

Optimization of Extraction Yield and Phytochemical Characterization of Crude Methanolic Extract and Its Fractions of *Mitragyna Speciosa* Leaves

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Research article

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Abstract

In this study, Response surface methodology (RSM) was applied to optimize the yield of crude methanolic extract of *Mitragyna speciosa* leaves using Ultrasound-assisted extraction (UAE). The crude methanolic extract and its fractions were quantified in terms of total phenolic content and total flavonoid content, along with characterized using Fourier-transform infrared and Gas chromatography–mass spectrometry. The results showed the maximum yield of 49.72% at the optimal conditions (temperature, 34 °C; time, 25 min; and volume of solvent, 166 mL). The recovery crude methanolic extract for TPC and TFC were 137.3 ± 15.7 mg GAE/g and 90.3 ± 15.3 mg RE/g, respectively.

Introduction

The development of drugs based on the medical plant has gained new attention due to a great source of therapeutic phytochemicals. Phytochemical compounds such as phenolics and flavonoids are the secondary metabolites that produce abundantly from plant which have been reported to have antioxidant, antibacterial, anticancer, anti-inflammatory properties which is a useful candidate for medical and pharmaceutical industry [1,2]. In addition, natural-based formulation products are now become more demanding compared to the synthetic-based product due to its efficiency and safety.

The extraction method is a crucial step in the route of phytochemical production for the discovery of bioactive constituents from plants [3]. Lately, a green method such as ultrasound-assisted extraction (UAE), microwave-assisted (MAE), and supercritical fluid extraction (SFE) has been growing rapidly as these methods are fast as compared to conventional like maceration and soxhlet methods. Further, these promising methods are environmental friendly and comparable yield to conventional and even higher [4,5].

Among several green extraction methods, UAE requires reasonable investment in terms of solvent and energy. Moreover, the instrument is simple thus easy to handle, safe, economical and reproducible as it can operate under ambient temperature and at atmospheric pressure [6,7]. Phytochemicals from plants can be extracted by acoustic cavitation that produced from ultrasound waves through the solvent and cause damaging the plant's cell walls [8]. Besides, mass transfer across cell membranes and penetration of solvent can be improved hence greater extract yields produced [9]. Numerous successful studies employed in UAE have been done for the plant samples [10-14]. Extraction times, temperatures, temperature, solid to solvent ratios, solvent concentrations, and volume of solvent are the parameters to be optimized to achieve the optimum extraction conditions. These parameters can be optimized by using mathematical and statistical tool viz. response surface methodology (RSM) where it able to considered interaction effects between parameters at one time compared to classical approach and thus shorten the time by reducing the number of experiments needed [15-17].

Liquid-liquid extraction (LLE) or also known as partitioning is one of the techniques used in the subsequence of the extraction process. As the crude plant extract consists of plentiful phytochemical with countless chemical properties, fractionation by different polarities of the solvent can be considered

because it can improve the quality of chemical characteristics ^[18]. This technique is favorable because it required a simple apparatus and procedure compared with the solid-phase extraction technique.

Mitragyna Speciosa Korth (Rubiaceae family) is a tropical plant that can be found in Southeast Asia countries. *M. speciosa* is commonly known in Malaysia as 'ketom' or 'biak-biak' and 'kratum' in Thailand. *M. speciosa* has traditionally used as an herbal remedy to relieve tiredness and muscle fatigue, and to treat some common illnesses such as diarrhea, coughing, muscle pain, diabetes, wound, and hypertension. In addition, it is also used as a substitute for opium or morphine in the treatment of drug addicts ^[19]. Numerous studies have revealed several biological activity of *M. speciosa* such as anti-inflammation, antinociceptive, antioxidant, antimicrobial ^[20-23] that are vital in a good healing process.

Due to the great beneficial value of this plant, research is needed to optimize the extraction process to ensure high pharmaceutical quality. To the best of our knowledge, there have been no studies to optimize the extraction yield from the *M. Speciosa* leaves extract. The current study is to optimize the ultrasound-assisted extraction conditions of the *M. speciosa* leaves extract to achieve maximum extraction yield extract by using RSM. Under optimize parameters, the crude methanolic extract was fractionated by liquid-liquid extraction to obtain its fractions. The crude methanolic extract and its fractions were quantified in terms of total phenolic content (TPC) and total flavonoid content (TPC), as well as characterized using Fourier-transform infrared (FTIR) and Gas chromatography–mass spectrometry (GC-MS).

Materials And Methods

Materials

All the chemicals and reagents used were of analytical grade. Methanol, Folin–Ciocalteu phenol reagents, AlCl₃, potassium acetate, gallic acid and rutin were obtained from Sigma-Aldrich (Germany). Sodium carbonate (Na₂CO₃) was purchased from Merck (Darmstadt, Germany). Distilled water was purified in our laboratory. Fresh leaves of *M. speciosa* were collected from Perak, Malaysia. The plant was identified by botanist and the voucher specimen MFI 0121/19 has been deposited in the herbarium of the Institute of Bioscience, Universiti Putra Malaysia.

Optimization of Extraction Process

Sample Preparation

Fresh leaves of *M. speciosa* were thoroughly washed with running tap water. The leaves were cut into small pieces and subjected to freeze-drying method by kept overnight under -80 °C after which the frozen leaves were lyophilized. The leaves were milled into a fine powder using a laboratory grinder and the ground leaves were stored in an airtight jar.

Ultrasound-Assisted Extraction

About 50.0 g of finely powdered of *M. speciosa* was placed in a conical flask and mixed with methanol. The solution was transferred to an ultrasonic bath (Power Sonic 405, Hwashin Technology Co., Seoul, Korea). The extract was than filtered and concentrated using a rotary evaporator at approximately 40 °C until the excess solvent was completely removed. Percentage of extraction yields of each run were calculated based on total dry weight according to the Equation (1):

$$\text{Extraction yield (\%)} = \frac{\text{weight of extract}}{\text{total dry weight}} \times 100 \quad (1)$$

Experimental design

Response Surface Methodology (RSM) was used to optimize the crude extract from the extraction of *M. speciosa*. Based on Central Composite Design (CCD), 20 experimental runs, with three factors at five levels including six central point were constructed for the optimization process. The CCD was generated by Design Expert Software (Version 11, Stat. Ease Inc., Minneapolis, USA). The influence of independent variables such as the temperature (A), time (B) and volume of solvent (C) on the extraction process were evaluated towards the dependent response (extraction yield). Quadratic model was developed from RSM which describing the extraction process. Table 1 shows the independent variables with the range of its parameters.

Table 1. The independent variables with the range of its parameter.

Symbol	Independent variables	Units	Coded levels				
			-1.68	-1	0	+1	+1.68
A	Temperature	°C	30	34	40	46	50
B	Time	min	15	24	38	51	60
C	Volume of solvent	mL	50	80	125	170	200

Statistical Analysis

Analysis of variance (ANOVA) is a statistical tool that used to analyze which independent variable is the most significantly affects the percentage yield. All analyses were considered significant at $p < 0.05$. Model analysis, lack of fit test, coefficient of determination (R^2), adjusted R^2 and Predicted vs Actual graph were

determined for the model adequacy. When R^2 values are closer to 1, the model is considered accurate (Yolmeh et al., 2014). The design was expressed by polynomial regression as shown in Equation (2):

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_{12}X_1X_2 + A_{13}X_1X_3 + A_{23}X_2X_3 + A_{11}X_1^2 + A_{22}X_2^2 + A_{33}X_3^2 \quad (2)$$

Where Y is the dependant variable (extraction yield); X_1 , X_2 and X_3 are the independent variables (extraction temperature, extraction time and volume of solvent); A_0 is the regression coefficient at the centre point; A_1 , A_2 and A_3 are linear coefficients; A_{12} , A_{13} and A_{23} are second order coefficients; and A_{11} , A_{22} and A_{33} are quadratic coefficients.

Verification of model

Some random extractions were prepared in order to validate the predicted value. The experimental and predicted value was compared to determine the validity of the model. The percentage of residual standard error (RSE) was calculated for each response. The RSE Equation (3):

$$\text{RSE (\%)} = \frac{(\text{actual value} - \text{predicted value})}{\text{Predicted value}} \times 100 \quad (3)$$

Fractionation

Fractionation was conducted by using liquid-liquid extraction. The optimized conditions obtained from RSM were used to get the crude methanol extract. The crude methanol extract was partitioned between n-hexane and water. The aqueous layer was fractionated by using different polarities based solvents such as n-hexane, dichloromethane, ethyl acetate and butanol successively. Four solvent fractions were collected and concentrated with vacuum rotary evaporator.

Quantification of total phenolic content (TPC) and total flavonoid content (TFC)

TPC

By using the Folin-Ciocalteu assay, the total phenolic content of the extracts was determined using method by [24] with several modifications. Each extract of 100 μL at a concentration of 1 mg mL^{-1} or gallic acid standard solution was mixed with 0.5 mL Folin-Ciocalteu reagent. The mixture was then

incubated for 3 min at room temperature and afterwards 1 mL of 7.5% sodium carbonate was added. After the mixture was heated for 1 min at 95 °C and allowed to cool at room temperature, blue complex formed. Absorbance was measured at 765 nm using spectrophotometer. The total phenolic compound concentration in extract was expressed in mg of gallic acid equivalent per gram of dry weight (mg GAE/g) extract.

TFC

Total flavonoid content was ascertained based on the method by Sepulveda *et al.* [25] with some modification. In brief, 0.5 mL of sample was mixed with 0.1 mL of 10% AlCl₃, 0.1 mL potassium acetate and 4.3 mL of distilled water, followed by 30 min incubation at room temperature. Absorbance was measured at 415 nm using a spectrophotometer. The total flavonoid content was expressed as milligram of rutin equivalents (RE) per gram dry matter of extract.

Characterization

Fourier-Transform Infrared Spectroscopy (FTIR)

Each extract were mixed with KBr salt, using mortar and pestle, and compressed into a thin pellet. The absorption spectrum of a chemical compound was determined by infrared spectroscopy. Infrared spectra were recorded on a Perkin-Elmer FTR SPECTRUM BX spectrophotometer between 4 000 – 400 cm⁻¹.

Gas Chromatography- Mass Spectroscopy (GC-MS)

Crude methanolic extract and its fractions were subjected to GC-MS analysis using the model instrument, GCMS-QP2010 Ultra (Shimadzu Co., Japan) attached with a capillary column DB-1 (0.25 μm film × 0.25 mm I. d. × 30 m length). Analysis was performed by injecting 1 μL of the sample with a split ratio of 20 : 1. Helium gas (99.9 %) was used as the carrier gas at a flow rate of 1 mL/min. The analysis was performed in the EI (electron impact) mode with 70 eV of ionization energy. The injector temperature was maintained at 250 °C (constant). The column oven temperature was set at 50 °C (held for 3 min), raised at 10 °C per min to 280 °C (held for 3 min), and finally held at 300 °C for 10 min. The compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST and WILEY libraries).

Results And Discussion

Optimization using Response Surface Methodology (RSM)

RSM was used to optimize the experiment that was designed using Central Composite Design (CCD) via Design Expert 11 Software. RSM was not only optimized, RSM can also investigate the relationship between parameters and its response. There were 20 experiments (three factors five levels) that have been carried out to determine the optimum condition that will produce the highest amount of yield. Table 2 shows the design matrices of the actual and predicted value for extraction of *M. speciosa*. The actual

value for the response (extraction yield) was from 21.34 - 50.73%. The predicted values were corresponding to the actual values since achieved from the model fitting technique. Figure 1 illustrates the correlation between predicted and actual values of extraction yield. Most of the points were closed to the line where indicates that the optimum condition for achieving high percentage yield able to determine by the predictability of the model.

The quadratic polynomial model can be expressed by the response after employing multiple regression analysis on the actual. The relationship between the extraction yield, Y with the three independent variables can be described in Equation (4).

$$Y (\%) = 38.62 - 3.30A + 5.18B + 7.99C + 10.56AB - 0.7575AC - 1.27BC - 0.2568A^2 - 0.8224B^2 - 5.03C^2 \quad (4)$$

Where A is extraction temperature (°C), B is extraction time (min), and C is volume of solvent (mL).

For the statistical analysis, the validity of the model was determined by using analysis of variance (ANOVA) as shown in Table 3. The model that was developed suggested as a quadratic model and significant due to the *F*-value and *p*-value were 146.46 and <0.0001 respectively. There is only 0.01% chance that the *F*-value can lead to the noise. *F*-value was obtained when an ANOVA test was run and to determine the means between the populations is significantly different, besides *p*-value also can be determined. The *F*-value must be used in combination with *p*-value in order to evaluate the result significantly.

Table 2. The design matrices of the actual and predicted value for extraction of *M. speciosa*

Run	Independent variables			Response variable	
	A	B	C		
	Temperature (°C)	Time (min)	Volume of solvent (mL)	Extraction yield (%)	
				Actual	Predicted
1	40	38	50	35.57	34.75
2	30	38	125	44.11	43.45
3	40	38	125	39.06	38.62
4	34	24	170	50.66	51.21
5	40	60	125	45.24	45.01
6	46	24	170	21.34	21.97
7	34	51	80	36.45	33.51
8	40	38	125	38.56	38.62
9	40	38	200	38.73	37.84
10	46	51	170	50.73	50.92
11	46	24	80	34.26	28.05
12	40	38	125	37.97	38.62
13	40	38	125	38.97	38.62
14	40	38	125	37.90	38.62
15	34	24	80	30.75	31.19
16	40	15	125	28.24	27.58
17	46	51	80	38.92	38.99
18	40	38	125	39.12	38.62
19	50	38	125	32.57	32.34
20	34	51	170	37.29	37.92

The value of the coefficient of determination (R^2) and the adjusted coefficient determination (Adj. R^2) were found high, which were 0.9947 and 0.9879, respectively, indicating a satisfactory correlation between the independent variables and response. The differences between both R^2 were only less than 0.2 which revealed the effectiveness of the model. A model that contains a high value of the coefficient of determination which is more than 0.9 represents the model was a good fit with high correlation [26]. Adeq precision was used to measure the signal to noise ratio. In this study, adeq precision was found greater than 4, which was 47.0226 that indicated an adequate signal. Hence, the designed model can be used to plot the design space.

Apart from that, the “lack-of-fit” of the model should also take into count. The lack-of-fit of this model was not significant which verified the accuracy of the model. According to Quanhong & Caili [27], they reported that the variation can be predicted accurately based on the lack-of-fit of the model where the value of F -value lowers than p -value.

Table 3. ANOVA for quadratic polynomial model developed for extraction yield of *M. speciosa*

Source	Sum of Squares	DF	Mean square	F-value	p-value
Model	866.84	9	96.32	146.46	<0.0001
A-Temperature	87.28	1	87.28	132.71	<0.0001
B- Time	214.83	1	214.83	326.68	<0.0001
C-Volume of solvent	256.63	1	256.63	390.24	<0.0001
AB	456.94	1	456.94	694.84	<0.0001
AC	2.08	1	2.08	3.16	0.1186
BC	5.82	1	5.82	8.85	0.0206
A ²	0.8980	1	0.8980	1.37	0.2808
B ²	9.21	1	9.21	14.01	0.0072
C ²	134.38	1	134.38	204.34	<0.0001
Residual	4.60	7	0.6576		
Lack -of- fit	3.10	2	1.55	5.13	0.0614
Pure Error	1.51	5	0.3015		
Cor Total	871.45	16			
R ²	0.9947				
Adjusted R ²	0.9879				
Predicted R ²	0.8870				
Adeq Precision	47.0226				

Effect of Independent Variables

Effects of temperature and time

Based on Figure 2, the volume of the solvent was being fixed at 125 mL and interaction on the extraction yield of *M. speciosa*. When the extraction temperature of 40 °C and the extraction time at 15 min, the extraction yield was found to be the lowest yield (28.24%), Meanwhile when the time at the maximum of 60 min and temperature at 40 °C, the highest extraction yield (45.24%) was achieved. These indicated that as the extraction time increases with extraction temperature, a higher amount of production able to obtain due to having significant conditions that able to release all the metabolites from the plant. Cares *et al.* [28] has reported that UAE was able to disrupt the biological membranes which enhance the release of

compounds from plant and also able to improve mass transfer. Furthermore, thermally unstable compounds can obtain from using UAE [29].

Effects of temperature and volume of solvent

As shown in Figure 3, the effects of temperature and volume of solvent towards the extraction yield of *M. speciosa* were evaluated. The response surface plot was generated with the fixed extraction time at 38 min and the interaction on response. The highest yield of extraction (44.11%) was obtained when the temperature was at 30 °C and used 125 mL amount of solvent. However, when the higher temperature was applied which was 50 °C and used the same volume of solvent (125 mL), the percentage yield was 32.57%. Thus, from these findings, it represented that the compounds in *M. speciosa* were heat-sensitive; hence will lowering the production yield as temperature increases. Another study by Sheng *et al.* [30] stated that some thermo-sensitive compounds such as flavonoids will degrade as exposed to high temperatures. Meanwhile, at the temperature of 40 °C and 200 mL of solvent used, the yield was 38.73% which lower compared to a similar condition (40 °C) and has 150 mL of solvent used.

Effects of time and volume of solvent

Figure 4 illustrates the effect of time and volume of solvent towards the extraction yield. At the fixed temperature of 40 °C, an increasing volume of solvent will influence the yields where 38.73% yield was obtained by using 200 mL of solvent for 38 min for the extraction process. In contrast, similar conditions, with different volumes of solvent used (125 mL) results in 39.06% yield. This can be concluded that the volume of solvent has little effect on extraction yield.

Optimum condition of extraction process of *M. speciosa*

In order to obtain the optimum condition for the extraction process, the desirability function was evaluated. The highest amount of yields able to achieve from the optimum condition by considering all of the independent variables. From the study, the maximum extraction yield (49.72%) was optimized at 34 °C of extraction temperature, in 25 min and the volume of solvent was 166 mL. The RSE percentage of the optimum condition was below 5% and represented the model was in good concurrence. The optimum conditions for the extraction yield of *M. speciosa* were tabulated in Table 4. There is no work reported on % yield crude methanolic extract. However, Orio *et al.* [31] revealed that % crude extract in methanol: water, 1:1 was 24.8% where the conditions were at 25 °C for 1 hour.

Table 4. Optimum conditions for extraction yield

temperature (°C)	time (min)	Volume of solvent (mL)	yield			Desirability
			Actual	Predicted	RSE	
34	25	166	49.72	50.77	2.07	1.000

Verification of the models

Three randomized validation sets were carried out to verify the model and to determine the competency of the model. The actual results were compared to the predicted value by measure the residual standard error (RSE) percentage, where the RSE percentage need to below than 5%. To reveal the model was adequate for the extraction process, the RSE percentage must not have significant differences between actual values and predicted values. As shown in Table 5, the model was verified since all of the set experiments contain RSE% below than 5. The equation of RSE% was shown in Equation (3).

Table 5. Predicted and actual response value for the verification of model

Set	temperature (°C)	time (min)	Volume of solvent (mL)	Extraction yield (%)		
				Actual	Predicted	RSE
1	36	26	160	45.24	46.20	2.08
2	35	25	166	47.11	48.73	3.32
3	45	49	140	47.80	48.68	1.81

Quantification of TPC and TFC

Table 6 shows the TPC and TFC for crude methanolic extract and its fractions of *M. Speciosa* leaves. The phenolic content was found to be more in ethyl acetate fraction. The order of TPC of *M. speciosa* extracts was: ethyl acetate fraction (277.7 ± 10.1 mg GAE/g) > dichloromethane fraction (184.3 ± 18.8 mg GAE/g) > methanol crude (137.3 ± 15.7 mg GAE/g) > hexane fraction (100.2 ± 7.8 mg GAE/g) > butanol fraction (72.4 ± 7.3 mg GAE/g). The flavonoid content was found to be more in methanol. The order of TFC was: methanol crude (90.3 ± 15.3 mg RE/g) > ethyl acetate fraction (75.7 ± 17.8 mg RE/g) > dicholoromethane fraction (54.9 ± 19.0 mg RE/g) > butanol fraction (46.3 ± 5.7 mg RE/g) > hexane fraction (28.1 ± 10.7 mg RE/g).

In the present study, the TFC of crude methanolic extract was similar with the previous study done by Parthasarathy *et al.* [23] which was 90.3 mg RE/g and 91.1 mg RE/g respectively. However, variation between TPC in the *M. speciosa* with those reported by previous studies was observed. TPC obtained from crude methanolic extract was more (137.3 mg GAE /g) compared to previous studies reported by Parthasarathy *et al.* [23] which was 105.58 mg GAE/g while a lower TPC (24.02 mg GAE/g) was reported by Lee, S.T. *et al.* [32]. These differences might be due to the usage of modern extraction which was ultrasound-assisted extraction (UAE) and environment conditions where it influences the amount of TPC

[33]. A study by Zhou *et al.*, [34] on *Melastoma sanguineum* shows that UAE improved the extraction efficiency and saved a lot of time compared with maceration extraction. Additionally, the present plant was collected from a different location. For most of the plants, change in one of environmental factors such as light, soil water, temperature, soil fertility, and salinity may affect the accumulation of secondary metabolites [35]. However there is no investigation has been conducted towards hexane, dichloromethane, ethyl acetate, and butanol extract of *M. speciosa*.

Table 6. TPC and TFC of crude methanolic extract and its fractions of *M. Speciosa* leaves

Extract	TPC (mg GAE/g)	TFC (mg RE/g)
Crude methanolic extract	137.3 ± 15.7	90.3 ± 15.3
Hexane fraction	100.2 ± 7.8	28.1 ± 10.7
Dicholoromethane fraction	184.3 ± 18.8	54.9 ± 19.0
Ethyl Acetate fraction	277.7 ± 10.1	75.7 ± 17.8
Butanol fraction	72.4 ± 7.3	46.3 ± 5.7

Characterization of crude methanolic extract and its fractions

FTIR analysis

Variation of peaks shows in Figure 5 indicated the various functional groups are obtained in the crude methanolic extract and its fractions of *M. speciosa* leaves.

Figure 5a represents the FTIR spectrum of crude methanolic crude extract. At 3336.85 cm⁻¹, the presence of hydroxyl group was found with strong absorption. N-H stretching was found at the absorption band of 2924.09 cm⁻¹. Furthermore, at the absorption band of 1732.08 cm⁻¹, C=O stretching was identified. The aromatic ring was identified at 1695.43 cm⁻¹. At 1367.83 cm⁻¹ of absorption peak, C-N stretching was found, while C-O stretching was presence at 1062.78 cm⁻¹.

Figure 5b illustrates the FTIR spectrum of hexane extract. Based on the FTIR spectrum, O-H stretching was presence at the absorption peak of 3385.07 cm⁻¹. Next, N-H stretching was found at 2924.09 cm⁻¹. C=O stretching, C=C stretching and C-O stretching were presence at the absorption peak of 1735.93 cm⁻¹, 1622.13 cm⁻¹ and 1058.92 cm⁻¹, respectively.

FTIR spectrum of dichloromethane extract was shown in Figure 5c. At the highest absorption peak, O-H stretching was found at 3282.84 cm⁻¹, followed by N-H stretching was at the absorption peak of 2931.80

cm^{-1} . C=O and C=C stretching were presence at the region of 1600 cm^{-1} to 1700 cm^{-1} . Furthermore, C-O stretching was presence at 1244.09 cm^{-1} . Lastly, C-N stretching was found at the absorption peak of 1103.28 cm^{-1} .

Figure 5d represents FTIR spectrum of ethyl acetate extract. At 3223.05 cm^{-1} , O-H stretching was detected at the absorption peak. N-H stretching was found at 2941.44 cm^{-1} , C=O stretching was found at 1681.93 cm^{-1} and N-O stretching was found at 1516.05 cm^{-1} . Besides, N-O stretching and C-N stretching were presence at the absorption peak of 1516.05 cm^{-1} and 1278.81 cm^{-1} , respectively.

FTIR spectrum of n-butanol crude extract of was shown in Figure 5e. Based on the figure, the highest absorption was at 3317.56 cm^{-1} due to the presence of N-H stretching. Besides, C=C stretching was exist at 1602.85 cm^{-1} . In addition, O-H bending was presence at 1406.11 cm^{-1} , followed by C-N stretching that can be found at 1047.35 cm^{-1} .

The FTIR analysis crude methanolic extract and its fractions of *M. speciosa* leaves showed the presence of multiple functional groups such as alcohols, carboxylic acids, alkenes, amides, esters which may represent different phytochemical classes which not limited to phenols and flavonoids only. Thus, the compounds can be verified through the result of GC-MS.

GC-MS analysis

The crude methanolic extract and its fractions of *M. Speciosa* leaves were further characterized using GC-MS. A total of 64 phytocompounds were found in the crude methanolic extract and its fraction contained more than 1% area (Table 7). These compounds belong to different chemical classes, including alcohol, esters of fatty acids, steroids/triterpenes, aldehydes, ketones, and amides, Among the crude fractions, hexane fraction have more phytocompounds same with crude methanolic extracts such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol, hexadecanoic acid, phytol, 9,12,15-octadecatrienoic acid, squalene, campesterol, and stigmasterol. Ethyl acetate and dichloromethane fractions have no phytocompound same with the crude methanolic extract.

The presence of various bioactive compounds detected by GC-MS such as stigmasterol, campesterol, phytol, squalene, hexadecanoic acid, benzenesulfonamide, and hydroquinone justifies the used of the *M. speciosa* for various skin ailments treatment. In addition, stigmasterol is one the types of sterol found in the most plant has been studied for its pharmacological potential, including cytotoxic, antimutagenic, antioxidant, antitumoral, among other herbal approaches to pathological states in principles and practice of phytotherapies [36].

Table 7. Main phytocompounds found in the crude methanolic extract and its fractions of *M.speciosa* leaves by GC-MS.

Extract	Compounds	Retention time (min)	Area (%)
Crude methanol extract	Glycerin	4.053	2.73
	Ethanol	6.821	1.40
	2-cyclohexen-1-one	6.963	1.35
	1,2,3,5-cyclohexanetetrol	11.748	13.30
	beta-D- Mannofuranoside	12.182	5.72
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	12.645	1.11
	Hexadecanoic acid	12.891	2.32
	Scyllo-Inositol	13.384	1.55
	9,12-Octadecadienoic acid	16.324	1.19
	9,12,15-Octadecatrienoic acid	13.769	2.06
	Phytol	13.815	1.04
	2-hydroxy-1-(hydroxymethyl)ethyl ester	15.600	2.68
	Squalene	16.765	5.17
	5-beta-Cholest-24-en-12-one	17.735	1.60
	2H-1-Benzopyran-6-ol	18.662	1.01
	Campesterol	19.660	1.79
	Stigmasterol	19.878	1.13
Gamma-Sitosterol	20.484	3.36	
Hexane fraction	1-Dodecanol	10.447	55.70
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	12.433	4.26
	n-Hexadecanoic acid	13.177	1.77
	Phytol	13.822	1.73
	9,12,15-Octadecatrienoic acid	14.052	1.72
	Squalene	16.770	1.14
	E,Z-1,3,12-Nonadecatriene	18.659	1.03
	Methyl (Z)-5,11,14,17-eicosatetraenoate	18.744	1.64
	Campesterol	19.700	1.35
	Stigmasterol	19.906	1.03

	Beta-Sitosterol	20.561	3.68
Dichloromethane fraction	2-methyl-2-pentanol	3.567	1.31
	3-methyl-3-pentanol	3.808	3.09
	1-Hydroxy-1-methylcyclopentane	4.306	14.69
	3-Methylcyclopentanol	4.927	1.91
	7-Oxabicyclo[4.1.0]heptane	12.322	1.66
	2-Phenylquinoline-4-carboxylic acid	15.845	1.10
	1-(Diethylamino)-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile	16.760	1.49
	3-Methyl-3H-cyclonona[def]biphenylene	17.742	1.72
	Cholest-24-en-12-one	17.764	5.07
	Speciofoline	18.726	4.05
	Dibenzo[a,h]cyclotetradecene	18.850	2.08
	Spiro[3H-indole-3,4'-[4H-1,6]methanoquinoline]-4'a(5'H)-carboxylic acid	19.029	5.94
	Mitragynine	21.421	12.95
	Silane	21.680	6.31
	Corynan-16-carboxylic acid	21.933	4.00
Benzoic acid	21.942	1.23	
Urs-12-en-28-oic acid	24.617	1.80	
Ethyl Acetate fraction	Phenol	6.711	1.76
	2-Oxabicyclo[3.2.0]hepta-3,6-diene	6.969	1.33
	2,5-Dimethyl-2,3-dihydro-5H-1,4-dioxepine	7.186	1.81
	O-Methoxyphenol	7.558	1.19
	Silane	8.550	1.71
	1,2-Benzenediol	8.566	9.07
	Benzofuran	8.681	5.78
	1,4:3,6-Dianhydro-alpha-d-glucopyranose	8.721	2.55
	Phosphonic acid	8.828	8.78
	2-Methoxy-4-vinylphenol	9.332	1.60

	D-Allose	10.882	6.84
	3',5'-Dimethoxyacetophenone	10.983	2.26
	N-Decanoic acid	11.811	16.38
	2(3H)-Naphthalenone	11.994	1.89
	Beta-D-Mannofuranoside	12.179	4.60
	Benzenesulfonamide	12.351	1.34
	Oxirane	12.417	2.23
Butanol fraction	Furfural	4.850	1.11
	4H-Pyran-4-one	8.230	1.37
	2-Furancarboxaldehyde	9.086	8.03
	Hydroquinone	9.425	2.06
	1,2,3,5-Cyclohexanetetrol	12.841	28.99
	6-Ethoxy-6-methyl-2-cyclohexenone	13.497	9.22
	4-O-Methylmannose	14.238	28.15
	Inositol	15.187	9.54

Conclusions

In this study, the optimization of the crude methanolic extract from the extraction process of *M. speciosa* leaves was successfully achieved by using RSM utilizing UAE method. The optimized conditions were: temperature (34 °C), time (25 min) and volume of solvent (166 mL) with optimized percentage yield obtained was 49.72%. The extraction yield of crude extract was similar to the RSM's predicted value. The RSE percentage for validation sets were all below 5%, which indicated the model was verified. Under these conditions, the recovery of crude methanolic extract for TPC and TFC were 137.3 ± 15.7 mg GAE/g and 90.3 ± 15.3 mg RE/g, respectively. However, ethyl acetate fraction showed the highest (277.7 ± 10.1 mg GAE/g) and TFC (75.7 ± 17.8 mg RE/g) compared to other fractions. Based on the present study, *M. speciosa* leaves extract could potentially be used in the development of drug formulation for targeted therapeutic as it contained higher phenolic and flavonoid content as well as other valuable phytochemicals.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: available

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Figures

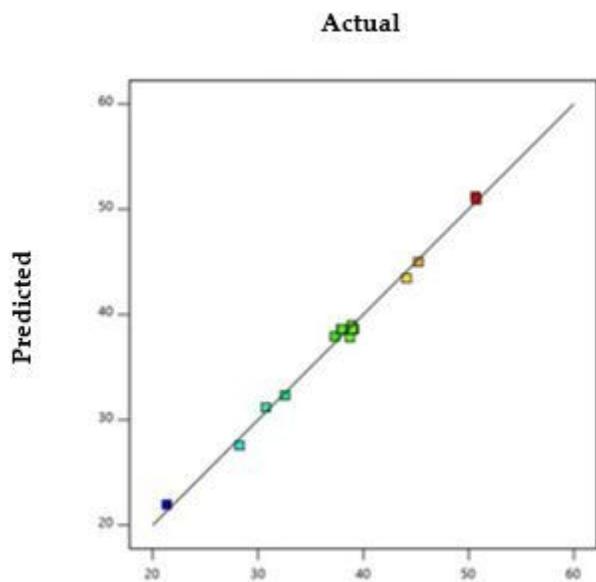


Figure 1

Correlation of predicted and actual values of extraction yield

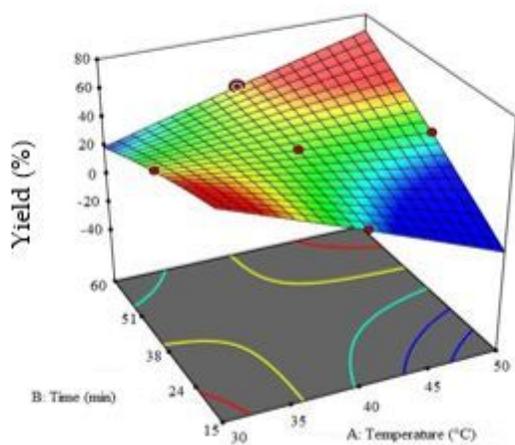


Figure 2

Three-dimensional plot as a function of time, temperature, and interaction on the extraction yield of *M. speciosa*.

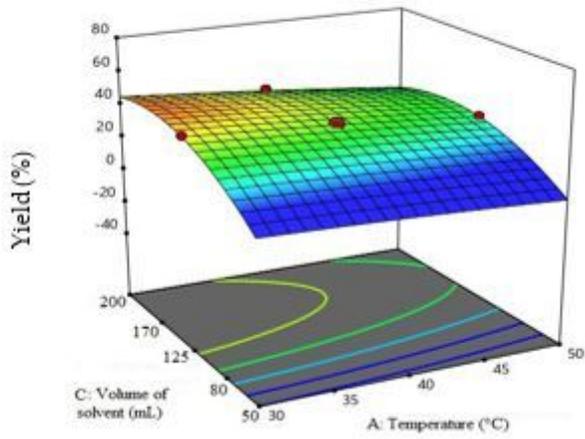


Figure 3

Three-dimensional plot as a function of temperature and volume of solvent and interaction on the extraction yield of *M. speciosa*.

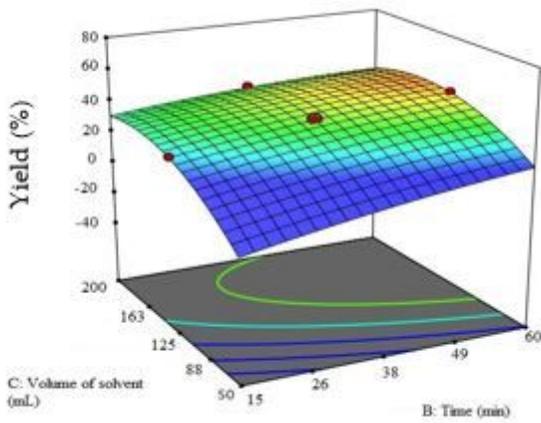


Figure 4

Three-dimensional plot as a function of time, volume of solvent, and interaction on the extraction yield of *M. speciosa*.

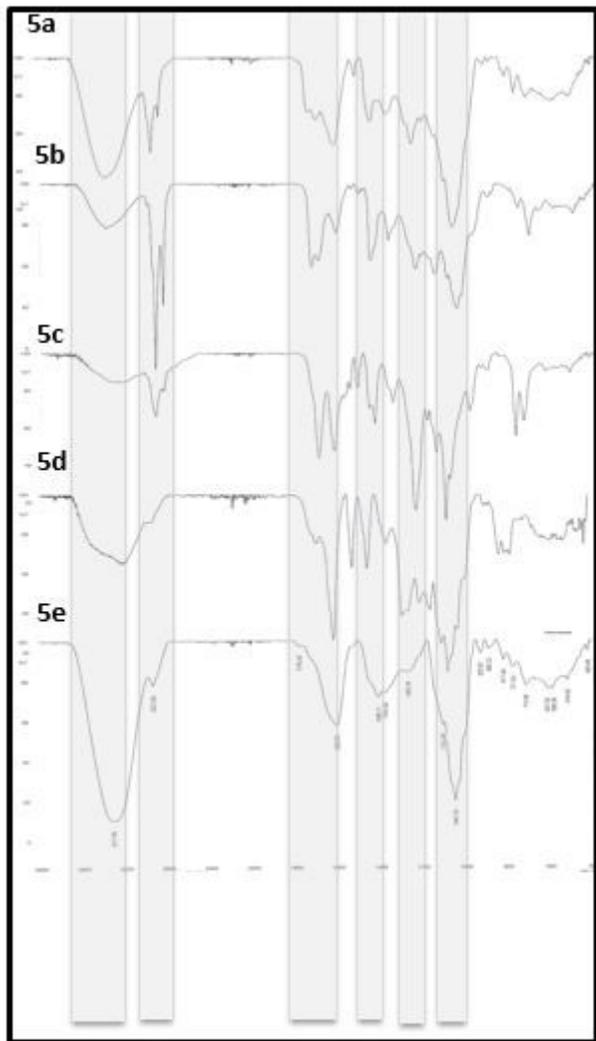


Figure 5

FTIR Spectrum of *M. speciosa* crude methanolic extract and its fractions. 5a) crude methanolic extract. 5b) hexane fraction. 5c) dichloromethane fraction. 5d) ethyl acetate fraction. 5e) butanol fraction.