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Optimization of Microwave Assisted Extraction of Phenolic Compounds and Antioxidant Activities of The Roots of *Ximenia Americana* with Response Surface Methodology

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***Abstract*:- The optimal conditions of Microwave-Assisted Extraction (MAE) of phenolic compounds and antioxidants from *X. americana* roots determined. A second-order regression for central composite design (CCD) was used to investigate the effects of four independent variables, namely extraction time (s), irradiation power (W), liquid-to-solids ratio (mL/g) and methanol concentration (%) on the responses. A polynomial model of the TPP, TF and % DPPH of the root of *X. americana* was deducted from statistical analysis of experiments. Pareto analysis indicated that the influences of the main factors (X1, X2, X3 and X4) on the Total Polyphenols (TPP) of the root of *X. americana* were 38.81 %, while the interaction effects (X1X3, X1X4, X3X4 and quadratic effects (X1X1, X2X2, X3X3, X4X4) were 17.03 and 53.19 % respectively. The influences of the main factors (X1, X2, and X3) on the Total Flavonoids (TF) of the root of *X. americana* were 20.90 %, while the interaction effects (X1X3, X1X4, X2X3, X3X4 and quadratic effects (X1X1, X2X2, X3X3, X4X4) were 23.43 and 51.26 % respectively. The influences of the main factors (X1, X3, and X4) on the % DPPH activities of the root of *X. americana* were 20.90 %, while the interaction effect X1X4 and quadratic effects (X1X1, X2X2, X3X3 and X4X4) were 4.48 and 46.02 % respectively. The interaction effect X1X4 simultaneously influencing the TPP, TF and %DPPH therefore a compromise between the three responses were desirable. To gain a better understanding of the two variables for optimal microwave assisted extraction performance at fixe power 600W and liquid solid ratio 20 mL/g, the model was presented as both 3-D response surface and 2-D contour graphs. The optimal individual conditions for extraction of total polyphenols, total flavonoids and antioxidant activity from the roots of *X americana* were 600 W, 20 mL/g, 70 sec, 70 %; 600 W, 20 mL/g, 76 sec, 77 %; and 600 W, 20 mL/g, 72 sec, 69 %, respectively. As a compromise for the simultaneously removal of maximum amounts of 7872 µg GAE/ gDM total polyphenols (TPP), 189 µg QE/g DM total flavonoids (TF) and with an antioxidant %DPPH of 84 %, the optimum conditions were found with 77.64 % methanol concentration at an extraction time of 83 sec. Close agreement between experimental and predicted values was found. The obtained extracts might be used as natural bioactive compounds in several industrial applications**

Keywords: Total polyphenols, total flavonoids, antioxidant activity, X. americana, RSM, Microwave -assisted extraction.

INTRODUCTION

Ximenia americana L. is widely used in folk medicine in Africa to treat various disorders such as inflammation, pain, fever, diarrhoea, wounds and intoxications (Onifade et *al*., 2011; Le et *al*., 2012; Kenmogne et *al*., 2014; Kidik et *al*., 2015). Different parts of the plant are used as infusion, maceration or decoction. Published scientific reports of the biological activities of the plant are scanty and include antitoxic, anticancer, analgesic and antimicrobial (Maikai et *al*., 2016; Kenmogne et *al*., 2014; Onifade et *al*., 2011). Renewed interest in traditional pharmacopeias is increasing worldwide most especially among African people who are becoming reliant on herbal medicines for their health care needs. This is because medicinal plants are more accessible and affordable (Alzeer et *al*., 2014; Maikai et *al*., 2015; Kenmogne et *al*., 2017). Therefore, it seems important to study the phenolic compounds and antioxidant activities of extracts of medicinal plants such as *Ximenia americana*, plant used for traditional treatment.

Microwave-assisted extraction (MAE) is one of the most inexpensive, simple, rapid, and efficient green extraction techniques compared with conventional extraction (Kenmogne et *al*., 2000: Kenmogne et *al*., 2014; Rolly et *al*., 2016; Gertrude et *al* 2016;)and has been applied to extract bioactive compounds from different materials due to its high reproducibility at shorter time, simplified manipulation, significant reduction in solvent consumption, and temperature, in respect to other classic methods (Pang et *al*., 2017). Therefore, the microwave technology has been used in some industries, such as food industry, chemical industry, and material industry (Belwat et *al*., 2018).

Response surface methodology (RSM), an effective statistical technique for modeling and optimization of complex processes, has been used increasingly to optimize processing parameters owing to more efficient and easier arrangement and interpretation of experiments compared to others (Gan et *al*., 2010; Gan et *al*., 2011a,b; Humal et *al*., 2017). The advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions (Prasad et *al*., 2011; Gan et *al*., 2011a,b; Rolly et *al*., 2016; Gertrude et *al*., 2016; Humal et *al*., 2018).Therefore, it is widely used in optimizing the extraction parameters from different materials.

Even the microwave treatment has so many advantages, there is no information published about the optimization of microwave assisted extraction of phenolic antioxidants from *X. americana* roots using response surface methodology (RSM). Therefore, the objective of this study was to determine the best microwave assisted extraction conditions for *X. americana* roots, in order to maximize simultaneously the total polyphenolic (TPP), total flavonoids and antioxidant activities as measured by the DPPH by using the composite center design (CCD) combined with the RSM.

1. MATERIALS AND METHODS

1.1. Sample materials and chemicals

The roots of *Ximenia americana* were collected from Ngaoundere in the region of North Cameroun and authenticated by Professor Mapongmetsem, botanist and professor in the Department of Biological Sciences Faculty of Science, University of Ngaoundere. The material was allowed to dry naturally, cut, ground using a mill (Model 14, Hamilton Beach, USA) and then sieved with mechanical shaker analysen sieb, Model NFX 11-501, Germany, through a standard set of stainless steel sieves. The powder was kept in sealed polyethylene bags at room temperature.

1.2. Chemicals reagents

Gallic acid, Folin –Ciocalteus phenol reagent, sodium acetate, and methanol reagent were purchased from *Sigma-Aldrich* and were used as received. All other chemicals organics solvent used in the study were of analytical grade.

1.3. Microwave-assisted extraction process

Two point five grams of *Ximenia americana* powder roots of 200 µm diameter were placed in a tube and mixed with 50mL of methanol-water (80:20, v/v). The extraction process was performed with domestic microwave (ER- 696 ETE, 2450MHz, and Toshiba, Japan) equipped with a digital timer and a power controller. After microwave extraction, the sample was filtrate with a wattman paper and the microwave extract was evaporated by rotary evaporation to remove solvent. Three replicates were performed in each extraction. Samples (the microwave extracts) were stored in 4 °C prior to analysis. The irradiation extraction time was assessed as shown in the results and the desired temperature was maintained through the timer.

1.4. Determination of total polyphenolic content in the roots of Ximenia americana

Determination of total polyphenolic content (TPP) in the roots of *Ximenia americana* was determined using the Folin–Ciocalteau method. Microwave extracts were mixed with 0.2 ml of Folin–Ciocalteau reagent (pre-diluted at a ratio of 1:16 with distilled water) and allowed to stand at room temperature for 5 min, and then 0.2 ml of sodium bicarbonate (1M) was added to the mixture. After standing for 20 min at room temperature, absorbance was measured at 760 nm. Results were expressed as µg Gallic acid equivalents/ dry weight sample (µg GAE per g of dry weight (DW)) (Singleton et *al*., 1999; Kumazawa et *al*., 2002).

1.5. Determination of total flavonoid content (TF)

The determination of flavonoids was performed according to the colorimetric assay of Dowd. A methanolic solution of aluminum chloride (0.5 ml, 2% w/v) was mixed with the methanol extract solution (0.5 ml, 0.1 mg/mL). After ten minutes the optical densities were read at 415nm against the blank (0.5 mL of methanol extract solution and 0.5 mL of methanol) and compared with the calibration curve of quercetin (0.1 mg/mL) (Singleton et *al*., 1999; Kumazawa et *al*., 2002).

1.6. Antioxidant assay: 1,1-diphenyl-2-picrylhydrazyl assay (DPPH)

The DPPH radical scavenging capacity assay was based on a previously described method (Liu et *al*., 2009) with some modification. Briefly aliquots of each extracts (1mL) were added to 1 mL of methanolic DPPH solutions (100 µM). Discolorations were measured at 517 nm after incubation for 20 min at30°C in the dark. The % DPPH which was scavenged (% DPPH) was calculated using the formula: (%) = $[A_0-(A_1-A_S)]/A_0]*100$ where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only. All samples were tested in triplicate.

1.7. Central Composite Design (CCD)

A central composite design (CCD) was used to identify the relationship existing between the response functions and the process variables, as well as to determine those conditions that optimized the extraction process of total phenolic content and antioxidant capacity. The independent variables or factors studied obtained from a preliminary experiments (data not shown) using single-factor tests determined the required range of extraction time (X1: 70-90 sec) microwave power (X2: 500-700 w) methanol concentration (X3: 65–95 %), and solvent-to-material ratio (X4: 15/1–20/1 mL/g) (Kenmogne et *al*., 2020). Once the desired value ranges of the variables had been defined, they were coded to lie at ±1 for the factorial points, 0 for the center points, and $\pm\alpha$ for the axial points. The codes were calculated as functions of the range of interest of each factor, as shown in Table 1. The regression analysis of experimental data was performed to establish the empirical second order polynomial models. Shown in Eq. (1),

$$\gamma_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Where γ_{is} the measured response variable (TPP, TF and %DPPH), β_0 is a constant, β_{iis} the linear coefficient (main effect) βii is the quadratic coefficient, βij is the two factors interaction coefficient, X_i and X_j are independent variables.

Software: Response surfaces were drawn and coefficients for the regression model were calculated using Modde 4 software package (Modde 4.0).

Tableau 1: Independent variables and their levels in central composite circumscribed (CCD) design.

Symbol	Independent variable	Factor level				
		–α	–1	0	+1	+α
X1	Extraction time (sec)	63.93	70.00	80.00	90.00	96.07
X2	Microwave power (w)	439.28	500.00	600.00	700.00	760.78
X3	Liquid-solid ratio (mL/g)	11.64:1.00	15.00:1.00	20.00:1.00	25.00:1.00	28.36:1.00
X4	Methanol concentration (%)	63.93	65.00	80.00	95.00	96.07

RESULTS AND DISCUSSION

III.1. Analysis of the process factors

Using the central composite experimental designs (CCD) of 28 runs shown in Table 2 (which includes the design, observed responses and the predicted values), the models of the extraction process were obtained. It was postulated a second-order responses models. The coefficients of the models presented in Table 3 were estimated by least square multiple regression method. The sign of the effect indicates its direction. The positive or negative sign indicates the positive or negative effect of the factor on the responses. The importance of these effects was graphically represented on Fig. 4. The higher the effect of a factor (absolute value), the more significant it was on the extraction process of total polyphenols of the roots of *Ximenia americana*. The responses resulting TPP, TF or % DPPH will depend therefore, on the relative sum of the weight of each factor on each response.

III.1.1. Total polyphenols microwave assisted extraction (TPP)

It was observed according to Pareto diagram (Fig.1-A) that 11 effects significantly influence at the 5% level. 5 contribute to increasing TPP (the direct effects of time (X_1), power (X_2), solvent-material ratio (X_3), the interactions effects of time-ratio ($X_1 * X_3$) and time - methanol concentration ($X_1 * X_4$), while 6 tend to decrease TPP extraction (.the methanol concentration (X_4), the interactions effects of time-ratio ($X_2 * X_4$) and all the quadratic effects ($X_1^{2,}$ X_2^2, X_3^2, X_4^2). It was noted that the quadratic effects of the liquid: solid ratio (X_3^2, 19.83 %) and methanol concentration (X_4^2, 16.00 %), linear effects of methanol concentration (X_4, 12.21 %) and liquid: solid ratio (X_3, 12.21 %) strongly influence on the microwave extraction of total polyphenols. Thus attention must be paid not only on main effects but also on quadratic factors. The equation of the model for the response, including the interaction and quadratic terms of each controlled factor were:

$$PPT*10^{-2} = 78.8002 + 1.5012X_1 + 2.0309X_2 + 9.1976X_3 – 5.8438X_4 – 4.3574X_1^2 – 0.0662X_1X_2 + 1.3812X_1X_3 + 2.2012X_1X_4 + 0.7375X_2X_3 + 0.7200X_2X_4 – 4.565X_3X_4 – 3.9528X_2^2 – 9.4929X_3^2 –7.6578X_4^2. \quad (2)$$

III.1.2. Total flavonoids microwave assisted extraction (TF)

For TF, 11 effects significantly influence at the 5 % level the extraction of total flavonoids and also 5 contribute to increasing TF (the direct effects of time (X_1), power (X_2), the interactions effects of methanol concentration ($X_1 * X_4$), power- liquid: solid ratio ($X_2 * X_3$) and solvent-material ratio- methanol concentration ($X_3 * X_4$)) while 6 tend to decrease TF extraction the linear X3, only the interactions effects of time-ratio liquid: solid ($X_1 * X_3$) and all the quadratic effects ($X_1^{2,}$ X_2^2, X_3^2, X_4^2). The linear effect of methanol concentration (X_4) had no effect on flavonoids microwaved extraction, but this factor associated to the ratio liquid: solid (12.00 %) or in the quadratic form (12.40 %) greatly influence the extraction of TF. The quadratic effect of time X_1^2 showed a strong influence (20.43 %). Thus attention must be paid not only on main effects but also on quadratic factors. The equation of the model for the response, including the interaction and quadratic terms of each controlled factor were:

$$FT = 185.66 + 4,4002X_1 + 16.6248X_2 – 8.5354X_3 – 2.8008X_4 + 2.0169X_1 X_2 – 6.2418X_1X_3 + 7.0144 X_1 X_4 + 13.9332X_2X_3 – 1.3381X_2X_4 + 5.9582X_3X_4 – 28.8784X_1^2 - 9.0932X_2^2 – 16.9639X_3^2 – 17.5311X_4^2$$

III.1.3. antioxydant activity (% DPPH)

For % DPPH, 8 effects significantly influence at the 5% level and therefore 3 contribute to increasing DPPH radical scavenging activity % (irradiation time X_1, solvent-material ratio (X_3 and while 5 tend to decrease the DPPH radical scavenging activity (methanol concentration (X_3), quadratic effects of irradiation time X_1, irradiation power X_2, methanol concentration (X_3) and ratio liquid to solid X_4). The linear effect of irradiation power X_2 had no effect on DPPH radical scavenging activity It was noted that the quadratic effects of the liquid: solid ratio (X_3^2, 13.13 %) and liquid: solid ratio (X_4^2, 21.70 %), the linear effects of liquid: solid ratio (X_3, 17.14 %) strongly influence the DPPH radical scavenging activity. Thus attention must also be paid not only on main effects but also on quadratic factors. The equation of the model for the response, including the interaction and quadratic terms of each controlled factor were:

$$\% DPPH = 84.0963 + 2.3848X_1 + 1.5669X_2 + 5.7673X_3 – 8.0214X_4 + 0.72562X_1 X_2 – 0.5756X_1X_3 + 2.0994X_1X_4 - 1.7494X_2X_3 + 0.7431X_2 X_4 – 1.6156X_3X_4 - 2.3309X_1^2 – 2.9077 X_2^2 -6.1462X_3^2 - 10.1551X_4^2. \quad (4)$$

Table 2: Experimental and calculated yield of the microwave assisted extraction of TPP, TF and antioxidant activities as measured by DPPH of the root of Ximenia americana

Coded values				**Real values**				**Laboratory sample**								
								TPP (µg QE/ g DM)			**TF (µg QE/ g DM)**			**% DPPH**		
X1	**X2**	**X3**	**X4**	**U1**	**U2**	**U3**	**U4**	**EXP**	**PRE**	**RES**	**EXP**	**PRE**	**RES**	**EXP**	**PRE**	**RES**
-1	-1	-1	-1	70	500	15	65	44.53	46.862	-2.332	120.241	124.848	-4.607	57.96	60.4862	-2.5262
1	-1	-1	-1	90	500	15	65	43.68	42.8319	0.8481	126.09	128.069	-1.979	60.03	60.757	-0.727
-1	1	-1	-1	70	700	15	65	46.92	48.1414	-1.2214	131.12	128.873	2.247	65.24	64.1813	1.0587
1	1	-1	-1	90	700	15	65	57.14	43.8463	13.2937	153.4	140.162	13.238	70.63	67.3545	3.2755
-1	-1	1	-1	70	500	25	65	70.79	70.1498	0.6402	103.65	80.4778	23.1722	80.46	79.9021	0.5579
1	-1	1	-1	90	500	25	65	71.67	71.6447	0.0253	54.51	58.7318	-4.2218	79.98	77.8704	2.1096
-1	1	1	-1	70	700	25	65	74.88	74.3792	0.5008	135.76	140.236	-4.476	79.17	76.5997	2.5703
1	1	1	-1	90	700	25	65	67.71	75.6092	-7.8992	130.95	126.558	4.392	71.89	77.4705	-5.5805
-1	-1	-1	1	70	500	15	95	39.28	38.4619	0.8181	92.19	95.977	-3.787	45.35	41.9895	3.3605
1	-1	-1	1	90	500	15	95	41.57	43.2368	-1.6668	139.99	127.256	12.734	48.61	50.6578	-2.0478
-1	1	-1	1	70	700	15	95	41.43	42.6213	-1.1913	107.13	94.6503	12.4797	47.07	48.6571	-1.5871
1	1	-1	1	90	700	15	95	45.41	47.1312	-1.7212	111.43	133.997	-22.567	57.45	60.2279	-2.7779
-1	-1	1	1	70	500	25	95	41.03	43.4897	-2.4597	70.46	75.4398	-4.9798	52.19	54.943	-2.753
1	-1	1	1	90	500	25	95	53.93	53.7897	0.1403	80.11	81.7515	-1.6415	58.03	61.3088	-3.2788
-1	1	1	1	70	700	25	95	48.67	50.5992	-1.9292	132.43	129.846	2.584	53.12	54.613	-1.493
1	1	1	1	90	700	25	95	61.80	60.6341	1.1659	157.09	144.225	12.865	66.93	63.8813	3.0487
-1.60717	0	0	0	63.93	600	20	80	68.54	65.1324	3.4076	94.08	103.996	-9.916	85.88	84.0963	1.7837
1.60717	0	0	0	96.07	600	20	80	78.03	69.9578	8.0722	114.33	118.139	-3.809	84.83	74.2429	10.5871
0	-1.60717	0	0	80	439	20	80	70.75	65.326	5.424	130.48	135.454	-4.974	76.57	74.0673	2.5027
0	1.60717	0	0	80	760.78	20	80	73.91	71.8542	2.0558	198.51	188.892	9.618	79.23	79.1039	0.1261
0	0	-1.60717	0	80	600	11.64	80	54.66	39.4979	15.1621	154.9	155.56	-0.66	59.38	58.9515	0.4285
0	0	1.60717	0	80	600	28.36	80	74.38	69.0623	5.3177	115.06	128.125	-13.065	79.69	77.4897	2.2003
0	0	0	-1.60717	80	600	20	63.93	68.69	68.4121	0.2779	131.77	144.879	-13.109	69.5	70.7574	-1.2574
0	0	0	1.60717	80	600	20	96.07	57.83	49.6281	8.2019	135.26	135.876	-0.616	48.86	44.9737	3.8863
0	0	0	0	80	600	20	80	78.86	78.8002	0.0598	183.63	18566	-2.03	82.34	81.9083	0.4317
0	0	0	0	80	600	20	80	77.98	78.8002	-0.8202	179.13	185.66	-6.53	79.91	81.9083	-1.9983
0	0	0	0	80	600	20	80	76.13	78.8002	-2.6702	182.82	185.66	-2.84	82.53	81.9083	0.6217
0	0	0	0	80	600	20	80	76.30	78.8002	-2.5002	185.14	185.66	-0.52	77.95	81.9083	-3.9583

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Table 3: Regression coefficients for mains factors and their interactions

	Factor/ interaction		Regression coefficient		
			YTPPx10^{-2}	**YFT**	**%DPPH**
	Mean/ interaction b0		78.8002	185.66	84.0963
X1	Irradiation time (L1) β_1		1.5012	4.4002	2.3847
:	Irradiation time (Q11) β_{11}		-4.3574	-28.8784	-2.3308
X2	Irradiation power (L2) β_2		2.0309	16.6248	1.5669
	Irradiation power (L22) β_{22}		-3.9528	-9.0932	-2.9077
X3 :	Solvent to solid ratio (L3) β_3		9.1976	-8.5354	5.7673
	Solvent to solid ratio (Q33) β_{33}		-9.4929	-16.964	-6.1462
X4	Solvent concentration (L4) β_4		-5.8438	-2.8008	-8.0214
	Solvent concentration (Q44) β_{44}		-7.6578	-17.5311	-10.1551
	X1 X2	β_1 β_2	-0.0662	2.0169	0.7256
	X1 X3	β_1 β_3	1.3812	-6.2418	-0.5756
	X1 X4	β_1 β_4	2.2012	7.0144	2.0994
	X2 X3	B_2 β_3	0.7375	13.9332	-1.7494
	X2 X4	B_2 β_4	0.7200	-1.3380	0.7431
	X3 X4	B_3 β_4	-4.5650	5.9582	-1.6156

L= linear, Q= quadratic

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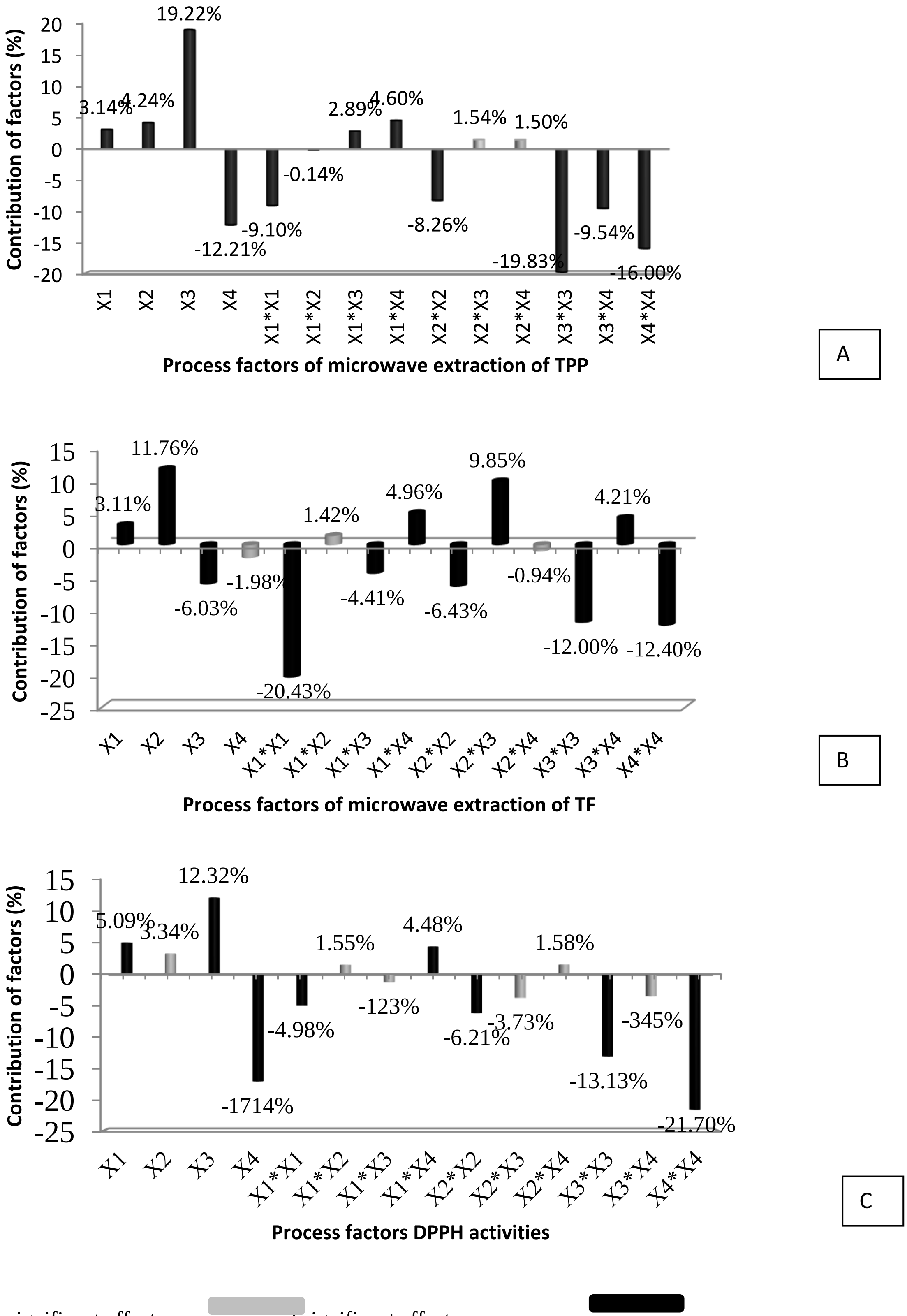

Non significant effect and significant effect

Figure 1: Pareto analysis of the process factors of the Yield of microwave assisted extraction of: (A) the Total Polyphenols (TPP), (B) the Total flavonoids and (C) the DPPH activities

3.2. Validation of the Models

It is usually necessary to check the fitted model to ensure it provides an adequate approximation to the real system. Graphical and numerical methods, as a primary tool and confirmation for graphical techniques were used to validate the models in this study (Deming *al*., 1993; Mathieu et *al*., 1997). The graphical method characterizes the nature of residuals of the models. If the model represents the phenomenon in the studied domain, the difference between the experimental results and the theoretical results calculated by the model (Table 2) was due solely to experimental error. The coefficient of determination obtained (R^2 = 0.89 for TPP, R^2 = 0.93 for TF and R^2=0.94 for % DPPH) when plotting the experimental responses versus the theoretical responses (Fig. 2) indicated that the variations of 89 % for TPP, 93% for TF and 94 % for % DPPH were all attributed to the independent variables, irradiation time, irradiation power, liquid: solid ratio and methanol concentration. We admitted in using this method of validation that the experimental results do not contain errors since they were the average of results of the three replications and that even if there were an error, it was constant and did not depend on the variation of parameters involved. Admitting this, it can be ascertained that in the overall, the models used represents the studied phenomenon in the variation domain. R^2 values insure a satisfactory adjustment of the quadratic models to the experimental data. Therefore, the regression models explained the microwave extraction efficiency well.

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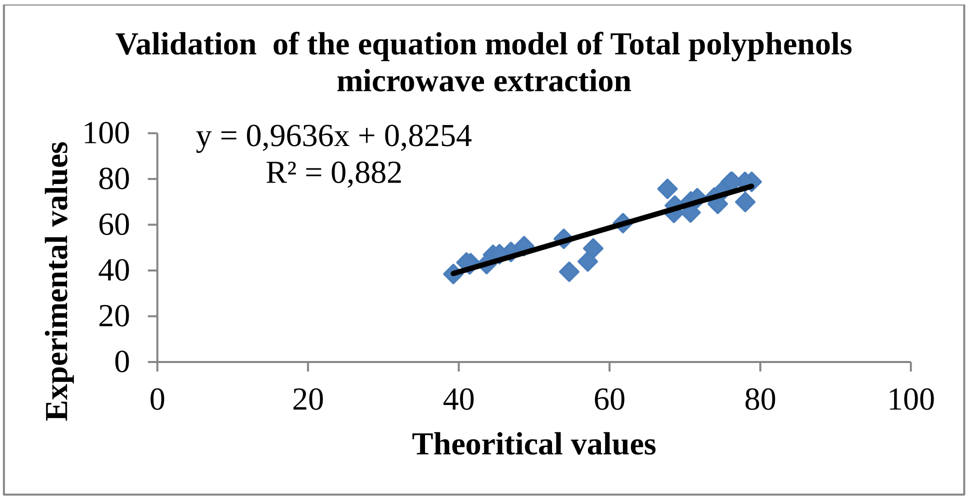

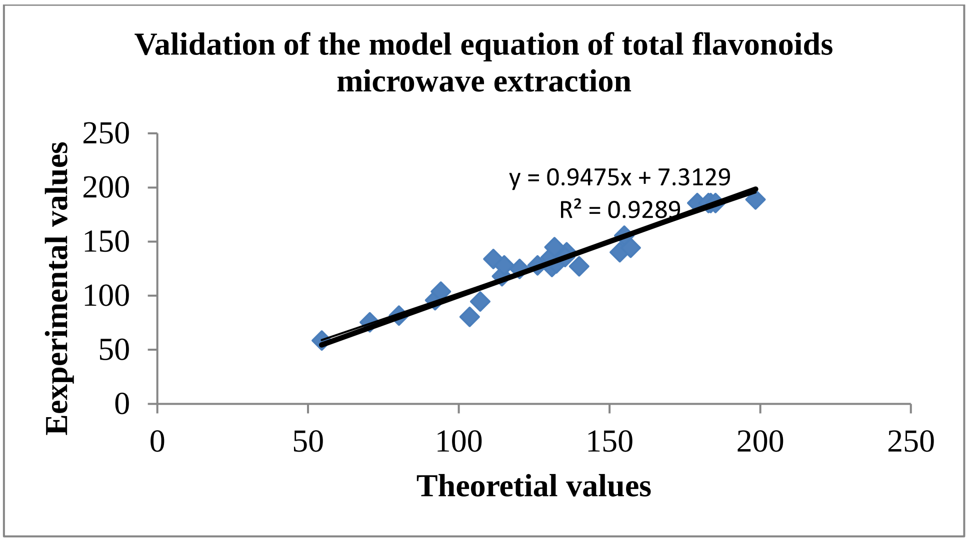

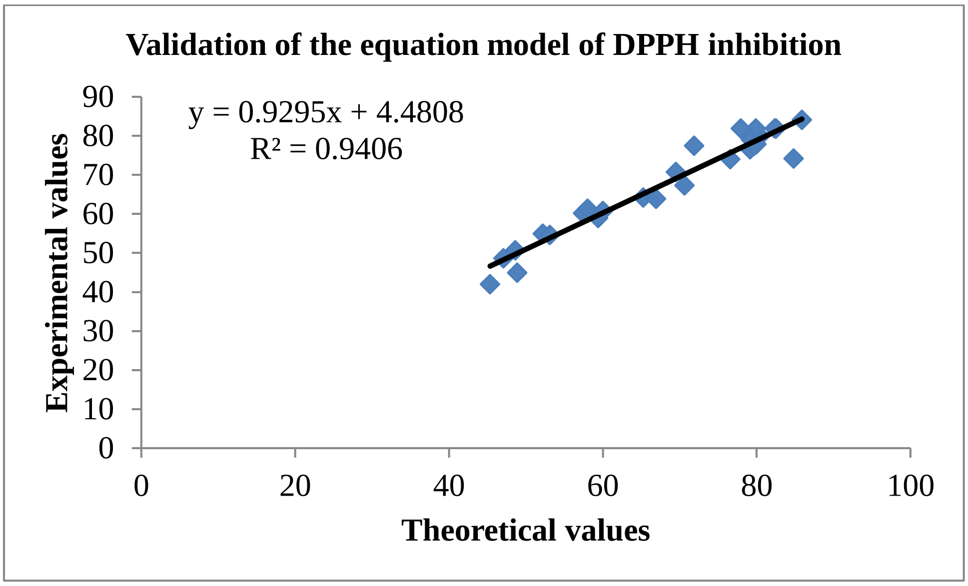

Figure 2:validation of the models equations

3.3. Optimal extraction conditions:

It was observed from table 1 that the yield of TPP and TF ranged from 3928 to 7886 µg GAE/g.DM and 54.51 to 190.82 µg QE/g.DM respectively. The highest TPP (7886 µg EAG/gMS) was obtained under the experimental conditions of X_1 = 70 sec, X_2 = 530 W, X_3 = 20/1 mL/g and X_4 = 40 %; whereas the highest TF was obtained under conditions of X_1 = 80sec, X_2 = 600 W, X_3 = 20 ml/g and X_4 = 80 %. A wide range of antioxidant activity (% DPPH) was also found from 45.35 to 84.83 % and the maximum point was found under the conditions of X_1= 96,07 sec, X_2= 600 W, X_3= 20 mL/g and X_4 = 80 %. Therefore, an optimization process was investigated, in order to obtain desirable antioxidant contents and activity. The result of the optimal conditions for each individual response as well as combination of all responses with the predicted and experimental values is presented in table 4 and 5. The target was to obtain high phenolic compounds yields with high antioxidant activity within the extraction parameters, where consideration of the efficiency, the energy conservation and the feasibility of the experiment were taken into account. Optimal conditions for TPP were irradiation time of 82.68 sec, irradiation power of 653.13 W, solvent-to-solids ratio of 23.16 mL/g and methanol concentration of 74.61 %. On the other hand, optimal conditions for TF were irradiation time of 81.74 sec, irradiation power of 808.40 W, solvent-to-solids ratio of 20.73 ml/g and methanol concentration of 79 %, whereas optimal conditions for % DPPH were irradiation time of 85.73 sec, irradiation power of 621.06 W, solvent-to-solids ratio of 22.19 mL/g and methanol concentration of 75.99 %.These conditions gave TPP, TF and %DPPH values of 8366.15 µg GAE/ gDM, 193.995 µg QE/ gDM and 87.55 %, respectively.

Tableau 4: optimal conditions for each individual response (TPP,TF or %DPPH)with the predicted and experimental values

Responses	Process variables				Predicted value	Experimental value[a]
	X1 (s)	X2 (w)	X3 (mL/g)	X4 (%)		
TPP[b]	82.68	653.13	23.16	74.61	7998.71	8366.15±94.65
TF[c]	81.74	808.40	20.73	79.22	188.68	193.99±19.98
%DPPH[d]	85.73	621.06	22.19	75.99	94.37	87.55±08.84

[a] Mean of triplicate determination. [b] Total polyphenol (µg GAE/ gDM). [c] Total flavonoids (µg QE/ gDM). [d] Antioxydant activity (%).

From Equations 2, 3 and 4, the TPP, TF or % DPPH of the roots of *Ximenia americana* at any point in the interval of our experimental domain can be predicted. The 3D response surface and the 2D contour plot are the graphical representations of the regression equation. The response surface curves allow visualizing the simultaneous evolution of the TPP, TF or %DPPH of the roots of *Ximenia americana* with the change of the process factors. The 3D response surface (Fig. 1A, 1B or 1C) of TPP, TF or % DPPH of the roots of *Ximenia americana* as a function of irradiation time and methanol concentration at a fixed irradiation power level of 600 w and solvent- to-solid ratio level of 20 mL/g indicates that all the graphics have not the same tendencies. The increase in times or methanol concentration led to a gradual increase of TPP and TF then diminishes. While the only increases in methanol concentration led to gradual increase of % DPPH then diminishes. Thus, the time and methanol concentration in the extraction medium had a significant influence on the Total polyphenols and antioxidant properties of the roots of *Ximenia americana* extracts.

Figure 3A', 3B' and 3C' show the contours of estimated responses surfaces (estimated iso-response lignes) of TPP, TF and % DPPH as a function of time and methanol concentration. The use of contour plots is to best indicate technically and economically the values of process factors for obtaining a maximum yield of TPP, TF and % DPPH of the roots of *ximenia americana*. These iso-responses-lignes allow us to choose several combinations between times and methanol concentration to reach the same TPP, TF and % DPPH. For instance, extraction at 76 sec with a 70 % methanol concentration or at 84 sec with a 81 % methanol concentration permit to obtain the same TPP of 7500 µg GAE/g DM. In this work, the technically and economically value of process factors for obtaining a maximum yield of TPP, TF and % DPPH of the roots of *Ximenia americana* were taken into consideration. Therefore, for the economical feasibility of an industrial process, the optimal extraction conditions (indicated by violet arrows on the curve) of the three individual responses, selected graphically according to the contour plots with a fixe irradiation microwave power of 600 w and solvent-to-solids ratio of 20 ml/g were: an irradiation time of 70 sec and methanol concentration of 70 % for TPP (fig 3A'), an irradiation time of 76 sec and methanol concentration of 77 % for TF (fig 3B') and an irradiation time of 72 sec and methanol concentration of 69 % for % DPPH (fig 3C') of the roots of *Ximenia americana*. As can be seen in table 6, the predicted results and experimental results obtained demonstrate that the mathematical model represents very well the studied phenomenon in the experimental domain indicating the robustness of the method used. The optimizations of the three individual responses were achieved under different optimal conditions. Therefore a compromise between their extraction conditions was desirable.

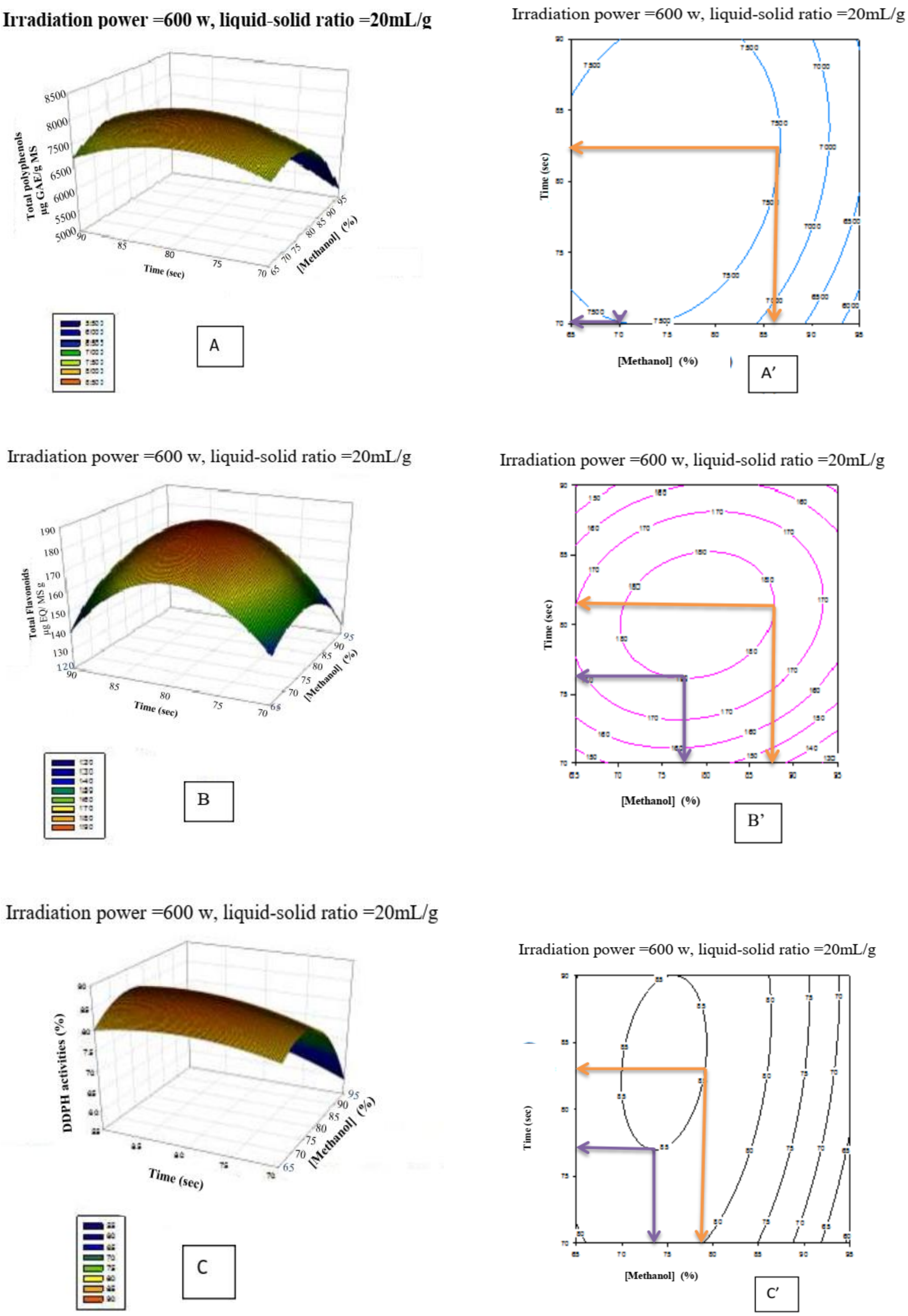

Figure 3: 3D response surface and 2-D contour graphs of individual responses TPP (A,A') TF (B,B') and % DPPH (C,C') between two independent variables while the remaining independent variable kept at its zero level.

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3.4. Multi-combined optimization

The optimum conditions for the simultaneous extraction of TPP, TF and %DPPH can be visualized graphically by superimposing the contours (fig 3A', 3B' and 3C') for various response surfaces in an overlay plot (fig 4). The optimum area is irradiation time of 77-85 sec and methanol concentration 70-78 %. Base on the overlain contour, a compromise for 7809.24 µg EAG/g MS TPP and 182.5 µg EQ/g MS TF yield with 90.48 % DPPH can be met at 82.51 sec irradiation time and 77.64 % methanol concentration (table 5).

To confirm the agreements of the results achieved from the model and experiments, additional experiments were conducted by applying the irradiation time and methanol concentration in the optimum region. As shown in Table 5, the TPP, TF YIELD and %DPPH obtained from the additional experiments were 7872.24 ±64.65 µg EAG/g MS, 189.70 ± 11.43 µg QE/g DM respectively with 84.04 ± 07.15 % DPPH. It could be observed that only small deviations were found between the experimental values and predicted values in Table 5, implying that the RSM approach was appropriate for optimizing the conditions of the microwave extraction process.

Table 5: optimum conditions based on combination of responses (TPP, TF and % DPPH)

Process variables				Predicted value			Experimental value[a]		
X1 (s)	X2 (w)	X3 (mL/g)	X4 (%)	TPP	TF	%DPPH	TPP	TF	%DPPH
82.51	600.00	20.00	77.64	7809.24	182.50	90.48	7872.24 ±64.65	189.70±11.43	84.04 ±07.15

[a] Mean of triplicate determination. [b] Total polyphenol (µg GAE/ gDM). [c] Total flavonoids (µg QE/ gDM). [d] Antioxydant activity (%).

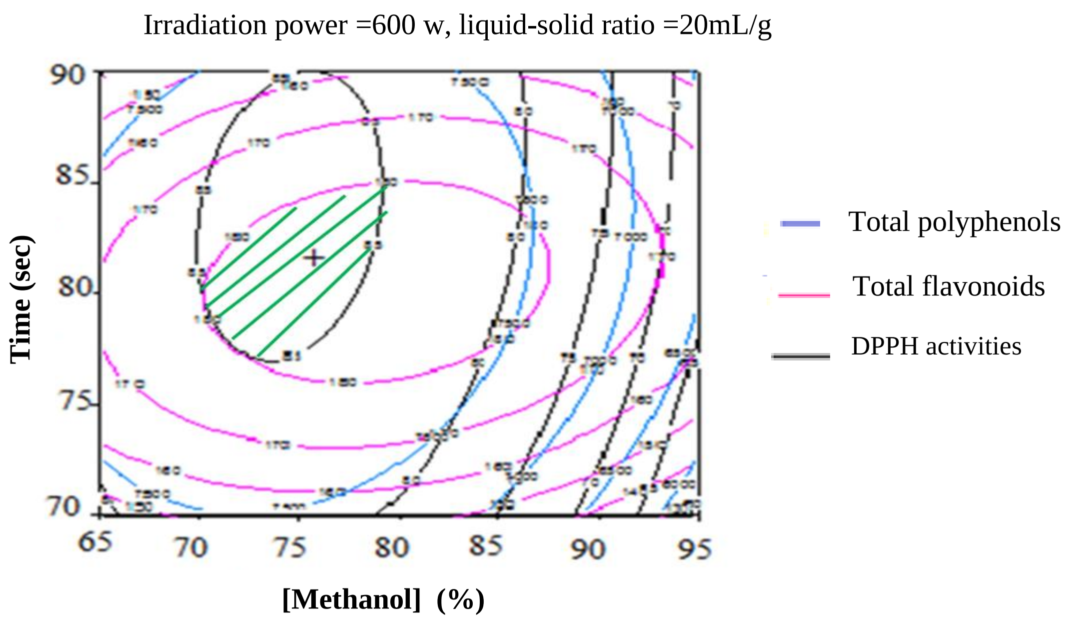

Figure 4: Superposition plots showing the best experimental parameters that maximize all the responses (TPP,TF and % DPPH).

4. CONCLUSION

This work has demonstrated the application of RSM in seeking optimal conditions for microwaved assisted extraction of the phenolic compounds of the roots of *Ximenia americana*. Simultaneous extraction of TPP, TF and % DPPH were investigated. In order to gain a better understanding of the four factors for optimal microwave extraction performance, the models were presented as 3D response surface and 2-D contour graphs. The following conclusions were obtained:

1) From Pareto analysis, the interaction effect X_1X_4 (irradiation time-methanol concentration) simultaneously influencing the TPP, TF and % DPPH of the root of *X. americana*

2) The optimal individual conditions for extraction of total polyphenols, total flavonoids and antioxidant activity (the economically value of process factors) from the roots of *X. americana* were 600 W, 20 mL/g, 70 sec, 70 %; 600 W, 20 mL/g, 76 sec, 77 %; and 600 W, 20mL/g, 72 sec, 69 %, respectively.

3) To simultaneously remove 7872 µg EAG/g MS TPP and 189 µg EQ/g MS, TF with 84 % of % DPPH, 83 sec irradiation time and 78 % methanol concentration were selected based on the overlain contour.

4) The results of a confirmation experiment were found to be in good agreement with the values predicted by the model. This demonstrates that to obtain a maximum amount of information in a short period of time, with the least number of experiments, RSM and CCD can be successfully applied for modeling and optimizing the microwave extraction process. Future studies can be oriented on the isolation and identification of compounds involved in anti-antioxydant activity.

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REFERENCES

[1] **Alzeer J, Vummidi BR, Arafeh R, Rimawi W, Saleem H,Luedtke N, W,** 2014. The influence of extraction solvents on the anticancer activities of Palestinian medicinal plants. J. Med. Plants Res., 8(8):408-415.

[2] **Belwal, T., Ezzat, S.M., Rastrelli, L., Bhatt, I.D., Daglia, M., Baldi, A., Devkota, H.P., Orhan, I.E., Patra, J.K., Das, G., Anandharamakrishnan, C., Gomez-Gomez, L., Nabavi, S.F., Nabavi, S.M., Atanasov, A.G.,** Trends in Analytical Chemistry, 2018, 100, 82-102.

[3] **Deming, S.N. and S.L. Morgan,** 1993. Experimental design: a chemometric approach Amsterdam, Elsevier.

[4] **Gan, C. Y., Abdul Manaf, N., & Latiff, A. A.** (2010). Optimization of alcohol insoluble polysaccharides (AIPS) extraction from the Parkia speciosa pod using response surface methodology (RSM). Carbohydrate Polymers, 79, 825–831.

[5] **Gan, C. Y., & Latiff, A. A.** (2011a). Extraction of antioxidant pectic-polysaccharide from

[6] mangosteen (Garcinia mangostana) rind: Optimization using response surface methodology. Carbohydrate Polymers, 83, 600–607.

[7] **Gan, C. Y., & Latiff, A. A.** (2011b). Optimisation of the solvent extraction of bioactive compounds from Parkia speciosa pod using response surface methodology. Food Chemistry, 124, 1277–1283.

[8] **G.T. Djiobie , R .K.T. Tsatsop, Kenmogne Sidonie Beatrice**, M.B. Ngassoum., 2016. Optimization of Microwave Assisted Extraction of Bioactive >Compounds from Flavorings Roots of Plant *Carissa Edulis*. Scholars Journal of Engineering and Technology (SJET). Vol 4(3) PP 127-136.

[9] **Huma ZE, Jayasena V, Nasar-Abbas SM, Imran M, Khan MK,** (2017) Process optimization of polyphenol extraction from carob (Ceratonia siliqua) kibbles using microwave-assisted technique. J Food Process Preserv

[10] **Huma ZE, Jayasena V, Nasar-Abbas SM, Imran M, Khan MK** (2018) Process optimization of polyphenol extraction from carob (Ceratonia siliqua) kibbles using microwave-assisted technique. J Food Process Preserv 42(2):e13450

[11] **Kenmogne Sidonie Béatrice, Xiu Deng**, 2000. Household Microwave Modification: Application on *Costus* Essential Oil. La Ravista Italiana Delle Sostanze Grasse. Vol LXXVII-November, PP 761-764.

[12] **Kenmogne Sidonie Beatrice, Ngassoum M, Tchatchueng J. B.n Vardamides J.C. and Dongmo A.** (2014). Microwave Assisted Extraction of Analgesic Compounds of the Root of *Ximenia Americana* (Olacaceae)Research Journal of Chemical Sciences. Vol 4(7), pp 1-6, July .

[13] **Kidik P, M, C, Ngene J, P, Ngoule C, C, Mvogo O, P, B, Ndjib R, C, Dibong S, D, Mpondo M, E,** 2015. Caractérisation des plantes médicinales à flavonoïdes des marchés de Douala (Cameroun). Int. J. Biol. Chem. Sci., 9(3): 1494-1516.

[14] **Le NHT, Malteruda KE, Diallob D, Paulsena BS, Nergårda CS, Wangensteen H.** 2012, Bioactive polyphenols in *Ximenia americana* and the traditional use among Malian healers. J Ethnopharmacol. 139(3): 858-862.

[15] **L. Liu, Y. Sun, T. Laura, X. Liang, H. Ye and X. Zeng,** (2009). Determination of polyphenolic content and antioxidant activity of kudingcha made from *Ilex kudingcha* C.J. Tseng. Food Chemistry, 112 : 35–41.

[16] **Maikai B, V, Maikai V, A,** 2015. Effects of treatment with fraction IV extract of Ximenia americana on the survival rate, packed cell volume and total plasma proteins of Trypanosoma congolense infected mice.J. Parasitol. Vector Biol., 7(5):101-107.

[17] **Mathieu, D., R. Phan-tan-Luu and G. Droeskeke, 1997.** Approache Methodologique des Surfaces de Reponses, Plans d'experiences. Applications a l'entreprise, Saporta (Eds.) Technip, Paris, pp: 211.

[18] **M. Pinelo, A. G. Tress, M. Pedersen, A. Arnous, A. S. Meyer,** (2007). Effect of cellulases, solvent type and particle size distribution on the extraction of chlorogenic acid and other phenols from spent coffee grounds, Am. J. Food Technol. 2: 641–651.

[19] **Onifade A, O, Ouedraogo M, Ouedraogo M, Zongo F, E,Kafando E, Lompo M, Guissou I, P,** 2011. Acute toxicity and anti-inflammatory activity of aqueous ethanol extract of root bark of *Ximenia americana* (Olacaceae). Afr. J. Pharm. Pharmacol., 5(7): 806-811.

[20] **Pang SF, Lau MZ, Yusoff MM & Gimbun J (2017),** Microwave irradiation induced fast simultaneous extraction of methoxylated and hydroxylated phenolic compounds from Orthosiphon stamineus leaves. Material Science Forum, 155-158.

[21] **Prasad KN, Fouad AH, Bao Y, Kin WK, Ramakrishnan NR, Azrina A, Ismail A** (2011) Response surface optimization for the extraction of phenolic compounds and antioxidant capacities of under utilised *Mangifera pajang* Kosterm. Peels. Food Chem 128:1121–1127

[22] **R .K.T. Tsatsop, G.T. Djiobie, Béatrice Sidonie Kenmogne** , K.R. Regonne, M.B. Ngassoum., 2016. Optimization of Microwave Assisted Extraction of Bioactive >Compounds from *Anogeissus Leiocarpus* Guill & Perr. Stem Bark Using Response Surface Methodology. International Journal of Scienctific & Technology Research. Vol5 Issue 05 May.

[23] **V. L. Singleton, J. A. Rossi Jr,** (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents, Am. J. Enol.Vitic.16 : 144–158.