the release of fatty acids and esters compounds into the solvent (Noor et al., 2018). Other than that, the amounts of fatty acids and esters compounds that diffuse into the solvent increases proportionally with the concentration of the ethanol, because the ethanol has the same polarity with the fatty acids and esters compounds. Therefore, both compounds can dissolve in ethanol.

3.5 Statistical analysis

The experimental results for each response which are the yields, the mass of fatty acids, and mass of esters were evaluated by the analysis of variance (ANOVA) for the model of extracted kaffir lime essential oil from which three factors were considered and analysed. The suggested model produced by Design Expert software for both responses of yield and mass of fatty acids is a quadratic model. Meanwhile, for the response mass of esters, the model suggested is two-factor interaction (2FI).

3.5.1 Yield of extracted oils

The ANOVA results of the yield of extracted kaffir lime leave essential oils are presented in Table 3. The Model *F*-value of 3.52 and *p*-values less than 0.05 implies that the model suggested is significant. There is only a 4.17% chance that an *F*-value this large could occur due to noise. The lack of fit *F*-value of 0.27 implies that the lack of fit is not significant relative to the pure error. There is a 90.77% chance that a lack of fit *F*-value this large could occur due to noise. From the regression analysis, the R^2 and standard deviation values are 0.73 and 2.76, respectively. An equation was then formed to estimate the yield of extracted essential oils. The equation accounts for the relationship between yield (Y_1, %), particle size (X_1, μm), extraction time (X_2, min) and percentage of ethanol (X_3, %). The yield of extracted oils can be estimated by using Eq. (2),

$$Y_1 = -0.0923 + 0.0410\,X_1 - 0.6128\,X_2 + 1.7016\,X_3 + 0.0861\,{X_2}^2 - 0.1118\,{X_3}^2 - 0.0054\,X_1X_2 + 0.1330\,X_2X_3 \quad (2)$$

3.5.2 Mass of fatty acids and esters

The ANOVA results of the mass of fatty acids and esters in extracted kaffir lime leave essential oils are presented in Table 4 and Table 5, respectively. Based on Table 5, the Model *F*-value of 4.02 and *p*-values less than 0.05 implies that the model suggested is significant. There is only a 4.02% chance that an *F*-value this large could occur due to noise. The lack of fit *F*-value of 1.52 implies that the lack of fit is not significant relative to the pure error. There is a 33.88% chance that a lack of fit *F*-value this large could occur due to noise. From the regression analysis, the R^2 and standard deviation values are 0.84 and

Table 3: Analysis of variance for yield of extracted oils

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	
Model	188.11	7	26.87	3.52	0.0417	significant
Particle size (X_1)	15.04	1	15.04	1.97	0.1910	
Extraction time (X_2)	28.48	1	28.48	3.73	0.0856	
Percentage of ethanol (X_3)	20.32	1	20.32	2.66	0.1374	
X_1X_2	29.88	1	29.88	3.91	0.0794	
X_2X_3	44.49	1	44.49	5.82	0.0391	
${X_2}^2$	19.57	1	19.57	2.56	0.1440	
${X_3}^2$	32.99	1	32.99	4.32	0.0675	
Residual	68.77	9	7.64			
Lack of fit	17.40	5	3.48	0.2709	0.9077	not significant
R^2	0.73					

Table 4: Analysis of variance for mass of fatty acids in extracted oils

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	
Model	6.37×10^5	9	70801.36	4.02	0.0402	significant
Particle size (X_1)	15676.47	1	15676.47	0.89	0.3771	
Extraction time (X_2)	16033.01	1	16033.01	0.91	0.3720	
Percentage of ethanol (X_3)	66764.66	1	66764.66	3.79	0.0927	
X_1X_2	1.37×10^5	1	1.37×10^5	7.76	0.0271	
X_1X_3	1767.06	1	1767.06	0.10	0.7608	
X_2X_3	14426.27	1	14426.27	0.82	0.3958	
${X_1}^2$	1.69×10^5	1	1.69×10^5	9.60	0.0174	
${X_2}^2$	300.2		300.20	0.02	0.8999	
${X_3}^2$	1.95×10^5		1.95×10^5	11.08	0.0126	
Residual	1.23×10^5	7	17631.01			
Lack of fit	65735.2	3	21911.73	1.52	0.3388	not significant
R^2	0.84					

Table 5: Analysis of variance for mass of esters in extracted oils

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	*p*-value	
Model	2.07×10^6	9	3.44×10^5	3.72	0.0330	significant
Particle size (X_1)	13874.22	1	13874.22	0.15	0.7069	
Extraction time (X_2)	89774.04	1	89774.04	0.97	0.3481	
Percentage of ethanol (X_3)	3.41×10^5	1	3.41×10^5	3.68	0.0840	
X_1X_2	8.50×10^5	1	8.50×10^5	9.17	0.0127	
X_1X_3	23848.04	1	23848.04	0.26	0.6229	
X_2X_3	7.47×10^5	10	92645.49	8.06	0.0176	
Residual	9.27×10^5	6	65329.68			
Lack of fit	3.92×10^5	4	1.34×10^5	0.49	0.7932	not significant
R^2	0.69					

Table 6: Multi response optimisation of extracted oils

Particle size (μm)	Extraction time (min)	Percentage of ethanol (%)	Yield (%)	Mass of fatty acids (μg fatty acids/g sample)	Mass of esters (μg esters/g sample)
400	5	13.44	16.08	750	1873.3

132.78, respectively. An equation is then formed to estimate the mass of fatty acids in extracted essential oils. The final quadratic equation for the mass of fatty acids (Y_2, μg fatty acids/g sample) is described as in Eq. (3).

$$Y_2 = 8707.2670 - 23.9320\,X_1 - 151.1132\,X_2 - 237.2503\,X_3 + 0.0201\,{X_1}^2 - 0.3378\,{X_2}^2 + 8.6175\,{X_3}^2 + 0.3699\,X_1X_2 + 0.0420\,X_1X_3 - 2.4022\,X_2X_3 \quad (3)$$

Table 6 shows that the model *F*-value of 3.72 and *p*-values less than 0.05 implies that the model suggested is significant. There is only a 3.30% chance that an *F*-value this large could occur due to noise. The lack of fit *F*-value of 0.49 implies that the lack of fit is not significant relative to the pure error. There is a 79.32% chance that a lack of fit *F*-value this large could occur due to noise. From the regression analysis, the R^2 and standard deviation values are 0.69 and 304.38, respectively. An equation is then formed to estimate the mass of esters in extracted essential oils. The final 2FI equation for the mass of esters (Y_3, μg esters/g sample) is described as fin Eq. (4).

$$Y_3 = 4081.7600 + 0.9218\,X_1X_2 + 0.1544\,X_1X_3 - 17.2858\,X_2X_3 - 11.1187\,X_1 - 222.8345\,X_2 + 136.9337\,X_3 \quad (4)$$

3.5.3 Multi-response optimisation

The multi-response optimisation of kaffir lime essential oils suggested by RSM is shown in Table 6. This multi-response optimisation resulted in a desirability of 59.60%.

Based on Table 6, the multi-response optimisation suggested by RSM includes particle size of 400 μm, extraction time of 5 minutes, and percentage of ethanol of 13.44% resulting in yield of 16.08%, mass of fatty acids of 750.01 μg fatty acids/g sample and mass of esters of 1873.02 μg esters/g sample. These optimisations were made by setting the three parameters in range and maximising all three responses.

4.0 Conclusions

The objective of this study was to obtain the optimum parameter of particle size, extraction time, and percentage of ethanol for obtaining the highest yield of extracted essential oils from kaffir lime leaves. These parameters were optimised using the Box-Behnken design. Based on the data analysis on the yield of extracted oils from experiments, the optimal settings suggested by RSM for maximising the yield of kaffir lime leaves essential oils to include particle size of 400 μm, extraction time of 14.95 min, and percentage of ethanol of 14.07%, resulting in a yield of 23.54%. For the analysis of the volatile compound in extracted oils, GC-MS resulted in the identification of almost 94 compounds for each the sample. The identified major compounds from kaffir lime leave oils were esters and followed by fatty acids. It is suggested that the quadratic model is used to characterise both the yield of extracted essential oils and the mass of fatty acids. Meanwhile, for the response mass of esters, the model suggested is 2FI.

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