Trop J Nat Prod Res, June 2024; 8(6):7474-7482 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Optimization Microwave-Assisted Extraction of *Moringa oleifera* Leaves Using Response Surface Methodology Focused on Extracting Phenolic And Flavonoid With Antioxidant Activity

Feda A. Makkiyah[1], Dhigna L.C. Pradana[1], Raihan P. Putra[2], Waras Nurcholis[2,3]*

[1]*Faculty of Medicine, Universitas Pembangunan Nasional Veteran, Jakarta, Indonesia*
[2]*Department of Biochemistry, Faculty of Mathematics and Natural Science, IPB University, Jl. Meranti, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia*
[3]*Tropical Biopharmaca Research Center, IPB University, Jl. Taman Kencana, Kampus IPB Taman Kencana, Bogor 16128, West Java, Indonesia.*

ARTICLE INFO

Article history:
Received 20 May 2024
Revised 27 May 2024
Accepted 31 May 2024
Published online 01 July 2024

ABSTRACT

Moringa oleifera is a plant with high levels of metabolites that possess pharmacological effect such as antioxidant. Therefore, it is crucial to study the extraction conditions of this plant to obtain the optimal amount of metabolites and pharmacological benefits. This research aimed to determine the optimal extraction time, ethanol concentration, and solid-solvent ratio for *M. oleifera* extraction using MAE method based on RSM to generate the highest total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), and cupric ion-reducing antioxidant capacity (CUPRAC). RSM was designed using Design Expert version 13.0. TPC and TFC were measured using the calorimetry method. Antioxidant capacities were evaluated using DPPH, FRAP, and CUPRAC method. RSM resulted in a quadratic model for all dependent variables. The highest TPC, TFC, and CUPRAC was found with extraction time (A) of 3 minutes, ethanol concentration (B) of 60%, and solid-solvent rasio (C) of 15 mL/g while the DPPH and FRAP methods were found with 1 minute, 60% ethanol, and 15 mL/g. The optimum formulation was obtained with 2.119 minutes, 57.618%, and 1:15 g/mL. The verification results showed that the optimum formulation was accurate with %RSE of <10%. RSM succeeded in optimizing the extraction conditions of *M. oleifera* by varying the extraction time, ethanol concentration, and solid-solvent ratio. The optimal conditions obtained for the extraction of *M. oleifera* can be applied to the exploration of the potential development of *M. oleifera* as a medicinal plant.

***Keywords*:** Antioxidant, flavonoid, *Moringa oleifera*, phenolic, response surface methodology

Introduction

Moringa oleifera L., often known as "Kelor" in Indonesia, is a plant with high nutritional value and low antinutritional content. Studies on the plant's pharmacology suggest that it possess anticancer, antioxidant, anti-obesity, local anaesthetic, and anti-allergic properties.[1] Various parts of *M. oleifera* such as leaves, roots, seeds, bark, fruit, flowers, and pods can be used as antioxidants, diuretics, antihypertensives, cholesterol-lowering agents, and antispasmodics.[2,3] Previous research have identified nine compounds in the ethanol extract of *M. oleifera* leaves: tannins, carbohydrates, saponins, glycosides, reducing sugars, steroids, terpenoids, flavonoids, and alkaloids, whereas fresh *M. oleifera* leaves contain carotenoids such as lutein, β-carotene, and zeaxanthin.[4] The ethanol extract of *M. oleifera* leaves also contains minerals, such as Mn and Cu, which act as catalytic cofactors and activators of antioxidant enzymes, such as superoxide dismutase.[5]

*Corresponding author. E mail: wnurcholis@apps.ipb.ac.id
Tel: +62-8179825145

Citation: Makkiyah FA, Pradana DLC, Putra RP, Nurcholis W. Optimization Microwave-Assisted Extraction of *Moringa oleifera* Leaves Using Response Surface Methodology Focused nn Extracting Phenolic And Flavonoid With Antioxidant Activity. Trop J Nat Prod Res. 2024; 8(6): 7474-7482. https://doi.org/10.26538/tjnpr/v8i6.21

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

The polyphenol content of these plants varies depending on the region of the plant, with phenolics and flavonoids being the principal components.

Phenolic acids have antioxidant and anticancer properties while flavonoids have the ability to remove oxygen-free radicals in the body.[6,7] Gallic acid, ellagic acid, ferulic acid, and chlorogenic acid are phenolic group components extracted from *M. oleifera*, whereas myricetin, quercetin, isorhamnetin, and kaempferol are flavonoid group compounds.[8,9]

Optimization of the extraction of secondary metabolites from plants is crucial and involves the use of independent variables, such as solid-solvent rasio,[10] extraction time,[11] and solvent type.[12] The effect of these independent variables can be improved to create extracts with the maximum metabolite content; one of the approaches is the response surface methodology (RSM). RSM is a popular approach for optimizing extraction parameters, such as the solid-solvent ratio, solvent concentration, and extraction duration. Rodríguez-Pérez *et al.*[13] discovered the best solvent concentration and temperature extraction formula from *M. oleifera* using the RSM approach. Furthermore, the RSM approach was used to determine the optimum formula for independent variables, such as the solid-solvent ratio, solvent concentration, simplicia size, and extraction time from *C. cristata*.[14] However, no research has been conducted to optimize microwave-assisted extraction (MAE) using independent variables, such as solid-solvent ratio, solvent concentration, and extraction duration, to yield dependent variables, such as total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *M. oleifera* based on RSM. This research aimed to determine the optimal extraction time, ethanol concentration, and solid-solvent ratio for *M.*

oleifera extraction using the MAE method based on RSM to generate the highest TPC, TFC, and antioxidant activities of 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric-reducing antioxidant power (FRAP), and cupric ion-reducing antioxidant capacity (CUPRAC).

Materials and Methods

Materials

Ethanol (pro-analysis), ammonium acetate buffer, $CuCl_2$, neocuproine, $AlCl_3$, Folin-Ciocalteu, sodium carbonate (Na_2CO_3), quercetin, and HCl were obtrained from Merck-Millipore (Darmstadt, Germany). Trolox, glacial acetate acid, and DPPH were obtained from Sigma-Aldrich (St. Louis, USA). Gallic acid ($C_7H_6O_5.H_2O$), 2,4,6-tripyridyl-s-triazine (TPTZ), and $FeCl_3$ were obtained from Sisco Research Laboratories Pvt. Ltd. (Maharashtra, India).

Plant material and sample preparation

Moringa oleifera leaves (BMK0128092016) were collected in March 2023 from the Tropical Biopharmaca Research Center, IPB University, West Java, Indonesia (6°35'15.7"S 106°48'05.0"E). The leaves were washed and dried in an oven at 50°C for three days. Simplicia is created by blending dried leaves and filtering them through a 60-mesh sieve. Subsequently, simplicia was employed for the extraction stage.

Experimental design and extraction

The experimental design for the response surface methodology (RSM) was created using Design Expert® 13.0 software (Stat-Ease Inc., Minneapolis, USA). The variables studied included extraction time (minutes), ethanol concentration (%), and solid-to-solvent ratio (mL/g), with the comparison according to Table 1. Based on the model, these factors were investigated to produce the optimum total phenol content (TPC), total flavonoid content (TFC), and antioxidant activity.

The extraction process was carried out by dissolving 2 gram of dried *M. oleifera* leaves with ethanol in an Erlenmeyer flask in proportion according to the RSM design that had been designed (Table 1). The microwave assisted extraction (MAE) method was used to extract the components by placing the Erlenmeyer flask in to the microwave (Sharp R-21D0(S)-IN) 135 W. The solution was then filtered and the volume was calibrated according to the initial extraction volume.

Total phenolic content (TPC)

Moringa oleifera's total phenolic content is determined using a standard calibration of gallic acid ($y = 0.0044x - 0.0078$ and $R^2 = 0.9936$) and follows Putra *et al*.[15] with slight modification. A 96-well microplate (BiologiX) was prepared and 20 µL of the sample was added, followed by 120 µL of 10% (v/v) Folin-Ciocalteu. The solution was incubated for 5 minutes, after which 80 µL of Na_2CO_3 10% (b/v) was added and the solution was incubated for 30 minutes in the dark. A nano spectrophotometer (SPECTROstarNano BMG LABTECH, Germany) was used to measure the absorbance of the solution at 750 nm. Measurement were carried out with three repetitions. The results are expressed in milligrams gallic acid equivalent per gram of dried weight (mg GAE/g DW).

Total flavonoid content (TFC)

The total flavonoid content in *M. oleifera* was determined using aluminium chloride calorimetry as reported by Nurcholis *et al.*[16] with a standard calibration of quercetin ($y = 0.0014x + 0.0157$ and $R^2 = 0.9852$). With three repetitions, 120 µL of water was mixed with 10 µL of sample, 10 µL of 10% (b/v) alumunium chloride ($AlCl_3$), 10 µL of glacial acetic acid, and 50 µL of ethanol pro analysis. The absorbance was measured at 415 nm using a nano spectrophotometer after incubation at room temperature and in the dark for 30 min. The results are expressed in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

Determination of antioxidant capacity

The antioxidant activity analyzed in this research is the radical scavenging of DPPH, the reducing power of Fe^{3+} ions to Fe^{2+} (FRAP assay), and the reducing power of Cu^{2+} to Cu^{+} (CUPRAC assay) with three repetitions, which is similar to Nurcholis *et al.*[16] with slight adjustments. All procedures used the Trolox standard, with findings expressed as micromol Trolox equivalent per gram of dried weight (µmol TE/g DW). DPPH free radical scavenging was measured by mixing 100 µL of sample with 100 µL of 125 µM DPPH on a 96-well microplate. After 30 minutes of incubation in a dark room, the absorbance at 515 nm was measured with a nano spectrophotometer.

FRAP reagent was prepared by mixing acetate buffer (pH 3.6), $FeCl_3$ (20 mM), and 10 mM tripyridyl-s-triazine (TPTZ) (in HCl 40 mM) in a ratio of 10:1:1 (v/v/v). Antioxidant activity was determined by pipetting a 10 µL sample into a 96-well microplate and adding 300 µL FRAP reagent. After 30 minutes, the absorbance of the mixture was measured with a nano spectrophotometer at a wavelength of 593 nm. For CUPRAC assay, 50 µL of the sample was added to 96-well microplate with 50 µL of $CuCl_2.6H_2O$ 10^{-2} M, 50 µL of 7.5×10^{-3} M neocuproine, and 50 µL of ammonium acetate buffer (pH 7). After 30 minutes of incubation in the darkroom, the absorbance of the mixture was measured at 450 nm using a nano spectrophotometer.

Statistical analysis

Data analysis was conducted in accordance with the method described by Makkiyah *et al.*[17] with slight adjustment. The responses data were analyzed using OneWay ANOVA ($\alpha = 0.05$) and Tukey's HSD follow-up test ($\alpha = 0.05$) using IBM SPSS Statistics 25. The results of Design Expert optimization (Stat-Ease Inc., Minneapolis, MN) were chosen as the most optimal based on the highest desirability value. The optimization results were confirmed through three repetitions based on the per cent residual standard error (RSE) value.

Table 1: Experimental design of response surface methodology (RSM) with three independent variables: extraction time, ethanol concentration (%), and solvent ratio (mL/g).

Run	Independent variables		
	Extraction time (minutes)	Ethanol concentration (%)	Solid-solvent ratio (mL/g)
1	1	60	5
2	2	80	5
3	1	60	15
4	2	80	15
5	2	60	10
6	3	60	15
7	3	40	10
8	2	60	10
9	2	60	10
10	3	80	10
11	3	60	5
12	1	40	10
13	2	60	10
14	2	40	5
15	2	60	10
16	2	40	15
17	1	80	10

Results and Discussion

Optimization of extraction by response surface methodology

Response surface methodology (RSM) is a design of experiment optimization method that uses statistical methods and mathematical equations to determine the impacts of numerous independent variables

being investigated on the creation of the expected dependent variable values.[18] The optimization concept integrates independent variable data and experimental data to construct an equation that produces the theoretical value of the dependent variable being researched based on a new independent variable formula. This strategy during the optimization stage saves time and allows for more efficient laboratory testing.

Table 2 shows the results of the experimental testing of the independent variable formula based on RSM, which produces the dependent variable value. The highest TPC (17.8977 mg GAE/g DW), TFC (5.6454 mg QE/g DW), and CUPRAC (64.415 µmol TE/g DW) were recorded in experiments on *M. oleifera* leaf extract using 60% ethanol, a solid-solvent ratio of 15 mL/g, and an extraction time of 3 min (R6). The highest levels of DPPH radical scavenging and FRAP method were produced in extracts with a proportion of 60% ethanol, solid-solvent ratio of 15 mL/g, and extraction time of 1 min (R3), with values of 1.2555 and 72.7148 µmol TE/g DW, respectively. In contrast, extracts with a solvent ratio of 5 mL/g and too high or too low ethanol concentration produced the lowest TPC (R2), TFC (R14), DPPH (R1), FRAP (R2), and CUPRAC (R14).

Fitting models

A suitable model for each dependent variable was selected by analyzing it using analysis of variance (ANOVA). The ANOVA results were used to evaluate the model that met the requirements at the 95% confidence interval (Table 3 and 4). Some parameters used to evaluate the model statistically were *p*-value, *F*-value, R^2 value, and Adeq precision.

The implementation of the experimental design generated a statistical model that represented the impact of the independent variable on the dependent variable. The R^2 value of the model describes excellent prediction efficiency, approaching 1.0 (10). In this research, all dependent variables created a significant quadratic model (p-value < 0.05; F-value > p-value), with R^2 values of 0.9091 (TPC), 0.8923 (TFC), 0.8408 (DPPH), 0.9696 (FRAP), and 0.9644 (CUPRAC). An F-value greater than the p-value indicates a more significant coefficient.[19] The Adeq precision was used to measure the noise ratio, which compares the range of predicted values at design points with an average prediction error of an ideal value greater than four.[20] In this research, all models of the response variables had an Adeq precision of > 4.

The quadratic model for TPC depicts the positive effect of the independent variables, including ethanol concentration (B), solid-solvent ratio (C), extraction time interaction with ethanol concentration (AB), and extraction time interaction with solid-solvent ratio (AC). In contrast, the quadratic model in TFC represents only a positive effect on the variable solid-solvent ratio (C), extraction time interaction with the solid-solvent ratio (AC), and ethanol concentration interaction with the solid-solvent ratio (BC). The antioxidant activity tests showed a positive quadratic model with the variables of solid-solvent ratio (C) and extraction time interaction with solid-solvent ratio (AC) (Table 4). The impact of independent variables on antioxidant activity was categorised according to the mechanism. The extraction time (A), solid-solvent ratio (C), extraction time interaction with solid-solvent ratio (AC), square of extraction time (A^2), and square of solid-solvent ratio (C^2) all had favourable effects on DPPH. This model contrasts with the quadratic models produced by the FRAP and CUPRAC methods, which show that the solid-solvent ratio (C), extraction time interaction with solid-solvent ratio (AC), and ethanol concentration interaction with extraction time (BC) have a positive effect on antioxidant activity.

Table 2: Response surface methodology for *M. oleifera* optimization extraction of TPC, TFC, and antioxidant capacity

Run	A	B	C	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	Antioxidant Activity (µmol TE/g DW)		
						DPPH	FRAP	CUPRAC
1	1	60	5	8.7841 ± 0.51^{fgh}	3.0389 ± 0.03^{de}	0.2187 ± 0.001^{k}	33.6229 ± 1.392^{hi}	31.5383 ± 1.037^{h}
2	2	80	5	6.1932 ± 0.21^{h}	2.2782 ± 0.12^{f}	0.4192 ± 0.005^{j}	25.5844 ± 1.413^{ij}	24.8217 ± 0.609^{ij}
3	1	60	15	15.4295 ± 0.35^{abc}	5.5168 ± 0.13^{ab}	1.2555 ± 0.007^{a}	72.7148 ± 1.056^{a}	63.3150 ± 1.502^{a}
4	2	80	15	12.7614 ± 0.34^{bcd}	4.9382 ± 0.22^{b}	0.5199 ± 0.012^{fgh}	61.3494 ± 1.006^{cd}	61.3650 ± 0.826^{ab}
5	2	60	10	14.6894 ± 1.06^{bc}	5.2779 ± 0.21^{ab}	0.6312 ± 0.018^{d}	53.5535 ± 1.038^{def}	52.2100 ± 2.207^{cd}
6	3	60	15	17.8977 ± 0.62^{a}	5.6454 ± 0.07^{a}	1.2377 ± 0.036^{a}	70.2917 ± 3.122^{ab}	64.4150 ± 1.540^{a}
7	3	40	10	11.4318 ± 0.69^{def}	3.6921 ± 0.14^{cd}	0.8199 ± 0.001^{c}	46.3612 ± 1.037^{fg}	40.7433 ± 0.203^{fg}
8	2	60	10	14.0530 ± 0.49^{bcd}	5.0993 ± 0.08^{ab}	0.5903 ± 0.007^{def}	58.2458 ± 2.082^{cd}	55.8767 ± 2.150^{bc}
9	2	60	10	15.6591 ± 0.59^{ab}	5.6350 ± 0.10^{a}	0.6286 ± 0.016^{de}	62.2073 ± 3.073^{bc}	58.0100 ± 0.666^{abc}
10	3	80	10	7.5076 ± 0.20^{gh}	2.6850 ± 0.05^{ef}	0.4403 ± 0.004^{hij}	38.3612 ± 2.035^{gh}	36.2767 ± 0.674^{gh}
11	3	60	5	7.9053 ± 0.43^{gh}	2.7425 ± 0.112^{ef}	0.1197 ± 0.011^{l}	29.6613 ± 0.437^{ij}	30.5550 ± 0.492^{hi}
12	1	40	10	13.7803 ± 0.79^{bcd}	4.1636 ± 0.10^{c}	0.5033 ± 0.0134^{ghi}	47.9381 ± 0.329^{ef}	44.5767 ± 1.593^{fg}
13	2	60	10	14.4621 ± 0.41^{bcd}	5.3064 ± 0.16^{ab}	0.5495 ± 0.013^{efg}	55.7073 ± 0.674^{cde}	53.6767 ± 1.093^{cd}
14	2	40	5	6.4205 ± 0.48^{h}	1.4746 ± 0.03^{g}	0.4279 ± 0.016^{ij}	23.3344 ± 0.234^{j}	21.1050 ± 0.535^{j}
15	2	60	10	13.4318 ± 0.59^{bcd}	4.8636 ± 0.19^{b}	0.5468 ± 0.021^{fg}	53.5150 ± 1.812^{def}	52.3100 ± 1.436^{cd}
16	2	40	15	12.3977 ± 0.89^{cde}	2.6239 ± 0.03^{ef}	1.1289 ± 0.015^{b}	56.0994 ± 0.173^{cde}	47.5650 ± 0.541^{ef}
17	1	80	10	10.5227 ± 0.76^{efg}	4.1493 ± 0.1^{c}	0.2965 ± 0.014^{k}	46.4765 ± 1.231^{fg}	45.3433 ± 1.717^{fg}

Description: Numbers in the same column followed by the same letter indicate no significant differences ($p > 0.05$) based on Tukey's HSD test ($\alpha = 0.05$). The data are presented as mean ± standard error. A: extraction time (minutes), B: ethanol concentration (%), C: solid-solvent ratio (mL/g), TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picryl hydrazyl, FRAP: ferric reducing antioxidant power, CUPRAC: cupric ion-reducing antioxidant capacity, GAE: gallic acid equivalent, QE: quercetin equivalent, TE: Trolox equivalent, DW: dry weight.

Effect of extraction time, ethanol concentration, and solid-solvent ratio on TPC and TFC

The TPC and TFC of *M. oleifera* were presented in Figure 1 and 2, where the highest content (17.8977 mg GAE/g DW in TPC and 5.6454 mg QE/g DW in TFC) were obtained at a solid-solvent ratio of 15 mL/g with 60% ethanol (Figure 1a and 2a), solid-solvent ratio of 15 mL/g with an extraction time of 3 minutes (Figure 1b and 2b), and 60% ethanol with an extraction time of 3 minutes (Figure 1c and 2c).

An increase in the solid-solvent ratio (mL/g) and extraction time led to higher TPC and TFC in the *M. oleifera* extract (Figure 1 and 2). Conversely, an increase in ethanol concentration does not always result in higher levels of these response variables. According to Dent *et al*.,[21] the extraction of polyphenolic compounds depends on the type and polarity of the solvent used, and the solubility of these compounds. As the ethanol concentration increased, the polarization of the solvent decreased, leading to consistent polarization of the phenolic group.[22]

These findings are consistent with prior research, which found that the best conditions for total phenolic extraction from red grapes[23] and *Padina australia*[11] were obtained using 60% ethanol as a solvent. In a study by Le *et al.*[22] on maximising total phenolic and total flavonoids of *Docynia indica* fruit using the microwave-assisted extraction method, the best formula was produced utilizing 65% ethanol concentration.

A solvent mixture of ethanol and water has been found to produce the best results in extracting polyphenols, with water acting as a plant-swelling agent and ethanol acting to destroy the interactions between solutes and the plant matrix.[24] Appropriate quantities of water and ethanol can improve the extraction efficiency of bioactive compounds.[25] This is corroborated by Dahmoune *et al*.,[26] who argue that the solvent concentration gradient in the plant matrix is the driving force for the extraction process, with the compounds developing equilibrium between the solvent and the plant tissue. Increasing the solid-solvent ratio can increase the extraction yield by preventing saturation of the extraction media. An increase in the solid-solvent ratio also increases the concentration gradient, which increases the compound's diffusion rate from the sample to the solvent.[14]

In the time extraction variable, the longer *M. oleifera* leaves extract was exposed to micro-radiation, the higher the total phenolics and flavonoids. However, excess extraction time reduces extraction yields owing to the increased breakdown of the polymer matrix and increased viscosity, which encapsulates the extracted chemicals.[27] These findings are consistent with those of Nurcholis *et al*.,[16] who found that exposure to micro-radiation for 3 min delivered the best results compared to exposure for 1 and 2 min in extracted *A. compactum*.

Effect of extraction time, ethanol concentration, and solid-solvent ratio on antioxidant capacity

Antioxidants play a significant role in neutralising free radicals. Antioxidant analysis can be divided into two types based on the principle of total antioxidant capacity (TAC): hydrogen atom transfer (HAT) (for example, DPPH) and single-electron transfer (SET) (example, FRAP and CUPRAC). Antioxidant chemicals neutralise synthetic radicals by donating hydrogen during HAT. The SET mechanism uses a reduction-oxidation (redox) interaction between oxidants and antioxidants as a signal for an endpoint reaction.[28] Because of this difference in mechanisms, it is not appropriate to rely solely on one approach to screen for antioxidant activity, as one chemical reaction method does not provide realistic results when compared to a set of experiments that include different chemical reactions.[29]

The highest DPPH radical scavenging and FRAP were found at 60% ethanol concentration (Figure 3a and Figure 4a), solid-solvent ratio of 15 mL/g (Figure 3b and Figure 4b), and 1-minute extraction time (Figure 3c and 4c). These results are in line with the research of Wen *et al.,*[30] who optimized MAE for blackberries' antioxidant activity using the DPPH method, which improved antioxidant activity by utilizing 40%–60% ethanol solvent but required a 3-minute extraction time. The compounds recovered from the sample may be responsible for the discrepancy in extraction time. On the other hand, the antioxidant activity of *M. oleifera* was evaluated using the CUPRAC method, showing the maximum activity at a 60% ethanol solvent (Fig. 5a), solid-solvent ratio of 15 mL/g (Fig. 5b), and a 3-minute extraction time (Fig. 5c), but not significantly different from the extraction time of 1 min (Table 2). As shown in Fig. 5a, b, and c, the highest activity was 64.4150 µmol TE/g DW based on these independent variables. The same results were reported by Doldolova *et al.,*[31] where the reducing power of *Curcuma longa* L. increased with increasing extraction time and solid-solvent ratio.

Optimum formulation and confirmation

RSM used the Design Expert 13.0 software to formulate 14 independent variable formulas to optimize the extraction of *M. oleifera* leaves. The optimal formula was selected based on the maximum desirability (approximately 1.0), indicating the validity of the optimum formula results. The results of numerical analysis, aiming to maximise the value of dependent variables, generated the best formula with a desirability of 0.936, which suggests an accuracy of 93.6% (Fig. 6). Moreover, the independent variables obtained from this optimization was verified using the same *M. oleifera* simplicia.

The RSM method successfully integrates independent variable data with experimental data, thereby producing predictive values for the dependent variable. The optimum formula for the independent variable obtained was an extraction time of 2.119 min, a solid-solvent ratio of 15 mL/g, and 60% ethanol. This optimum formula can predict the value of the dependent variable of 16.9660 mg GAE/g DW (TPC), 5.3500 mg QE/g DW (TFC), 1.1160 µmol TE/g DW (DPPH), 70.6460 µmol TE/g DW (FRAP), and 64.7140 µmol TE/g DW (CUPRAC), with desirability value of 0.936. The verification results were evaluated based on the residual standard error (RSE) value, which according to Salbi *et al.,*[32] is below 10%. The verification results (Table 5) show that all dependent variables met the established tolerance limits, indicating that the formula for the independent variable resulting from the optimization of RSM *M. oleifera* extraction can be considered reliable for further research on this plant extract as a natural herbal remedy.

Table 3: Results of analysis of variance (ANOVA) response variables on optimizing the extraction of *M. oleifera*

	TPC	TFC	DPPH	FRAP	CUPRAC
			Quadratic models		
F	7.77	6.45	4.11	24.80	21.05
p-value	0.0065	0.0113	0.0379	0.0002	0.0003
R^2	0.9091	0.8923	0.8408	0.9696	0.9644
Adjusted R^2	0.7921	0.7539	0.6362	0.9305	0.9186
Adeq precision	9.2264	7.7123	6.8136	16.5839	14.5234

Description: TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picryl hydrazyl, FRAP: ferric reducing antioxidant power, CUPRAC: cupric ion-reducing antioxidant capacity.

Table 4.: Equations of response surface analysis for the investigated TPC, TFC, and antioxidant capacity

Response	Model	Equation
TPC	Quadratic	$Y = -1949.47 - 2016.68A + 41.28B + 47.09C + 21.93AB + 22.44AC - 0.0454BC - 517.68A^2 - 3.53B^2 - 1.29C^2$
TFC	Quadratic	$Y = -273.69 - 280.66A - 14.13B + 7.31C - 7.45AB + 3.19AC + 0.378BC - 70.54A^2 - 1.49B^2 - 0.922C^2$
DPPH	Quadratic	$Y = -19.30 + 18.07A - 2.66B + 1.55C - 1.30AB + 0.6090AC - 0.1501BC + 4.34A^2 - 0.0791B^2 + 0.1138C^2$
FRAP	Quadratic	$Y = -3223.34 - 3332.80A - 95.05B + 40.84C - 49.04AB + 11.54AC + 0.75BC - 846.34A^2 - 10.92B^2 - 4.13C^2$
CUPRAC	Quadratic	$Y = -6665.39 - 6903.59A - 74.15B + 46.29C - 39.25AB + 15.62AC + 2.52BC - 1773.01A^2 - 10.71B^2 - 4.99C^2$

Description: TPC: total phenolic content, TFC: total flavonoid content, DPPH: 2,2-diphenyl-1-picryl hydrazyl, FRAP: ferric reducing antioxidant power, CUPRAC: cupric ion-reducing antioxidant capacity.

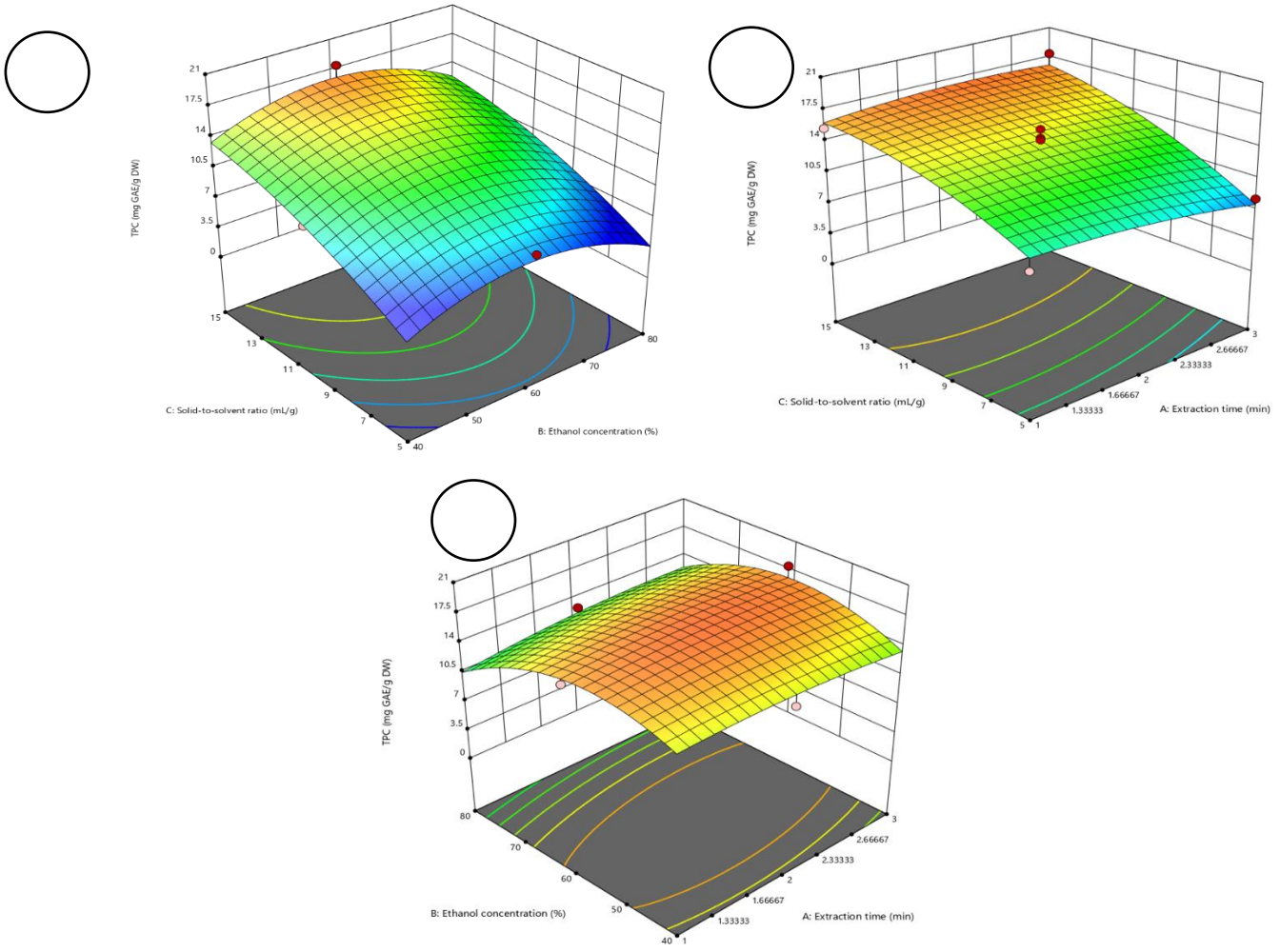


Figure 1: The interplay effect of the independent variables ethanol concentration (%) with solvent-solid ratio (mL/g) (A), solvent-solid ratio (mL/g) with extraction time (min) (B), and ethanol concentration (%) with extraction time (min) (C) on total phenolic content (TPC)

.

Table 5: Verification results on TPC, TFC, and antioxidant capacity under optimal extraction conditions

	A	B	C	TPC	TFC	DPPH	FRAP	CUPRAC	*Desirability*
Prediction	2.119	57.618	15.00	16.9661	5.3496	1.1155	70.6460	64.7107	
Actual	2.120	57.618	15.00	17.5265	5.4561	1.1975	71.6571	70.2483	0.936
%RSE				3.30	1.99	7.35	1.43	8.56	

Conclusion

Response surface methodology was used to formulate the independent variables (extraction time, ethanol concentration, and solid-solvent ratio) to optimize the dependent variables (TPC, TFC, DPPH, FRAP, and CUPRAC) of *M. oleifera* leaves extraction. According to Design Expert 13.0, the optimum formula used an ethanol concentration of 57.618% and a solid ratio of 15 mL/g, with an extraction time of 2.119 min. The effect of the optimum formula for the independent variable on the dependent variable is very good, based on the verification results with an analysis of the %RSE value < 10%, which indicates a good level of optimization accuracy. The optimized extraction conditions for *M. oleifera* obtained in this study can be utilized to explore its potential as a medicinal plant.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The author gratefully acknowledges the internal grant provided by the Institute for Research and Community Research of Universitas Pembangunan Nasional Veteran Jakarta (267/UN.61.0/ HK.02/LIT.RISCOP/2022).

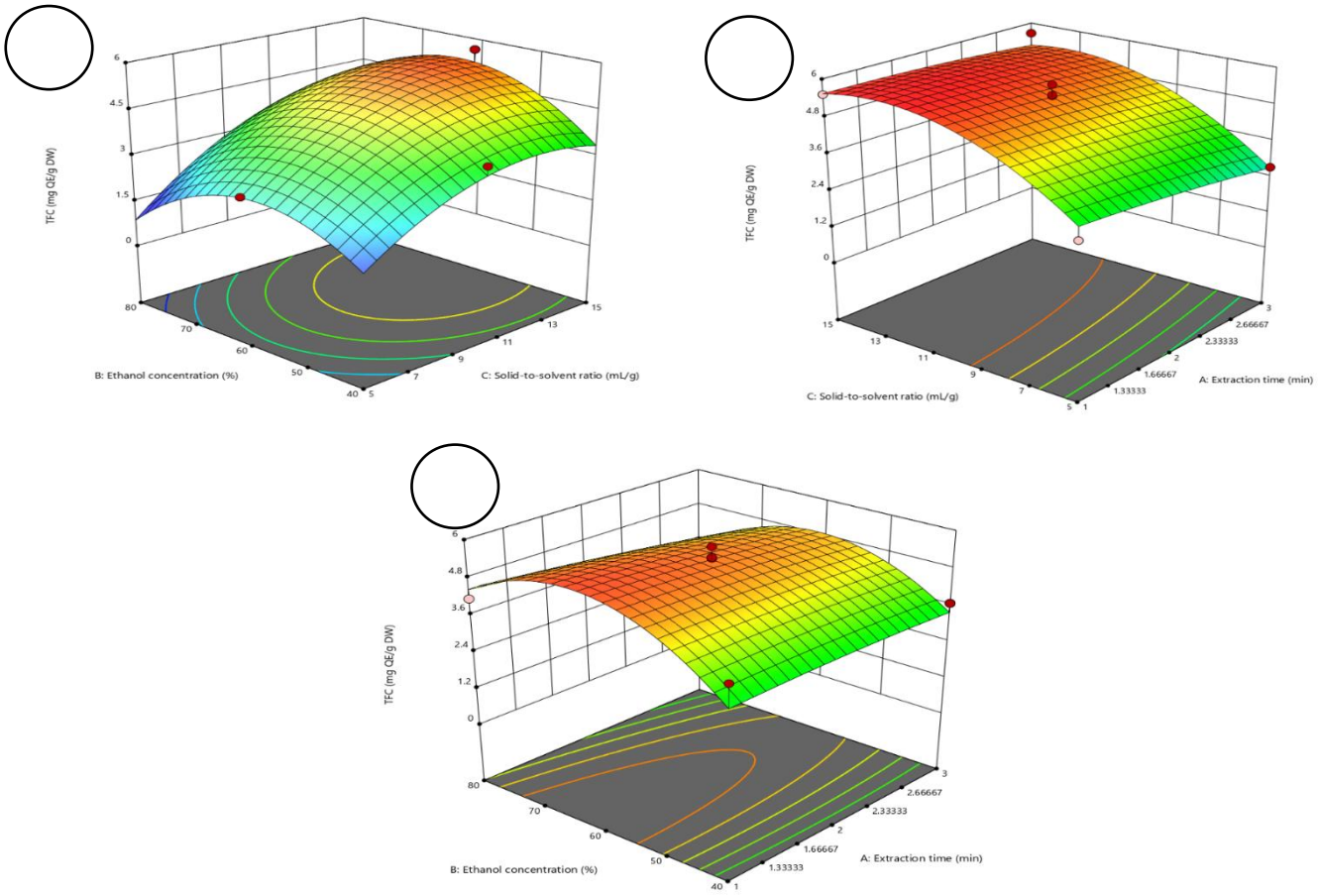
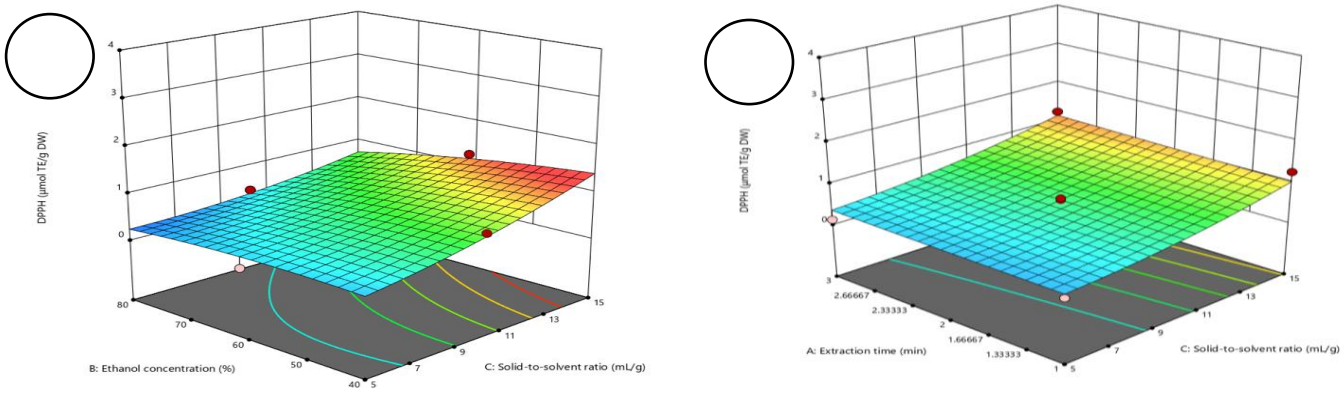


Figure 2: The interplay effect of the independent variables ethanol concentration (%) with solvent-solid ratio (mL/g) (A), solvent-solid ratio (mL/g) with extraction time (min) (B), and ethanol concentration (%) with extraction time (min) (C) on total flavonoid content (TFC).



ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

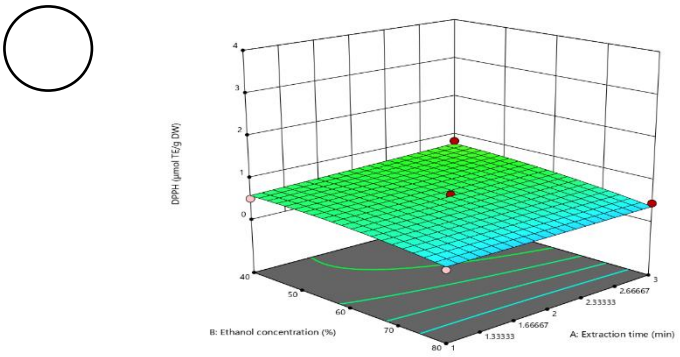


Figure 3: The interplay effect of the independent variables ethanol concentration (%) with solvent-solid ratio (mL/g) (A), solvent-solid ratio (mL/g) with extraction time (min) (B), and ethanol concentration (%) with extraction time (min) (C) on DPPH

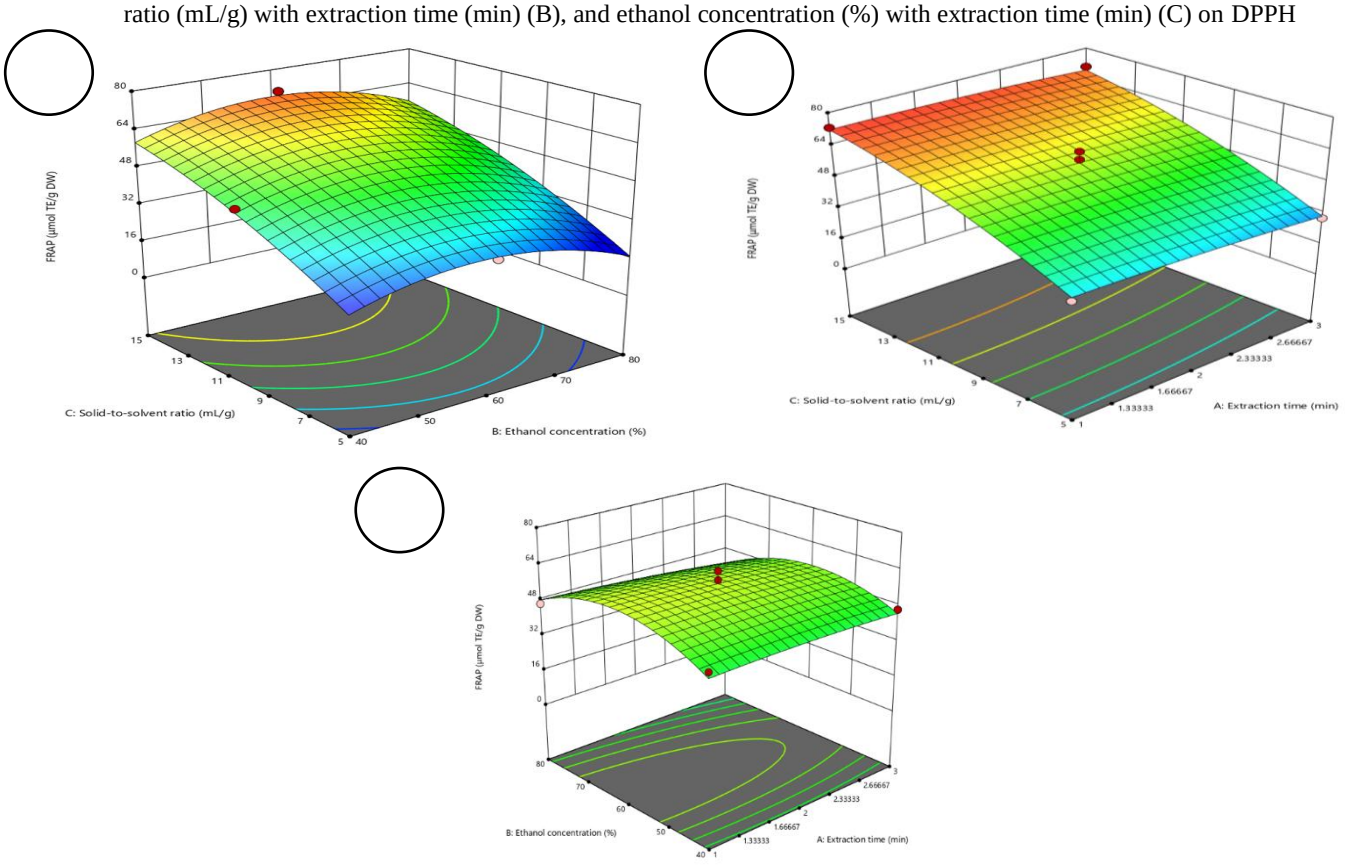
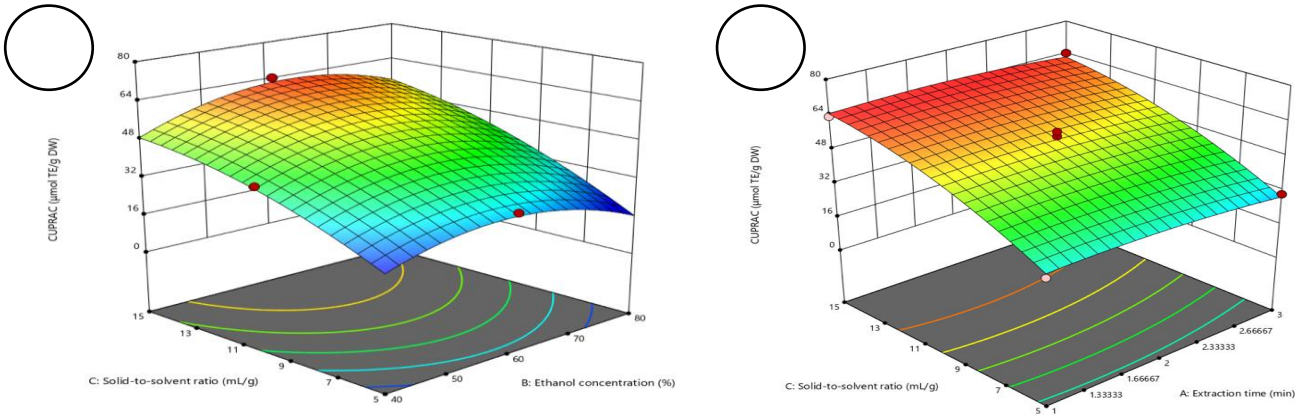


Figure 4: The interplay effect of the independent variables ethanol concentration (%) with solvent-solid ratio (mL/g) (A), solvent-solid ratio (mL/g) with extraction time (min) (B), and ethanol concentration (%) with extraction time (min) (C) on FRAP



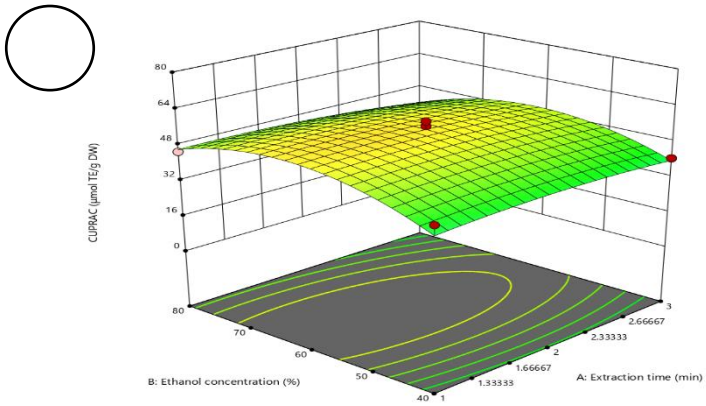


Figure 5: The interplay effect of the independent variables ethanol concentration (%) with solvent-solid ratio (mL/g) (A), solvent-solid ratio (mL/g) with extraction time (min) (B), and ethanol concentration (%) with extraction time (min) (C) on CUPRAC

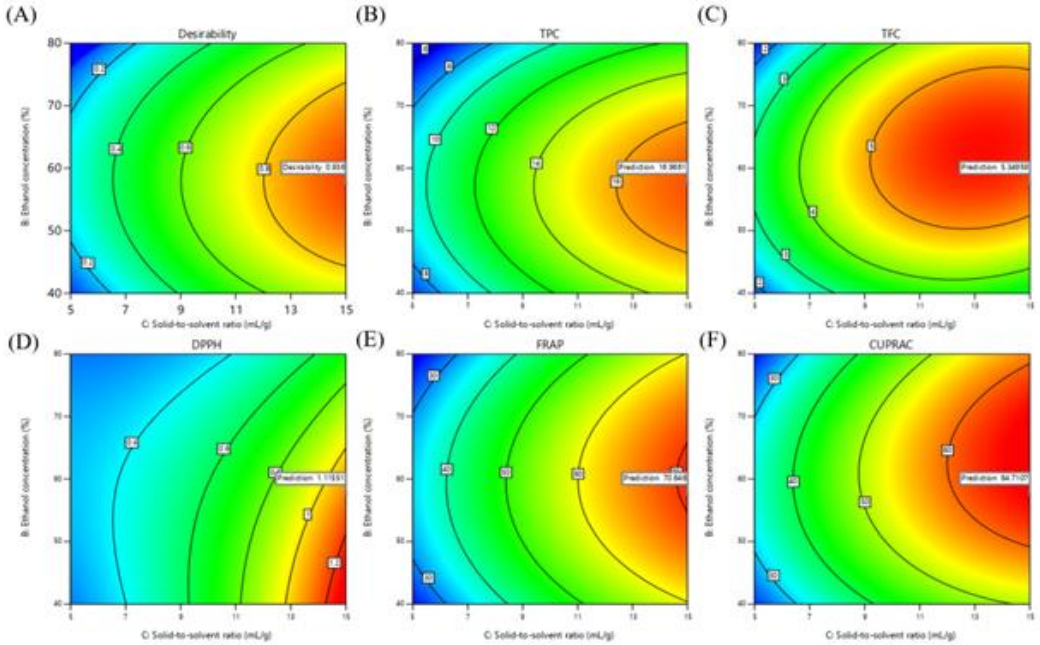


Figure 6: Contour plot showing the desirability of the optimum extraction in *M. oleifera* (A), TPC (B), TFC (C), DPPH (D), FRAP (E), and CUPRAC (F).

References

1. Bhattacharya A, Tiwari P, Sahu PK, Kumar S. A review of the phytochemical and pharmacological characteristics of *Moringa oleifera*. J Pharm Bioallied Sci 2018;10(4):181.
2. Nunes C dos R, Arantes MB, de Faria Pereira SM, da Cruz LL, de Souza Passos M, de Moraes LP, et al. Plants as sources of anti-inflammatory agents. Molecules2020;25(16): 3726.
3. van den Berg J, Kuipers S. The antibacterial action of *Moringa oleifera*: A systematic review. South African Journal of Botany 2022;151:224–33.
4. Batmomolin A, Ahsan A, Wiyasa IWA, Santoso S. Ethanolic extract of *Moringa oleifera* leaves improve inflammation, angiogenesis, and blood pressure in rat model of preeclampsia. J Appl Pharm Sci 2020;10(8):52–7.
5. Karthivashan G, Kura AU, Arulselvan P, Isa NM, Fakurazi S. The modulatory effect of Moringa oleifera leaf extract on endogenous antioxidant systems and inflammatory markers in an acetaminophen-induced nephrotoxic mice model. PeerJ 2016;2016(7): e2127.
6. Verma S, Singh A, Mishra A. Gallic acid: molecular rival of cancer. Environ Toxicol Pharmacol 2013;35(3):473–85.
7. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci 2016;5:1–15.
8. Nizioł-ŁUkaszewska Z, Furman-Toczek D, Bujak T, Wasilewski T, Hordyjewicz-Baran Z. *Moringa oleifera* L. Extracts as bioactive ingredients that increase safety of body wash cosmetics. Dermatol Res Pract 2020;2020.
9. Zhu Y, Yin Q, Yang Y. Comprehensive investigation of *Moringa oleifera* from different regions by simultaneous determination of 11 polyphenols using UPLC-ESI-MS/MS. Molecules 2020;25(3): 676.
10. Juliana D, Aisyah SI, Priosoeryanto BP, Nurcholis W. Optimization of cardamom (*Amomum compactum*) fruit extraction using the Box–Behnken design focused on polyphenol extraction with antioxidant activity. J Appl Pharm Sci 2022;12(6):194–209.
11. Hassan IH, Pham HNT, Nguyen TH. Optimization of ultrasound-assisted extraction conditions for phenolics, antioxidant, and tyrosinase inhibitory activities of Vietnamese brown seaweed (*Padina australis*). J Food Process Preserv 2021;45(5).
12. Nurcholis W, Marliani N, Asyhar R, Minarni M. Optimized solvents for the maceration of phenolic antioxidants from

Curcuma xanthorrhiza rhizome using a simplex centroid design. J Pharm Bioallied Sci 2023;15(1):35–41.
13. Rodríguez-Pérez C, Gilbert-López B, Mendiola JA, Quirantes-Piné R, Segura-Carretero A, Ibáñez E. Optimization of microwave-assisted extraction and pressurized liquid extraction of phenolic compounds from *Moringa oleifera* leaves by multiresponse surface methodology. Electrophoresis 2016;37(13):1938–46.
14. Yatma F, Falah S, Ambarsari L, Aisyah SI, Nurcholis W. Optimization of extraction of phenolic and antioxidant activities from *Celosia cristata* seeds using response surface methodology. Biointerface Res Appl Chem 2023;13(2): 1–16.
15. Putra RP, Aisyah SI, Kurniatin PA, Nurcholis W. Optimization of solvent sonication-maceration for enhancing total phenolic content and antioxidant activity of *Portulaca oleracea* L. extract using the simplex centroid design method. Trop J Nat Prod Res 2024;8(3):6558–62.
16. Nurcholis W, Alfadzrin R, Izzati N, Arianti R, Vinnai BÁ, Sabri F, Kristóf E, Artika IM. Effects of methods and durations of extraction on total flavonoid and phenolic contents and antioxidant activity of java cardamom (*Amomum compactum* Soland Ex Maton) Fruit. Plants 2022;11(17): 2221.
17. Makkiyah FA, Rahmi EP, Susantiningsih T, Marliani N, Arista RA, Nurcholis W. Optimization of *Graptophyllum pictum* leaves extraction using a simplex centroid design focused on extracting flavonoids with antioxidant activity. J Appl Pharm Sci 2023;13(05):214–21.
18. Breig SJM, Luti KJK. Response surface methodology: A review on its applications and challenges in microbial cultures. In: Materials Today: Proceedings. Elsevier Ltd; 2021. page 2277–84.
19. Yi S, Su Y, Qi B, Su Z, Wan Y. Application of response surface methodology and central composite rotatable design in optimizing the preparation conditions of vinyltriethoxysilane modified silicalite/polydimethylsiloxane hybrid pervaporation membranes. Sep Purif Technol 2010;71(2):252–62.
20. Behera SK, Meena H, Chakraborty S, Meikap BC. Application of response surface methodology (RSM) for optimization of leaching parameters for ash reduction from low-grade coal. Int J Min Sci Technol 2018;28(4):621–9.
21. Dent M, Dragovic-Uzelac V, Penic M, Brncic M, Bosiljkov T, Levaj B. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in dalmatian wild sage (*Salvia officinalis* L.) extracts. Food Technol Biotechnol 2013; 51(1):84-91.
22. Le XD, Nguyen MC, Vu DH, Pham MQ, Pham QL, Nguyen QT, Nguyen T, Pham V, Bach L, Van NT, Tran Q. Optimization of microwave-assisted extraction of total phenolic and total flavonoid contents from fruits of *Docynia indica* (Wall.) decne. using response surface methodology. Processes 2019;7(8): 485.
23. Morelli LLL, Prado MA. Extraction optimization for antioxidant phenolic compounds in red grape jam using ultrasound with a response surface methodology. Ultrason Sonochem 2012;19(6):1144–9.
24. Şahin S, Şamli R. Optimization of olive leaf extract obtained by ultrasound-assisted extraction with response surface methodology. Ultrason Sonochem 2013;20(1):595–602.
25. Alara OR, Abdurahman NH, Olalere OA. Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. J King Saud Univ Sci 2020;32(1):7–16.
26. 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;166:585–95.
27. Weremfo A, Adulley F, Adarkwah-Yiadom M. Simultaneous optimization of microwave-assisted extraction of phenolic compounds and antioxidant activity of avocado (*Persea americana* Mill.) seeds using response surface methodology. J Anal Methods Chem 2020;2020.
28. Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations. Antioxidants 2020;9(8):1–39.
29. Opitz SEW, Smrke S, Goodman BA, Yeretzian C. Methodology for the measurement of antioxidant capacity of coffee: a validated platform composed of three complementary antioxidant assays. Processing and Impact on Antioxidants in Beverages 2014;253–64.
30. Wen Y, Chen H, Zhou X, Deng Q, Zhao Y, Zhao C, Gong X. Optimization of the microwave-assisted extraction and antioxidant activities of anthocyanins from blackberry using a response surface methodology. RSC Adv 2015;5(25):19686–95.
31. Doldolova K, Bener M, Lalikoğlu M, Aşçı YS, Arat R, Apak R. Optimization and modeling of microwave-assisted extraction of curcumin and antioxidant compounds from turmeric by using natural deep eutectic solvents. Food Chem 2021;353.
32. Salbi NM, Muhammad N, Abdullah N. Optimizing flavonoid-rich quranic mixed food (QMF) formulation with simplex-centroid mixture design. Food Res 2021;5(2):80–7.