International Scholars Journals

International Journal of Biochemistry and Biotechnology ISSN 2169-3048 Vol. 7 (4), pp. 800-809, April, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Optimization of microwave-assisted extraction of total phenol content and total flavonoids content from *Anacardium occidental L.* (Anacardeaceae) using response surface methodology

MADJITOLOUM BETOLOUM Salomon[1,4*], TALLA Emmanuel[2*], NGASSOUM Martin Benoît[1], TSATSOP TSAGUE Roli Karole[1], NYEMB Jean Noël[2], MAHMOUT Yaya[3]

[1]Department of Applied Chemistry, National Advanced School of Agro-Industrial Sciences, University of Ngaoundere (UN), Cameroon.
[2]Department of Chemistry, Faculty of Science, University of Ngaoundere (UN), Cameroun.
[3]Laboratory of Research on Natural Substances, University of N'Djamena (UN), Chad.
[4]Department of Chemistry, Faculty of Sciences and Techniques, University Adam Barka of Abeche (UNABA), Chad.

Accepted 03 February, 2018

The optimal conditions of Microwave-Assisted Extraction (MAE) of total phenol content and total flavonoids content from *A. occidentale L*. stem bark were 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), solvent-to-solids ratio (mL/g) and methanol concentration (%) on the responses. The second-order regression for CCD consisted of 24 experimental points and 4 replications at the central point. Data were analyzed using Statgraphics software. The optimal conditions based on combination responses were: extraction time of 83 s, irradiation power of 620 W, solvent-to-solids ratio of 33.4 mL/g and methanol concentration of 63.17% according to the analysis of response surface. These optimum conditions yielded total phenolic contents (TPC) of 674.58 mg Gallic Acid Equivalent (GAE)/ 100g DM and total flavonoid content (TFC) of 85.38 mg Quercetin Equivalent (QE)/100g DM. Close agreement between experimental and predicted values was found.

Keywords: *Anacardiu moccidentale L.*, total phenols and total flavonoids, Microwave-Assisted Extraction, Optimal conditions.

INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The Cashew (*A. occidentale*) is a tree in the family of the flowering plant *Anacardiaceae*. The family contains 73 genera and about 600 species. *Anacardium* contains 8 species, native to tropical America, of which the cashew is by far the most important economically. It is a multipurpose tree that grows up to 8-10 m high. It has a thick and tortuous trunk with branches so winding that they frequently reach the ground (Anyin *et al.,* 2016). The cashew tree produces many resources and products. The cashew nut has international appeal and market value as food. Even the shell oil around the nut is used medicinally and has industrial applications in

Corresponding author: salomonbeto@yahoo.fr; Tel.+23566305267; +237674863715;+237 699506524

plastics and resin industries for its phenolic content. The pseudo-fruit, a large pulpy and juicy part, have a fine sweet flavor and is commonly referred to as the “cashew fruit” or the “cashew apple”.

In Chad, it is also used to treat diabetes, weakness, muscular debility, urinary disorders, asthma, eczema, psoriasis, scrofula, dyspepsia, genital problems, bronchitis, cough, intestinal colic, venereal diseases, as well as impotence, and syphilis-related skin disorders. In addition to being delicious, cashew fruit is a rich source of vitamins, minerals, and other essential nutriments. Several clinical studies have shown that anacardic acid, a component of cashew, with highest concentration in the nutshells curb the darkening effect of aging by inhibiting tyrosinase activity, and that they are toxic to certain cancer cells. Moreover, anacardic acid causes allergies and dermatitis (André *et al.*, 2012).

Phytochemical studies revealed that the stem barks of the plant contain tannins and flavonoids (Chen *et al.,* 2011, Honoré *et al.,* 2017 and John *et al.*, 2017). Resorcinolic acid, ascorbic acid, carotenoids, vitamin C and phenolic compounds were also identified in the apples and the stem barks (Jothi *et al.,* 2013). In 2007, Edy Sousas de Brito et *al.* (2007) quantified from the methanol/water extracts of the apples, total flavonoids. From the ethanolic extract, phenolics were showed as the main components (Honoré *et al.,* 2017). Glucosyl quercetin, derivative of amentoflavone and a tetramere of proanthocyanidin were also identified (André *et al.,* 2012).

Due to the fact that *A. occidentale* is very useful, as found by above mentioned reports, there is a need to find out more about the potentiality of this plant as an anti-oxidant agent. The objective of the present work is to point out the best process of extraction of the phenolic and flavonoid compounds.

Extraction is the initial and the most important step in the recovery and purification of bioactive compounds from plant materials. Such traditional methods as mechanical agitation, soxhlet extraction, which have been used for many decades, are very time-consuming and require relatively large quantities of solvents (Huanhuan *et al.,* 2012). There is an increasing demand for new extraction technique to shorten the extraction time, reduce organic solvent consumption, and to prevent environmental pollution. Novel extraction method including Microwave-Assisted Extraction (MAE) is fast and efficient for extracting chemicals from solid plant matrixes (Martins *et al.*, 2007, Aguilar *et al*., 2010 and Huanhuan *et al.,* 2012,). However, many factors such as extraction time, irradiation power, solvent-to-solids ratio and solvent composition influence the extraction efficiency, phenolic content (Pinelo *et al.,* 2007, Kiassos *et al.,* 2009, Chee-Yuen and Latiff, 2011 and Mussatto *et al.,* 2011). Hence, it is necessary to optimize the extraction conditions to obtain the highest phenolic recovery. The aim of the present study was to describe the response surface optimization of solvent extraction of phenolic compounds from *A. occidentale* stem bark for the enhanced recovery of total phenolic content (TPC) and total flavonoids content (TFC).

MATERIALS AND METHODS

Plant material

Samples of stem barks, leaves and Cashew nuts were collected in the southwestern part of Chad (Pala, region of Mayo-Kebbi) in April, 2014. The species were identified and voucher specimen was deposited at the National Institute of Research for the Development (IRED), Farcha, N’djamena, Chad (n° 1344). The air-dried materials were ground, and then pulverized. Obtained powders were kept in closed containers until needed.

Preparation of *A. occidentale* bark extracts

The process of MAE was performed with the use of a household microwave (DAE WOO, KOG-360, Combi Grill, AHYEON-DONG MAPO-GU SEOUL, KOREA) with CAVITY DIMENSIONS (WXHXD) of 290X290X220mm. Extraction of polyphenolic compounds from *A. occidentale bark* powders was carried out using aqueous methanol solvent. Desired weight of a dried bark powder and solvent were extracted at different periods (X_1), irradiation power (X_2) after mixed at different solvent-to-solids ratio (X_3) in each extraction with the corresponding concentration of solvent (X_4) (Table 1). The mixtures were then filtered and the filtrates were collected as the extracts. Four replicates were performed in each extraction.

Determination of total phenolic content (TPC)

Total phenolic content of *A. occidentale* extracts obtained was determined according to the method of Prasad *et al.,*(2011). Total phenolic phytochemical content was measured using the Folin-Ciocalteu. Briefly, 0.03 mL of each extract, 1.37 mL of distilled water were mixed to 0.2 mL of the reagent of dilute Folin-Ciocalteu 0.2 g/L (1:16 dilution) and then shaken and left to stand for a few minutes at room temperature to allow for the reagent to react completely with the oxidible substances or phenolates. 0.4 mL of Na_2CO_3 (20% in water) were added to destroy the residual reagent. Absorbances were measured at 760 nm, using a spectrophotometer (spectrophotometer UV Rayleigh Vis-723N) after incubation for 20 min 40°C against distilled water as a blank. Total phenolic contents of the samples determined from the calibration curve equation

($y = 0.1236x$ $R^2 = 0.9831$) were expressed in mg Gallic Acid Equivalents (GAE)/100 g of dry material. All measurements were performed in three replications.

Determination of total flavonoid content (TFC)

Total flavonoid content of the extracts were evaluated using UV spectroscopic method. A volume of methanolic solution of aluminum chloride (0.5 mL, 2% w/v) was mixed with the methanolic solution of the extract (0.5mL, 0.1 mg/mL). After 10 min., absorbances were measured at 415 nm. Results, determined from de the calibration curve equation ($y = 0,009x$, $R^2 = 0,9963$) were calculated as follows and expressed in mg of Quercetin Equivalents (QE)/100g dry material (DM) (Chia-Pu et al., 2012):

$F = \frac{0.05 \times Aext}{Aq \times Cext} \times 100$, where F: content of total flavonoids (mg[] QE/100g DM);

A_{ext}.: absorbance of the extract; Aq.: absorbance of quercetin; C_{ext}.: concentration of plant extract (10 mg /mL).

All measurements were performed in three replications.

Experimental design

The extraction parameters were optimized using response surface methodology (RSM). A central composite design (CCD) was employed in this regard. Irradiation time (X_1), irradiation power (X_2), solvent-to-solids ratio (X_3) and methanol concentration (X_4) were chosen for independent variables. The range and center point values of two independent variables presented in Table 1 were based on the results of preliminary experiments, a modified design from Pompeu *et al.*(2009). The experimental design consists of sixteen factorial points, eight axial points at a distance of ±1.60717 from the center and four replicates of the central point. TPC and TFC were selected as the responses for the combination of the independent variables given in Table 2. Four experiments were carried out at each experimental design point and the main values were stated as observed responses. Experimental runs were randomized, to minimize the effects of unexpected variability in the observed responses. The variables were coded according to the equation:

$$X = \frac{Xi - X0}{\Delta X} \quad (1)$$

Where X is the coded value, Xi is the corresponding actual value, Xo is the actual value in the center of the domain and ΔX is the increment of Xi corresponding to a variation of 1unit of x. The mathematical model corresponding to the composite design is:

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j + \varepsilon \quad (2).$$

Where Yi is the dependent variables (TPC and TFC), βo is the model constant, βi, βii and βij are the model coefficients, and ε is the error. They represent the linear, quadratic and interaction effects of the variables. Analysis of the experimental design data and calculation of predicted responses were carried out using STATGRAPHICS centurion software (Version XVI.I). Additional confirmation experiments were subsequently conducted to verify the validity of the statistical experimental design.

Statistical analysis

Comparison of means was performed by one-way analysis of variance (ANOVA) followed by Duncan's test. Statistical analyses ($p < 0.05$) were performed using STATGRAPHICS centurion software (Version XVI.I). The optimal extraction conditions were estimated through regression analysis and three-dimensional (3D) response surface plots (obtained using Sigmaplot 12.0 software) of the independent variables and each dependent variable.

RESULTS AND DISCUSSION

The effects of the four process variables, i.e., time (X_1: 70–90 s), irradiation power (X_2: 570–670 w), solvent-to-solids ratio (X_3: 25–35 ml/g), and methanol concentration (X_4: 50-70%) were investigated during the study. The two responses of interest were TPC and TFC. The results of 28 runs using CCD design are shown in Table 3, which include the design, observed responses and the predicted values. A close agreement between experimental and predicted values was found. In addition, it was observed that the yield of TPC and TFC ranged from 425.187 to 643.042 mg GAE/100 g DM and 54.394 in 80.472 mg QE/100 g DM respectively. The highest TPC (643.042 mg GAE/100 g DM) and the highest TFC (80.472 mg QE/100 g DM) were obtained under the experimental conditions of X_1= 80s, X_2= 620 W, X_3 = 30 ml/g and X_4 = 60%. Therefore, an optimization process was investigated, in order to obtain desirable phenolic contents.

Model fitting

Table 3 shows the results of fitting quadratic models to the data. The results of analysis of variance (ANOVA) indicate that the contribution of the quadratic model was significant. The fitted quadratic models for TPC and TFC in coded variables are given in equations (3) and (4), respectively. In order to obtain simpler quadratic regression model insignificant factors and their interactions have been omitted from the equations. The significance of each coefficient was determined using the F-test and p-value in Table 3. This table shows only

Table 1. Experimental domain of central composite design (CCD).

Xj	Factor levels				
	-1.60717	-1	0	1	1.60717
Time (s)	64	70	80	90	96
Irradiation power	540	570	620	670	700
Solvent-to-solids ratio (mL/g)	22	25	30	35	38
Solvent concentration	44	50	60	70	76

Table 2.ANOVA for response surface quadratic model: estimated regression model of relationship between response variables and independent variables (X_1, X_2, X_3, and X_4).

	Y_{TPC}			Y_{TFC}		
	Coeff.	**Quadratic average**	**P-value**	**Coeff.**	**Quadratic average**	**P-value**
X_1	16.667	5879.77	**0.0000**	2.429	124.882	**0.001**
X_2	-12.596	3358.35	**0.0001**	-1.597	53.951	**0.005**
X_3	6.952	1022.9	**0.0005**	0.731	11.298	**0.040**
X_4	18.360	7134.41	**0.0000**	1.816	69.833	**0.003**
X_1^2	-41.102	22542.2	**0.0000**	-4.326	249.756	**0.001**
X_1X_2	-3.255	169.501	**0.0068**	-0.324	1.681	0.272
X_1X_3	-3.729	222.543	**0.0046**	0.113	0.203	0.673
X_1X_4	-0.475	3.605	0.4002	-0.264	1.118	0.354
X_2^2	-55.194	40649.6	**0.0000**	-6.386	544.297	**0.000**
X_2X_3	1.275	26.002	0.0786	0.196	0.616	0.476
X_2X_4	-0.705	7.957	0.2422	0.682	7.449	0.067
X_3^2	-52.779	37169.9	**0.0000**	-8.027	859.806	**0.000**
X_3X_4	8.151	1062.91	**0.0005**	0.730	8.528	0.057
X_4^2	-30.055	12053.3	**0.0000**	-3.095	127.849	**0.001**

those which are significant. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller (Bocco *et al.*, 2017). Lack of fit was also given in Table 2 in order to check the quality of the fitted models.

$$Y_{TPC} = 650.3 + 16.7X_1 - 12.6X_2 + 6.9X_3 + 18.4X_4 - 3,3X_1X_2 - 8.2X_3X_4 - 41.1X_1^2 - 55.2X_2^2 - 52.8X_3^2 - 30.1X_4^2 \quad (3)$$

$$Y_{TF} = 81.2 + 2.4X_1 - 1.6X_2 + 0.7X_3 + 1.8X_4 - 0.3X_1X_2 + 0.1 - 0.7X_3X_4 - 4.3X_1^2 - 6.4X_2^2 - 8.1X_3^2 - 3.1X_4^2 \quad (4)$$

The effect of extraction time (X_1), irradiation power (X_2), liquid to solid ratio (X_3) and solvent concentration (X_4), was significant ($p < 0.05$) both in first-order linear effect (X_1,X_2.X_3 and X_4) and second-order quadratic effect (X_1^2, X_2^2, X_3^2 and X_4^2) as shown in table 2. These results obtained suggest that the change of different independent variables had significant effects ($p < 0.05$) on the yield of TPC and TFC. Interaction terms X_1X_2, X_1X_3 and X_3X_4 were equally significant ($p > 0.05$). These results are similar to the research findings by Prasad *et al.,*(2011). The coefficient of determination (R^2) of the predicted models in this response was 96.66 % for TPC and 96.77 % for TFC. However, p-value for lack of fit was 0.0068for TPC and 0.040 for TFC, which suggests a relative good fit to the mathematical model Equations (3) and (4).

Influence of extraction parameters on phenolic compounds

The influence of four independent variables towards total phenolic content was reported through the significant ($p < 0.05$) coefficient of the second-order polynomial regression equation. 3D response surfaces curves in Figures 1 and 2 demonstrated the effects of the independent variables and their mutual interactions

Table 3. CCD with the observed responses and predicted values for TPC and TFC.

N°	Coded variables levels				Observed responses		Predicted responses	
	X_1	X_2	X_3	X_4	Y_{TPC} (mg GAE/100 g MS)	Y_{TFC} (mg QE/100g MS)	Y_{TPC} (mg GAE/100 g MS)	Y_{TFC} (mg QE/100g MS)
1	70	570	25:1	50	425.187	55.868	436.816	57.153
2	90	570	25:1	50	502.977	64.574	501.504	62.963
3	70	570	25:1	50	435.06	54.394	441.151	52.852
4	90	670	25:1	50	455.131	58.474	451.074	57.365
5	70	570	35:1	50	450.466	55.295	442.696	56.537
6	90	570	35:1	50	432.456	62.432	440.407	62.797
7	70	670	25:1	50	455.456	55.036	445.643	53.020
8	90	670	35:1	50	460.881	57.560	466.584	57.984
9	70	570	25:1	70	495.382	58.079	480.434	58.490
10	90	570	25:1	70	440.701	61.217	446.61	63.243
11	70	670	25:1	70	470.971	57.274	480.731	56.918
12	90	670	25:1	70	489.089	60.781	481.137	60.374
13	70	570	35:1	70	519.902	59.675	519.513	60.794
14	90	570	35:1	70	471.079	63.619	465.153	65.997
15	70	670	35:1	70	504.496	57.560	509.685	60.006
16	90	670	35:1	70	529.124	65.188	517.985	63.912
17	64	620	30:1	60	583.370	67.372	572.274	66.157
18	96	620	30:1	60	557.766	74.058	562.913	73.965
19	80	540	30:1	60	481.929	71.397	484.331	67.305
20	80	700	30:1	60	512.307	59.389	503.956	62.173
21	80	620	22:1	60	539.864	58.120	521.123	59.328
22	80	620	38:1	60	535.308	64.192	548.101	61.676
23	80	620	30:1	44	634.254	68.081	641.864	70.321
24	80	620	30:1	76	642.716	79.708	641.864	76.160
25	80	620	30:1	60	638.811	80.295	641.864	81.236
26	80	620	30:1	60	643.042	82.205	641.864	81.236
27	80	620	30:1	60	642.066	80.472	641.864	81.236
28	80	620	30:1	60	641.864	80.117	641.864	81.236

on the TPC and TFC values respectively. They were obtained by keeping two of the variables constant. The constant was equal to the natural value of zero level. As evidence, the figures 1 and 2 show that at lower irradiation power, increasing power led to a gradual increase in the TPC and TFC values over time. This phenomenon is considered to be caused by the low rate of mass transfer at low temperatures, which would require more time for the phenolic compounds to dissolve from the raw materials into the solution. These results are similar to the research findings by Karabegovic *et al.* (2013). Moreover, the microwave irradiation accelerates cell rupture by sudden temperature rise and internal pressure increase inside the cells of plant sample, which promotes the destruction of sample surface and in turns the exudation of the chemical substance within the cells into the surrounding solvents takes place (Zhang *et al.,* 2008; Hayat *et al.,* 2009). At higher irradiation power, however, dissolution of the phenolic compounds can reach the equilibrium in a shorter time then decreased by changes in the extraction time. This suggests that a higher irradiation power and a short extraction time are more effective in extracting and oxidative phenolic compounds from *A. occidentale* bark using MAE. The decrease observed at higher values of irradiation power and long period of extraction may be due to thermal degradation of the phenolic compounds (Chen *et al.,* 2007). The total phenolic content increased with increasing liquid-to-solid ratio. When the liquid-to-solid ratio increased from 8:1 to 16:1, the total phenolic content also increased, which was probably due to the fact that more solvent could enter cells while more phenolic compounds could permeate into the solvent under the higher solid-to-liquid ratio conditions (Prasad *et al.*, 2009; Zheng *et al.*, 2009). With further increase in liquid-to-solid ratio, a decline in TPC and TFC were observed (Figures 1 and 2). Pompeu *et al.* (2009) have reported that extraction of phenolic compounds was highly dependent on liquid/solid ratio. They have reported liquid/solid ratio of 40:1 (mL/g) was sufficient to extract high quantities of phenolic compounds from fruits of *E. oleraeceae*. Gan and Latiff (2011) reported that liquid/solid ratio (20 mL/g) played a significant role

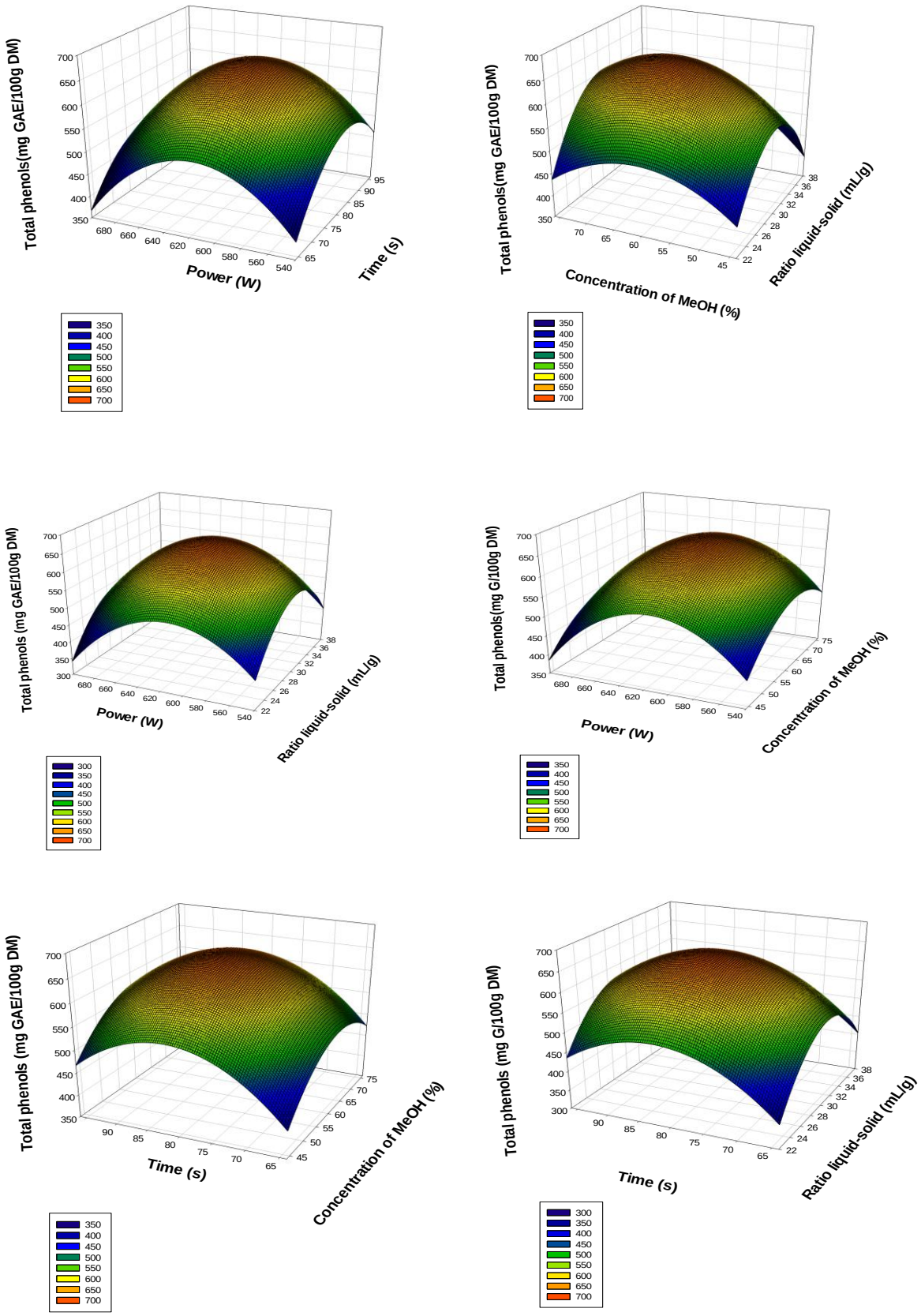

Figure 1. 3D response surface plots showing the effects of variables on TPC.

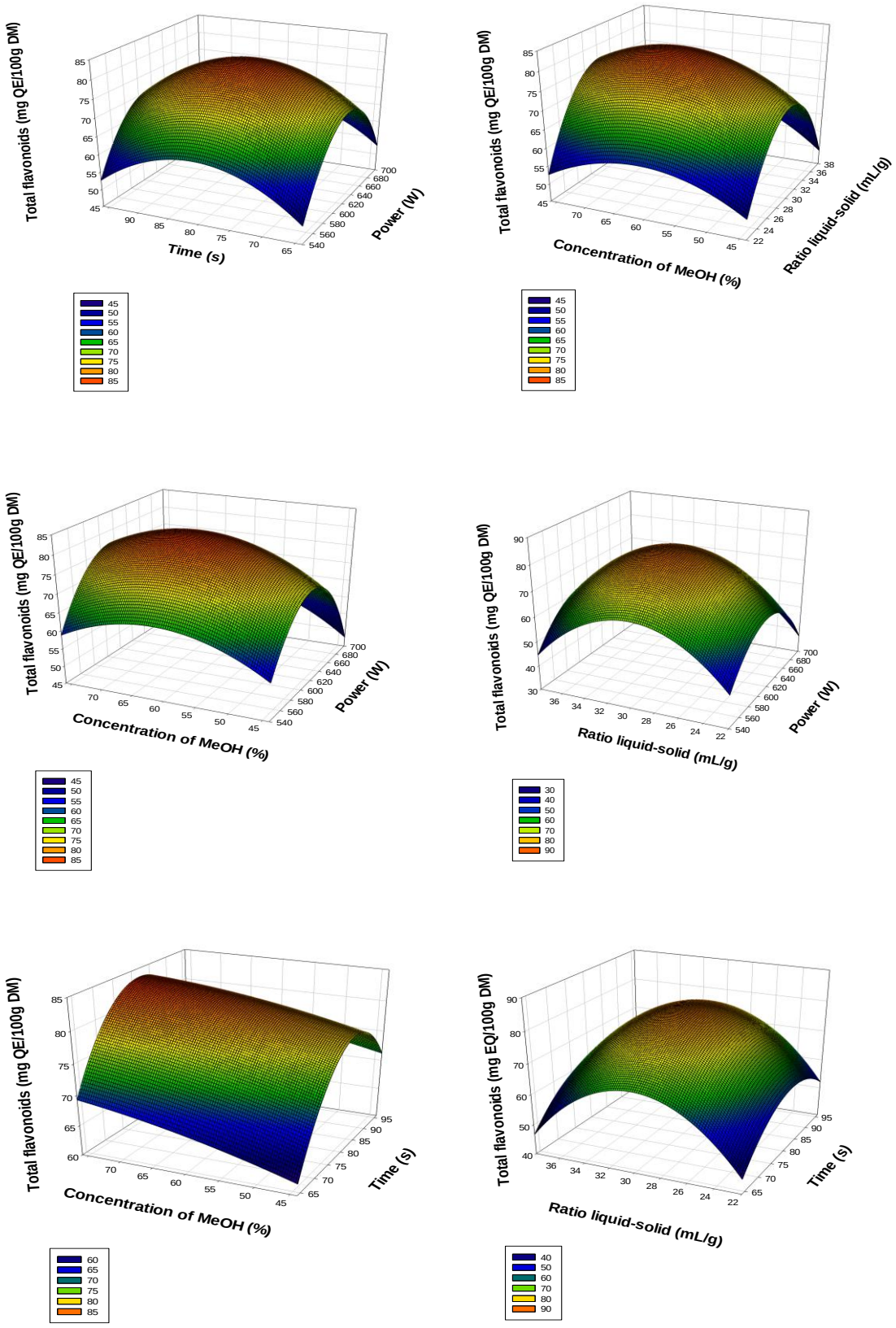

Figure 2. 3D response surface plots showing the effects of variables on TFC.

Table 4. Predicted and experimental values under optimum conditions based on both individual and combination of all responses.

	Values real				Answers		
	Time (s)	Power (W)	Ratio liquid-solid (mL/g)	Concentration of MeOH (%)	Calculated	Test 1	Test 2
TPC (mg GAE/100g DM)	81	620	30.40	63.17	655.90	673.90	675.26
TFC (mg QE/100g DM)	83	620	30.30	62.76	81.94	84.93	85.82

in the yielding of phenolic compounds, while extraction temperature did not make any significant contribution towards TPC and TFC. When methanol concentration increased from 31 to 50 %, increase in the phenolic content from 443 to 544 mg GAE/g DM, was observed (Fig. 1). This is probably due to the increased solubility of phenolic compounds in the mixture of methanol and water. The methanol concentration was also an important variable contributing to the extraction of phenolic compounds from other natural sources, such as *Mashua tubers* (Chirinos *et al.,* 2007), brewer's spent grains (Meneses *et al.,* 2013). Mussatto *et al.* (2011) showed that both of solvent-to-solids ratio and concentration of methanol had significant effects on extraction of phenolic compounds from spent coffee grounds.

Verification of predictive models

Based on the above findings, an optimization study was performed to evaluate the optimal operating conditions for individual response as well as combination of all responses. The target was to obtain high phenolic compounds yields with high antioxidant activities within the extraction parameters, where consideration of the efficiency, the energy conservation and the feasibility of the experiment were taken into account. Table 4 shows the optimal conditions for each individual response with the predicted and experimental values. Optimal conditions for TPC were extraction time of 81 s, incubation power of 620W, solvent-to-solids ratio of 30.40 ml/g and methanol concentration of 63.17%. On the other hand, optimal conditions for TFC were extraction time of 83 s, incubation power of 620W, solvent-to-solids ratio of 30.30 ml/g and methanol concentration of 62.76%.

These conditions gave TPC and TFC values of 673.90 to675.26 mg GAE/100 g DM and84.93 to85.82 mg QE/100 g DM, respectively. Table 4 shows the four optimum conditions based on combination of all responses. These optimal conditions extraction are time of 83 s, irradiation power of 620 W, solvent-to-solids ratio of 30.4 ml/g and methanol concentration of 63.17%, yielded TPC and TFC of 674.58 mg GAE/100g DM and 85.38 mg QE/100g DM, respectively. It could be observed that only small deviations were found between the experimental values and predicted values in Table 4.

CONCLUSION

The response surface methodology (RSM) was used to determine the optimum process parameters that yield high phenolic compounds. ANOVA (The F-test and P-value) indicated that the effects of extraction time, irradiation power, solvent-to-solids ratio and methanol concentration were significant in TPC yield and TFC. Whereas the effect of power was not significant in phenolic compounds. Quadratic models were used in predicting all the responses. The optimal conditions based on both individual and combination of all responses were determined. Based on the combination of all responses, these optimal conditions of extraction time of 83s, irradiation power of 620 W, solvent-to-solids ratio of 30.4 ml/g and methanol concentration of 63.17% yielded TPC and TFC of 674.58 mg GAE/100g DM and 85.38 mg QE/100gDM, respectively. Results showed that predicted and experimental values were not significantly different. Therefore, it is suggested the models obtained can be used to optimize the process of bioactive compounds extraction from *A. occidentale* stem bark.

ACKNOWLEDGEMENTS

This research was supported by the National Commission of Formation of the Formative (CONFOFOR) of the Ministry of the Higher education and the Scientific Research (MESRS) of Chad and the University Adam Barka of Abéché (UNABA)-Chad. The authors are thankful to them.

REFERENCES

Aguilar, Rodriguez I. G., Mussatto S. I., Teixeira J. A., 2010.Kinetic study of nordihydroguaiaretic acid recovery from Larrea tridentata by microwave-assisted extraction, J. Chem. Technol. Biotechnol. Vol. 85. p. 1142–1147.

André G., Mirko F., Virgínia M. M., Carlos F. H. M. et

Friedhelm M., 2012. Changements de la composition phénolique, de l'acide ascorbique et de la capacité antioxydante de la pomme cajou (*Anacardium occidentale* L.) au cours de sa maturation.*Fruits,* 2012, vol. 67, p. 267–276 © 2012 Cirad/EDP Sciences.

Anyin Chen1, Wenjun Xiang, Dan Liu, Changlu Liu, Li Yang (2016). Determination of Total Flavonoids and Its Antioxidant Ability in *Houttuyniacordata. Journal of Materials Science and Chemical Engineering, 2016, 4, 131-136*.

Bocco R., Gandonou C. B., Gbaguidi F. and Ahouansou A. C., 2017. Phytochemical screening and quantitative variation of some secondary metabolites in five cultivated rice varieties. *Journal of Applied Biosciences* 113: 11146-11157.

Chen C. N., Hsiao C. J., Lee S. S., Guh J. H., Chiang P. C., Huang C. C. Huang W. J., 2011.Chemical modification and anticancer effect of prenylated flavanones from Taiwanese propolis. *Nat. Prod.*, 4: 73-78.

Chen Y., Xie M. Y. and Xiao F. G., 2007. Microwave-assisted extraction used for the isolation of total triterpenoid saponins from Ganodermaatrum. Journal of Food Engineering, 80: 162-172.

Chirinos R., Rogez H., Campos D., Pedreschi R., Larondelle Y., 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolumtuberosum Ruíz & Pavón) tubers, Sep. Purif. Technol. 55: 217–225.

Edy S. B., Manuela C. P. A., Long-Ze L. and James H., 2007.Determination of the flavonoid components of cashew apple (*Anacardium occidentale*) by LC-DAD-ESI/MS. *Food Chemistry*, 105.1112-1118.

E. Kiassos, Mylonaki, S. D. P. Makris and P. Kefalas, 2009. Implementation of response surface methodology to optimise extraction of onion (Alliumcepa) solid waste phenolics. Innovative Food Science and Emerging Technologies 10: 246–252.

Gan C. Y. and Latiff A. A., 2011. Optimization of the solvent extraction of bioactive compounds from Parkiaspeciosa pod using response surface methodology. Food Chemistry 124: 1277–1283.

Chia-Pu L., Zong-Tsi C., Pei-Ying Y., Yin-Chuan W. and Pin-Der D., 2012. Identification of bioactive compounds and comparison of apoptosis induction of three varieties of sugarcane leaves. *Journal of Functional Foods,* 4: 391-397.

Hayat, Hussain S., Abbas S., Farooq U., Ding B., Xia S., Jia C., Zhang X. and Xia W., 2009. Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation and Purification Technology, 70: 63–70.

Honoré N. T. M., Dieudonné N. and Remy M., 2017. Anthelmintic Activity, Acute Toxicity of *Anacardium occidentale L*. (Anacardiaceae) on *Onchocercaochengi* and *Caenorhabditiselegans. Asian Journal of Medicine and Health5(3): 1-12, 2017; Article no.AJMAH.34454. ISSN: 2456-8414*

John O. O., Gabriel I., Peter Y. and Felicia O., 2017. Comparative Studies of the Phytochemical, Antioxidant and Antimicrobial Properties of Cashew Leaf, Bark and Fruits Extracts. *American Journal of Food and Nutrition, Vol. 5, No. 4, 115-120.*

Jothi V., Vijay K. T., Vasudev B. and Giliyar S., 2013. Antimicrobial effect of *Anacardium occidentale* leaf extract against pathogens causing periodontal disease. Advances in Bioscience and Biotechnology, 2013, 4, 15-18.

Karabegovic´ T., Stojicˇevic´ S. S., Velicˇkovic´ D. T., Nada Cˇ. Nikolic´ and Lazic M. L., 2013. Optimization of microwave-assisted extraction and characterization of phenolic compounds in cherry laurel (Prunus laurocerasus) leaves. Separation and Purification Technology, 120: 429–436.

Martins S., Markom C. N M., Hasan M., Daud W. R. W., Singh H. and Jahim J. M., 2007. Extraction of hydrolysable tannins from Phyllanthus niruri Linn. Effects of solvents and extraction methods, Sep. Purif. Technol, Vol. 52. p. 487–496.

Meneses N. G. T., Martins S., Teixeira J. A., Mussatto S. I., 2013. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. Separation and Purification Technology 108: 152–158.

Mussatto S. I., Ballesteros L. F., Martins S. and Teixeira J. A., 2011. Extraction of antioxidant phenolic compounds from spent coffee grounds. Separation and Purification Technology 83: 173–179.

Prasad K. N., Fouad A. H., Bao Y., Kin W. K., Ramakrishnan N. R., Azrina A. and Amin I., 2011. Response surface optimisation for the extraction of phenolic compoundsand antioxidant capacities of *underutilised Mangifera pajang Kosterm*. Peels. *Food Chemistry*128 : 1121–1127.

Prasad N. K., Chun Y., En Y., Zhao M. and Jiang Y., 2009. Effects of high pressure on the extraction yield, total phenolic content and antioxidant activity of long a fruit pericarp. Innovative Food Science and Emerging Technologies, 10: 155–159.

Pinelo M., TressA. G., Pedersen M., Arnous A. and MeyerA. S., 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. Vol. 2. p. 641–651.

Pompeu D. R., Silva E. M. and Rogez H., 2009.Optimization of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using response surface methodology. Bio-resource Technology, 100: 6067–6082.

Huanhuan Y., Xinxuan D., Lei Y., Wenjie W., Fengjian

Y., Chunjian Z., Xiangdong M., Lin Z. and Yuangang Z., 2012. Microwave-Assisted Method for Simultaneous Extraction and Hydrolysis for Determination of Flavonol Glycosides in *Ginkgo Foliage* Using Brönsted Acidic Ionic-Liquid $[HO_3S(CH_2)_4mim]HSO_4$ Aqueous Solutions. *Int. J. Mol. Sci.* 13, 8775-8788

Zhang, Yang R. and Liu C. Z., 2008.Microwave-assisted extraction of chlorogenic acid from flower buds of Lonicera japonica Thunb, Sep. Purif. Technol. 62: 480–483.

Zheng X., Wang X., Lan Y., Shi J., Xue S. J., Liu C., 2009. Application of response surface methodology to optimize microwave-assisted extraction of silymarin from milk thistle seeds. Separation and Purification Technology, 70: 34–40.