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Research Article

Optimization of Microwave-Assisted Extraction of Bioactive Compounds from Flavorings Roots of Plant *Carissa edulis*

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Abstract: The present study reports the extraction of bioactive compounds from *Carissa edulis* roots. Composite Center Design (CCD), a widely used form of Response Surface Methodology(RSM), was used to investigate the effect of process variables on the Microwave–Assisted Extraction(MAE). Four independent variables including extraction time (s), microwave power (W), methanol concentration (%), solvent-to-material ratio (mL/g), were studied on the response (total phenolic compounds, total flavonoids, total antioxidant activity and 1,1-diphenyl-2-picrylhydrazyl assay (DPPH). Analysis of variance showed that all factors were significant at the 5% on the measured effects. The results showed that the optimal MAE condition was obtained with an extraction time of 67.75s, microwave power of 536.41W, a methanol concentration of 43.82% and a solvent-to-material ratio of 12.55ml/g for a combination of (total phenolic compounds (5482.15 µg Gallic acid equivalents/g of Matter (µg GAE/g)), total flavonoids (206.89 µg Quercetinequivalents/g of Matter (µg QE/g) total antioxidant activity (12094.5 µg equivalents of Ascorbic Acid/g of Matter (µg EAA/g) and DPPH radical scavenging (75.10%)). Close agreement between experimental and predicted values was found. This methodology could be applied in the extraction of bioactive compounds in the natural product of industry.
Keywords: *Carissa edulis*, microwave –assisted extraction, phenolic compounds, antioxidant activities.

INTRODUCTION

Carissa edulis belongs to the family Apocynaceae. It was formerly known as Carissa pubescence [1]. The plant is commonly known among Haoussa people in Northern Cameroon as "ya 'iré". The plants parts are used. The roots and fruits are edible: Fruits are sweet and pleasant to eat; vinegar can be made from them by fermentation. The root is used to impart an agreeable flavor to food and drinks [2]. They are put into water gourds to impart an agreeable taste and are added to soups and stews for the same reason. Health benefits of *Carissa edulis* roots may be attributed to its high level of phenolic compounds. Epidemiological evidences have suggested that food phenolic may have protective effects against degenerative diseases [3]. Most the beneficial characteristics of phenolic compounds have been ascribed to their antioxidant activity which is a fundamental property important to life [4]. We know that antioxidants are compounds that protect the body's cells against damage caused by free radicals. These are highly reactive molecules that are involved in the aging of the skin as well as in the development of cardiovascular disease [5]. Several techniques are currently available for the extraction of natural antioxidants from plants, including hydrodistillation, soxhlet and organic solvent extraction. However, these methods have several disadvantages, including long extraction time, potential loss of volatile constituents, degradation of compounds, high energy expense and few adjustable parameters to control the selectivity of the process [6]. Recently, interest in microwave-assisted extraction (MAE) has shown high extraction efficiency and low energy and solvent consumption. MAE is a process of using microwave energy to effectively heat solvents so that the analytes can be readily partitioned from the sample matrix into the solvent. MAE can significantly reduce both extraction time and solvent consumption [7]. In the present study, the applicability of MAE to the extraction of phenolic compounds in a root of *Carissa edulis* and optimized conditions for antioxidant activities as measured by the DPPH and antioxidant capacity assays were investigated using the composite center design (CCD) combined with the RSM. To the best of our knowledge, no work has been published on MAE of natural antioxidants from *C. edulis* species. In the present work, MAE of natural antioxidants from *Carissa edulis* was studied. The effects of the main operating parameters, namely extraction time, microwave power, methanol concentration and solvent-to-material ratio on the extraction of bioactive compounds of *Carissa edulis*

were investigated. Additionally, the antioxidant properties of the extracted under optimized conditions were evaluated by means of total polyphenolic contents, total flavonoids contents, DPPH, and antioxidant capacity.

MATERIALS AND METHODS

Sample materials and chemicals

The roots of *Carissa edulis* 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 and cut, and then ground using a mill (Model 14, Hamilton Beach, USA). The powder was kept in sealed polyethylene bags at room temperature.

Chemicals reagents

Gallic acid, ascorbic acid, Folin–Ciocalteu's phenol reagent, sodium acetate, ferric chloride hexahydrate, sodium phosphate monobasic, quercetine and methanol reagent were purchased from VWR. All other chemicals organics solvent used in the study were of analytical grade.

Microwave-assisted extraction process

Thirty five grams of *Carissa edulis* powder were placed in a tube and mixed with methanol. The extraction process was performed with domestic microwave (DAE WOO, KOG-360, Combi Grill) equipped with a digital timer and a power controller. After microwave extraction, the sample was filtrate with a wattman paper and the extract was evaporated by rotary evaporation to remove methanol. Three replicates were performed in each extraction. Samples (the extracts) were stored in 4°C prior to analysis. The extraction time, microwave power methanol concentration and solvent-to-material ratio were assessed as shown in the results.

Determination of total polyphenolic content (TPC)

TPC was determined using the Folin–Ciocalteau method. Extracts were mixed with 0.2 ml of Folin–Ciocalteau reagent (pre-diluted at a ratio of 1:16 with distillated 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 mg Gallic acid equivalents/sample (µg GAE/sample)[8].

Determination of total flavonoid content (TFC)

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, 0.1mg/ml). After ten minutes the optical densities were read at 415nm against the blank (0.5 ml of methanol extract solution and 0.5ml of methanol) and compared with the calibration curve of quercetine(0.1 mg/ml).

Antioxidant assay

1. 1,1-diphenyl-2-picrylhydrazyl assay (DPPH)

The DPPH radical scavenging capacity assay was based on a previously described method [9] with some modification. Briefly aliquots of each extracts (1 ml)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.

2. Antioxidant capacity by phosphomolybdenum method

Antioxidant capacities (AC) were determined by the method of [10]. An aliquot (0.4 mL) of the sample fractions was mixed with 2 mL of reagent solution (0.6 M sulphuric acid, 0.28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was covered and incubated at 95°C for 90 min. After the mixture was cooled, the absorbance was measured at 695 nm against blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for dissolving the sample, and it was incubated under the same conditions. The antioxidant activity was expressed as the absorbance value at 695 nm.

Experimental design

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 were extraction time (X_1: 50-70s)microwave power (X_2:350-530w) methanol concentration (X_3: 40–70%), and solvent-to-material ratio (X_4: 15/1–25/1 mL/g), while response variables were total phenolic content (Y_1), total flavonoid content (Y_2), antioxidant capacity (Y_3), and DPPH (Y_4). The selection and range of these four factors was based on our preliminary experimental data(data not shown). Each variable to be optimized was coded at three levels -1, 0, +1 (Table 1). Twenty-seven randomized experiments including four replicates as the center points were assigned based on CCD. The regression analysis of experimental data was performed to establish the empirical second order polynomial models. Shown in Eq.(1),

$$Y = b_0 + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum \sum_{j=i=1}^{4} b_{ij} X_i X_j$$

Where Yis the measured response variable (TPC, TFC, DPPH, and antioxidant capacity value), $\beta_0$is a constant, $\beta_i$is the linear coefficient (main effect) β_{ii} is the quadratic coefficient, β_{ij} is the two factors interaction coefficient, X_i and X_j are independent variables.

Verification of the model

Experimental data for the contents of phenolic compounds and antioxidant activities were obtained according to the recommended optimum conditions. The phenolic compounds and antioxidant activities were determined after extraction under optimal conditions. The experimental and predicted values were compared in order to determine the validity of the model.

Statistical analysis

Results were expressed as mean of three independent extractions. One-way analysis of variance (ANOVA) was used to compare the means. A response surface analysis and ANOVA were employed to determine the regression coefficients, statistical significance of the model terms and to fit the mathematical models of the experimental data that aimed to optimize the overall region for both response variables. Differences in the different responses were considered significant Atp< 0.05. Statistical analyses were performed with STATGRAPHICS Centurion XV.II. RSM was performed using Sigma plot.

RESULTS AND DISCUSSION

Table-1: CCD with the observed responses and predicted values for TPC, TFC, %DPPH and antioxidant capacity

N°	Level of variables				Observed responses				Predicted value			
	X_1	X_2	X_3	X_4	Y_{TPC} (mgEAG/gM	Y_{TFC} (µgEQ/gM)	$Y_{AC*1000}$ (µgEVC/gM)	Y_{DPPH} (%)	$Y_{TPC*100}$ (mgEAG/gMr	Y_{TFC} (µgEQ/gM)	Y_{AT} µgEVC/gM	Y_{DPPH} (%)
1	-1	-1	-1	1	3029,62	63,45	6155,24	57,18	2977,34	68,63	6022,01	54,71
2	0	1,6071	0	0	4970,37	141,91	15843,35	36,11	5041,91	153,53	14478,5	42,35
3	1	-1	-1	-1	4658,88	146,46	8190,20	67,30	4607,15	150,93	8161,33	63,61
4	1	1	1	1	3686,71	81,179	8125,87	44,74	3771,32	90,22	8067,3	44,01
5	0	0	-1,6071	0	5666,06	116,83	9681,81	67,73	5844,1	111,59	9700,17	72,72
6	0	0	0	0	5457,80	127,86	16875,79	48,18	5704,42	128,77	16934,7	45,29
7	1	1	1	-1	3792,63	183,70	9856,64	42,99	3673,05	168,59	10707,3	41,72
8	-1,6071	0	0	0	3536,80	95,28	6752,44	55,14	3538,29	112,41	6494,27	55,75
9	-1	-1	1	-1	3568,22	123,31	7653,84	31,27	3548,15	120,06	8519,99	34,00
10	0	0	0	0	5565,52	127,86	16234,96	48,18	5704,42	128,77	16934,7	45,29
11	1	1	-1	1	5870,73	120,75	10288,11	75,10	5718,94	114,07	10139,4	68,63
12	-1	-1	1	1	1862,65	114,18	10993,00	31,92	1904,04	106,93	10029,6	31,71
13	-1	1	1	-1	3298,92	152,10	8545,45	39,29	3473,71	153,49	8624,05	35,48
14	-1	1	-1	-1	4780,07	184,80	9155,24	62,49	4690,63	181,63	9621,66	60,44
15	-1	-1	-1	-1	4160,68	112,33	5955,94	58,54	4182,04	107,68	5802,57	58,14
16	0	0	0	1,6071	3859,96	73,98	14514,68	34,70	4051,27	85,96	15192,7	36,98
17	1	-1	1	1	3119,38	126,12	7531,46	41,87	3036,96	119,36	7782,47	40,17
18	1,6071	0	0	0	4742,36	160,44	8787,34	61,29	4842,95	151,90	8262,76	68,22
19	0	0	1,6071	0	3845,60	88,56	11019,86	30,97	3769,63	102,38	10218,7	33,53
20	-1	1	1	1	2692,99	98,73	7479,02	34,03	2572,87	84,32	8225,32	33,97
21	0	-1,6071	0	0	4012,56	120,56	10598,60	36,11	4043,1	117,52	11180,7	37,42
22	-1	1	-1	1	4236,98	92,10	8043,35	56,19	4229,2	86,54	7932,72	57,79
23	1	1	-1	-1	5116,69	206,71	13318,18	68,41	5181,27	218,35	14069,6	67,49
24	1	-1	1	-1	3568,22	131,741	8615,38	41,39	3681,96	141,69	8514,07	38,66
25	0	0	0	0	6229,80	137,97	17200	47,85	5704,42	128,77	16934,7	45,29
26	0	0	0	0	5709,15	133,57	16318,88	47,63	5704,42	128,77	16934,7	45,29
27	0	0	0	-1,6071	5029,62	183,70	18598,60	32,64	4940,38	180,31	17137,8	37,90
28	1	-1	-1	1	4470,37	99,69	6430,06	61,29	4401,55	102,69	6139,52	63,98

The effects of the four process variables, i.e extraction time (X_1: 50-70s) microwave power (X_2:350-530w) methanol concentration (X_3: 40–70%), and solvent-to-material ratio (X_4: 15/1–25/1 mL/g), were investigated during the study. These parameters, which gave the highest yield of antioxidant with desired antioxidant activities, were selected during the preliminary study. The four responses of interest were TPC, TFC, antioxidant capacity (AC) and %DPPH. The results of 27 runs using Composite Center Design (CCD) are shown in Table 1, 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 1863–6230 mg GAE/g powder and 63.45–206.7 µgQE/g powder, respectively. The highest TPC (6230mgGAE/g) was obtained under the experimental conditions of X_1=60s, X_2=440W, X_3=20/1 ml/g, X_4=55% whereas the highest TFC (206,7 µg QE/g powder) was obtained under conditions of X_1=70s, X_2=530W, X_3=15/1 ml/g, X_4=40%; A wide range of antioxidant activities were also found (% DPPH: 30.97–75.11%, AC 5955.94–18598.60 µg EVC/ g powder).The highest % DPPH (75.11%) was obtained under the experimental conditions of X_1=70s, X_2=530W, X_3=15/1 ml/g, X_4=70% whereas the highest antioxidant capacity AC (18600 µg EVC/g powder) was obtained under conditions of X_1=60s, X_2=440W, X_3=20/1 ml/g, X_4=31; Therefore, an optimization process was investigated, in order to obtain desirable phenolic contents and activities

Model fitting

The Statistical analysis of these data revealed a significant influence ($p< 0.05$) of all the studied variables on the extraction results (total phenolic and antioxidant activity). The fitted quadratic models for TPC, TFC, % DPPHsc, AC in coded variables are given in sum different equation. The significance of each coefficient was determined using the F-test and p-value in Table 2. The corresponding variables would be more significant if the absolute F-value becomes greater and the p-value becomes smaller [11].

$$Y_{TPC}= 5704.42 + 405.889\times X_1 + 310.737\times X_2 - 645.379\times X_3 - 276.609\times X_4 - 586.063\times X^2_1 + 16.3824\times X_1\times X_2 - 72.8232\times X_1\times X_3 + 249.776\times X_1\times X_4 - 449.831\times X_2^2 - 145.759\times X_2\times X_3 + 185.817\times X_2\times X_4 - 347.484\times X_3^2 - 109.852\times X_3\times X_4 - 467.903\times X_4^2$$

$$Y_{TFC} = 128.779 + 12.2874\times X_1 + 11.2028\times X_2 - 2.86587\times X_3 - 29.3528\times X_4 + 1.30796\times X_1^2 - 1.63272\times X_1\times X_2 - 5.4073\times X_1\times X_3 - 2.29986\times X_1\times X_4 + 2.61295\times X_2^2 - 10.1299\times X_2\times X_3 - 14.0098\times X_2\times X_4 - 8.43598\times X_3^2 + 6.47823\times X\times X_4 + 1.68858\times X_4^2$$

$$Y_{DPPH} = 45.2923 + 3.87762\times X_1 + 1.53451\times X_2 - 12.1927\times X_3 - 0.285896\times X_4 + 6.46439\times X_1^2 + 0.393962\times X_1\times X_2 - 0.20177\times X_1\times X_3 + 0.948935\times X_1\times X_4 - 2.09141\times X_2^2 - 0.204726\times X_2\times X_3 + 0.194678\times X_2\times X_4 + 3.03406\times X_3^2 + 0.286829\times X_3\times X_4 - 3.03689\times X_4^2$$

$$Y_{AC} = 16934.7 + 550.187\times X_1 + 1025.98\times X_2 + 161.334\times X_3 - 605.136\times X_4 - 3699.65\times X_1^2 + 522.29\times X_1\times X_2 - 591.171\times X_1\times X_3 - 560.315\times X_1\times X_4 - 1589.28\times X_2^2 - 928.759\times X_2\times X_3 - 477.098\times X_2\times X_4 - 2700.45\times X_3^2 + 322.552\times X_3\times X_4 - 297.887\times X_4^2$$

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

SOURCE	SUM OF SQUARE	DF	MEAN SQUARE	F VALUE	P VALUE
TPC					
X_2	2,60415E6	1	2,60415E6	22,26	0,0180
X_1X_1	4,58317E6	1	4,58317E6	39,18	0,0082
X_2X_2	2,70009E6	1	2,70009E6	23,08	0,0172
X_3X_3	1,61119E6	1	1,61119E6	13,77	0,0340
X_4X_4	2,92139E6	1	2,92139E6	24,97	0,0154
LACK OF FIT	237588,	10	23758,8	0,20	**0,9764**
PURE ERROR	350928,	3	116976,		
TOTAL(Corr)	3,05434E7	27			
TFC					
X_3	1770,68	1	1770,68	73,50	0,0033
X_4	2882,61	1	2882,61	119,66	0,0016
X_1X_3	467,823	1	467,823	19,42	0,0217
X_2X_3	1641,84	1	1641,84	68,15	0,0037
X_2X_4	3140,41	1	3140,41	130,36	0,0014
X_3X_3	949,618	1	949,618	39,42	0,0082
X_3X_4	671,479	1	671,479	27,87	0,0133
LACK OF FIT	1947,43	10	194,743	8,08	**0,0560**
PURE ERROR	72,2697	3	24,0899		
TOTAL	33432,3	27			

DPPH					
X_1	47,5929	1	47,5929	655,42	0,0001
X_2	56,6662	1	56,6662	780,37	0,0001
X_4	1,01379	1	1,01379	13,96	0,0334
X_1X_1	557,614	1	557,614	7679,10	0,0000
X_1X_2	2,4833	1	2,4833	34,20	0,0100
X_1X_4	14,4076	1	14,4076	198,41	0,0008
X_2X_2	58,3658	1	58,3658	803,78	0,0001
X_3X_3	122,836	1	122,836	1691,62	0,0000
X_3X_4	1,31634	1	1,31634	18,13	0,0238
X_4X_4	123,066	1	123,066	1694,78	0,0000
LACK OF FIT	292,877	10	29,2877	403,33	**0,0002**
PURE ERROR	0,217844	3	0,0726145		
TOTAL	4691,49	27			
AC					
X_2	3,4007E7	1	3,4007E7	160,62	0,0011
X_3	2,02428E7	1	2,02428E7	95,61	0,0023
X_4	5,08393E6	1	5,08393E6	24,01	0,0163
X_1X_1	1,82642E8	1	1,82642E8	862,66	0,0001
X_1X_2	4,36459E6	1	4,36459E6	20,62	0,0200
X_1X_3	5,59174E6	1	5,59174E6	26,41	0,0143
X_1X_4	5,02324E6	1	5,02324E6	23,73	0,0165
X_2X_2	3,37039E7	1	3,37039E7	159,19	0,0011
X_2X_3	1,38015E7	1	1,38015E7	65,19	0,0040
X_2X_4	3,64196E6	1	3,64196E6	17,20	0,0255
X_3X_3	9,73083E7	1	9,73083E7	459,61	0,0002
LACK OF FIT	1,00714E7	10	1,00714E6	4,76	**0,1130**
PURE ERROR	635156,	3	211719,		
TOTAL	3,96623E8	27			

The coefficient of determination (R) of the model DPPH was 0.9375
The coefficient of determination (R2) of the model AC was 0.97306.
The coefficient of determination (R2) of the model TPC was 0.9607.
The coefficient of determination (R2) of the model TFC was 0.9395.

1. TPC, TFC

It can be seen that a factor X_2, different quadratic term X_1X_1, X_2X_2, $X_3X_3X_4X_4$ have an effect on extraction yield of TPC(table 2). These results suggest that the change of time, power, ratio liquid solid, and methanol had significant effect ($p \leq 0.05$) on the yield of TPC.

According to TFC the factor X_3, X_4 interaction $X_1X_3X_2X_3X_2X_4$ X_3X_4 and quadratic term X_3X_3 have an effect on extraction yield.(table 2), because they had significant effect ($p \leq 0.05$) on the yield of TFC

The coefficient of determination (r^2) of the predicted models in TPC, TFC were 0.9607, 0.9395 respectively whereas p-value for lack of fit were 0.9764, 0.0560. The predicted models can reasonably represent the observed values Thus, the responses were sufficiently explained by the models.

2. Antioxidants assay (AC, DPPH)

In term of antioxidant activities, it can be observed that all factors except a time, all quadratic time except a methanol concentration, an interaction X_1X_2,X_1X_3,X_1X_4, X_2X_3,X_2X_4 gave a significant effect ($p \leq 0.05$).According to DPPH the factor $X_1X_2X_4$, quadratic term X_1X_1 $X_2X_2X_3X_3$ X_4X_4 and the interaction X_1X_2 ,X_3X_4 have an effect on extraction yield. The coefficient of determination (r^2) of the predicted models in AC, DPPH were 0.9375 and 0.97306 respectively. The fitness of the model was investigated through the lack of fit test ($p > 0.05$), which indicated the suitability of models to accurately predict the variation. However p-value of DPPH for lack of fit was 0.0002 which suggesting not a good fit to the model.

Interpretation of response surface model

3D response surfaces and the contour plots of TPC, TFC and AC are given inFig. 1 3D plot of DPPH was not given due to the lack of fit to the model.

1. TPC

3D response surfaces and contour plot shown in Fig-1. As the extraction and separation of phenolic compounds depends largely on the polarity of solvents and the compounds, a single solvent might not be effective for the isolation of a bioactive compound [12]. Hence, a combination of alcohol with water is more effective in extracting phenolic compounds than alcohol alone [13]. When methanol concentration increased from 50% to 69%, increase in the phenolic content from 4800 to 5600 mg GAE/g, was observed (Fig. 1a). This is probably due to the increased solubility of phenolic compounds in the mixture of methanol and water [14, 15]obtained high phenolic content when 42% ethanol

was used to extract polyphenols from *Myrtus communis* L. leaves. However, when methanol concentration was higher than 68% in the present investigation, the phenolic content decreased.This may be attributed to the difference in dielectric properties of the solvent towards microwave heating, because it plays an important role in microwave extraction, facilitating heat distribution throughout the sample [15]

The total phenolic content decreased with increasing liquid-tosolid ratio.(Fig 1.a) Lower solvent-to-material ratio (16:1–19:1), resulted in lower TPC ranging from 4500-5000 µg GAE/g. With further increase in solvent-to-material ratio, a decline in TPC content was observed (Fig. 1b).[16]. Reported liquid/solid ratio (20 mL/g) played a significant role in the yield of phenolic, while extraction temperature did not make any significant contribution towards TPC.

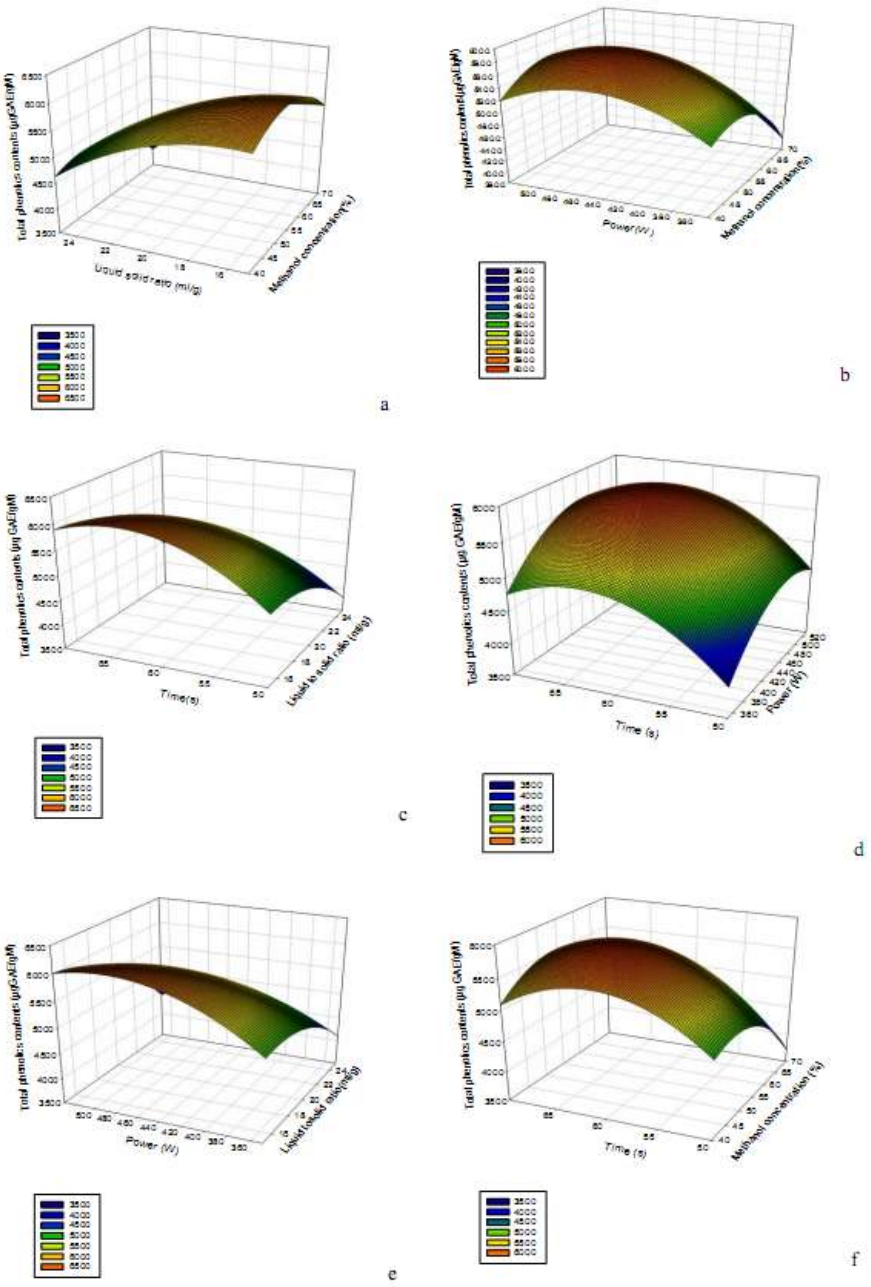

Fig. 1: Three dimensional responses surface model of total polyphenol content

2. TFC

A liquid to solid ratio is a factor that would influence the extraction efficiency of flavonoids contents. Fig. 2a presents the interaction of methanol concentration and solvent-to-material ratio. The increased extraction yield of total flavonoids was observed with an increased solvent-to-material ratio from 20 to 23 ml/g. This is probable due to the fact more solvent can enter cells while more phenolic compounds can permeate into the solvent under the higher solvent-to-material ratio conditions [17].

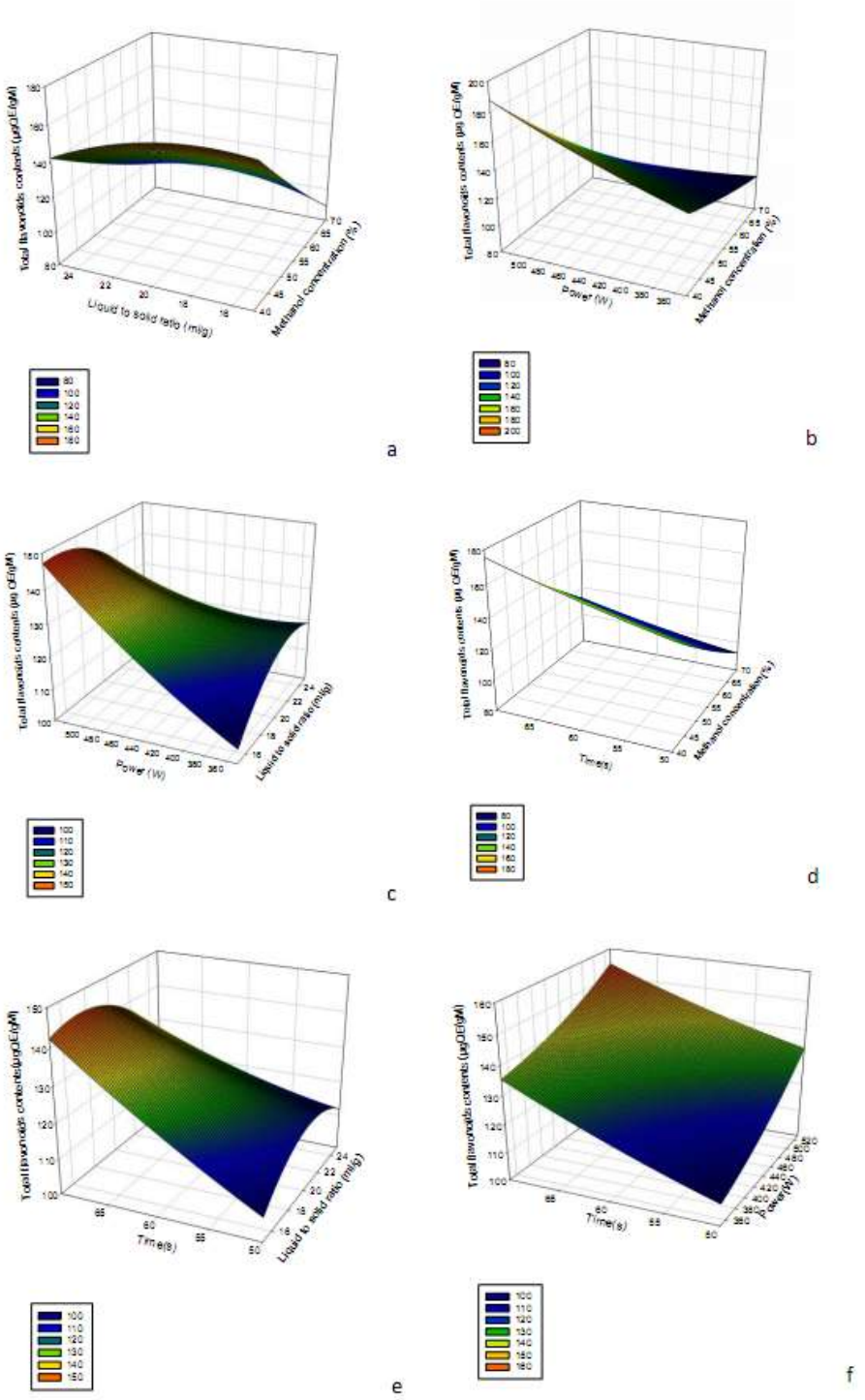

Fig. 2: Three dimensional responses surface model of total flavonoids contents

3. AC

As evident in Fig. 3, the AC value increased as the methanol concentration increased. This suggests an enhanced degree of breakage of cell membranes in the raw material by the increasing methanol concentrations. Increasing extraction power led to a gradual increase in the AC value over time. This phenomenon is considered to be caused by the low rate of mass transfer, which would require more time for the phenolic compounds to dissolve from the raw materials into the solution (Fig. 3b). At higher power, however, dissolution of the phenolic compounds can reach the equilibrium in a shorter time thus are not readily affected by changes in the extraction time. This suggests that a higher microwave power and a short extraction time are more effective in extracting antioxidant phenolic compounds from roots of *Carissa edulis* using MAE. This is favorable as the extraction time is extended at a higher microwave power may lead to thermal degradation of the phenolic compounds.

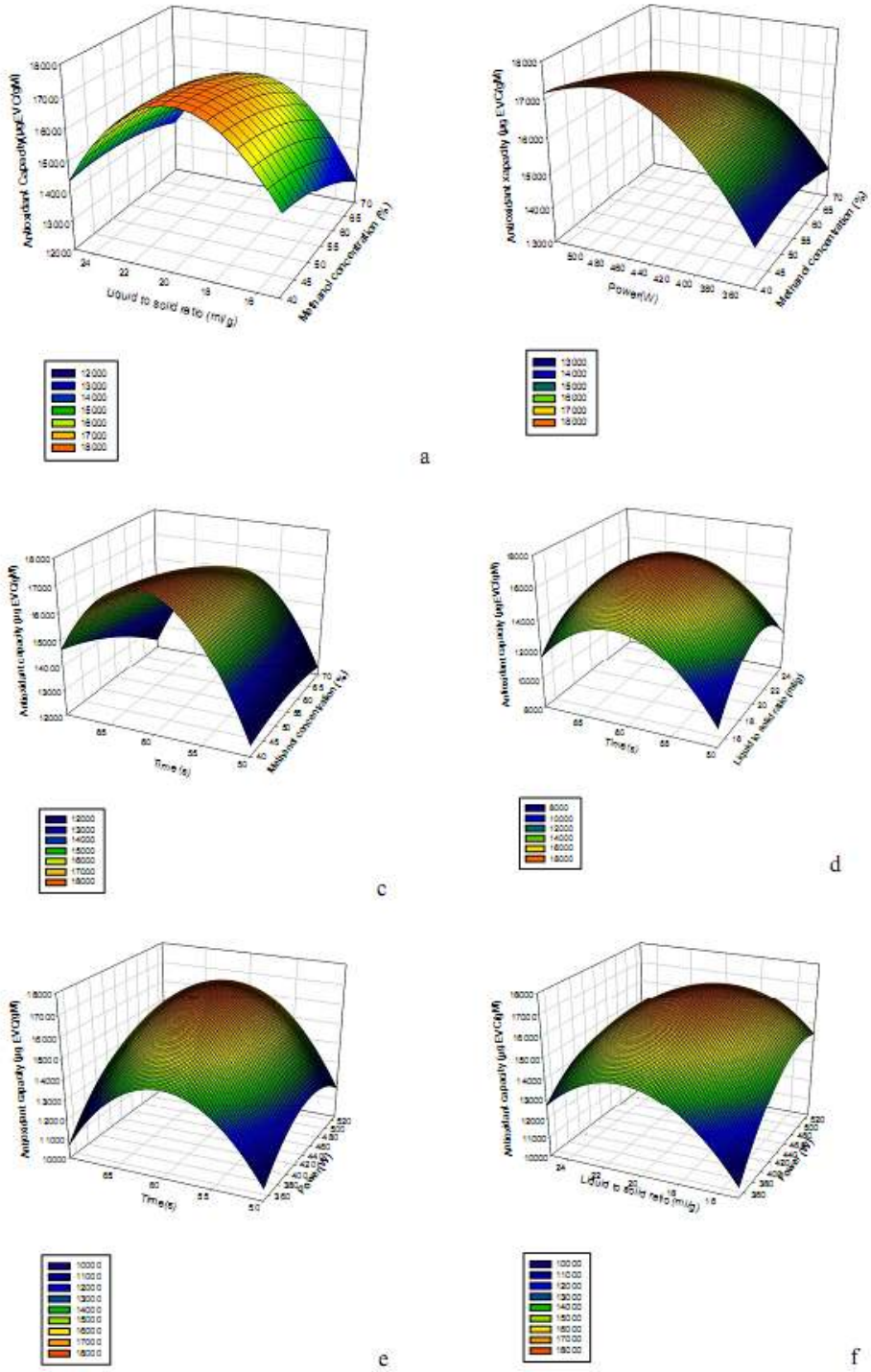

Fig 3: Three dimensional responses surfaces model of antioxidant capacity

Optimization of phenolic and antioxidant capacity and verification of the models

To obtain an extract with high phenolic and antioxidant capacity, the optimal level of extraction parameters were generated based on four single response variables and their combination. Numerical optimizations were run for determining the optimum levels of independent variables with desirable response goals. (Table 3)

Table 3: Combination of parameters for optimization for model PT, FT, AAT and % DPPH

Factors	Level		Optimal	
	Low	**Hight**	**Coded variables**	**Real Variables**
Time (s)	-1,60717	1,60717	0,775048	67.75
Microwave Power (W)	-1,60717	1,60717	1,16041	536.41
solvent-to-material ratio (ml/g)	-1,60717	1,60717	-1,48961	12.55
Methanol Conc. (%)	-1,60717	1,60717	-0,74535	43.82

The response surface models were verified using experimental and predicted values. No significant difference ($p > 0.05$) was found between the experimental and the predicted values for phenolic content (E= 0.017%) and total antioxidant activity (E= 0.731%). The antioxidant activity of *Carissa edulis* extract was in moderate agreement with the amount of phenolic found in it. Statistical correlations between total phenolic content and antioxidant capacity were determined (R^2=0.5902). A similar finding of low correlation between total phenolic and antioxidant activity was reported[18]. Thus, the content of phenolic compounds in the present study could not be used as an indicator of antioxidant capacity. There are several antioxidant compounds like flavanoids, carotenoids and polysaccharides which are not determined in the present study, which might also contribute to antioxidant activity.

CONCLUSION

In this work, it was a question for us to study the optimization of solvent extraction of bioactive substances from the roots of *Carissa edulis* plant by evaluating the antioxidant properties of crude extracts through the centered composite design. This study showed that the effect of microwave power and the time have a considerable influence on the extraction of polyphenol and antioxidant activities. Optimal conditions for releasing the maximum TPC and TFC have a AC and the highest DPPH inhibition are 67.75s, 536.41W, 12.55ml/g and 43.82% of methanol. Results showed that predicted and experimental values were not significantly different. To conclude, MAE is a suitable process for rapid extraction of bioactive compounds and this methodology could be applied in the extraction of bioactive compounds in the natural product industry.

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