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Optimization of the Extraction of Polyphenols and Flavonoids from *Argania spinosa* Leaves using Response Surface Methodology

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Abstract – To our knowledge, this is the first study aiming to optimize the extraction conditions of total phenolic compounds (TPC) and total flavonoids contents (TFC) from *Argania spinosa* leaves using Response Surface Methodology (RSM) with a Box–Behnken design (BBD). The optimal conditions obtained were 5% (*w/v*) solvent-to-solid ratio, 72.33% ethanol concentration, and 10h ours as an extraction time, which resulted in an extract with maximum TPC (131.63 mg GAE/g dw) and TFC (10.66 mg QE/g dw). Under the optimal extraction conditions, the antioxidant activity of the extracts of leaves of argan tree showed a moderate antiradical capacity of DPPH (IC_{50} = 0,130 mg/mL) and ABTS (IC_{50} = 0.198 mg/mL). However, the leaves of argan tree showed a very interesting reducing power of Iron (IC_{50} = 0.448 mg/ml) which is similar to that of the ascorbic acid (IC_{50} = 0.371 mg/mL).
Key words – *Argan spinosa* leaves, optimization, response surface methodology, phenolic compounds, antioxidant activities

Introduction

Argania spinosa L. Skeels is a tree endemic to Morocco that grows in the arid and semi-arid zones of the southwest. The argan tree constitutes the second forest species of the country after the holm oak with a total area of 830,000 ha. In recent years, it has been the subject of special attention from the scientific community. This interest was aroused after its classification in 2014 as intangible cultural heritage of humanity by UNESCO, as well as its recognition in 2018 by the FAO as a world agricultural heritage system.Currently, the argan forest is a fragile ecosystem due to overexploitation of forest resources by the local population, demographic pressure, pastoral activity and desertification. To ensure the sustainable management of this unique agroforestry system, the preservation of the argan tree and its ecosystem has become a national priority. Thus, the economic valuation of the argan tree through its products is a way to sustainably revive an integrated rural forestry. In addition to the nutritional, cosmetic and medicinal quality of argan oil already known internationally, the leaves of the argan tree are also used for therapeutic purposes in the form of infusion to treat gastritis, diarrhea, fever, migraines and as a poultice in case of sprains, super infected wounds or scabies even for animals. These multiple uses in traditional medicine have prompted several researchers to conduct research to promote its use in the pharmaceutical industry. It has been reported that the leaves of argan are rich in phenolic compounds. The latter are bioactive molecules widely spread in the plant kingdom and known for their beneficial and preventive effects on human health. Indeed, their role as natural antioxidants is gaining interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases.[1] They are also used as additives for the food, pharmaceutical and cosmetic industries. Currently, the extraction of high-value phenolic compounds from plants constitutes a new and promising pathway for antioxidant molecules. In addition, the extraction process is vital for qualitative and quantitative analysis of active compounds. However, the extraction rate and composition of phenolic compounds are influenced by various factors, including the extraction method, solvent, time, temperature, pH, particle size, and others.[2]

For decades, most of the research efforts were focusing on the argan kernel and its oil, the majority of the research

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works aimed the argan oils uses in food and cosmetics. Recently, many researchers started showing interest on the argan tree other parts such as the fruit pulp and the leaves, however, to our knowledge no study has been done on optimizing the extraction of polyphenols from argan leaves.

The response surface methodology (RSM) is a mathematical and statistical method that significantly minimizes the number of experimental trials in order to evaluate the interactions between several factors and to find the optimal conditions for the experiment. This methodology has been widely used in the extraction of active compounds from plants.[3]

The objective of the present study is to optimize the extraction parameters of phenolic compounds from argan leaves (solvent concentration sample/solvent ratio and maceration time) using the RSM, and then to evaluate the antioxidant activity under the optimal conditions. compounds. This study is carried out for the first time on leaves of trees planted in the region of the Doukkala region of Morocco.

Experimental

Chemicals and Reagents – 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin Ciocalteau reagent, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), ferric chloride ($FeCl_3$), potassium persulphate ($K_2S_2O_8$), potassium ferricyanide ($K_3Fe(CN)_6$), and all solvents were obtained from Merck Life Science (Darmstadt, Germany). Ascorbic acid,quercetine and trichloracetic acid (TCA) were obtained from Scharlau (Barcelona, Spain).

Plant materials – Leaves of *Argania spinosa* L. Skeel were collected at the end of March 2021 in the region of Doukkala, commune of Sidi Abed in Douar Rouahla (32°59'24.7 "N 8°36'52.5 "W) on trees planted in 2006. The species was identified by Professor Rachid TALAL, a botanist from our institute, where a collection of voucher specimens was deposited.

Sample preparation – After harvesting, leaves were washed with distilled water and then air-dried under hot air ventilation at 40 °C for two days. The dried leaves were then finely ground using a blender and sieved, the resulting fine powder was stored at 4 °C until use.

Extraction of phenolic compounds – For each experimental trial, quantities of 0.25 to 1.25 g of powder were macerated under magnetic stirring in a final volume of 5 ml of a hydromethanolic solution at various concentrations of 40-100% and at time intervals ranging from one hour to 10 hours. In order to limit the risk of oxidation of the phenolic compounds, the extraction processes are performed in the dark and at room temperature. After completion of the extraction cycle, the liquid extracts were separated from the solid residues by filtration on filter paper and then stored at -20 °C.

Experimental design for RSM – Based on the literature and the results of our preliminary experiment, appropriate ranges of the process independent variables including sample to solvent ratio (X_1:5-25%, w/v), solvent composition (X_2: 40-100%, v/v, methanol water) and extraction time (X_3: 1-10hour), were chosen. The optimization of the extraction of phenolic compounds in argan leaves by the RSM approach was performed using the Design expert software (version 11). A Box-Behnken design (BBD) with three factors (X_1, X_2, X_3) at three levels (–1, 0, +1) (Table 1) and 15 combinations with three replicates in the center was used randomizing the order of the experiments. The response variables were total polyphenol (TPC) and total flavonoid (TFC) contents.

The generalized second-order polynomial model used in the surface analysis response and to calculate predicted responses is represented by the following mathematical equation.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{12} X_2^2 + \beta_{13} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where Y is the response; β_0, β_1, β_2, β_3, β_{11}, β_{22}, … are the regression coefficients for intercept, linear, quadratic and interaction terms; X_1, X_2 and X_3 are the non-coded values for Solvent-to-solid ratio, methanol concentration, and extraction time, respectively.

The adequacy of the generated mathematical models was evaluated regarding their determination coefficients R^2; R^2 adj; predicted R^2, (CV%), and adequate precision values.

Table 1. Independence factors and corresponding levels

Independent factors	Symbols	Values of coded levels		
		-1	0	1
Solvent-to-solid ratio % (w/v)	X_1	5	15	25
Méthanol concentration % (v/v)	X_2	30	70	100
Extraction time (hours)	X_3	1	5.5	10

The analysis methodology consists of three main steps. First, the model was tested for validation and fit by an analysis of variance (ANOVA) at the 95% confidence level for each response variable. Next, the experimental data are fitted to a second-order polynomial model to obtain the regression coefficients (βi and βij), which determine the magnitude of the effect of each independent factor on the response. These coefficients were examined statistically by calculating the (F-value) at a probability (p) significance ($p \leq 0.05$) parameters. Finally, regression analysis and plotting of the reply surface plot were performed to establish optimal settings to optimize the experimental conditions for the TPC and TFC contents.

Total phenolic content – Total phenolic content (TPC) was measured using the method of Folin Ciocalteu.[4] Briefly, 0.125 mL of extract aliquot was transferred to a test tube and then mixed thoroughly with 0.5 mL of Folin-Ciocalteu reagent (FCR) diluted 1/10 with distilled water. After shaking for 3 min, 1 mL of sodium carbonate (7.5%, w/v) was added and mixed thoroughly. The mixtures were then allowed to stand for 30 min in the dark at room temperature before measuring absorbance at 760 nm against methanol blank. Gallic acid was used as the standard for the calibration range. Tests were carried out in triplicate and results were expressed as mg of gallic acid equivalent (GAE) per g dry weight (DW) of Argan leaves.

Total flavonoids content – Total flavonoids content (TFC) was determined using the aluminium chloride colorimetric method as described by Ahn et al,[5] 1 mL of $ALCl_3$ solution at 2% was added to 1 mL of sample or standard. After 1 h at room temperature, the absorbance was measured at 420 nm. Quercetin was used as the standard for the calibration range. Tests were carried out in triplicate and results were expressed as mg of quercetin equivalents per g dry weight of leaves (mg QE/g dw)

In vitro antioxidant activities

DPPH radical scavenging assay – The free radical scavenging activity towards DPPH radical is measured according to the method of *Barros L* et al.[6] To 50 µl of each extract at different concentrations (0.1–1 mg/mL), 1950 µl of a methanolic solution of DPPH was added. The mixture was subjected to vortexing. After incubation in the dark for 30 minutes and at room temperature, the absorbance reading is taken at 515 nm. The blank is represented by the extraction solvent, and 1950 µL of the methanolic solution of DPPH. The DPPH radical scavenging capacity (%) was calculated using the following formula:

$$\text{DPPH scavenging effect (\%)} = (1 - A_0 / A_1) \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extract samples or standard.

Ascorbic acid was used as positive control. All determinations were performed in triplicate. The half-maximal inhibitory concentration (IC_{50}) was calculated as the concentration of extract causing a 50% inhibition of DPPH radical and expressed as the mean ± standard deviation (SD) in mg/mL

$ABTS^{\bullet+}$ radical scavenging activity – The radical cation $ABTS^{\bullet+}$ is generated by mixing one volume of the aqueous solution of ABTS (7 mM) with the same volume of potassium persulfate at 2.45 mM (final concentration in the mixture), the whole is kept protected from light and at 4 °C for 16 h before use. The resulting solution is diluted with ethanol to give an absorbance of 0.7 ± 0.020 at 734 nm. ABTS radical scavenging activity was measured according to the protocol described by Dorman and Hiltunen.[7] In brief, 1980 µL of the ABTS ethanolic solution and 20 µL of each extract or ascorbic acid (as reference standard) at different concentrations were introduced into test tubes. After vortexing, the tubes are placed in the dark at room temperature for 5 minutes. The reading is done by measuring the absorbance at 734 nm against a blank (methanol).

The $ABTS^{+}$ scavenging capacity was calculated as:

$$ABTS^{+} \text{ scavenging effect (\%)} = (1 - A_0 / A_1) \times 100$$

where A_0 is the absorbance of the control A_1 is the absorbance of the extract samples or standard.

All determinations were performed in triplicate (n = 3). The IC_{50} values were calculated and expressed as the mean ± SD in mg/mL

Ferric ion reducing antioxidant power (FRAP) – The reducing power of the extract was evaluated according to Oyaizu.[8] 0.5 mL of samples solution at different concentrations (0.1–1 mg/mL) were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) solution of potassium ferricyanide $[K_3Fe(CN)_6]$. The mixture was incubated at 50 °C for 20 min. After incubation, 2.5 mL of 10% (w/v) trichloroacetic acid (TCA) solution was added to the mixture and centrifuged at 3000 g for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% (w/v) ferric chloride ($FeCl_3$). The absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicated reducing power. The sample concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against the simple con-

centrations. The analyses were done in triplicate. Ascorbic acid was used as positive control.

Statistical analysis – The data were presented as mean values ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test using MINITAB software (version 16). Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

At present, medicinal plants rich in phenolic compounds are much studied because of their various pharmacological activities. Thus, it seems important to establish a rapid, simple, accurate, and fast technique by an optimization method to extract phenolic compounds. In this study, the Box-Behnken method (BBD) was used. Based on our knowledge, there is no available research concerning the extraction optimization of polyphenols from Argania spinosa leaves.

The decoded values and the responses of each independent variable are listed in Table 2. TPC in argan leaves extracts varied from 20,11-131.63 mg GAE/g dw. TFC ranged from 2.13-10.66 mg/g dw. The highest values for TPC and TFC were observed with 5% ratio and 70% methanol for 10 hours. The design matrix is shown in Table 2 and consists of 15 tests with their observed and predicted responses, with showing their comparison.

Optimization of extraction process was carried out by applying second order polynomial equation. Response surface 3D plots were generated for each response variable TPC and TFC, whose regression equations and statistical parameters (ANOVA) are presented in Table 3.

The results of the ANOVA for the predicted responses revealed that the coefficients of determination R^2 for the experimental factors TPC and TFC were 0.98 and 0.99 respectively (Table 3) indicating good representation of the variability of the parameters by the models. According to Le Man et al.[9] a model is adequate when $R^2 > 0.75$. In addition, the fit of the models was tested using Fisher's test which is significant for $p < 0.05$ (Table 3), these results showed that the models established for TPC, and TFC are validated and indicate that were reproducible and ready for optimization the extraction parameters of phenolic compounds from argan leaves. A good precision is described as a signal to noise ratio greater than 4, which is considered desirable.[10] The values of adequate precision are 23.22 and 41.64 for TPC and TFC, respectively, demonstrating an adequate signal. Simultaneously, the smaller values of coefficient of variation (C.V.%) (9.24 and 3.39% for TPC and TFC, respectively, indicate better precision and reliability of experimental values.

The regression coefficients for dependent variables were obtained by multiple linear regressions as shown in

Table 2. Box-Behnken Design framework of the independent variables (X_1, X_2 and X_3) and the experimental results total polyphenols (TPC) and total flavonoids (TFC) contents

		Independent variables			Experimental results	
Std	Run	X_1: Ratio (%)	X_2: Methanol (%)	X_3: Temps (h)	TPC (mg EAG/g of dw)	TFC (mg EQ / g of dw)
9	1	15	40	1	44.82	8.33
10	2	15	100	1	50.82	8.33
2	3	25	40	5.5	20.11	3.06
12	4	15	100	10	53.21	7.65
5	5	5	70	1	118.95	10.49
4	6	25	100	5.5	25.47	5.07
13	7	15	70	5.5	64.47	6.13
15	8	15	70	5.5	68.47	6.22
3	9	5	100	5.5	84.84	10
11	10	15	40	10	46.16	5.03
14	11	15	70	5.5	67.51	6.33
8	12	25	70	10	45.95	2.13
1	13	5	40	5.5	77.26	8.93
7	14	5	70	10	131.63	10.44
6	15	25	70	1	52.09	7.07

X_1: sample/solvent ratio (% w/v), X_2: méthanol concentration (% v/v), X_3: maceration time (h), TPC: total polyphenol content mg GAE/g dry matter TFC: total flavonoid content in mgQE/g dry matter.

Table 3. Analysis of variance (ANOVA) for the quadratic polynomial mode

Factor	TPC (mgGAE/g dw)			TFC (mgQE/g dw)		
	Coefficient Estimate	F value	Prob>|t|	Coefficient Estimate	F value	Prob>|t|
Intercept, X_0	66.82	41.74	0.0004	6.23	175.40	< 0.0001
Linear						
X_1	-33.63	263.23	< 0.0001	-2.82	1124.44	< 0.0001
X_2	3.25	2.46	0.1779	0.7125	71.97	0.0004
X_3	1.28	0.3835	0.5629	-1.12	178.24	< 0.0001
Cross Product						
$X_1.X_2$	-0.55	0.0358	0.8573	0.2350	3.91	0.1048
$X_1.X_3$	-4.71	2.58	0.1694	-1.22	105.94	0.0001
$X_2.X_3$	0.26	0.0080	0.9321	0.6550	30.41	0.0027
Quadratic						
X_1^2	11.75	14.84	0.0120	0.3679	8.86	0.0309
X_2^2	-26.65	76.28	0.0003	0.1704	1.90	0.2265
X_3^2	8.59	7.92	0.0374	0.9379	57.56	0.0006
Lack Of Fit		12.47	0.0751		8.71	0,10
R^2	0.98			0.99		
Predicted R^2	0.80			0.95		

X_1, X_2, and X_3 represents the linear effects (solid/liquid ratio, methanol concentration and time extraction, respectively); $X_1.X_2$, $X_1.X_3$ and $X_2.X_3$ are the different interactions and X_1^2, X_2^2 and X_3^2 the quadratic effects.

Table 3. The negative linear effect of solvent to sample ratio (X_1) and quadratic effect X_1^2 and the quadratic effect of the extraction time X_3^2 was found to be significant for all response variables.

The model showed high significance ($p < 0.001$) with the experimental data, while the analysis of variance (ANOVA) showed a significant linear and quadratic effect of solvent to sample ratio (X_1) (with $p < 0.0001$and $p < 0.01$ respectively) as well as a significant quadratic effect of methanol concentration (X_2) and times extraction (X_3) with $p < 0.001$ and $p < 0.01$ respectively) on TPC (Table 3). Based on regression coefficient values, effect of X_1 showed major negative effect followed by negative quadratic effect of X_2^2 and X_1^2. quadratic effect of X_3^2 remains the least significant. The non-significant variables were removed and the fitted second order polynomial equation showed as:

$$TPC = 66.82 - 33.63X_1 + 11.75X_1^2 - 26.65X_2^2 + 8.59\,X_3^2$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The non-significant value of lack of fit ($F = 12.47$) indicating that the developed model adequately explains the relationship between the independent variables and responses with good prediction ($R^2 = 0.98$, Adj. $R^2 = 0.80$).

The 3D surface plots (Fig. 1) illustrate the interactive effect of independent variables X_1, X_2 and X_3 on the TPC. Generally, as shown in Fig. 1a. shows the impact of methanol concentration and solvent/solid ratio on the extraction of TP from the Argan leaves for a fixed time of 5.5h. The result shows that the TPC is increasing with decreasing ratio and increasing methanol concentration. In fact, with a minimal value of ratio (5%) the TPC is increasing with increasing solvent concentration up to 72.125% to reach a maximum value of 112.338 mg/g DW and then it decreases. Fig. 1b. shows that at a methanol concentration of 70%, as the ratio decreases and the extraction time increases, the TP content increases to 126.776 mg/g DW. Fig. 1c. illustrates the quadratic effect of the solvent concentration. Indeed, from 40 to 71.970% methanol and with a fixed ratio of 15%, the TPT increases with increasing time until a peak of 76.801 mg/g DW and then decreases. This phenomenon is most likely due to Fick's second law of diffusion principle revealing that the final equilibrium between the solution concentration in the solid matrix and solvent will be attained after a particular duration, leading to deceleration in the extraction yield of target compounds.[11]

Diluted methanol was more effective in the extraction of argan leaves phenolic compounds; it revealed that a mixture of solvents and water are more efficient than the mono-solvent system in polyphenols extraction.[12]. In fact, the addition of water to organic solvents increases the

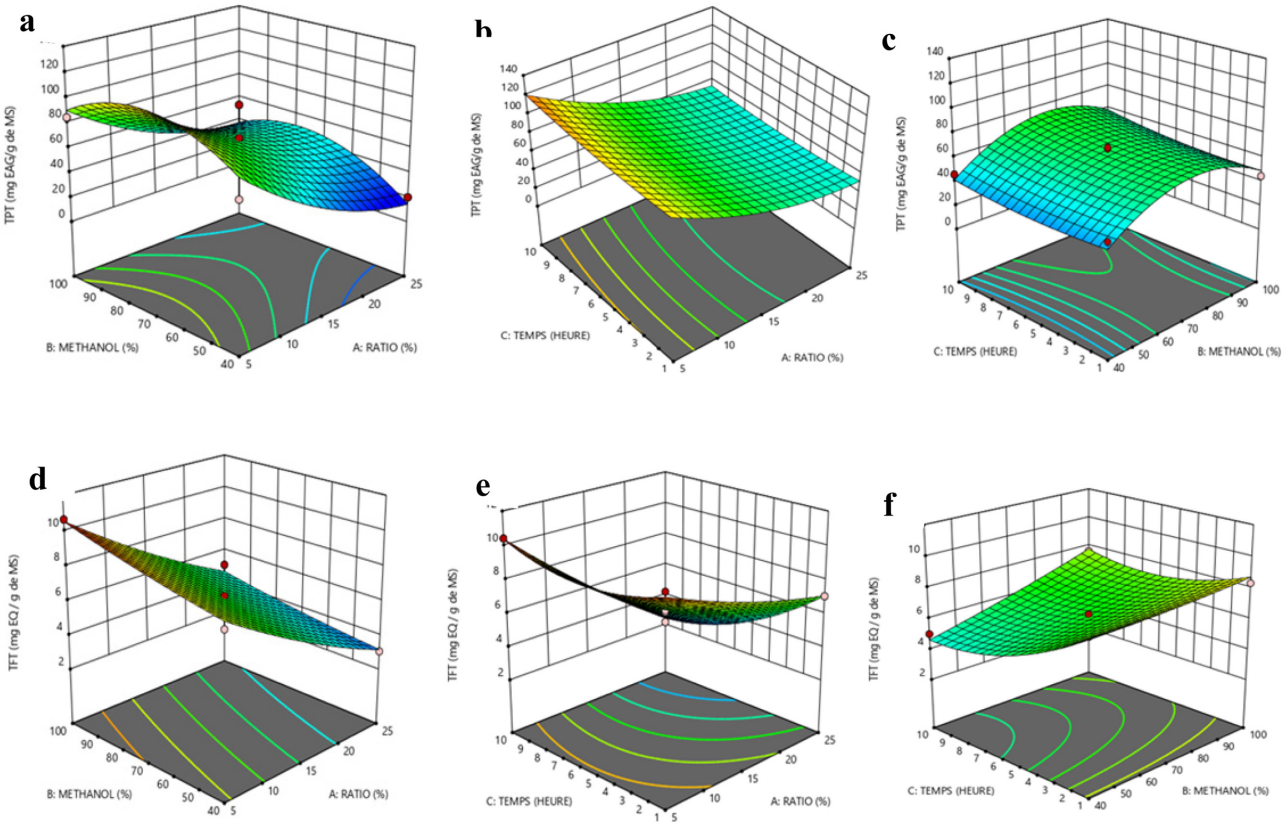

Fig. 1. Response surface plots showing interaction effects of process variables; Extraction time (h), sample/solvent ratio (% w/v) and methanol concentration (% v/v) on the responses TFC (a,b,c) and TFC (d, e, f).

solubility of polyphenols by modulation of the polarity of the organic solvent.[13] This increase may be due to the weakening of hydrogen bonds in aqueous solutions. It could also be due to the increase of the basicity and ionization of polyphenols in such solutions.[14] The solubility of polyphenols depends mainly on the number of hydroxyl groups, molecular weight and chain length and the length of the carbon chain of the backbone.[15]

Linear effect of solvent to sample ratio (X_1), methanol concentration (X_2), extraction time (X_3) and their interaction effect (X_1X_3) and (X_2X_3) and quadratic effects X_1^2 and X_3^2 showed significant effect on TF content (Table 3). Among these, TF content depends more on X1followed by X_1X_3, X_3, X_3^2, X_2, X_2X_3 and X_1^2 having regression coefficient values as mentioned in Table 3. The non-significant variables were removed and the fitted second order polynomial equation showed as:

$$\mathbf{TFC} = 6.23 - 2.82X_1 + 0.71X_2 - 1.12X_3 - 1.22X_{13} + 0.65X_{23} + 0.36X_1^2 + 0.93X_3^2$$

The non-significant value of lack of fit ($F = 8.71$) showed the model is fitted with good prediction ($R^2 = 0.95$, Adj. $R^2 = 0.99$)

The influence of the factor X_1 and X_2 on the content of flvonoids are represented in Fig. 1d. It results that at a fixed time of 5.5 h, the TFC increases when the % of methanol increases while the solide/solvant ratio decreases. The highest value (10.059 mg/DW) is obtained with 100% methanol and a low ration of 5%. Fig. 1e. shows the interaction of the extraction time and the solid/solvent ratio on the TFC. In fact, by extracting with 70% methanol for 10 hours, a value of 10.450 mg/g dw can be reached with a low ratio of 5%. Fig. 1f. shows that by using a ratio of 15%, the TFC increased with the increasing of the solvent concentration and slightly with the decreasing of the time. Indeed, at a time course of 1h the more we increase the concentration of the solvent from 40 to 100% the more the TFC increases up to 8.541 mg/g dw. In other words, the difference with TPC, there is an improvement of the extraction even beyond 70% of methanol in the first hours.

The aim of the optimization was to determine the extraction conditions that would provide simultaneously the highest TPC and TFC. Design expert software was

used to carry out optimization. The BBD proposed the optimal sample to solvent-ratio, methanol concentration, and time to be 5%, 72.33% and 10 h, respectively, for the extraction of *A. spinosa* leaves polyphénols. Methanol concentration around 70% is generally used in the extraction of flavonoids, phenolic acids, their derivatives and several other subgroups of flavonoids.[16] At this optimal point, the predicted TPC and TFC were 120.49 mg GAE/g dw and 10.55 mg QE/g dw, respectively (Table 4). A validation of the predictive capacity of the models was performed experimentally under the optimal conditions obtained from RSM. Experiments were carried out in triplicate under the obtained conditions, and the mean TPC and TFC were 131.63 ± 0.29 GAE mg/g dw and 10.66 ± 0.09 QE/g dw, respectively.

The experimental values of investigated responses were comparable and in line with those of predicted values, which confirmed that the models were sufficient to reflect the expected optimization. The results obtained by hydromethanolic maceration after optimization of the extraction conditions for TPC and TFC are superior to those reported by EL ADIB et al.[16]

The antioxidant activity of phenolic compounds is mainly due to its reducing properties and chemical structure. These characteristics play an important role in the neutralization or sequestration of free radicals and in the chelation of transition metals, acting both at the initiation and in the propagation of the oxidative process.[17]

The parameter IC_{50} has been used to compare the effective concentration of extract that causes 50% of inhibition. Therefore, the extracts with the lowest IC_{50} have the largest antioxidant property. The antioxidant activity was screened with three different methods (FRAP, DPPH, and ABTS). Since the antioxidant activity of a substance is usually correlated directly to its reducing capacity.

The antioxidant activities of the hydromethanolic extract of argan leaves resulting from the optimal conditions were determined in comparison with ascorbic acid (a strong synthetic antioxidant) by means of the DPPH and ABTS radical scavenging tests as well as the reducing activity of iron (FRAP). the results are shown in Table 5.

The scavenging potential of an extract is frequently associated with its ability to scavenge stable free radicals, which is due to its capacity as a hydrogen donor. The results are depicted as the IC_{50} value (Table 5), which is the concentration of the sample at which the percent inhibition reaches 50%. The antioxidant activity of extracts of argan leaves showed a moderate anti-free radical capacity of DPPH with an IC_{50} value of 0.130 mg/mL in comparison with ascorbic acid (IC_{50} of 0.033 mg/mL)

The ABTS trapping test is widely used as an index to inform and study the antioxidant capacity of pure compounds as well as natural extracts.[18] the free radical scavenging activity of ABTS from hydromethanolic extract (IC_{50} = 0.198 mg/ml) is slightly lower than that of ascorbic acid (IC_{50} = 0.160 mg/mL).

The reduction of Fe^{3+} is often used as an indicator of electron donor activity, which is an important mechanism of phenolic antioxidant action.[19] The leaf extract exhibits an IC_{50} of 0.448 mg/mL very close to that of ascorbic acid (IC_{50} = 0.371 mg/mL) with a non-significant difference which indicates a powerful anti-free radical activity of the argan leaves. These results agree with results reported by Lfitat et al.[20] who have been able to demonstrate that argan leaves have a very interesting antioxidant power**.** Previous studies have shown that plant phenolic contents and their antioxidant activities depend on biological factors (genotype, organ and ontogeny), as well as environmental (temperature, salinity, water stress and light inten-

Table 4. Experimental data of the validation of predicted values at optimal extraction conditions

Extraction Variables			TPC (mg GAE/g of dw)		TFC (mg QE/g of dw)	
X_1 (sample/solvant,%)	X_2 (méthanol ,%)	X_1 (times h)	Predicted Value	Experimental Value *	Predicted Value	Experimental Value*
5	72.33	10	126.93	131.63 ± 0.29	10.55	10.66 ± 0.09

TPC and TFC represent total phenolic content and total flavonoid content, respectively. *Means of triplicate determination.

Table 5. Antioxidant activity of methanolic extract of argan leaves

Simple	IC50 (µg mL^{-1})		
	DPPH	FRAP	ABTS
Argan leaf extract	0.130 ± 0.022^{a}	$0.198 \pm 0.006_{a}$	$0.448 \pm 0.034_{a}$
Standard **(**Ascorpic acid)	0.032 ± 0.002^{b}	$0.160 \pm 0.012_{b}$	$0.371 \pm 0.165_{a}$

The data shown in the table as mean ± standard deviation (n = 3); Lettering (a,b,c,d) indicated the significant difference in the means ($p < 0.05$) using a one-way analysis of variance (ANOVA) and Tukey's test.

sity…) conditions.[21]

In conclusion, the results of RSM showed the solvent to sample ratio affected the measured responses significantly. Under the optimal condition 5% of solvent to sample ratio, 72.33% methanol and 10 hours the maximum values were found to at 131.63 g GAE/g and 10.66 mg QE/g dw respectively for TPC and TPC. Overall, our study suggests that the model obtained in the present study can be applied for large scale production of polyphenols for further use in pharma/food industries. This may ease the fortification/supplementation of bioactive compounds in various food formulations designed to combat oxidative-stress mediated health problems by their biochemical and physiological processes. These findings indicate that *Argania spinosa* leaf extracts could be considered as a good source of natural antioxidants.

Conflict of interest

The authors declare that there is no conflict of interest.

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