Bull. Chem. Soc. Ethiop. **2022**, 36(2), 261-275.
DOI: https://dx.doi.org/10.4314/bcse.v36i2.2

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM FRESH *MORINGA OLEIFERA* LEAVES WITH A RESPONSE SURFACE METHODOLOGY AND COMPARISON WITH THE SOXHLET EXTRACTION METHOD

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(Received July 27, 2021; Revised April 8, 2022; Accepted April 29, 2022)

ABSTRACT. The present study evaluated the optimum conditions of ultrasound-assisted extraction (UAE) and Soxhlet extraction (SE) of antioxidant capacities and total phenolics from fresh *Moringa oleifera* leaves, using the response surface methodology. Spectrophotometric method with Folin–Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagents was used to determine the total phenolic content (TPC) and the antioxidant activity (AA), respectively. The four models obtained showed the non-linear and quadratic dependences of both measured responses (TPC and AA) which were influenced significantly by all control variables including the acetone solvent (70%, v/v) to solid (SS) ratio, extraction time, and extraction temperature of both extraction methods. Furthermore, at the same extraction temperature, the extraction efficacy of UAE was better than SE as significantly shorter extraction time, less extraction solvent, but higher bio-active content was experienced. The optimal UAE conditions included a SS ratio of 31:1 (mL/g), extraction time of 26 min, and extraction temperature of 59 ºC, resulting in the maximum TPC (34.36 mg GAE/g dry weight, DW) and AA (491.9 µmol TE/g DW) in the extracts. In addition, the models proposed were considered to be accurate and reliable for predicting the TPC and AA of fresh *M. oleifera* leaf extract. The research findings also imply potential applications for ultra-sonication extraction to produce the extracts from fresh *M. oleifera* leaves for pharmaceutical industry as well as food technology.

KEY WORDS: Antioxidant activity, Herbal plant, Model, Polyphenols, RSM

INTRODUCTION

Moringa oleifera belongs to the *Moringaceae* family and occurs primarily in the tropical and sub-tropical places of the world, especially in Asia and Africa. This plant is often called the drumstick tree, the ben oil tree, or the horseradish tree [1] and widely recognized as a multi-purpose, potential, and precious species. Furthermore, all parts of the *M. oleifera*, such as its leaves, flowers, toasted seeds, and green pods have been consumed as vegetables by humans and animals for centuries due to extreme richness in nutritional and biochemical compounds. Besides, its roots are consumed as spices, while oil in its seeds is used in cosmetic products [2].

Medicinal plants are traditionally recognized to be safe products as less side-effects are experienced with plant-based treatments; therefore, people can consume these plants without any prescription. Presently, healing with medicinal plants is increasing rapidly in both developed and developing countries. Regarding *M. oleifera*, the local citizens usually use it as folk medicine [3], this plant can be used to cure various diseases, protect tissues (liver, kidneys, heart, testes, and lungs), analgesic, antiulcer, antihypertensive, radioprotective, and immunomodulatory actions [4]. In addition, it is also widely used by patients affected by diabetes, hypertension, or HIV/AIDS in Africa [5]. Importantly, no serious effects regarding human health were claimed [4].

Furthermore, scientists also point out the presence of a large range of phytochemical compounds in *M. oleifera*, such as vitamin A, C, calcium, potassium, iron, and protein [6]. Moreover, Atawodi *et al*. [7] and Waterman *et al*. [8] demonstrated that *M. oleifera* is rich in phenolic compounds (chlorogenic acid, rutin, quercetin glucoside or isoquercetin, and kaempferol

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rhamnoglucoside), various derivations of salicylic acid, gallic acid, coumarin acid, and caffeic acid; all of them present a high level of AA. These reports showed that *M. oleifera* is a potential material to isolate bioactive compounds, especially phenolic compounds, and apply them in the food technology or the pharmaceutical industry. In Vietnam, *M. oleifera* has been widely cultivated in many regions, but it is only used as a simple medicine and food; people are rarely interested in its nutritious components.

Nowadays, many modern extraction methods can isolate phytochemical compounds in herbal plants, such as Soxhlet extraction (SE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE). Among them, UAE is widely considered a green extraction technique due to the reduction in extraction time and solvent consumption; furthermore, this method is completely suitable for lab-scale and industry-scale production due to the ease of handle and reasonable cost of the equipment [9, 10]. For the SE method, it is quite simple, requires a relatively cheap apparatus, and has high phenolic extraction rates [11]. In summary, UAE presents several advantages over conventional techniques. In particular, the optimal conditions of UAE are rather simple due to the smaller quantity of control factors in comparison with the other techniques [10]. Hence, UAE and SE are selected and compared in this study to recover the total polyphenols and antioxidant activity from fresh *M. oleifera* leaves.

The response surface methodology (RSM) plays an important role in many areas of science, especially food, medicine, and the mechanical fields. The regression equation obtained from RSM can show the interactions between variables for response and exactly predict the experimental results. Until now, there have been no reports in the literature of UAE and SE of phenolic compounds and antioxidant capability from fresh *M. oleifera* leaves using the RSM and comparing the extraction efficacy of both methods. In this study, we used the RSM to optimize the extraction processes, including the independent variables (SS ratio, extraction time, and extraction temperature) and the responses (TPC and AA). The central composite face (CCF) model was chosen to design the experiments. The results obtained from these models could provide important data for future studies.

EXPERIMENTAL

Chemicals and reagents

Gallic acid (≥ 97.5%), Trolox reagent (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) (97%), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (> 90%), and Folin-Ciocalteu (FC) reagents were purchased from Sigma-Aldrich (Germany). All organic solvents and other chemicals were of analytical reagent grade.

Sample preparation

The fresh *M. oleifera* was harvested in March in Phan Rang-Thap Cham City, Ninh Thuan province (South central coast region, Vietnam), location of about 11°35'58.4"N; 108°54'45.1"E. The fresh leaves had a green color and no physical damage, and no pest contamination was observed on the leaves. The branches and twigs were removed, and the fresh leaves were separated by hand. The samples were washed, drained, and ground by a grinder (Philips, model HR1711, China) for 30 s. The samples obtained were separated with a sieve (hole diameter of 4 mm), packaged in polyethylene (PE) bags, and stored at 4ºC until further analyses.

Soxhlet extraction (SE) process

Aqueous acetone (70%, v/v) was used as a solvent for extracting polyphenols and determining the antioxidant activity (AA) from the fresh samples (2 g) in the Soxhlet system. The extraction

conditions consisted of an extraction temperature of 55-65 °C, a SS ratio of 30-40 mL/g, and an extraction time of 90-120 min. The extracts after the SE process were filtered through Whatman filter paper (No. 4) under vacuum to remove the residue and prepare them for the TPC and AA analysis.

Ultrasound-assisted extraction (UAE) process

The phenolic compounds in the samples (2 g) were extracted by using aqueous acetone as a solvent (70%, v/v). The effects of the SS ratio (25-35 mL/g), extraction time (20-30 min), and extraction temperature (55-65 °C) were examined. The UAE process was carried out in an ultrasonic bath (Elmasonic S60 H, 550 W, Germany). The TPC and AA were evaluated similar to the SE process.

Determination of total phenolic content (TPC)

The spectrophotometric method with the Folin–Ciocalteu reagent was done to determine the TPC of extracts obtained, using the Singleton–Rossi method performed by Messaoudi *et al*. with minor modifications [12]. Firstly, the extracts were made up to 250 mL of aqueous acetone (70%, v/v). Secondly, 0.1 mL of the diluted extracts was added to 1.5 mL of the FC reagent (10%, v/v). After that, the mixture was remained unchanged at room temperature for 5 min. Then, 4 mL of 20% Na_2CO_3 was added, and the volume was made up to 10 mL with distilled water. Finally, the mixture was kept in the dark for 30 min, and the absorbance was recorded at 738 nm using a spectrophotometer (Genesys 20, USA). The calibration curve was plotted using the gallic acid as a standard reagent. The TPC was expressed in mg of gallic acid equivalent weight (GAE) per gram of dry weight (DW).

Determination of antioxidant activity (AA)

The AA of the extracts obtained was measured according to the method described by Soto *et al.* with slight modifications, using DPPH free radical-scavenging capacity [13]. The diluted extracts were prepared as mentioned in the previous section. Briefly, 0.1 mL of the diluted extracts was added to 4 mL of the 0.1 mM DPPH solution. Then, the volume was made up to 5 mL with ethanol solution. The mixture was also kept in the dark for 30 min, and the absorbance was measured at 517 nm using a spectrophotometer (Genesys 20, USA). The calibration curve was plotted using Trolox as a standard reagent. The AA was expressed in µmol of Trolox equivalents (TE) per gram of dry weight (DW).

Experimental design

In this study, the response surface methodology (RSM) was used to determine the main effects of the process variables (SS ratio, extraction time, and extraction temperature) on the TPC and AA during SE and UAE of fresh *M. oleifera* leaves and to find the optimum parameters of the extractions. The experimental design adopted was a central composite rotatable design with three factors and three levels for each factor according to the second-order central composite face (CCF) design. Selection of the actual factor values was based on the preliminary experiments. All levels of independent variables are displayed in Table 1 and 2 including 17 experimental runs (three replications of the central point). The experimental data were fit to a second-order polynomial equation, and the regression equation was described as follows:

$$Y_r = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} . \sum_{j=2}^{n} \beta_{ij} x_i x_j$$

where β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients, Y_r is the response (TPC and AA), and x_i and x_j are independent variables (extraction temperature, extraction time, and SS ratio).

The experimental designs and data analysis were designed and analyzed through the analysis of variance (ANOVA) at $p < 0.05$, whereas the three-dimensional (3D) and response surface plots were plotted by Modde software (version 5.0, 1999, Umetrics AB, Umea, Sweden).

Table 1. Coding of the independent variables and their levels for central composite face (CCF) designed for the SE and UAE method.

Methods	Independent variables	Symbols	Coded levels		
			-1	0	1
SE	Extraction temperature (°C)	X_1	55	60	65
	SS ratio (mL/g)	X_2	30	35	40
	Extraction time (min)	X_3	90	105	120
UAE	Extraction temperature (°C)	X_1	55	60	65
	SS ratio (mL/g)	X_2	25	30	35
	Extraction time (min)	X_3	20	25	30

RESULTS AND DISCUSSION

Model fittings

Some main factors which significantly affected on the total phenolic content and the antioxidant ability of the extracts from fresh *M. oleifera* with SE and UAE method were implemented by the single-factor preliminary experiments. After that, on the basis of the previous results obtained, three critical influencing factors, including solvent to material ratio (mL/g), treatment temperature (°C), and extraction time (min), were chosen to optimize the extraction conditions for maximizing the TPC and AA obtained in the extracts using the RSM to design and run the experimental matrix (Table 1 and 2).

Based on the central composite face (CCF) design, the mathematical equations were formed using the second-order polynomial models, which expressed the relationships between the independent values (S_1, S_2, U_1, and U_2) and dependent variables. The basis for the fitted experimental models was based on data described in Table 3. The ANOVA results of the responses (TPC and AA) implied that four models were considerably significant ($p < 0.0001$ for all). The lack of fit (F) values were insignificant (0.057 and 0.128 for TPC and AA in SE; 0.062 and 0.396 for those in the UAE method, respectively). All aforementioned lack-of-fit values were more than 0.05, illustrating that the fitted models for anticipation of the measured variables were good.

All determination coefficients (R^2) and adjusted coefficients of determination (R^2_{adj}) were approximately 1 (from 0.964 to 0.988 for R^2 and from 0.917 to 0.973 for R^2_{adj}), indicating that more than 96.4% of the variability in the response variables could be explained by the models. In addition, there were feasible agreements between all R^2 and R^2_{adj}, as their difference was less than 0.2 [14]. Hence, there was a satisfactory between the predicted and experimental values. Furthermore, the Q^2 values for the TPC and AA were 0.732 and 0.857, respectively, in the Soxhlet method. Those values for the UAE method were 0.719 and 0.711, respectively. These results satisfied the standards ($Q^2 > 0.5$ and R^2-$Q^2 < 0.3$) suggested by Eriksson *et al*. [15]. Thus, the models indicated a reasonable goodness of precision and measured responses.

From the above-mentioned statistics and explanations, it could be concluded that four mathematical models were a good fit and could be applied to optimize the extraction conditions. After removing all irrelevant factors, the quadratic model expressions were expressed as coded values by the following models:

Table 2. Summary of the data regarding the response variables: TPC (S_1 and U_1), AA (S_2 and U_2), and independent factors (temperature, X_1; SS ratio, X_2; and time, X_3) in different experimental runs for both extraction processes.

Run	SE					UAE				
	X_1	X_2	X_3	S_1	S_2	X_1	X_2	X_3	U_1	U_2
1	55	30	90	22.911	445.823	55	25	20	30.973	480.681
2	65	30	90	21.701	432.152	65	25	20	28.351	470.132
3	55	40	90	24.312	442.675	55	35	20	31.395	483.321
4	65	40	90	21.861	439.723	65	35	20	29.543	476.703
5	55	30	120	23.731	448.142	55	25	30	31.301	483.582
6	65	30	120	24.232	443.174	65	25	30	30.077	477.246
7	55	40	120	24.571	448.372	55	35	30	32.138	481.972
8	65	40	120	24.922	449.154	65	35	30	32.453	483.683
9	55	35	105	25.901	454.542	55	30	25	33.194	488.134
10	65	35	105	24.231	450.635	65	30	25	33.293	487.008
11	60	30	105	25.062	452.792	60	25	25	33.072	487.291
12	60	40	105	25.893	455.632	60	35	25	33.648	489.483
13	60	35	90	24.631	454.076	60	30	20	33.143	487.725
14	60	35	120	25.602	457.193	60	30	30	33.734	487.672
15	60	35	105	26.644	458.951	60	30	25	34.275	491.961
16	60	35	105	26.742	458.582	60	30	25	34.054	493.868
17	60	35	105	26.508	459.644	60	30	25	33.991	491.215

For the SE method:

$$S_1 = 26.376 - 0.448X_1 + 0.392X_2 + 0.764X_3 - 1.119X_1^2 - 0.707X_2^2 - 1.068X_3^2 - 0.564X_1X_3$$
$$S_2 = 459.209 - 2.472X_1 + 1.347X_2 + 3.159X_3 - 6.733X_1^2 - 5.109X_2^2 - 3.687X_3^2 + 2.059X_1X_2 + 1.555X_1X_3$$

For the UAE method:

$$U_1 = 34.343 - 0.528X_1 + 0.540X_2 + 0.630X_3 - 1.277X_1^2 - 1.161X_2^2 - 1.082X_3^2 + 0.446X_1X_3$$
$$U_2 = 492.19 - 2.292X_1 + 1.623X_2 + 1.559X_3 - 4.499X_1^2 - 3.683X_2^2 - 4.372X_3^2 + 1.497X_1X_2 + 1.568X_1X_3$$

where S_1, U_1 are the TPC (mg GAE/g DW) and S_2, U_2 are the AA (μmol TE/g DW) received after the Soxhlet and UAE process, respectively.

According to the above-described mathematical formulas, all measured responses depended significantly on any of the three variables and followed the non-linear quadratic patterns. Furthermore, the square of the extraction temperature revealed the more obvious effect than the others, and all quadratic factors negatively affected the four responses.

In addition, the linear and quadratic effects of the processing temperature showed markedly negative influences on all the responses among the experimental factors, meaning that a decline in extraction temperature led to an increase in the amount of the TPC and AA attained. For example, with the processing temperature declining from 65 to about 59 °C, when the SS ratio grew from 30 to about 36 mL/g at the fixed extraction time of 105 min, the amount of the TPC and AA through SE increased from 24.14 to 26.48 mg GAE/g DW and from 441.5 to 457.7 μmol TE/g DW, respectively (Figure 1 and 2). In the case of UAE, there were an increase in the amount of the TPC from 30.79 to 34.07 mg GAE/g DW and that of AA from 477.9 to 491.4 μmol TE/g DW with an increase in the extraction time from 20 min to 26.4 min at the fixed ratio of 30/1 mL/g (Figure 3 and 4). This result is in agreement with Zhao *et al*. [16], who suggested that the TPC yield extracted gradually was reduced with the rising temperature factor.

Table 3. Analysis of variance (ANOVA) and regression equation coefficients of the models for the TPC and AA.

Factors	S_1		S_2		U_1		U_2	
	Coefficient	*p*	Coefficient	*p*	Coefficient	*p*	Coefficient	*p*
Constant	26.376	<0.0001	459.209	<0.0001	34.343	<0.0001	492.189	<0.0001
X_1	-0.448	0.011	-2.472	0.0004	-0.528	0.013	-2.292	0.004
X_2	0.392	0.019	1.347	0.011	0.540	0.011	1.623	0.020
X_3	0.764	0.0006	3.159	< 0.0001	0.630	0.005	1.559	0.024
X_1^2	-1.119	0.003	-6.733	< 0.0001	-1.277	0.004	-4.499	0.004
X_2^2	-0.707	0.026	-5.109	0.0003	-1.161	0.007	-3.683	0.009
X_3^2	-1.068	0.004	-3.687	0.002	-1.082	0.010	-4.372	0.004
X_1X_2	-0.174	0.270	2.059	0.002	0.289	0.148	1.497	0.042
X_1X_3	0.564	0.006	1.555	0.010	0.446	0.040	1.568	0.036
X_2X_3	-0.004	0.979	0.223	0.628	0.200	0.297	-0.548	0.395
Q^2	0.732		0.857		0.719		0.711	
R^2	0.968		0.988		0.964		0.966	
R_{adj}^2	0.927		0.973		0.917		0.922	
p	0.000		0.000		0.000		0.000	
Lack of Fit (F)	0.057		0.128		0.062		0.396	

ANOVA was set up with constraints set at $p < 0.05$ for the models to be significant and $p > 0.05$ for an insignificant lack of value.

On the contrary, the linear impacts of the extraction time and SS ratio were positive, while the quadratic levels of these factors presented negative influences on all the dependent responses. These above-mentioned tendencies are observed clearly in Figure 1-4, which are the 2D contour plots and 3D response surface curves describing the interactions and effects between the input factors on the output responses. Each plot was built with two input factors changing together while the rest factor is at a fixed level.

In terms of the interaction parameters on the responses, the extraction temperature interacted considerably with the time process in all cases, and this interaction had a positive effect on all variables except for the TPC over the SE. Furthermore, the antioxidant abilities were also positively affected by the interplay between the solvent to material ratio and the extraction temperature.

Similar results were also found in the report of Zhang *et al*. [17], who conducted the UAE of the AA from *Angelica keiskei* and revealed the interplay between the extraction time and temperature, SS ratio and treatment temperature. In addition, there was a dependence of the AA on both the first order and second-order level of variables, including ultrasonic temperature, ultrasonic time, solvent concentration, and SS ratio.

Furthermore, the results in this study quite resembled the suggestions of Zulkifli *et al*. [18], who affirmed that there were interactions between the processing time and temperature on the TPC and AA, and that both these responses depended on all linear and quadratic control factors.

Determination and validation of the optimal conditions

The main objectives in this study were to maximize the yield extraction of both the TPC and AA through the optimization of the major control factors. The optimal conditions during UAE of the control variables were an extraction temperature of 59.13 °C, extraction time of 25.97 min, and SS ratio of 30.99 mL/g. Furthermore, those values for SE were 59.43 °C, 110.3 min, and 36.03 mL/g, respectively (Table 4). However, to operate the extraction systems conveniently, the optimal above-mentioned parameters were adjusted as follows: extraction temperature of 59 °C, extraction time of 26 min, and acetone to material ratio of 31 mL/g for UAE. These values for SE were 59 °C, 110.3 min, and 36 mL/g, respectively.

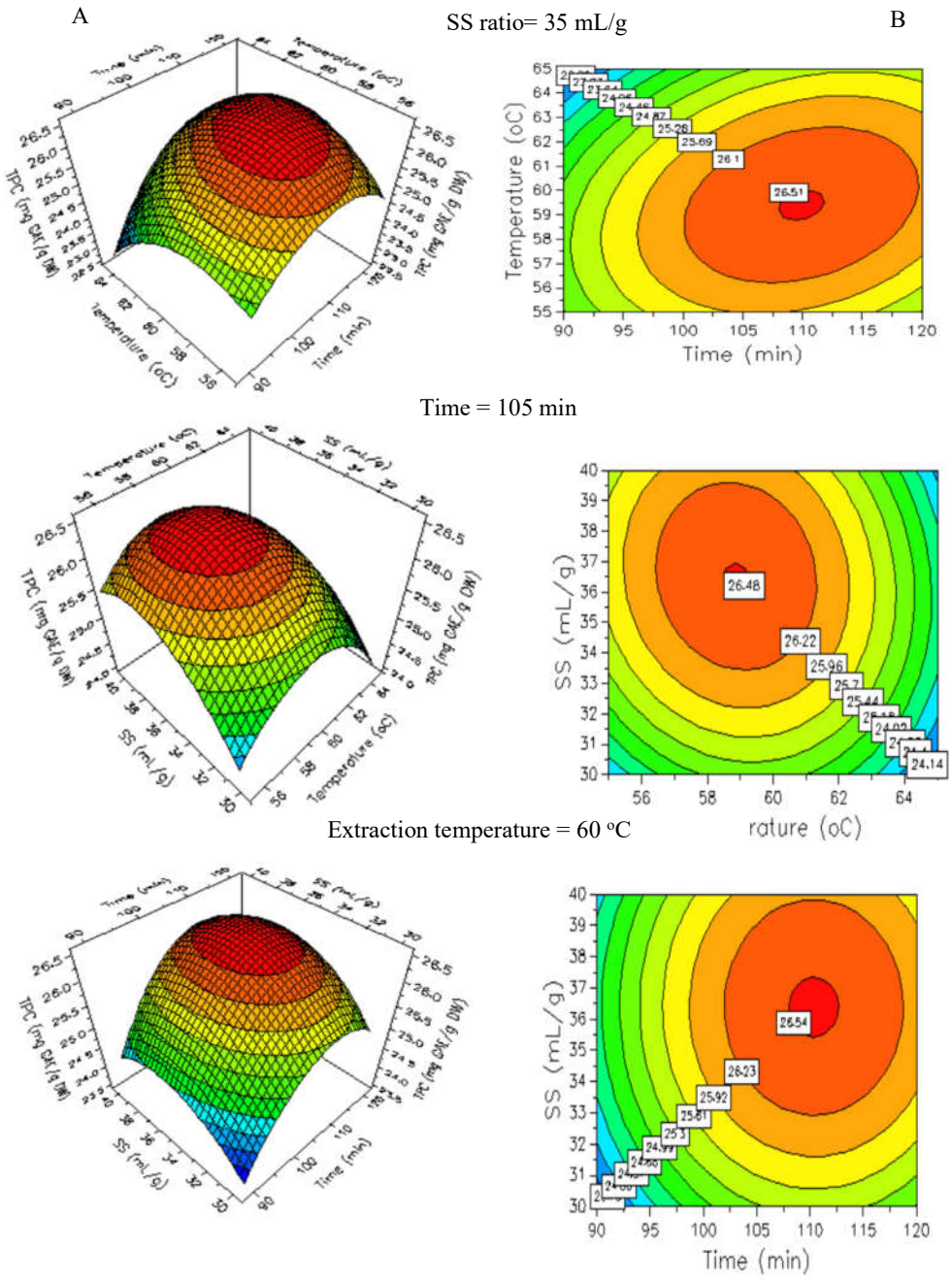

Figure 1. Response surfaces (A) and contour plots (B) of the TPC from *M. oleifera* at each center constant factor during the SE method.

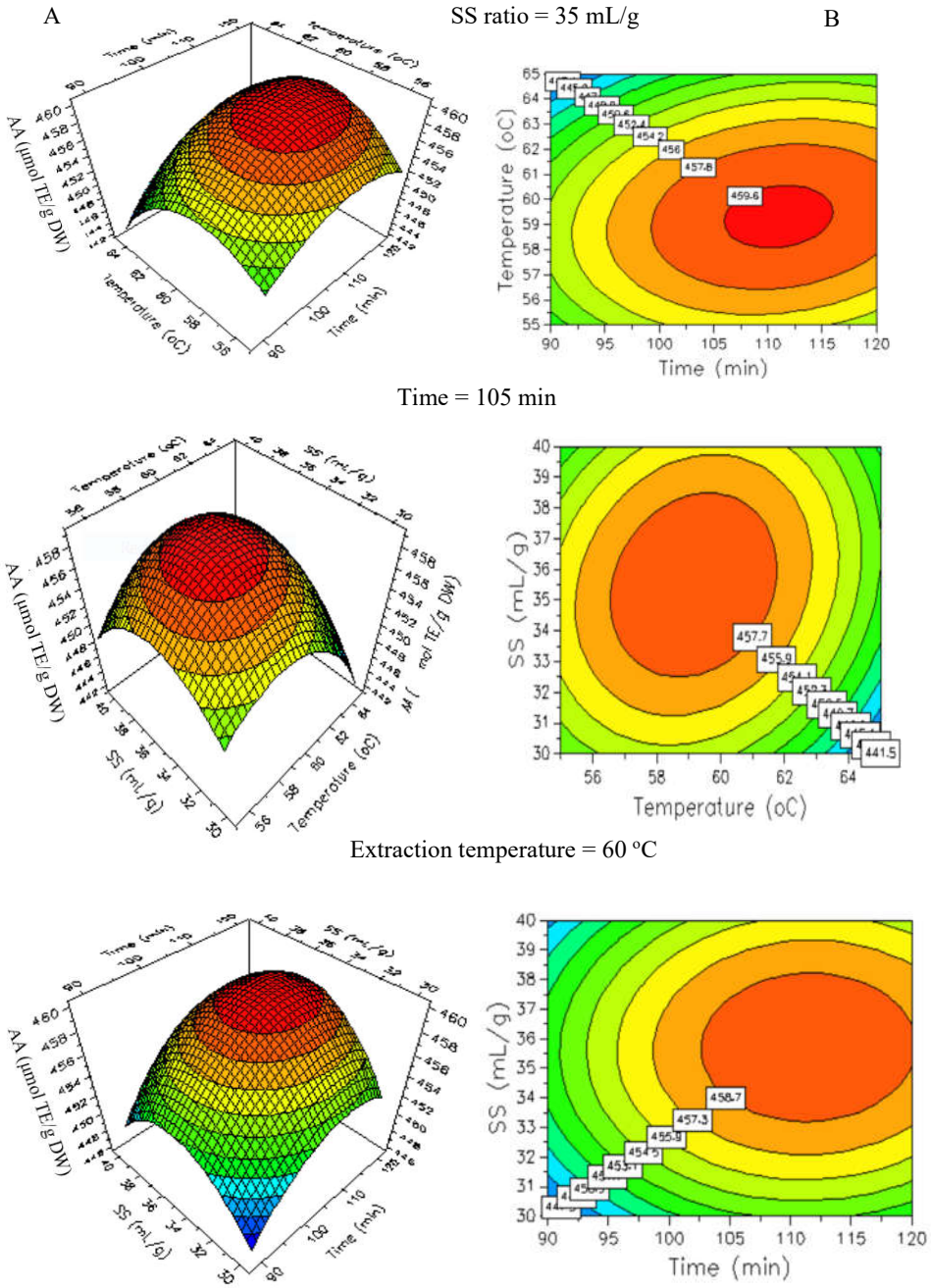

Figure 2. Response surfaces (A) and contour plots (B) of the AA from *M. oleifera* at each center constant factor during the SE method.

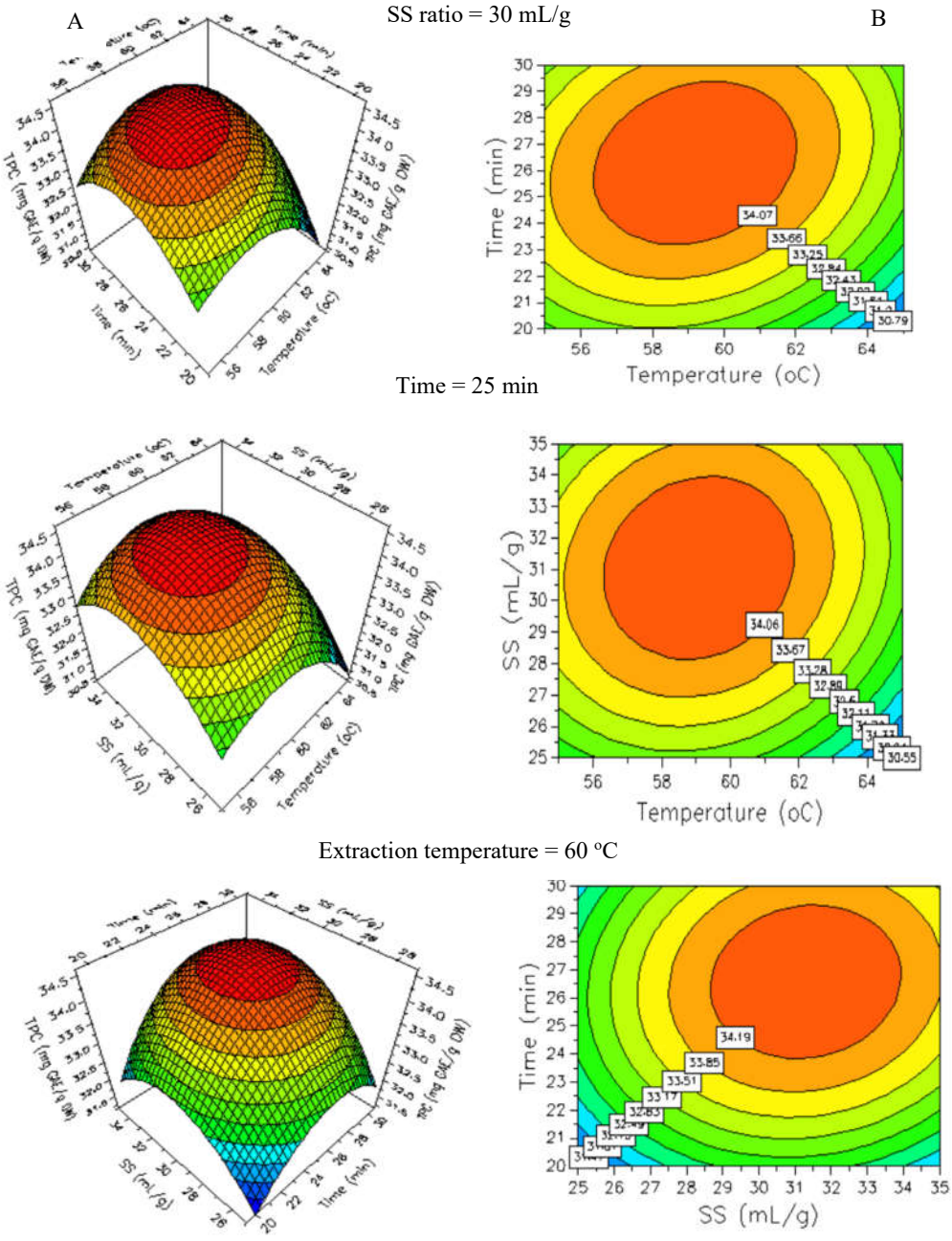

Figure 3. Response surfaces (A) and contour plots (B) of the TPC from *M. oleifera* at each center constant factor during the UAE method.

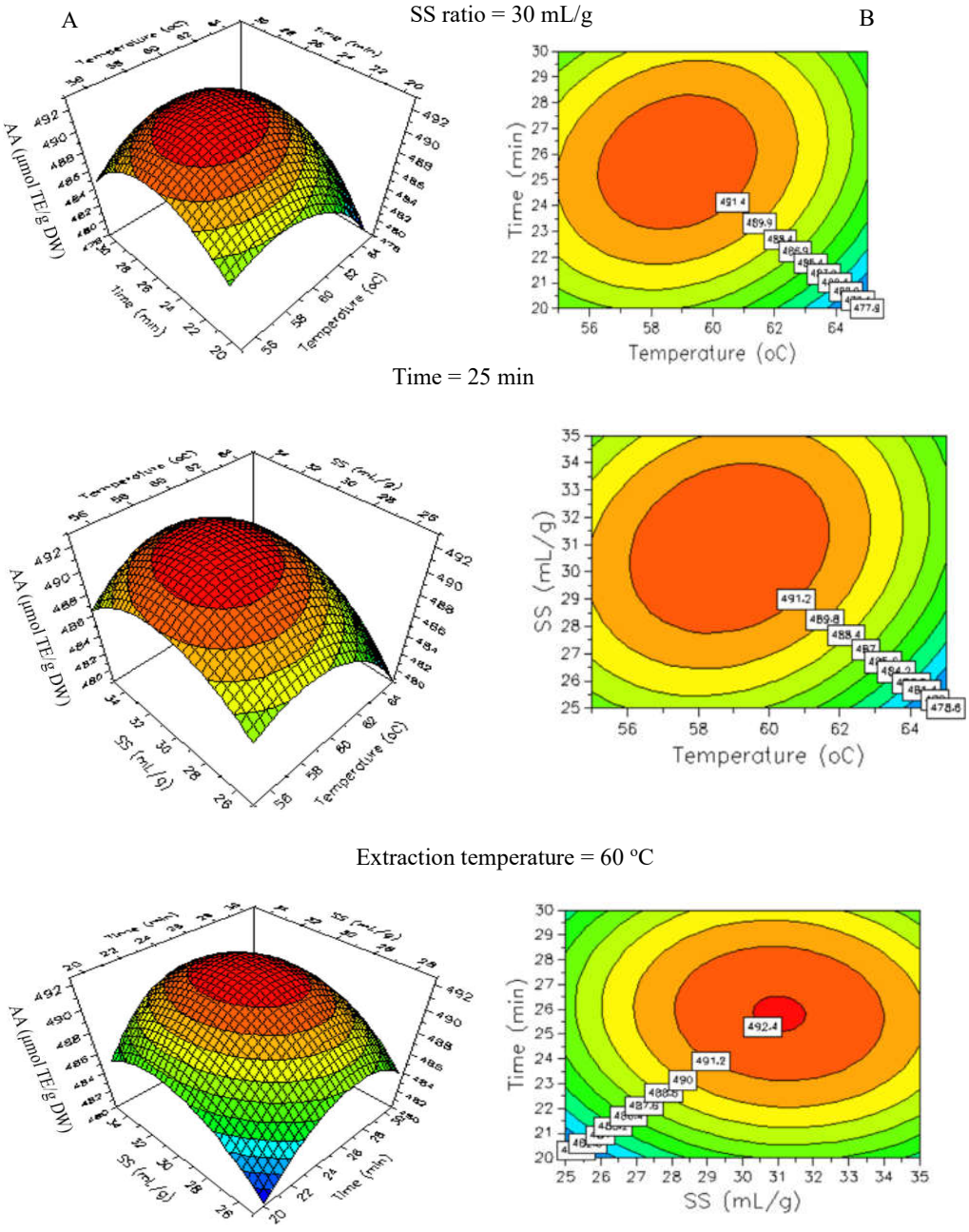

Figure 4. Response surfaces (A) and contour plots (B) of the AA from *M. oleifera* at each center constant factor during the UAE method.

The corresponding optimal response variable values were 34.52 mg GAE/g DW total polyphenol content and 492.6 µmol TE/g DW antioxidant activity for UAE, and those for SE were 26.58 mg GAE/g DW and 460.0 µmol TE/g DW, respectively.

Table 4. Response variables including experimental values and predicted values at optimum extraction conditions in two different extraction methods.

Extraction methods	SE		UAE	
	Experimental values	Predicted values	Experimental values	Predicted values
TPC (mg GAE/g DW)	26.07 ± 0.80	26.58	34.36 ± 0.23	34.52
AA (µmol TE/g DW)	459.2 ± 4.4	460.0	491.9 ± 4.6	492.6
Extraction conditions:				
Extraction temperature (°C)	59		59	
Extraction time (min)	110.3		26	
SS ratio (mL/g)	36		31	

Based on the identified optimal control variables, the experimentally obtained response variable for the case of UAE corresponded to 34.36 ± 0.23 mg GAE/g and 491.9 ± 4.6 µmol TE/g DW for the TPC and AA, respectively. Furthermore, those values for SE were 26.07 ± 0.80 mg GAE/g DW and 459.2 ± 4.4 µmol TE/g DW, respectively (Table 4).

The experiments were conducted to give evidence for the competence of the RSM models with the optimal control variables. The above-mentioned results were verified, as there were no significant differences between the experimental and predicted values. Hence, the fit models achieved were adequate to anticipate the optimal conditions.

The optimal temperature and time parameters in this study were similar to those in the statements of Savic *and* Gajic [19], who used the UAE technique to extract a TPC of 15.51 g GAE/100 g DW from wheatgrass (*Triticum aestivum* L.) under the optimal conditions, including 56% (v/v) ethanol, an SS ratio of 10 mL/g, an extraction temperature of 59 °C, and an extraction time of 28 min. However, with the same optimal SS ratio (31 and 36 mL/g), there was a lower extraction temperature and a longer processing time than those in the study by Zhang *et al*. [17], who extracted flavonoids and the AA of *Angelica keiskei* with the UAE method for 80, 4 min, SS ratio of 35 mL/g, and ethanol concentration of 78%. A different trend was observed in the study by Zhao *et al*. [16], who collected 2.44% of the TPC from *M. oleifera* leaves under optimal conditions including 70% ethanol concentration, 30:1 SS ratio, 50 °C extraction temperature, and 42 min extraction time. These different input factors could be because of the different kinds of materials and solvent phase.

Regarding the amount of the TPC and AA achieved from *M. oleifera,* the results in this research showed some slight differences compared to some other scientific studies. For instance, Rocchetti *et al*. [20] extracted the highest TPC of 31.84 mg/g DW and AA of 49.55 mg TE/g DW from dried *M. oleifera* using the homogenizer-assisted-extraction with methanol:water (50:50, v/v), while Rodríguez-Pérez *et al*. [21] used ethanol:water (50:50) as the solvent through UAE to isolate 47 mg GAE/ g DW from *M. oleifera* Lam leaves. These different outcomes could be due to the different kinds of materials (fresh and dried), species of *M. oleifera,* storage conditions, and extraction conditions.

Comparative efficiency of Soxhlet and ultrasound extractions

When it comes to UAE and SE, the former method achieved more efficient productivities in the main object extractions, including the TPC (34.52 mg GA/g DW) and AA (492.6 µmol TE/g DW) in comparison with the latter approach (26.58 mg GA/g DW and 460.0 µmol TE/g DW, respectively). Hence, the UAE procedure was considered a better extraction process in

comparison with the SE process. The more efficient extraction can be explained clearly that the ultrasound method facilitates considerably better the transport of biologically active substances like polyphenols from the deepest places, even in the vegetable core, to the surfaces through the cooperative phenomena, including cavitation, agitated mechanical reactions, and thermodynamics [22].

In terms of comparing the influences of the control parameters on both the AA and TPC, the extraction time demonstrated a more profound effect on the efficiency of SE in comparison with UAE. It was also the most significant factor among all the experimental factors, as its p coefficient on the antioxidant ability under the SE process was the lowest value ($p < 0.0001$). The second largest p belonged to the extraction time on the TPC ($p = 0.0006$) and the extraction temperature on the antioxidant ability ($p = 0.0004$) through SE. For the ultrasonic method, the process time had a significant impact on the TPC ($p = 0.005$) but a considerably weaker effect on the rest of the response variable ($p = 0.024$), whereas the process temperature affected more significantly the AA than the TPC ($p = 0.004$ compared to $p = 0.013$) (Table 3).

Furthermore, the optimal extraction time reduced remarkably to 25.97 min through the UAE method in comparison with that of 110.3 min for the SE process (Table 4), meaning that the ultrasonic time was reduced by more than 4 times compared to the Soxhlet time. The processing time plays a vital role in the extraction efficiency of the bioactive substances from plants, as it could decrease remarkably the electricity consumption [14, 22]. Moreover, a longer procedure time damages the biologically active substances like phenolics extracted because the cell-wall bound phenolic compounds held in the cells are released out along with the polymerization [23], and the oxidation reactions occur, leading to the destruction of phenolics as well as the antioxidant activity [24]. Furthermore, prolonged extraction under higher temperatures causes the degradation of the soluble substances extracted, especially the deterioration of the biological compounds contributing to the antioxidant activity of the extracts [25] and facilitation of an increase in the evaporation rate of the organic solvents, leading to a reduction in the extraction efficiency [26]. Hence, UAE with a shorter treatment time was considered the most cost-efficient method yet high-quality products compared to SE. This result quite resembled the study of Savic and Gajic [19], who declared that UAE was an economical treatment to extract polyphenols from wheatgrass *(T. aestivum* L.*)* due to a much shorter extraction time than SE extraction (28 min compared to 24 h).

For the second control parameter, the extraction temperature is considered one of the important factors for the extraction of plant-based bioactive compounds because an increase in the processing temperature can facilitate the rates of mass transfer and diffusion into the liquid-phase extraction while reducing the extract viscosity and surface tension, leading to highly favorable conditions for enhancing the extraction efficiency [14, 22–24]. Apart from the above-mentioned effects, an improvement in the treatment temperature also accelerates the formation of cavitation bubbles under UAE. However, an excessive temperature in UAE causes less of a difference in the vapor pressure formed between the inside and outside of the acoustic bubbles, resulting in a decrease in the explosive forces of the bubbles, which destroys the plant cell walls to release active substances [25]. Furthermore, the bioactive compounds obtained easily undergo oxidative degradation at high temperatures. Therefore, a proper range of extraction temperatures of plant-based active compounds should adapt to the solvent-sample relationship and satisfy both the main targets, including the yield of extraction and preservation of bio-active components [24].

Based on Table 3, the temperature extraction followed the same trend with the extraction time, as it experienced a dominant effect on the responses in SE compared to UAE (over Soxhlet process, p values were 0.0004 and 0.011 for the AA and TPC, while those of the UAE method were 0.004 and 0.013, respectively). The optimal temperatures achieved over the SE approach (59.43 ºC) were quite similar to the UAE process (59.13 ºC) with the same range of experimental temperatures (from 55 to 65 ºC). This phenomenon could be explained by the fact that the

experimental temperatures in the range of 55 to 65 °C were suitable for the extraction and preservation of the target compounds attained in both extraction processes.

On the other hand, the process temperature expressed a more marked influence on the antioxidant capacity than the TPC in all extraction processes. This result could be explained by the fact that the phenolics maintained thermal stability with temperatures below 70 °C [24], whereas some temperature-induced bioactive compounds extracted, such as vitamin C, vitamin E, etc., can deteriorate under the experimental temperature.

The lowest effect on all responses among the control parameters was the SS ratio, which had almost the same impact on both extraction processes. (*p* coefficients were from 0.011 to 0.024) (Table 3). However, there was a difference in the optimal SS ratio for the two methods (36.03 mL/g for the Soxhlet method and 30.99 mL/g for the UAE method) (Table 4), meaning less organic solvent was used through UAE while still achieving a higher extraction efficiency compared to SE. As a result, those results could take advantage of the environmental and economic efficiency as well. This phenomenon can be explained by the fact that the Soxhlet process takes a longer time under a high temperature than the UAE method, leading to an increase in solvent evaporation, thereby requiring more solvent volume. Hence, the application of the ultrasound technique could remarkably reduce the consumption of the extraction solvent and extraction time yet achieve a high extraction efficiency.

These finding were in accordance with the study of Zhao *et al*. [16], who indicated that the solvent-to-sample ratio revealed a more significant impact than the processing temperature after UAE of the TPC and AA from dried *M. oleifera* leaves.

From the aforementioned results, it could be concluded that the ultra-sonication treatment presented more effectively in terms of the TPC and AA than the Soxhlet technique in this study. This trend was in line with a report by Mohammadpour *et al*. [25], who concluded that ultrasonic treatment of *Moringa peregrina* oil improved more efficiently the chemical properties of oil extracted, including the AA, TPC, and peroxide value, compared to the Soxhlet method.

In summary, UAE presented a simple, cost-effective, eco-friendly and potential extraction to apply on the industrial scale. This suggestion was in accordance with the report of Wang *et al*. [26], who revealed an aqueous two-phase UAE as an efficient alternative to conventional methods for the extraction of polyphenols from olive leaves.

CONCLUSION

In this study, the UAE and SE for TPC and AA extraction from fresh *M. oleifera* were carried out and compared using the RSM to design and analyze the optimal experiments. All four models achieved followed the non-linear quadratic patterns, and 3 control parameters remarkably affected all measured variables in the two extraction treatments. At the optimum extraction conditions for UAE method (extraction temperature of 59 °C, time of 26 min, and SS ratio of 31 mL/g), the maximum values of the TPC and AA obtained were 34.36 mg GAE/g DW and 491.9 μmol TE/g DW, respectively, while those of SE were 26.07 mg GAE/g DW and 459.2 μmol TE/g DW under optimum conditions including temperature of 59 °C, time of 110.3 min, and SS ratio of 36 mL/g. Therefore, the ultrasound treatment was considered an efficient, environment-friendly, and commercially plausible extraction in comparison with the SE technique, as the former could significantly reduce both the solvent consumption and processing time yet enhance the yield of the TPC and AA. This finding referred that the ultrasound process could be considered a highly promising method towards furthering the extraction of biochemical compounds from herbal plants to produce plant-based functional foods. Future work needs to mitigate the consumption of extraction organic solvents to further enhance the safe, cost-effective, and valuable production of the extracts from *M. oleifera* leaves.

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