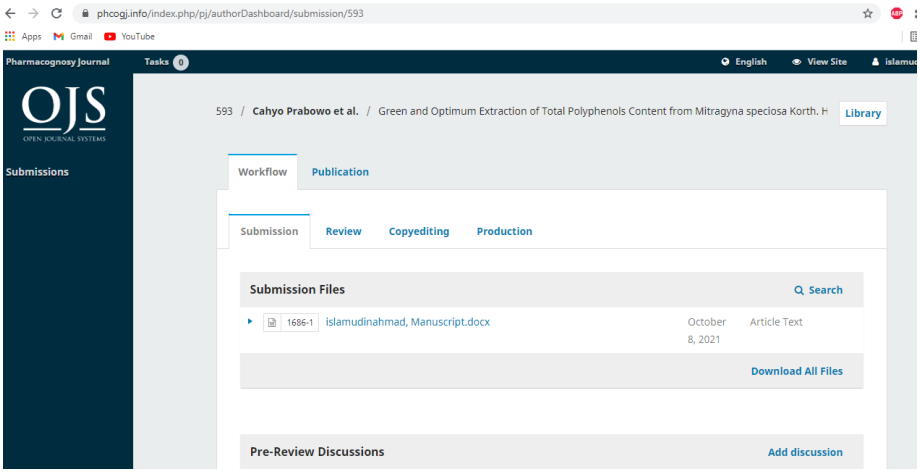
Bukti Submit



Bukti Accepted

From: Pharmacognosy Journal
Sent: Wednesday, 10 November 2021 14:56
To: Islamudin Ahmad
Subject: Manuscript Accepted for Publication - PJ-21-593

Wisnu Cahyo Prabowo, Risna Agustina, Yuspian Nur, Ramila Hidayati, Dewi Rahmawati, M. Arifuddin, Herman Herman, Neneng Siti Silfi Ambarwati, Reza Yuridian Purwoko, Abdul Mun'im, Islamudin Ahmad:

We have reached a decision regarding your submission to Pharmacognosy Journal, "Green and Optimum Extraction of Total Polyphenols Content from Mitragyna speciosa Korth. Havil Leaves using Micro-wave-Assisted Natural Deep Eutectic Solvent Extraction".

Our decision is to: Accept Submission

Minor comments:

For author and editor

- The authors need to perform English editing for this manuscript.

- The authors need to check the journal guidelines.

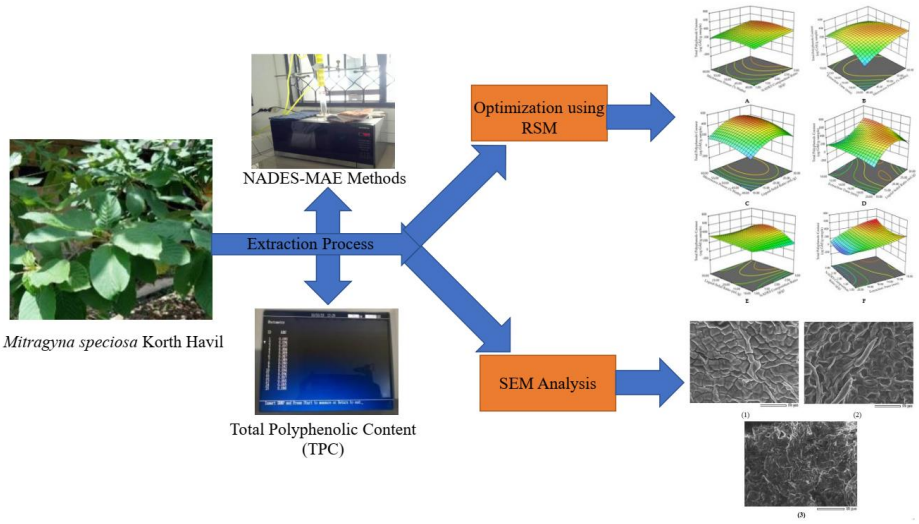
KIndly revise as per the suggestions.

Publication Charges - USD 1199 We request you to confirm your mode of payment 1) Credit Card or 2) Bank Transfer. An invoice can be raised accordingly. Awaiting your response.

Regards,
Emily Flora

\

GRAPHICAL ABSTRACT



Green and Optimum Extraction of Total Polyphenols Content from *Mitragyna speciosa* Korth. Havil Leaves using Microwave-Assisted Natural Deep Eutectic Solvent Extraction

Wisnu Cahyo Prabowo[1], Risna Agustina[2], Yuspian Nur[2], Ramila Hidayati[1], Dewi Rahmawati[1], M. Arifuddin[1], Neneng Siti Silfi Ambarwati[3], Reza Yuridian Purwoko[4], Abdul Mun'im[5], Islamudin Ahmad*[1,2]

[1]Laboratory of Pharmaceutical Research and Development of FARMAKA TROPIS, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, 75119 East Kalimantan, Indonesia.
[2]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, 75119 East Kalimantan, Indonesia.
[3]Department of Cosmetology, Faculty of Engineering, Universitas Negeri Jakarta, East Jakarta, 13220 Jakarta, Indonesia.
[4]Faculty of Military Medicine, Universitas Pertahanan RI, Bogor, 16810 West Java, Indonesia
[5]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Indonesia, Depok, 16424 West Java, Indonesia
*Corresponding Author. E-mail: islamudinahmad@farmasi.unmul.ac.id; Tel. +6281342205060.

ABSTRACT

The current study mainly aims to apply and optimize the microwave-assisted natural deep eutectic solvent extraction (MANDESE) method of total polyphenol content from *Mitragyna speciosa* (Korth.) Havil leaves using response surface methodology (RSM) and its extraction mechanism using scanning electron microscopy (SEM) imaging. The extraction process was performed using the maceration and MANDESE method. Total polyphenols content was examined using Folin-Ciocalteu reagent and spectrophotometer UV-Vis. The extraction mechanism was performed using SEM imaging. ~~For optimization using RSM, the extraction condition as experimental design variable~~ The~~factors~~ extraction condition as experimental design variable factors for optimization using RSM included NADES composition ratio, the liquid-solid ratio, extraction time, and microwave power. The results show that the MANDESE with some different combinations of NADES composition is more effective than a maceration. SEM imaging result shows that the levels of damage of cells and cell walls were more severe after extraction. The optimum extraction condition has obtained the NADES composition ratio of 3 g/g (choline chloride/sorbitol) and the liquid-solid ratio of 20 mL/g for 20 min extraction time

with 60% Watts microwave power. The scale-up confirmation test was obtained the total polyphenols content of 526.12 µg GAE/g sample. This finding demonstrated the optimum condition of the MANDESE method and performed efficiently, rapidly, safely, and environmentally friendly.

KEYWORDS: Microwave-assisted natural deep eutectic solvent extraction, *Mitragyna speciosa* (Kort.) Havil, response surface methodology, total polyphenols content

Introduction

Mitragyna speciosa (Korth.) Havil is a native to Southeast Asia and belongs to the Rubiaceae family, ~~known as~~ the coffee family. *M. speciosa* is abundant and found growing in riverbanks, swampy areas, and inundated areas by water in some countries, such as Indonesia, Malaysia, Thailand, the Philippines, and Papua New Guinea.[1] This plant has enormous potential as a source of raw materials for medicines with high export value. A local community traditionally uses the leaves of this plant to treat different diseases like fever, muscle pain, diarrhea, toughs, malarial, high blood pressure, diabetes mellitus, and worm diseases.[2] Some studies have reported a pharmacological activity of *M. speciosa* leaves such as antinociceptive,[3,4] anti-inflammation,[5] analgesic,[6,7] opioid-like effects,[8,9] and morphine withdrawal effect.[10,11] However, legality is still constrained in its utilization related to the sedative effect caused by the high content of indole alkaloids. The content of major compounds in indole alkaloids is about 0.088%, knowing that it can be separated using chloroform extraction up to 95%. It can even reach 99% with some optimizations ~~reported by Beng et al. (2011)~~Beng et al. (2011) reported in laboratory experiments.[12] This result proves that different extraction methods can avoid the unwanted effects of alkaloid compounds. Therefore, it is necessary to innovate to suppress alkaloids and increase the content of other beneficial metabolites, mainly polyphenols. Optimization needs to be done to increase the level of target secondary metabolite through a green extraction approach.

With the growth of “Green Chemistry” in recent years, green chemistry principles approaches have gained more traction. One of the most significant features of green technology is green

Formatted: Space After: 0 pt

solvents. Natural deep eutectic solvent (NADES) is a green solvent type made up of the primary metabolites of living organisms. It is often made up of ingredients abundant in our everyday diet, making it inexpensive, safe, and sustainable.[13,14] NADES has emerged as a new type of green solvent with ~~a lot of~~many ~~good~~ good advantages, including biocompatibility, low toxicity, sustainability, ecological efficacy, and incredibly considerable solvent power. The eutectic mixtures have melting points below their ~~individual~~ components, usually above 150°C.[15] NADES comprises at least one species of the hydrogen bond donor (HBD) and the hydrogen bond (HBA), which establishes strong interactions between hydrogen bonding after mixing, leading to eutectic mixtures that are usually liquid under room-close conditions. The most common NADES compositions and pharmaceutical excipients include citric acid, lactic acid, malic acid, choline chloride, sucrose, glucose, and sorbitol.

On the other hand, conventional extraction methods have been used for a long time, but this requires a longer time and a ~~larger~~ more significant number of solvents. This is the main weakness of the conventional method. Therefore, ~~the development of~~ new extraction methods with reduced solvent consumption shortened extraction time and increased attention to pollution prevention. In comparison with conventional methods, microwave-assisted extraction can improve extraction efficiency by reducing time and solvent consumption.[16] Thus, microwave-assisted natural deep eutectic solvent extraction (MANDESE) has ~~great~~ excellent potential for extracting polyphenols compounds from natural products. MANDESE has risen rapidly in the last decade, and for most applications, it has proven to be effective in all aspects compared with conventional extraction techniques. Some studies have reported the application of the MANDESE method in extracting secondary metabolites from natural products, ~~include~~ including extraction of alkaloids and polyphenols from *Peumus boldus* leaves,[17] a flavonoid from *Scutellaria baicalensis*,[18] polyphenols and caffeine from green coffee beans,[19] bioactive compounds from Cinnamon Bark and Sappan Wood,[20] anthocyanins from *C. roseus*.[21] The application and optimization of the non-conventional MANDESE method on total polyphenols content extraction from *M. speciosa* leaves have never been reported.

In the current study, optimization of extraction condition of non-conventional MANDESE method performed using response surface methodology (RSM). RSM is a multi-factor mathematical and statistical test for the time and number of experimental samples.[22] This method

analyzes the impact of extraction factors to determine the best model from an optimum process combination of four factors and three-level using Box-Behnken Design.[23] To obtain the optimum condition, we considered using four factors: NADES composition ratio, the liquid-solid ratio, extraction time, and microwave power that significantly contributed to the total polyphenol content extraction process based on the green extraction approach. RSM with Box-Behnken Design is very efficient to optimize the extraction conditions and to achieve the best predicted model.[24,25]

The purpose of this study, to apply and optimize the non-conventional MANDESE method on total polyphenols content from *M. speciosa* leaves. We also conducted scanning electron microscopy (SEM) imaging on both powder samples before and after the extraction process to ~~provide a better description and clarification of~~describe better and clarify the extraction mechanism.

MATERIALS AND METHODS

Materials and Equipment

The dried sample of *M. speciosa* leaves was obtained from Kota Bangun, Kutai Kertanegara, East Kalimantan, Indonesia. The voucher specimen (043/HKKC-LP/FF-UNMUL/II/2021) was identified and authenticated in the Laboratory of Dendrology, Faculty of Forestry, stored at Pharmaceutical Research~~,~~ and Development Laboratory of FARMAKA TROPIS, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. Choline chloride, citric acid, sorbitol sucrose, glucose, lactic acid, and malic acid (100% pure pharmaceutical excipient and food grade) were purchased from CV. Chlorogreen, Bandung, Indonesia. Folin-Ciocalteu reagent, sodium carbonate, and gallic acid were purchased from Sigma-Aldrich, Germany (PT. Elo Karsa, Indonesia). Aqua demineralization, methanol for analysis, ethyl acetate for analysis, and n-hexane were purchased from SmartLab Indonesia, Tangerang, Banten, Indonesia. The equipment used in this study include spectrophotometer UV-Vis double beam (Dynamica, Hallo DB 20S, UK), JEOL scanning electron microscope (JSM-5510LV), a modified domestic microwave (Modena® 900 Watts, USA), a domestic Food dehydrator (Wirastar®, Indonesia), micropipettes (10-100 µL and 100–1000 µL, Socorex, Switzerland), a rotary vacuum evaporator (Rotavapor® R-215, Buchi, Flawil, Switzerland), and a licensed Design Expert v12 software (Stat-Ease, Minneapolis, MN, USA).

Preparation of Natural Deep Eutectic Solvent (NADES) as a Green Solvent

NADES preparation was conducted using different pharmaceutical excipient types, including citric acid and glucose, lactic acid and sucrose, malic acid and glucose, choline chloride, and sorbitol. Each NADES composition was prepared by heating and stirring each combination component at a specific ratio (g/g) in a tube at 60-80 ^{o}C accompanied by agitation at a speed of 3000 rpm for 40 min constantly until a homogenous mixture was formed. Each mixture was added to aqua demineralization with a ratio of 1:1 v/v and ~~continues~~ continued ~~stirred~~ stirring to obtain a homogenous NADES solution. All NADES solutions obtained were stable in liquid form at room temperature storage.

Extraction Process

Conventional Maceration Procedures

The dried sample of *M. speciosa* leaves (5 g) was extracted by the conventional maceration method using 50 mL ethanol 96% at room temperature for 3x24 hours. Then the extractant and the residue were separated using the Buchner funnel. The extract solution was evaporated to obtain the thick/dry extract.

Non-conventional Microwave-Assisted Natural Deep Eutectic Solvent Extraction Procedures

~~The extraction process was performed using the non-conventional microwave-assisted natural deep eutectic solvent extraction (MANDESE) method according to some previous studies.~~According to some previous studies, the extraction process was performed using the non-conventional microwave-assisted natural deep eutectic solvent extraction (MANDESE) method with modification. Briefly, a dried sample of *M. speciosa* leaves was extracted using microwave-assisted extraction combined with each combination of NADES composition under various conditions (**Table 1**). After the extraction process, the extract solution was separated with its residue by filtration using 0.45 cellulose acetate membrane and evaporated to dry using a domestic food dehydrator. The dry extract was stored in a tightly closed container until ready to use.

Table 1. Design Experimental (with Box-Behnken Design) of Response Surface Methodology (RSM) with a green solvent of choline chloride-sorbitol.

Factors	Unit	Symbol	Range and Level		
			Low (-1)	Medium (0)	High (1)
NADES Composition	g/g	X_1	1:1	2:1	3:1

Ratio					
Liquid-Solid Ratio	mL/g	X_2	10	20	30
Extraction Time	Minutes	X_3	10	15	20
Microwave Power	% Watts	X_4	40	50	60

Determination of Total Polyphenols Content

T~~otal polyphenols contents were determined using Folin-Ciocalteu reagent by spectrophotometry at the maximum wavelength with a range of 730–760 nm regarding the previous study~~he previous study determined total polyphenols contents using Folin-Ciocalteu reagent by spectrophotometry at the maximum wavelength with a range of 730–760 nm.[26] Briefly, standard and sample solution (1 mL) was added to 0.5 mL of Colin-Ciocalteu reagent and 5 mL of distilled water. The mixture was homogenized for 1 min and then allowed to stand for 4 min. Then 1.5 mL distilled water~~,~~ and 2 mL sodium carbonate were added and homogenized for 1 min. Next, the mixture of the sample test was incubated for one h at room temperature. ~~The absorbance was measured by a spectrophotometer.~~A spectrophotometer measured the absorbance at 746 nm. Gallic acid solution with some different concentrations (12.5, 25, 50, 100, and 200 µg/mL) was used as a standard to obtain a linear regression equation (1), Y = 0.0022X – 0.00095, where R2 = 0.9977. Where coefficient correlation (R^2) value of 0.998, y is absorbance, and x is total polyphenols content. The total polyphenols content (in µg GAE/g sample) was determined using this equation.

Scanning Electron Microscopy Imaging

Scanning electron microscopy (SEM) imaging was carried out to study the extraction mechanism and compare changes in the structure of the sample before and after the extraction process related to works of literature.[27,28,29] Briefly, each dried powder of sample (after and before extraction) was plated on the carbon plate and coated with the spray of a nano-palladium-gold solution to form a conductive surface. Then, it was observed by SEM at 20 kV operating voltage and under high vacuum conditions.

Optimization of MANDESE Conditions using RSM

Optimization of MANDESE condition was performed using Box-Behnken Design with four factors and three levels (in **Table 1**), with independent variables including NADES composition ratio (X_1), liquid-solid ratio (X_2), Extraction time (X_3), and microwave power (X_4). In contrast,

the dependent variable is total polyphenols content as a response. Based on total polyphenols content extraction efficiency, all factors and levels were expected to achieve optimum MANDESE conditions significantly. The variation condition of MANDESE was simulated with RSM using Box-Behnken Design to obtain 29 experiment samples run. A total of 29 experiments were needed to build the math equation formula (2),[30] as follow:

$$Y = A_0 + \sum_{i=1} A_{ii} X_i^2 + \sum_{i=1}^{n} \sum_{=i+1}^{n} A_{ij} X_i X_j$$

The regression model was calculated based on 29 experiments data from the independent and dependent variables by the multilinear quadratic model using the licensed Design Expert v12 program. The best and significant model was determined according to the R^2 value and analysis of variance (ANOVA).

RESULTS

Selection of NADES Compositions and Total Polyphenols Content Determination

In the present study, the selection of solvent is an essential step in the extraction process. Some NADES compositions were used to determine the effectiveness of extraction on the total polyphenols content of *M. speciosa* leaves, compared with the conventional maceration extraction method using ethanol as a solvent (As can be seen in **Figure 1**). The extraction of the target secondary metabolites from the matrix sample of plants using several NADES compositions according to the solubility balance.

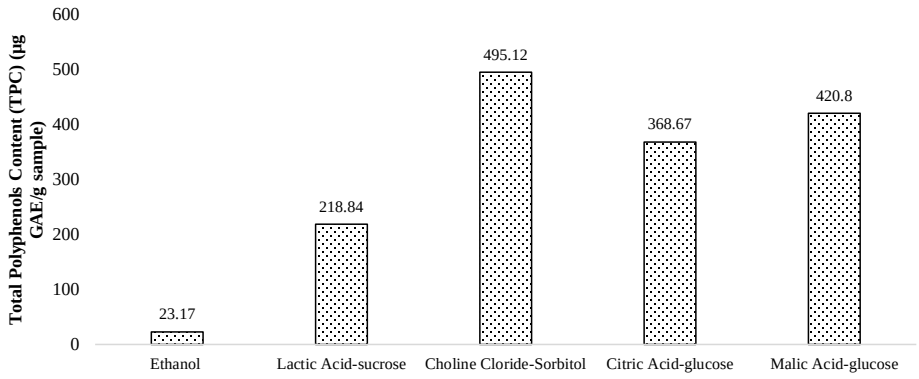


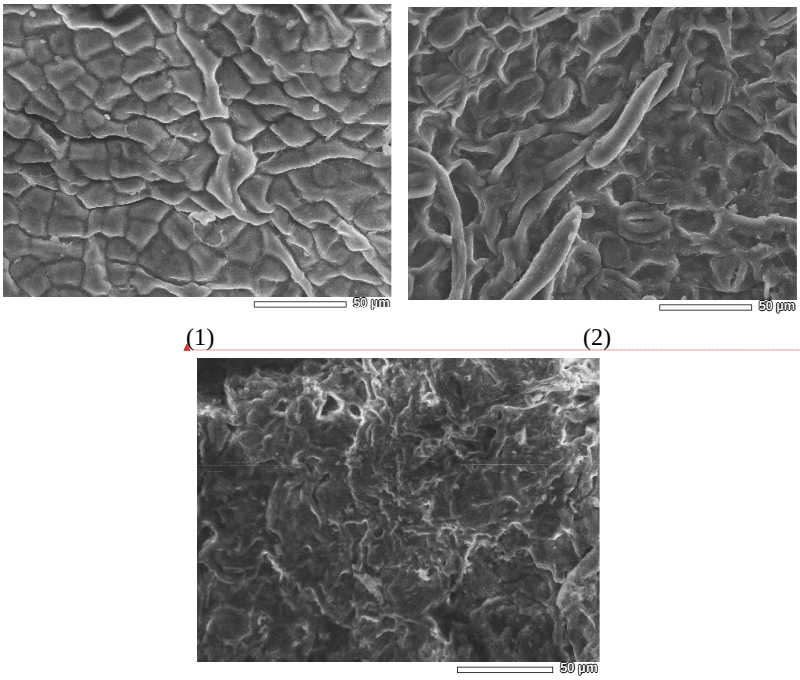
Figure 1. The results of total polyphenols content based on different NADES compositions for non-conventional MANDESE and ethanol for conventional maceration methods

Figure 1 demonstrated that the extraction process using the non-conventional microwave-assisted natural deep eutectic solvent extraction (MANDESE) method (with some different NADES composition) has a higher efficiency of extracting the target secondary metabolite than the conventional maceration method. Meanwhile, the use of the NADES composition with various combinations of pharmaceutical excipient mixtures at the same ratio (1:1 g/g) showed differences in the ability to extract target secondary metabolite (total polyphenols content), including choline chloride-sorbitol, malic acid-glucose, citric acid-glucose, and lactic acid sucrose, respectively. Therefore, the combination of choline chloride-sorbitol was used as the NADES composition of green solvent to optimize the extraction condition using the MANDESE method. In this study, total polyphenols contents (in µg GAE/g sample) were calculated using equation (1).

Scanning Electron Microscopy Imaging

Scanning electron microscopy imaging was conducted to examine the effect of conventional maceration and non-conventional MANDESE methods and understand the extraction mechanism. **Figure 2** shows the micrographs of the dried leave of samples before extraction (1), after extraction by maceration (2), and after extraction by the MANDESE method (3). The result

shows that the levels of damage of cells and cell walls were more severe after extraction using the MANDESE method than the maceration method.



(1) (2)

(3)

Figure 2. The Micrograph of SEM imaging results of the dried leaves powder of sample, (1) before treatment, (2) after maceration treatment, and (3) after MANDESE treatment

Optimization of MANDESE Method Using RSM

The MANDESE method was optimized using RSM to obtain the optimum extraction condition on total polyphenol content from *M. speciosa* leaves. The experimental design was built with four factors and three levels (Box-Behnken Design) using the licensed Design Expert v12 program, such as NADES ratio of choline chloride and sorbitol (1:1, 2:1, and 3:1 g/g), a liquid-solid ratio of NADES solution and dried leaves powder of sample (10, 20, and 30 mL/g), extraction time (10, 15, and 20 min), and microwave power (40, 50, and 60 %watts). In this work, a total of 29 experiments were carried out according to the experimental design (Box-

Formatted: Font: Times New Roman, 12 pt, Not Bold

Formatted: Font: Times New Roman, 12 pt

Behnken Design) of RSM to predict the optimum extraction condition of total polyphenols content from *M. speciosa* leaves (**Table 2**).

The extraction process According to the results obtained using the licensed Design Expert v12 program shows the highest total polyphenols content from *M. speciosa* leaves were obtained from run 24th with the yield of 495.12 µg GAE/g sample and the lowest the yield of 42.12 µg GAE/g sample (run 19th), as can be seen in **Table 2**. The results of 29 experiment data were calculated to predict the optimum extraction condition of the MANDESE method using multilinear regression analysis. The best equation prediction regression model was obtained based on the calculation results using equation (2), as follows:

$$Y = 352.89 + 97.98X_1 + 100.27X_2 + 56.74X_3 + 7.19X_4 - 62.65X_1X_2 + 29.87X_1X_3 + 15.16X_1X_4 - 51.04X_2X_3 + 90.31X_2X_4 + 21.15X_3X_4 - 37.11X_1^2 - 117.22X_2^2 - 14.27X_3^2 - 64.57X_4^2 + 19.98X_1^2X_2 + 18.28X_1^2X_3 - 64.45X_1^2X_2^2 - 2X_1X_3^2 - 21.1X_2^2X_3 + 54.41\ X_2^2X_4 - 228.85\ X_2X_3^2 + 147.21X_1^2X_2^2 + 29.11X_1^2X_3^2$$

Where coefficient correlation (R^2) value of 0.9994, Y is total polyphenols content (µg GAE/g sample), X_1 is NADES composition ratio (g/g), X_2 is extraction time (min), X_3 is a liquid-solid ratio (mL/g), and X_4 is microwave power (%Watts).

Table 2. Box-Behnken Design (four factors and three levels) with observed predicted responses value of total polyphenols content (µg GAE/g sample) from *M. speciosa* leaves.

Run	Factors Variable				Total polyphenol content (µg GAE/g)	
	X_1	X_2	X_3	X_4	Observed	Predicted
1	1 (-1)	20 (0)	10 (-1)	50 (0)	129.37	129.37
2	2 (0)	10 (-1)	15 (0)	40 (-1)	229.32	231.26
3	2 (0)	30 (1)	15(0)	40 (-1)	300.51	302.45
4	2 (0)	30 (1)	15 (0)	60 (1)	361.07	359.13
5	2 (0)	20 (0)	20 (1)	40 (-1)	119.46	119.46
6	3 (1)	10 (-1)	15 (0)	50 (0)	231.70	231.70
7	3 (1)	20 (0)	10 (-1)	50 (0)	321.71	321.71

8	2 (0)	20 (0)	15 (0)	50 (0)	355.19	352.89
9	3 (1)	20 (0)	15 (0)	40 (-1)	328.77	326.83
10	2 (0)	20 (0)	15 (0)	50 (0)	343.18	352.89
11	3 (1)	30 (1)	15 (0)	50 (0)	441.49	441.49
12	1 (-1)	20 (0)	15 (0)	40 (-1)	163.14	161.20
13	2 (0)	20 (0)	15 (0)	50 (0)	364.78	352.89
14	2 (0)	10 (-1)	15 (0)	60 (1)	205.30	203.36
15	1 (-1)	20 (0)	15 (0)	60 (1)	143.32	145.26
16	2 (0)	20 (0)	15 (0)	50 (0)	349.34	352.89
17	2 (0)	30 (1)	20 (1)	50 (0)	77.42	77.42
18	2 (0)	10 (-1)	10 (-1)	50 (0)	263.30	263.30
19	2 (0)	20 (0)	10 (-1)	60 (1)	42.12	42.12
20	1 (-1)	30 (1)	15 (0)	50 (0)	369.80	269.80
21	1 (-1)	10 (-1)	15 (0)	50 (0)	279.49	279.49
22	3 (1)	20 (0)	15 (0)	60 (1)	369.61	371.54
23	2 (0)	10 (-1)	15(0)	50 (0)	108.21	108.21
24	1 (-1)	20 (0)	20 (1)	50 (0)	495.12	495.12
25	2 (0)	20 (0)	10 (-1)	40 (-1)	99.533	99.53
26	2 (0)	20 (0)	15 (0)	50 (0)	351.97	352.89
27	3 (1)	20 (0)	20 (1)	50 (0)	436.88	436.88
28	2 (0)	20 (0)	20 (1)	60 (1)	423.28	423.28
29	2 (0)	30 (1)	10 (-1)	50 (0)	436.67	436.67

~~In~~ **Table 3**, based on the analysis of variance (ANOVA) from total polyphenols content, shows that the resulting model has a significant effect on the total polyphenols content with an F-value of 341.70 (refers to p-value < 0.05), where a 0.01% chance the F-value can occur due to an error or interference. In this case, X_1, X_2, X_3, X_4, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4, X_3X_4, X_1^2, X_2^2, X_3^2, X_4^2, $X_1^2X_2$, $X_1^2X_3$, $X_1X_2^2$, $X_1X_3^2$, $X_2^2X_3$, $X_2^2X_4$, $X_1^2X_2^2$, $X_1^2X_3^2$ are significant model term. On the other hand, the F-value of "Lack of Fit" was 0.47 (with a p-value of 0.5293), which indicated that the p-value > 0.005 was not significant to the pure error. There is a 52.93% chance that a Lack of Fit F-value this large could occur due to noise. In addition, the optimum condition from the equation model can be applied as navigation to design the desired optimum extraction condition.

Table 3. Analysis of Variance (ANOVA) for response surface by the regression model

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Model	0.00004470	23	19435.86	341.70	< 0.0001
X_1	38397.77	1	38397.77	675.07	< 0.0001
X_2	12878.28	1	12878.28	226.41	< 0.0001
X_3	40216.09	1	40216.09	707.04	< 0.0001
X_4	414.09	1	414.09	7.28	0.0429

X_1X_2	3568.63	1	3568.63	62.74	0.0005
X_1X_3	15697.71	1	15697.71	275.98	< 0.0001
X_1X_4	919.70	1	919.70	16.17	0.0101
X_2X_3	10419.82	1	10419.82	183.19	< 0.0001
X_2X_4	1788.74	1	1788.74	31.45	0.0025
X_3X_4	32620.51	1	32620.51	573.50	< 0.0001
X_1^2	2825.30	1	2825.30	49.67	0.0009
X_2^2	857.40	1	857.40	15.07	0.0116
X_3^2	57858.78	1	57858.78	1017.22	< 0.0001
X_4^2	17555.96	1	17555.96	308.65	< 0.0001
$X_1^2X_2$	668.48	1	668.48	11.75	0.0187
$X_1^2X_3$	796.80	1	796.80	14.01	0.0134
$X_1X_2^2$	16928.28	1	16928.28	297.62	< 0.0001
$X_1X_3^2$	8308.77	1	8308.77	146.08	< 0.0001
$X_2^2X_3$	0.00001047	1	0.00001047	1841.60	< 0.0001
$X_2X_3^2$	890.25	1	890.25	15.65	0.0108
$X_3^2X_4$	7893.59	1	7893.59	138.78	< 0.0001
$X_1^2X_2^2$	847.14	1	847.14	14.89	0.0119
$X_1^2X_3^2$	21670.93	1	21670.93	381.00	< 0.0001
Residual	284.40	5	56.88		
Lack of Fit	30.09	1	30.09	0.4732	0.5293
Pure Error	254.31	4	63.58		
Cor Total	0.00004473	28			

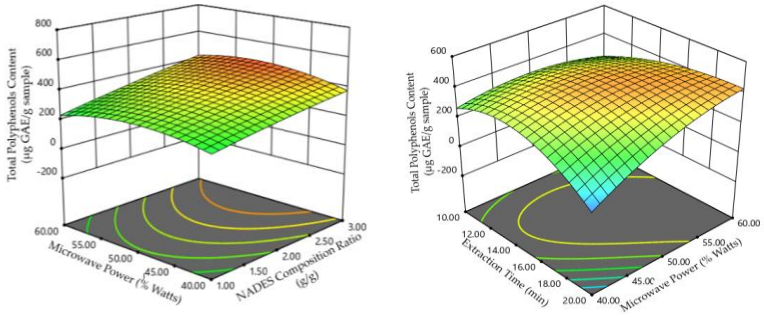
In **Table 4**, the coefficient estimates represent the expected change in response per unit in factor value when all other factors are constant. Orthogonal design intercept is the overall average response of all analyses. The coefficient fits the mean based on the coefficient set. If the factors are orthogonal, the variance inflation factor (VIF) is 1. VIF greater than 1 indicates multicollinearity, and the higher the VIF, the stronger the correlation of the factors. As a hard rule, a VIF below 10 is acceptable. The equation in phrases of codec factors can be applied to predict approximately the response for given tiers of every factor. By default, the excessive tiers of the factors are coded as +1, and low tiers are coded as -1. The codec equation is beneficial for figuring out the relative effect of the factor through evaluating the factor coefficients.

Table 4. Coefficient Estimate (CE), Standard Error (SE), Confidence Interval (CI), and Variance Inflation Factor (VIF) of the modified quadratic regression model

Factor	Coefficient Estimate (CE)	df	Standard Error (SE)	95% Confidence Interval (CI)		Variance Inflation Factor (VIF)
				Low	High	
Intercept	352.89	1	3.37	344.22	361.56	-
X_1	97.98	1	3.77	88.28	107.67	3.0000
X_2	56.74	1	3.77	47.05	66.43	3.0000
X_3	100.27	1	3.77	90.58	109.96	3.0000

X_4	7.19	1	2.67	0.3402	14.05	1.5000
X_1X_2	29.65	1	3.77	20.18	39.56	1.0000
X_1X_3	-62.65	1	3.77	-72.34	-52.95	1.0000
X_1X_4	15.15	1	3.77	5.47	24.68	1.0000
X_2X_3	-51.04	1	3.77	-60.73	-41.35	1.0000
X_2X_4	21.15	1	3.77	11.45	30.84	1.0000
X_3X_4	90.31	1	3.77	80.61	100.00	1.0000
X_1^2	-37.11	1	5.27	-50.65	-23.58	3.0000
X_2^2	-14.27	1	3.68	-23.72	-4.82	1.6700
X_3^2	-117.22	1	3.68	-122.67	-107.78	1.6700
X_4^2	-64.57	1	3.68	-74.02	-55.12	1.6700
$X_1^2X_2$	18.28	1	5.33	4.57	31.99	2.0000
$X_1^2X_3$	19.96	1	5.33	6.25	33.67	2.0000
$X_1X_2^2$	-92.00	1	5.33	-105.71	-78.29	2.0000
$X_1X_3^2$	-64.45	1	5.33	-78.16	-50.75	2.0000
$X_2X_3^2$	-21.10	1	5.33	-34.81	-7.39	2.0000
$X_2^2X_3$	-228.85	1	5.33	-242.56	-215.15	2.0000
$X_3^2X_4$	54.41	1	4.62	42.53	66.28	1.5000
$X_1^2X_2^2$	147.21	1	7.54	127.82	166.69	3.4500
$X_1^2X_3^2$	29.11	1	7.54	9.72	48.49	3.4500

The three-dimension (3D) response graph and contour plot for the effect of various extraction variable factors on total polyphenol content was presented in **Figure 3.** It demonstrated that the curvature of these plots indicates the interaction and relationship between each factor that are variable parameters of extraction condition, including microwave power and NADES composition ratio; microwave power and extraction time; microwave power and liquid-solid ratio; extraction time and liquid-solid ratio; liquid-solid ration and NADES composition ratio; and Extraction time and NADES composition ratio. In general, with view glance from the results, the efficiency of polyphenols extraction from this plant was strongly influenced by these various variable factors used.



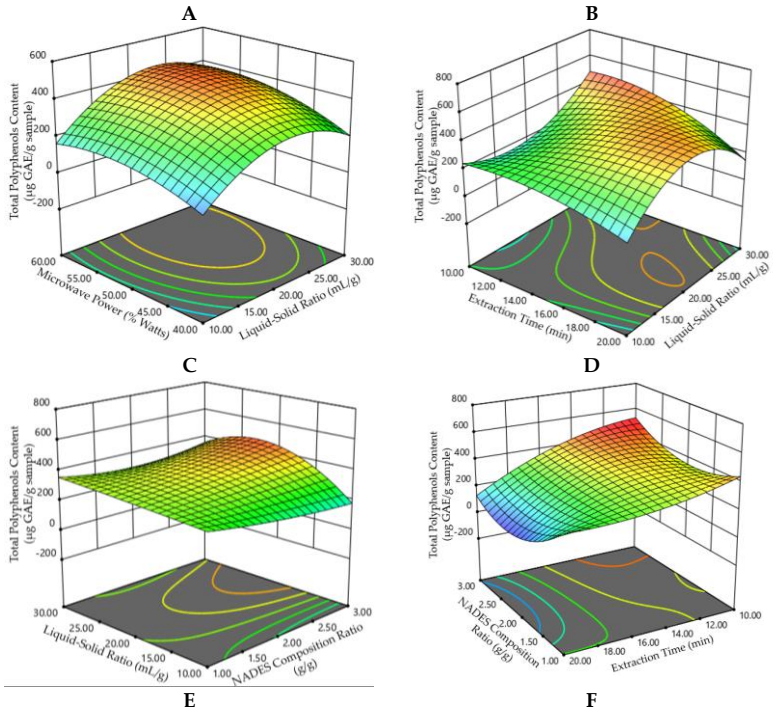


Figure 2. The influence of variable factors on TPC extraction from *M. speciosa* include power and NADES composition ratio (**A**); power and time (**B**); power and liquid-solid ratio (**C**); time and liquid-solid ratio (**D**); liquid-solid and NADES ratio (**E**); time and NADES ratio (**F**)

DISCUSSION

In the present study, the NADES composition ~~with a combination of~~combined with choline chloride and sorbitol as a green solvent extracted secondary metabolites with the highest total polyphenol content compared with conventional organic ethanol solvent and other NADES compositions. The above results show that a green solvent of NADES composition with choline

chloride-sorbitol was more effective than the others. This solvent is twenty times as strong as that of ethanol. Other parameters, such as microwave power, extraction time, and liquid-solid ratio, also significantly impact the extraction efficiency. As a result, it becomes the essential aspect to consider in selecting these four factors in optimizing the extraction conditions.

On the other hand, the non-conventional microwave-assisted natural deep eutectic solvent extraction (MANDESE) method provides ~~a number of~~several advantages, including non-volatile, low-toxicity, operator convenience and safety, and environmentally friendliness,[20,31,32] as well as being available in the laboratory. This result is in line with the results of SEM imaging, which shows that the level of cell wall damage in the sample matrix has a good correlation with the level of extraction efficiency. Each extraction method, which is compared to the sample matrix before the extraction procedure for both samples, can demonstrate physical, structural changes in the cell wall of the sample matrix. The effect of the extraction process on the extent of damage to the cell wall matrix of samples has been revealed in several papers.[29,33,34]

Based on our findings, the optimum condition of the MANDESE method was obtained regarding the RSM analysis result using the licensed Design Expert v12 software as follows: NADES composition ratio of 3 g/g (choline chloride/sorbitol) and the liquid-solid ratio of 20 mL/g for 20 min extraction time with 60% Watts microwave power with total polyphenols content prediction of 539.37 µg GAE/g sample of *M. speciosa* leaves. We performed a scale-up confirmation test to prove the accuracy of the prediction of the optimum conditions obtained. A 50 g dried sample of *M. speciosa* leaves was extracted under the recommended optimum conditions, providing the approximately total polyphenols content value of 526.12 µg GAE/g sample (with a standard deviation of 5.418 in triple repetition).

A combination of choline chloride and sorbitol with various ratios from 1 to 3 g/g was used in this study. The interaction impact of many aspects and sample characteristics contribute to the variation in optimum conditions. NADES composition with choline chloride and sorbitol was chosen and implemented in this study based on the selectivity efficiency in extracting total polyphenols content. Both materials ~~are a mix of~~mix solids and liquids, with sorbitol acting as a hydrogen-bonding donor (HBD) and choline chloride acting as a hydrogen bond acceptor

(HBA).[35,36,37] At temperatures less than 100^{o}C, the deep eutectic solvent in liquid was formed by adjusting the NADES composition ratio at a specific proportion (HBD and HBA mixture).[38,37]

Furthermore, different liquid-solid ratios, such as 10, 20, and 30 mL/g was used to test the effect. In this work, the increase in the liquid-solid ratio causes maximal direct contact between target secondary metabolites and the green solvent in the matrix sample under these conditions.[39] However, if it is too large or has a high ratio, it will cause a waste of material used. A low ratio can result in an inefficient extraction process.[40] The optimum condition of the MANDESE method with NADES composition and the liquid-solid ratio was 3 g/g (choline chloride-sorbitol) and 20 mL/g (NADES solution-sample).

While the influence of various conditions of the extraction equipment, such as microwave power and extraction time, dramatically affects the level of ~~an~~ extraction efficiency of the total polyphenol content. In this study, the effect of microwave power was tested at various levels extending from 40 to 60 % Watts. When the microwave power is increased, the temperature rises. A temperature increase that is too high can damage the sample matrix and generate a change in the target compound's structure.[16,26] ~~For extraction time, t~~The extraction process was carried out in different ~~variations of time~~time variations, including 10, 15, and 20 min, based on some previous studies.[41,42,43] This result shows that the highest total polyphenols content of *M. speciosa* leaves was achieved at 20 minutes for optimal extraction time. This situation shows that the dissolution process of the target secondary metabolite in the sample is in equilibrium [44]. Based on these two conditions, there is a tendency to increase the yield of the total polyphenol content. However, the increase in microwave power and extraction time was limited because the resulting temperature could not be regulated. Excessive temperatures should be avoided because most secondary metabolites decompose at high temperatures.

Overall, these findings indicate that ~~a variety of~~various variable factors used proved to significantly affect the extraction efficiency of total polyphenols from *M. speciosa* leaves. However, our findings should be interpreted with caution, given several limitations. First, the target secondary metabolites (total polyphenols content) are compounds measured using the UV-Vis spectrophotometry method with Folin-Ciocalteu reagent, calculated based on equivalence and only using standard gallic acid. Therefore, prospective studies using pure compounds as the

target to ensure the selectivity of green solvent media used with more sensitive analytical methods (e.g., HPLC) are needed to confirm our findings. Second, in this study, only four combinations of excipients were used as the composition of NADES, so that the best NADES composition used had not been compared with other materials that may have high selectivity potential when used as a solvent. Third, the selectivity of the target compound only focuses on the efficiency level concerning polyphenol levels, has not considered the possibility of other secondary metabolite groups, so further analysis is needed.

Despite these limitations, to the best of our knowledge, this is the first time reported regarding the use and optimization of the MANDESE method to increase the efficiency of polyphenol extraction from M. speciosa leaves. This finding adds to information related to the use of NADES as a green solvent and optimum medium for extracting target secondary metabolites (especially the polyphenol group), considering that this group of compounds from plants has not been widely studied ~~and~~ developed for its benefits.

ACKNOWLEDGMENTS

This research was funded by ~~**KEMENTERIAN PENDIDIKAN, KEBUDAYAAN, RISET DAN TEKNOLOGI RI (Formerly KEMENTERIAN RISET DAN TEKNOLOGI/ BADAN RISET DAN INOVASI NASIONAL, DEPUTI BIDANG PENGUATAN RISET DAN PENGEMBANGAN)**~~**Direktorat Riset dan Pengabdian Masyarakat Deputi Bidang Penguatan Riset dan Pengembangan Kementerian Riset dan Teknologi/Badan Riset dan Inovasi**, via Hibah Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) with a grant number: ~~036/E4.1/AK.04.PT/2021~~036/SP2H/LT/DRPM/2021 and 580/UN17.L1/PG/2021.

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

REFERENCES

1. Ridsdale CE. A revision of Mitragyna and Uncaria (Rubiaceae). Blumea. 1978; 24: 43-100.
2. Singh D, Narayanan S, Vicknasingam B. Traditional and non-traditional uses of Mitragynine (Kratom): A survey of the literature. Brain Res Bull. 2016; 126: 41-46.
3. Luliana S, Islamy MR. Antinociceptive activity of dichloromethane fraction of Kratom leaves (*Mitragyna speciosa* Korth.) by oral route in male swiss mice. Pharm Sci Res. 2018; 5(2): 58-64.
4. Hiranita T, Leon F, Felix JS, Restrepo LF, Reeves ME, Pennington AE, Obeng S, Avery BA, McCurdy CR, McMahon LR, Wilkerson JL. The effects of mitragynine and morphine on schedule-controlled responding and antinociception in rats. Psychopharmacol. 2020; 236(9): 2725-2734.
5. Tohar N, Shilpi JA, Sivasothy Y, Ahmad S, Awang K. Chemical constituents and nitric oxide inhibitory activity of supercritical carbon dioxide extracts from *Mitragyna speciosa* leaves. Arab J Chem. 2019; 12(3): 350-359.
6. Reanmongkol W, Keawpradub N, Sawangjaroen K. Effects of the extracts from *Mitragyna speciosa* Korth. leaves on analgesic and behavioral activities in experimental animals. Songklanakarin J Sci Technol. 2007; 29: 39-48.
7. Ya K, Tangamornsuksan W, Scholfield CN, Methaneethorn J, Lohitnavy M. Pharmacokinetics of mitragynine, a major analgesic alkaloid in kratom (*Mitragyna speciosa*): A systematic review. Asian J Psychiatr. 2019; 43: 73-82.
8. Kruegel AC, Uptery R, Grinnell SG, Langreck C, Pekarskaya EA, Le Rouzic V, Ansonoff M, Gassaway MM, Pintar JE, Pasternak GW, Javitch JA, Majumdar S, Sames D. 7-Hydroxymitragynine is an active metabolite of mitragynine and a key mediator of its analgesic effects. ACS Cent Sci. 2019; 5(6): 992-1001.
9. Kaye AD, Cornett EM, Patil SS, Gennuso SA, Colontonio MN, Latimer DR, Kaye AJ, Urman RD, Vadivelu N. New opioid receptor modulators and agonists. Best Pract Res Clin Anaesthesiol. 2018; 32(2): 125-136.
10. Hassan R, Pike See C, Sreenivasan S, Mansor SM, Müller CP, Hassan Z. Mitragynine attenuates morphine withdrawal effects in rats—A comparison with methadone and

buprenorphine. Front Psychiatry. 2020; 11: 1-13.
11. Saingam D, Assanangkornchai S, Geater AF, Lerkiatbundit S. Factor analytical investigation of krathom (*Mitragyna speciosa* Korth.) withdrawal syndrome in Thailand. J Psychoactive Drugs. 2016; 48(2): 76-85.
12. Beng GT, Hamdan MR, Siddiqui MJ, Mordi MN, Mansor SM. A simple and cost effective isolation and purification protocol of mitragynine from *Mitragyna speciosa* korth (ketum) leaves. Malaysian J Anal Sci. 2011; 15(1): 54-60.
13. Dai Y, Spronsen JV, G. W, Verporte R, Choi YH. Natural deep eutectic solvents as new potential media for green technology. Anal Chim Acta. 2013; 766: 61-68.
14. Savi LK, Dias MCGC, Carpine D, Waszczynskyj N, Ribani RH, Haminiuk CWI. Natural deep eutectic solvents (NADES) based on citric acid and sucrose as a potential green technology: a comprehensive study of water inclusion and its effect on thermal, physical and rheological properties. Int J Food Sci Technol. 2018; 54(3): 898-907.
15. Liu Y, Friesen JB, Mcalpine JB, Lankin DC, Chen S, Pauli GF. Natural deep eutectic solvents: Properties, applications, and perspectives. J Nat Prod. 2018; 81: 679-690.
16. Ahmad I, Yanuar A, Mulia K, Mun'im A. Ionic liquid-based microwave-assisted extraction: Fast and green extraction method of secondary metabolite on medicinal plant. Pharmacogn Rev. 2018; 12(23): 20-26.
17. Torres-Vega J, Gomez-Alonso S, Perez-Navarro J, Pastene-Navarrete E. Green extraction of alkaloids and polyphenols from Peumus boldus leaves with natural deep eutectic solvents and profiling by HPLC-PDA-IT-MS/MS and HPLC-QTOF-MS/MS. Plants. 2020; 9: p. 242.
18. Oomen WW, Begines P, Mustafa NR, Wilson EG, Verpoorte R, Choi YH. Natural deep eutectic solvent extraction of flavonoids of *Scutellaria baicalensis* as a replacement for conventional organic solvents. Molecules. 2020; 25: p. 617.
19. Ahmad I, Miftah A, Azminah A, Chany F, Mun A. Optimization of betaine-sorbitol natural deep eutectic solvent-based ultrasound-assisted extraction and pancreatic lipase inhibitory activity of chlorogenic acid and caffeine content from robusta green coffee beans. Heliyon. 2021; 7(8): p. e07702.
20. Ahmad I, Arifianti AE, Sakti AS, Saputri FC, Mun'im A. Simultaneous natural deep

eutectic solvent-based ultrasonic-assisted extraction of bioactive compounds of Cinnamon bark and Sappan wood as a dipeptidyl peptidase IV inhibitor. Molecules. 2020; 25: p. 3832.

21. Dai Y, Rozema E, Verpoorte R, Choi YH. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. J Chromatogr A. 2016; 1434: 50-56.
22. Dahmoune F, Nayak B, Moussi K, Remini H, Madani K. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chem. 2015; 166: 585-595.
23. Meeker W, Hahn G, Escobar L. *Statistical Interval, A Guide for Practioner and Researcher*. 2nd Editio. John Wiley and Sons, Inc; 2017.
24. Piepel G, Szychowski J, Leoppky J. Augmenting scheffe linear mixture models with squared and/or crossproduct terms. J Qual Technol. 2002; 34(3): 297-314.
25. Brusco MJ, Steinley D, Cradit JD. An exact algorithm for hierarchically well-formulated subsets in second-order polynomial regression. Technometrics. 2009; 51(3): 306-315.
26. Herman, Ibrahim A, Rahayu BP, Arifuddin M, Nur Y, Prabowo WC, Maryono, Ambarwati NSS, Rijai L, Ahmad I. Single factor effect of natural deep eutectic solvent citric acid-glucose based microwave-assisted extraction on total polyphenols content from *Mitragyna speciosa* Korth. Havil leaves. Pharmacogn J. 2021; 13(5): 1109-1115.
27. Liu T, Sui X, Zhang R, Yang L, Zu Y, Zhang L. Application of ionic liquids based microwave-assisted simultaneous extraction of carnosic acid, rosmarinic acid and essential oil from *Rosmarinus officinalis*. J Chromatogr A. 2011; 1218(47): 8480-8489.
28. Khademi A, Saatchi M, Mehdi M, Baghaei B. Scanning electron microscopic evaluation of residual smear layer following preparation of curved root canals using hand instrumentation of two engine-criven systems. Iran Endod J. 2015; 10(4): 236-239.
29. Ahmad I, Yanuar A, Mulia K, Mun'im A. Application of ionic liquid as a green solvent for polyphenolics content extraction of *Peperomia pellucida* (L) Kunth herb. J Young Pharm. 2017; 9(4): 486-490.
30. Peng Y, Khaled U, Al-Rashed AAAA, Meer R, Goodarzi M, Sarafraz MM. Potential application of Response Surface Methodology (RSM) for the prediction and optimization

of thermal conductivity of aqueous CuO (II) nanofluid: A statistical approach and experimental validation. Phys A Stat Mech its Appl. 2020; 554: 124353.

31. Paiva A, Creveiro R, Aroso I, Martins M, Reis RL, Duarte ARC. Natural deep eutectic solvents − solvents for the 21st century. Am Chem Soc Sustain Chem Eng. 2014; 2: 1063-1071.
32. Espino M, Fernández M de los Á, Gomez FJV, Boiteux J, Silva MF. Green analytical chemistry metrics: Towards a sustainable phenolics extraction from medicinal plants. Microchem J. 2018; 141: 438-443.
33. Ding X, Li L, Wang Y, Chen J, Huang Y, Xu K. Design of guanidinium ionic liquid based microwave-assisted extraction for the efficient extraction of Praeruptorin A from *Radix peucedani*. J Sep Sci. 2014; 37: 3539-3547.
34. Zhang Q, Zhao S, Chen J, Zhang L. Application of ionic liquid-based microwave-assisted extraction of flavonoids from *Scutellaria baicalensis* Georgi. J Chromatogr B. 2015; 1002: 411-417.
35. Duan L, Dou L, Guo L, Li P, Liu E. Comprehensive evaluation of deep eutectic solvents in extraction of bioactive natural products. ACS Sustain Chem Eng. 2016; 4(4): 2405-2411.
36. Leng KY, Suyin G. Natural deep eutectic solvent (NADES) as a greener alternative for the extraction of hydrophilic (Polar) and lipophilic (non-polar) phytonutrients. Key Eng Mater. 2019; 797(4): 20-28.
37. Farias FO, Pereira JFB, Coutinho JAP, Igarashi-Mafra L, Mafra MR. Understanding the role of the hydrogen bond donor of the deep eutectic solvents in the formation of the aqueous biphasic systems. Fluid Phase Equilib. 2020; 503: 112319.
38. Vian M, Breil C, Vernes L, Chaabani E, Chemat F. Green solvents for sample preparation in analytical chemistry. Curr Opin Green Sustain Chem. 2017; 5: 44-48.
39. Liu Y, Li J, Fu R, Zhang L, Wang D, Wang S. Enhanced extraction of natural pigments from *Curcuma longa* L. using natural deep eutectic solvents. Ind Crops Prod. 2019; 140: 111620.
40. Ozturk B, Parkinson C, Gonzalez-Miquel M. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents. Sep Purif Technol. 2018; 206: 1-13.

41. Pal CBT, Jadeja GC. Microwave-assisted deep eutectic solvent extraction of phenolic antioxidants from onion (*Allium cepa* L.) peel: a Box–Behnken design approach for optimization. J Food Sci Technol. 2019; 56(9): 4211-4223.
42. Gomez, A.V.; Tadini, C.C.; Biswas, A.; Buttrum, M.; Kim, S.; Boddu, V.M.; Cheng, H.N. Microwave-assisted extraction of soluble sugars from banana puree with natural deep eutectic solvents (NADES). LWT - Food Sci Technol. 2019; 107: 79-88.
43. Wang H, Ma X, Cheng Q, Xi X, Zhang L. Deep eutectic solvent-based microwave-assisted extraction of baicalin from *Scutellaria baicalensis* Georgi. J Chem. 2018; 2018: 1-11.
44. Sahin S, Samli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason Sonochem. 2013; 20(1): 595-602.