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Ultrasound-assisted extraction of phenolic compounds from *Moringa oleifera* leaves by response surface methodology

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ABSTRACT

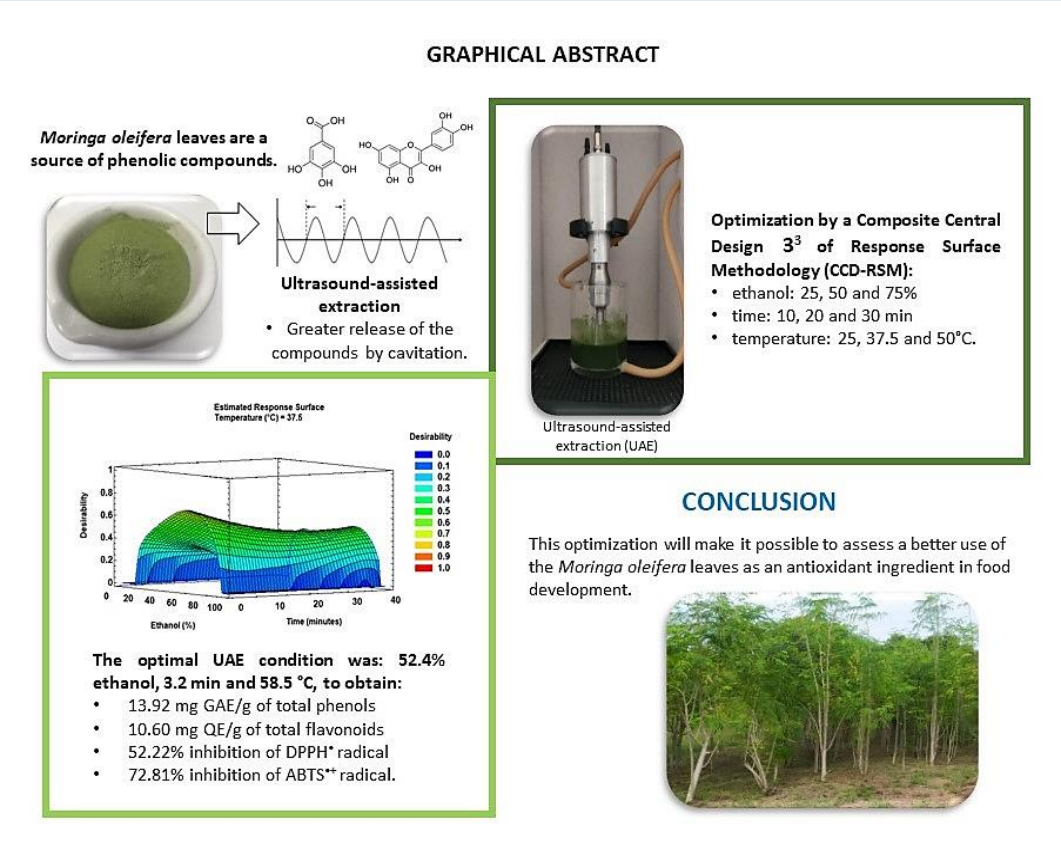
Background: *Moringa oleifera* is a tree that grows in tropical and subtropical areas around the world. Its leaves, seeds, bark, roots, and flowers are used as ingredients in meals and medicinal applications. Moringa leaf extracts have been studied to contain antioxidant compounds such as phenolic molecules. Recent extraction techniques such as ultrasound and microwaves are alternatives to increase the extraction performance of phenolic compounds while preserving their antioxidant activity.

Objective: The approach consisted of optimizing, using a Composite Central Design of Response Surface Methodology (CCD-RMS), the process conditions, for ultrasound-assisted extraction (UAE) of antioxidant phenolic compounds from *Moringa oleifera* leaves.

Methods: A 3^3 CCD-RMS was used; three independent variables were studied: ethanol concentration (25%, 50%, 75%), time (10, 20, and 30 min), and temperature (25, 37.5, and 50°C). The results showed that the optimal UAE conditions were an ethanol concentration of 52.4%, extraction time of 3.2 min, and temperature of 58.5°C; under these conditions the phenolic content was 13.92±0.21 mg GAE/g sample the total flavonoid content was 10.60±0.06 mg QE/g sample, and 52.22±2.01% and 72.81±1.58% of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals were inhibited, respectively.

Conclusion: This optimization will make it possible to assess better use of *M. oleifera* leaves as an antioxidant ingredient in functional food development.

Keywords: *Moringa oleifera*, composite central design, optimization, flavonoids, phenols, antioxidant activity.



INTRODUCTION

The tree *Moringa oleifera* Lam. is studied for its nutritional, phytochemical, and pharmacological properties. Moringa leaves contain a great diversity of active compounds, such as phenolic compounds with properties associated with antioxidant, antimicrobial, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, and cardioprotective effects [1,2]. Recent investigations have shown positive effects through the ingestion of the leaf extracts of *M. oleifera*, such as an increase in the activity of antioxidant enzymes and decrease in body weight, cholesterol, triglycerides, and blood glucose, as well as histological improvement of the heart and liver in rat models [3,4].

The appropriate method of extraction of natural antioxidants has a substantial effect on the recovery of phenolic compounds. Several studies have shown that ultrasound-assisted extraction (UAE) results in a higher yield, with less solvent consumption and time, compared to conventional methods. This is because ultrasound waves break plant cell walls, thus facilitating the introduction of the solvent through the plant tissue and favors the transfer of its components across the cell membrane [5-7]. However, more research on the optimum variable conditions during phenolic extraction by UAE, such as frequency, time, temperature, and power level for UAE extractions [8]. The objective of the present investigation was to optimize the yield of phenolic compounds and their antioxidant capacity during UAE from *M. oleifera* leaves by means of a Central Composite Design of Response Surface Methodology (CCD-RSM).

MATERIALS AND METHODS

Materials: Leaves were collected from an 18-month-old moringa tree in Huetamo, Michoacán, Mexico. The leaves

were washed, disinfected with 70% ethanol and dehydrated for 48 h in a solar dehydrator. Dried samples were ground and sieved to obtain particles with a size less than < 250 µm.

Methods

Extraction of phenolic compounds from dried leaves of *M. oleifera*: Sonics Vibra cell ultrasound equipment (model VC505 Sonics & Materials Inc., Newtown, CT USA) was employed to obtain phenolic compounds from *M. oleifera* leaves. An amplitude of 30% was used, with interval pulses of 50 s of ultrasound waves and 5 s of rest. The extraction in dried leaves was carried out using 3 g of *M. oleifera* leaves with 30 mL of solvent (1:10 w/v). A 3^3 Composite Central Design (CCD) was applied to evaluate the effect of ethanol concentration, 25, 50 and 75%, temperature, 25, 37.5 and 50°C, and time, 10, 20 and 30 min on the extraction of phenolic compounds. The CCD included two-star points. The extracts were subjected vacuum filtered through Whatman filter paper to get a particle size ≤ 25 µm and placed in Falcon tubes for the determination of total phenolic content, total flavonoid content, and the antioxidant activity by DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging.

Quantification of total phenols: The total phenolic content was determined by the Folin–Ciocalteu method [9]. A calibration curve with gallic acid was used as a standard. The absorbance was read at 750 nm in a UV/VIS spectrophotometer (VELAB®, model VE-5600UV, McAllen, TX, USA). Results were expressed in milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g). The determinations were carried out in three repetitions.

Quantification of total flavonoids*:* The determination of total flavonoid was carried out by the method proposed by Quettier-Deleu et al. [10] with slight modifications. A calibration curve with quercetin was used as a standard. The absorbance was evaluated in a UV/VIS spectrophotometer at 415 nm. The expression of the result was in milligrams of quercetin equivalents per gram of dry sample (mg QE/g). Three repetitions of the measurements were carried out.

Evaluation of DPPH$^{\bullet}$ scavenging capacity: The capacity of phenolic extracts from dried leaves of *M. oleifera* to scavenge the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) was determined by the method proposed by Randhir and Shetty [11]. The absorbance was read in a UV/VIS spectrophotometer at 517 nm and the results were expressed as the % inhibition of the DPPH$^{\bullet}$ radical and as gallic acid equivalents per gram of dry sample (mg GAE/g). The measurements were carried out in three repetitions.

Evaluation of ABTS$^{\bullet+}$ scavenging capacity: The effectiveness of *M. oleifera* extracts to eliminate the radical cation of 2,2'-azino-bis (3-ethylbenzothiazoline 6-sulfonic acid) (ABTS$^{\bullet+}$) was evaluated, according to what was described by Re et al. [12], with slight modifications. The absorbance at 734 nm was measured in a UV/VIS spectrophotometer. The results were expressed as the % inhibition of the ABTS$^{\bullet+}$ radical and as gallic acid equivalents per gram of dry sample (mg GAE/g). The determinations were carried out in triplicate.

Statistical analysis: Statgraphics Centurion XVII software was used to apply the CCD used to optimize the extraction of phenolic compounds and the antioxidant activity of *M. oleifera* leaves. The model presented three factors with three levels each, 3^3, which included 17 experimental runs, with three repetitions at the central point of the experimentation and two-star points. A regression analysis of variance (ANOVA) was performed at a significance level of $\alpha = 0.05$ to verify the validity of the model.

RESULTS AND DISCUSSION

The results obtained for total phenolic content, total flavonoid content, and DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays in the extracts of dried leaves of *M. oleifera* are presented in Table 1. Treatment 11 presented greater extraction of total phenolic compounds of 16.84 mg GAE/g and greater radical inhibition, 59.38% for DPPH$^{\bullet}$ and 70.44% for ABTS$^{\bullet+}$, compared to the other treatments. Treatment 17 showed the highest extraction of flavonoids of 12.98 mg QE/g. Derived from these results and using the CCD-RMS, it was possible to optimize the extraction conditions individually for phenols and flavonoids and perform a simultaneous optimization that included the four response variables: total phenols, total flavonoids, and antioxidant activity by capturing DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals.

Table 1. Experimental values of *Moringa oleifera* extracts.

Run	Ethanol (%)	Time (min)	Temperature (°C)	Phenol compounds (mg GAE/g)	Flavonoids (mg QE/g)	DPPH$^{\bullet}$ (% inhibition)	ABTS$^{\bullet+}$ (% inhibition)
1	8	20	37.5	15.38	5.11	21.15	45.89
2	25	10	25	12.97	5.07	35.20	54.78
3	25	10	50	16.12	6.50	41.32	57.62
4	25	30	25	13.13	4.14	31.24	47.96
5	25	30	50	16.59	6.15	45.05	60.21
6	50	3.2	37.5	16.49	6.23	52.86	64.81
7	50	20	16.5	14.08	5.63	45.45	61.29
8	50	20	37.5	14.73	4.53	55.48	59.17
9	50	20	37.5	15.30	4.46	55.59	58.40
10	50	20	37.5	14.73	4.53	55.48	57.47
11	50	20	58.5	**16.84**	6.77	**59.38**	**70.44**
12	50	36.8	37.5	15.82	4.96	50.35	70.34
13	75	10	25	12.75	7.91	47.38	46.93
14	75	10	50	10.22	11.40	56.24	61.60
15	75	30	25	12.31	5.80	44.87	50.34
16	75	30	50	13.45	7.46	48.83	56.33
17	92	20	37.5	8.74	**12.98**	35.78	38.81

Optimization in the extraction of total phenols: From the response values of phenolic compounds for the different combinations of variables, it was statistically demonstrated that they fit a second-order polynomial model. Quadratic equation obtained is shown below:

$$Phenols = 8.94629 + 0.237388*Ethanol - 0.264418*Time + 0.163064*Temperature - 0.00200863*Ethanol^2 + 0.00107505*Ethanol*Time - 0.00320628*Ethanol*Temperature + 0.00194566*Time^2 + 0.0039893*Time*Temperature - 0.000329423*Temperature^2$$

Pareto diagram shown in Figure 1 and ANOVA presented in Table 2, indicated that the ethanol concentration in linear and quadratic terms (A and AA) and the interaction of the ethanol concentration and temperature (AC) had negative and significant effects ($p \leq 0.05$), which indicated that lower ethanol

concentration favored the extraction of phenolic compounds. The temperature in a linear term (C) had positive and significant effect, showing that a higher temperature favors the extraction of phenolic compounds.

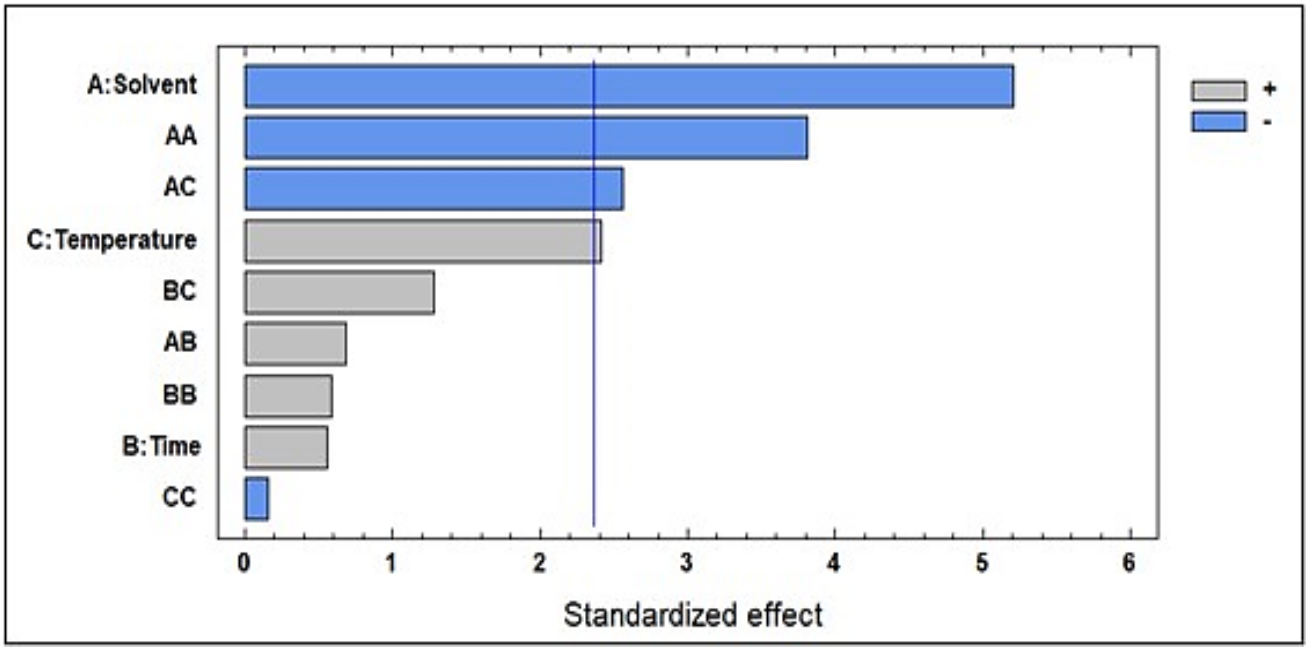


Figure 1. Pareto plots (P = 0.05) of standardized effects for the extraction of total phenolic content in *Moringa oleifera*.

The model had a satisfactory level of adequacy with an R^2 of 0.89 and the adjusted R^2 was 0.76 (Table 2), which indicated a strong agreement between the observed values and those predicted by the quadratic equation. The location of the stationary point represented the optimal extraction conditions according to the response surface, 22.2% ethanol, 36.8 min, and 58.5°C, to obtain a maximum concentration of total phenolic compounds of 19.84 mg GAE/g.

The values obtained in the prediction were confirmed experimentally; the results obtained under the same process conditions were 18.13±0.43 mg GAE/g of total phenolic compounds; the difference between the value predicted by the quadratic equation and the experimental tests was 8.6%, which confirmed the adequacy of the model (Table 3).

UAE was an efficient method to maximize the extraction up to 18.13 mg GAE/g of phenolic compounds with antioxidant activity from *M. oleifera* leaves. This result is greater than the value of 13.4 mg GAE/g of total phenolic found in extract of leaves of *M. oleifera* collected in Italy by using an optimization by UAE [13]. In another study done in Brazil was reported concentrations up to 26.6 mg GAE/g using UAE technology in *M. oleifera* leaves [14]. In Mexico, was obtained around 12 mg GAE/g of an extract of phenolic compounds from *M. oleifera* leaves [2].

Table 2. Analysis of variance for total phenolic compound content.

Source	Sum of squares	Gl	Square medium	F-ratio	P-value
A	33.0325	1	33.0325	27.06	0.0012*
B	0.383605	1	0.383605	0.31	0.5925
C	7.13608	1	7.13608	5.85	0.0462*
AA	17.767	1	17.767	14.56	0.0066*

Source	Sum of squares	Gl	Square medium	F-ratio	P-value
AB	0.577866	1	0.577866	0.47	0.5135
AC	8.03143	1	8.03143	6.58	0.0373*
BB	0.426766	1	0.426766	0.35	0.5729
BC	1.98931	1	1.98931	1.63	0.2424
CC	0.0298677	1	0.0298677	0.02	0.8801
Total error	8.54362	7	1.22052		
Total (corr.)	81.8827	16			
R-squared = 89.57%					
R-squared (adjusted by g.l.) = 76.16%					
Standard error of est. = 1.10					
Average absolute error = 0.55					
Durbin–Watson statistic = 1.16 (P = 0.05)					
Lag 1 residual autocorrelation = 0.40					

A = % ethanol; B = time (min); C = temperature (°C).

The differences found during the extraction of phenolic compounds in *M. oleifera* leaves differing greatly depending on the genetic and environmental conditions, harvest, processing conditions [15], and plant collection site. The total phenolic content optimized in this work is within the upper range reported in studies when was used UAE. UAE has been shown to be better in terms of cost, performance, and extraction time, compared to conventional methods, which have low efficiency and high solvent consumption [16].

Table 3. Optimization of individual responses in *Moringa oleifera* extracts.

Response variable	Predicted value	Experimental value	Process variable
Phenols (mg GAE/g)	19.84	18.13±0.43	Ethanol: 22.2% Time: 36.8 min Temperature: 58.5°C
Flavonoids (mg QE/g)	18.89	17.52±0.12	Ethanol: 90.4% Time: 3.2 min Temperature 58.5°C

The more efficient extraction of the bioactive compounds can be explained because UAE facilitates the transport of biologically active substances such as phenolic compounds from the deepest places, even in the plant nucleus, to the surfaces through cooperative phenomena, which include cavitation, agitated mechanical reactions, and thermodynamics, which increases the release of bioactive compounds from the plant material during the liquid extraction phase [17]. Likewise, the efficiency of the extraction of phenolic compounds is correlated with the polarity of the solvent, time, and extraction temperature, the parameters that were optimized in this study.

In this work, 22.2% ethanol used as solvent maximized the extraction of phenolic compounds; however, we used a lower ethanol concentration than that used by other authors, who reported a 70% [18] and 50% [19] of ethanol concentration for the extraction of

phenolic compounds from *M. oleifera* leaves. Most phenolic compounds have high or medium polarity, which could explain the use of hydroalcoholic mixtures, since too high a concentration of ethanol will lead to a decrease in the dissolution of phenolic acids due to the dissolution of some substances soluble in lipids and a high concentration of water will increase the dissolution of sugars and proteins, which will reduce their extraction rate [19].

The optimal extraction time and temperature were 36.8 min and 58.5°C, respectively. Therefore, the phenolic compounds remain stable in the upper limits evaluated, and their extraction is also favored. These conditions are similar to those reported by [13], 60 min and 60 °C, and by [18] who used 42 min and 50°C to optimize by UAE the extraction of phenolic compounds from *M. oleifera* leaves.

Optimization in the extraction of total flavonoid content: Based on the response values for flavonoids of the different combinations of process variables, the following quadratic equation was obtained:

$$\textbf{Flavonoids} = 10.3207 - 0.154128*Ethanol - 0.0324002*Time - 0.204483*Temperature + 0.00246345*Ethanol^2 - 0.0023849*Ethanol*Time + 0.0006824*Ethanol*Temperature + 0.00321399*Time^2 - 0.001232*Time*Temperature + 0.00342105*Temperature^2$$

Figure 2 shows the Pareto diagram and Table 4 the ANOVA related to the flavonoid content extracted from leaves of *M. oleifera*. The ethanol concentration and temperature in linear terms (A and C), as well as the quadratic contribution of the ethanol (AA) concentration, had positive and significant effects ($p \leq 0.05$) on the extraction of flavonoid compounds, which indicated that the extraction of flavonoid compounds is favored using a higher ethanol concentration and higher temperature. Time in a linear term had a negative and significant influence ($p \leq 0.05$), so a shorter time favored the extraction of these compounds. The quadratic equation presented an $R^2 = 0.92$ and the adjusted R^2 was 0.82. The stationary point, which indicated the maximum extraction point and therefore the optimal process conditions, was at 90.4% ethanol, 3.2 min, and 58.5°C to obtain 18.89 mg QE/g of flavonoids. The predicted values were confirmed experimentally. The results obtained under the same process conditions were 17.52±0.12 mg QE/g of flavonoids; the difference between the value predicted by the quadratic equation and that obtained in the experimental tests was 7.2%, which confirmed the adequacy of the model (Table 3).

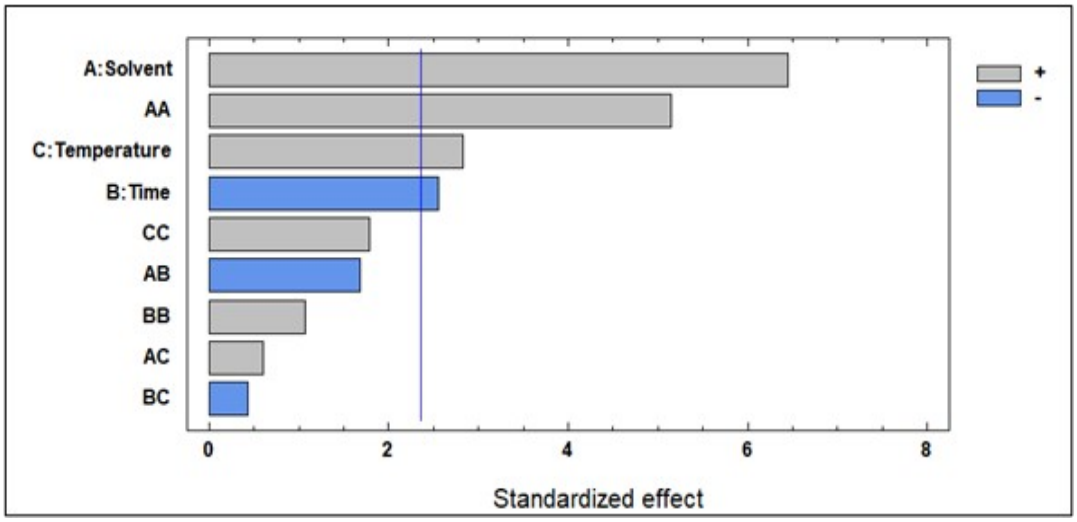


Figure 2. Pareto plots (P = 0.05) of standardized effects for the extraction of total flavonoids content in *M. oleifera*.

Table 4. Analysis of variance for total flavonoids content.

Source	Sum of squares	Gl	Square medium	F-ratio	P-value
A	41.9539	1	41.9539	41.62	0.0003*
B	6.556	1	6.556	6.5	0.0381*
C	8.09071	1	8.09071	8.03	0.0253*
AA	26.7241	1	26.7241	26.51	0.0013*
AB	2.84387	1	2.84387	2.82	0.1369
AC	0.363804	1	0.363804	0.36	0.5669
BB	1.16451	1	1.16451	1.16	0.3181
BC	0.189728	1	0.189728	0.19	0.6774
CC	3.22117	1	3.22117	3.2	0.117
Total error	7.05561	7	1.00794		
Total (corr.)	94.1556	16			
R-square = 92.50%					
R-square (adjusted by g.l.) = 82.87%					
Standard error of est. = 1.00					
Average absolute error = 0.55					
Durbin–Watson statistic = 2.07 (P = 0.53)					
Lag 1 residual autocorrelation = −0.04					

A = % ethanol; B = temperature (° C); C = time (min).

Table 5. Correlation between dependent variables in *M. oleifera* extracts.

Variable	Phenols	Flavonoids	DPPH$^{\bullet}$	ABTS$^{\bullet+}$
Phenols	1			
Flavonoids	−0.71	1		
DPPH$^{\bullet}$	0.24	0.03	1	
ABTS$^{\bullet+}$	0.62	−0.29	0.70	1

Knowledge of the most effective extraction conditions allows the recovery of bioactive compounds, such as flavonoids [20]. In the present investigation, the extraction by UAE of flavonoid compounds from *M. oleifera* leaves was optimized, 17.52 mg QE/g being obtained by using 90.4% ethanol for 3.2 min at 58.5°C.

These results are comparable with those from previous studies of optimization by UAE in *M. oleifera* leaves that reported concentrations of 25.2 mg QE/g of flavonoids, using 50% ethanol for 5 min [14], and of 14.16 mg QE/g of flavonoids, using 74.5% methanol, for 15 min at 11°C [21].

The extraction of flavonoid compounds was favored at higher concentrations of ethanol, 90.2%. Regarding the temperature, the extraction of both phenolic and flavonoid compounds was maximized at 58.5°C. On the other hand, we observe that the extraction time for flavonoid compounds was less than 3.2 min compared to

36.8 min for phenolic compounds. The times reported by other authors [14,21] to maximize the extraction of flavonoid compounds were 5 and 15 min, respectively; these values are lower than those reported to maximize the extraction of phenolic compounds of 42 and 60 min in other studies [13,18].

It has been pointed out that the longer the extraction time, the lower the extraction yield of some phenolic compounds, which may be due to oxidation that occurs when ultrasonic irradiation is used [22]. The flavonoid compounds obtained in the present study could present oxidation effects at a longer extraction time, so it is important to control this parameter at 3.2 min as indicated by the optimization. Likewise, it is important to use adequate temperatures since temperatures higher than 58.5°C could promote the degradation of the soluble substances extracted and therefore the yield of phenolic compounds [23].

According to the results of the optimization of individual response variables, the process conditions to maximize the yields of the phenolic and flavonoid compounds are different with respect to ethanol concentration and time. An analysis was carried out to determine the correlation of phenols and flavonoids with the antioxidant activity by capture of $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals. The correlation coefficients (R) are shown in Table 5. A strong positive correlation of 0.62 can be observed between phenols and inhibition of $ABTS^{\bullet+}$ radical. Although a weak correlation of 0.24 was obtained between phenols and inhibition of the $DPPH^{\bullet}$ radical, a high correlation of 0.70 was observed between the $ABTS^{\bullet+}$ radical and $DPPH^{\bullet}$, which indicated that the extraction of phenols favors the antioxidant activity of the extracts. A high correlation of phenols with the antioxidant activity of extracts from leaves of *M. oleifera* and other plants was also documented [24]. There was no correlation between the flavonoid compounds and the antioxidant activity by capturing the $DPPH^{\bullet}$ radical, since a value of 0.03 was obtained, and there was even a negative correlation between the flavonoids and the $ABTS^{\bullet+}$ radical, which was −0.29; this indicated that the greater the extraction of flavonoid compounds, the lower the antioxidant activity.

The correlation between phenols and flavonoids was −0.71; although this is a strong correlation, it is negative, which indicates that when the extraction of phenolic compounds increases, the extraction of flavonoids decreases and vice versa. The decrease in antioxidant activity when a greater number of flavonoids is extracted is due to the flavonoids in *M. oleifera* leaf extracts being found in their glycosidic forms [13], which possibly interferes with the antioxidant activity *in vitro*. However, when ingested these compounds present different structural transformations, due to acid hydrolysis in the stomach or due to different enzymes from microbiota present in the large intestine, resulting in smaller and biologically active molecules due to the deglycosylation of flavonoids and release in aglycones, which provide flavonoids their function as an antioxidant molecule among other cellular functions [25].

Flavonoids are molecules of great medical relevance due to their different beneficial functions in the body; however, it is also important that phenolic extracts have good antioxidant activity *in vitro*, since this is highly valued in the food industry, because phenolic extracts act as functional ingredients, fulfilling a double function, as an antioxidant in food and as an antioxidant in the body.

Therefore, to enhance the extraction of both flavonoid and phenolic compounds, all the responses were simultaneously optimized, to obtain a more balanced extract and thus enhance its effects.

Simultaneous optimization: The desirability function was used, which allowed determination of the optimal conditions for all the responses studied simultaneously [26]. The scale of the desirability function ranges from 0, a completely undesirable answer, to 1, a totally desired response [27]. A desirability value of 0.82 was obtained,

the response variables were simultaneously optimized, and optimal process conditions of 52.2% ethanol, 3.2 min and 58.7°C were obtained; these effects are shown in the three-dimensional response surface diagram (Figure 3), which allowed simultaneous maximization of all responses, and the model predicted values of 14.55 mg GAE/g for phenols, 10.15 mg QE/g for flavonoids, and 75.69% antiradical activity against $ABTS^{\bullet+}$ and 57% antioxidant activity against $DPPH^{\bullet}$ (Table 6).

The adequacy of the prediction model was evaluated by performing experiments in triplicate under optimized conditions and comparing the predicted values with the experimental ones. Table 6 shows that the observed and predicted values were consistent. The strong correlation between these results confirms the suitability of the model to reflect the intended simultaneous optimization, since the disparity between experimental and model-predicted values remains within a margin of close to 5% for all responses. Suggesting that the CCD-RSM methodology can be effectively used to optimize *M. oleifera* leaves extraction parameters.

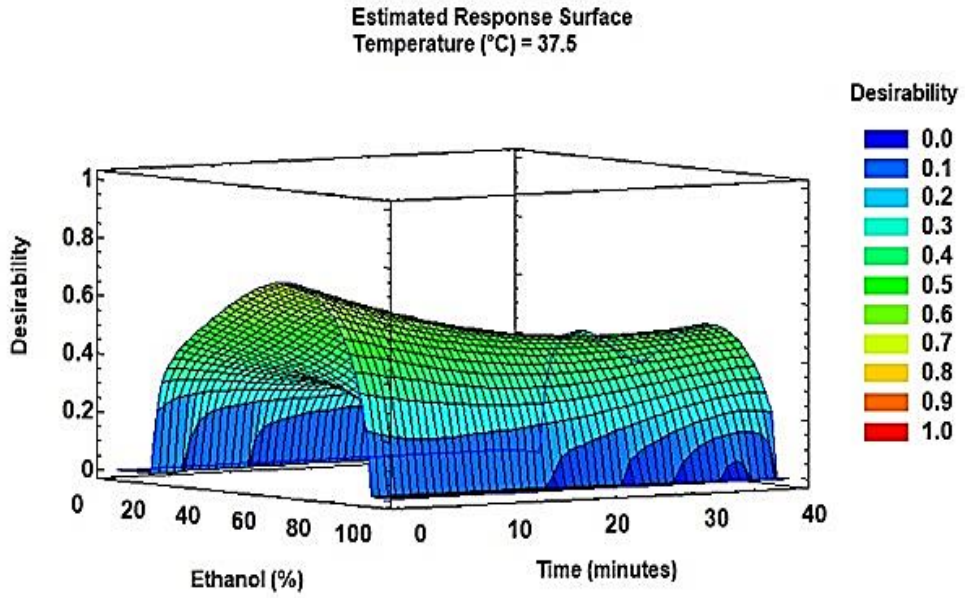


Figure 3. Response surface diagram for simultaneous optimization.

Table 6. Simultaneous optimization in *M. oleifera* extracts.

Response variable	Predicted value by simultaneous optimization1	Experimental value by simultaneous optimization[1,2]
Phenols (mg GAE/g)	14.55	13.92±0.21
Flavonoids (mg QE/g)	10.15	10.60±0.06
$ABTS^{\bullet+}$ (mg GAE/g)	7.95	7.71±0.17
% Inhibition (10 mg)	75.69	72.81±1.58
$DPPH^{\bullet}$ (mg GAE/g)	5.30	4.90±0.19
% inhibition (10 mg)	57.00	52.22±2.01

[1]52.4% ethanol; 58.5°C; 3.2 min. Desirability 0.82. [2]Data for phenols, flavonoids, $DPPH^{\bullet}$, and $ABTS^{\bullet+}$ are the average of triplicates.

The extracts obtained from the *M. oleifera* leaves in this work presented an important source of phenolic compounds; in addition, due to the simultaneous optimization, it was possible to obtain an extract with high antioxidant activity. This is the first study that simultaneously evaluates the effect of process variables

% ethanol, time, and temperature on the UAE of phenols and flavonoids, and in turn, how they influence and correlate with its antioxidant activity.

These types of studies provide important data for the use of *M. oleifera* leaves and in the future could contribute to generating value chains that allow the development of new products in the food sector, such as functional foods. These foods have currently acquired greater importance because their consumption is associated with "promoting optimal health and reducing the risk of chronic/viral diseases and controlling their symptoms"[28].

CONCLUSIONS

The use of an ultrasound-assisted process enhanced the extraction of the phenolic compounds. It was shown that the process variable that had the greatest influence on the extraction of phenolic compounds was the ethanol concentration, followed by the time, and finally the temperature, to maximize the extraction of antioxidant phenolic compounds from *M. oleifera* leaves. The findings presented in this work can contribute to the development of foods and/or nutraceuticals with high phenolic and antioxidant content, to counteract the increase in chronic-degenerative diseases and help maintain a good state of health in the population.

Abbreviations: $ABTS^{\bullet+}$:2,2'-azino-bis(3-ethylbenzothiazoline 6-sulfonic acid), CCD-RMS: Composite Central Design of Response Surface Methodology, $DPPH^{\bullet}$: 2,2-diphenyl-1-picrylhydrazyl radical, mg GAE/g: milligrams of gallic acid equivalents per gram of dry sample, mg QE/g: milligrams of quercetin equivalents per gram of dry sample, *M. oleifera: Moringa oleifera,* UAE: ultrasound-assisted extraction.

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Declaration of interest statement: The authors have no conflicts of interest to declare.

Author contributions: ETR and HEMF carried out the research and wrote the manuscript.

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