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Response Surface Optimization of Bioactive Compounds Extraction from *Buchholzia coriacea* Using D-Optimal Design

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Abstract

This study investigates the use of D-optimal design and response surface methodology for the optimization of bioactive compound extraction from Buchholzia Coriacea. After thorough sample preparation, the Soxhlet apparatus was employed to extract the bioactive compound from the dried Buchholzia coriacea seed in a batch-wise process. The D-optimal design was employed to generate 23 sets of experimental runs to determine the effect of temperature, extraction time, and the weight of the dried sample on the extraction yield. The temperature, extraction time, and the weight of the dried sample were found to significantly influence the extraction yield resulting in a maximum value of 41%. Optimum conditions of 80°C, 20 g, and 50 min representing temperature, the weight of the dried sample, and extraction time were obtained using the desirability analysis.

1. Introduction

Buchholzia coriacea also known as wonderful kola is a medicinal plant frequently used in traditional medicine across Tropical Africa [1]. Studies have shown that *Buchholzia coriacea* possesses medicinal properties that can be used as antimicrobial, anthelmintic, anti-trypanosomal, hypoglycaemic, and analgesic [2]. The plant has been widely investigated due to its several medicinal properties [3,4]. The antimicrobial properties of the *Buchholzia coriacea* seed have been reported by Ezekiel and Onyeoziri [5]. Hexane and methane were employed to extract the active components of the *Buchholzia coriacea* by soxhlet oil extraction method. The inhibitory properties of the extracts were tested using test organisms. The results revealed that the extracts displayed an inhibitory zone with the test bacteria. In a similar study reported by Umeokoli et al. [6], antimicrobial, anti-inflammatory, and chemical evaluation of *Buchholzia coriacea* were investigated. The extraction of the active components was performed using cold maceration and hexane. The study revealed that the aqueous extract recorded more pharmacological activities compared to n-hexane extracts of *Buchholzia coriacea* seeds. The analgesic and anti-inflammatory effects of *Buchholzia coriacea* seed extracts have been investigated by Olaleye et al. [7]. The study revealed that the ethanol extract of the *Buchholzia coriacea* seed shows a significant anti-inflammatory response using a cotton pellet test. Mbata et al. [8] employed methanol as the extracting solvent to obtain the active compound in *Buchholzia coriacea* seed. The result revealed that the extracts inhibited the growth of the bacterial isolates at varying concentrations. Besides the *Buchholzia coriacea* seed extract, the medicinal properties of the leave extract of *Buchholzia coriacea* plant have also been investigated. Ejikeugwu et al. [6] revealed that the extracts of *Buchholzia coriacea* leave displayed antibacterial properties by inhibiting pathogenic strains of S. aureus. Based on existing literature, there are myriads of studies on the use of various types of solvent to extract the active compounds in *Buchholzia coriacea*. Besides, the medicinal properties and potentials of these active compounds have been extensively investigated [1,2,9]. However, there is dearth of studies on the optimization of the extraction of the active compounds which is the main focus of this study. An optimal design and response surface methodology was employed to maximize the extract yields from *Buchholzia coriacea* seed.

2. Methodology

2.1 Materials

Fresh *Buchholzia coriacea* seed was obtained locally from Oregbeni market, Ugbowo Benin City, Edo State. The clean seeds were cut into a smaller piece to facilitate its drying. The seeds were dried continuously under sunlight for 3 weeks to reduce the moisture content and prevent further breakdown of materials by microbial action. The hull was then separated from the seed in other to increase the exposed surface to increase the drying rate.

2.2 Methods

The D-optimal experimental design was employed in other to optimize bioflavonoid extraction from the *Buchholzia coriacea* seed. A three-level-three-factor design was applied, which generated 23 experimental runs [10]. This included 6 factorial points, 6 axial points, and 5 central points to provide information concerning the interior of the experimental region, making it possible to evaluate the curvature effect [11]. Selected extraction process factors for the bioflavonoid separation from garcinia kola seed were mass of B. coriacea (g), extraction time (min), and extraction temperature (ºC). The details of the design values are summarized in Table 1 The Design Expert 7.0 (Stat-case, Inc. Minneapolis, USA), was used to develop the experimental design using the D-optimal Design. Soxhlet apparatus was employed to extract the bioactive compound from the dried *Buchholzia coriacea* seed in a batch-wise process [12]. For a normal run, 20 g of dried *Buchholzia coriacea* seed in powdery form was placed in the extractor section of the soxhlet apparatus with 300 ml of ethanol added. The experimental runs for that batch for conducted at 30ºC for 80 min. The vapour containing the bioactive component was trapped at the soxhlet extractor. The extract was collected from the distillation flask and subsequently analyzed using GC-MS to determine the various components.

Table 1: Details of the D-optimal experimental design parameters

Factor	Name	Units	Type	Low Actual	High Actual	Levels:	
A	Temperature	ºC	Categoric	30	80	3	
B	Weight of dried sample	g	Categoric	20	70	3	
C	Extraction Time	min	Categoric	50	80	3	
Response	Name		Analysis	Minimum	Maximum	Mean	Std. Dev.
Y1	Extraction yield	%	Factorial	35	40.89	37.1975	2.02951

3. Results and Discussion

3.1 Model performance

The experimental runs using the combinations of the three parameters (temperature, extraction time, and weight of the dried samples) produces different values of the extraction yield as shown in Table 2. This implies that the variation in the process conditions significantly influences the extraction yield. The three-dimensional plots and the interaction plots of the process variables are displayed in Figure 1. It can be seen that the extraction time, temperature, and weight of the dried sample played a significant role in the extraction of the active compound from the *Buchholzia coriacea* seed. As shown in Figure 1 (a), The maximum extraction yield of 41% tends to be favoured at extraction time and temperature of 80 min and 80 ^oC. The interaction effects between the extraction time and the temperature did not make much impact at all points. On the contrary, there is a more effect of interaction between the extraction time and the weight of the dried sample as shown in Figure 1 (b). the combination of the 70 g of the dried sample and 80 min of the extraction time produces the maximum effect on the extraction yield. The analysis of variance (ANOVA) summarized in Table 3 revealed that the individual effects of the temperature, weight of the sample and extraction time on the extraction yield are significant since the p-value is less than 0.005. Moreover, the interaction effects between the three parameters are also significant. AN R^2 of 0.999 obtained for the model implies that over 90% of the datasets can be described by the optimization model. The desirability analysis to determine the optimum condition is depicted in Figure 2 and the detail summarized in Table 4. The desirability analysis helps to rank the optimization solution according to their importance [13]. Based on the analysis, a maximum effect on the yield will be obtained at 80^{o}C, 20 g of the dried sample, and 50 min. These conditions can be validated and employ to design an extractor that can maximize the extraction yield of *Buchholzia coriacea* seed.

Table 2: Actual design of D-optimal approach of RSM optimization techniques

Temperature(^{o}C)	Weight of sample(g)	Extraction Time(min)	Extraction yield (%)
30	20	80	36.32
80	70	80	40.89
50	20	50	35.02
80	20	80	36.98
80	20	80	36.98
50	20	50	35.02
80	70	80	40.74
50	70	50	35.87
50	70	50	35.99
80	70	80	40.75
80	70	80	40.89
50	60	70	36.57
80	50	80	38.79
80	30	80	38.79
80	20	80	36.47
30	20	50	35.00
45	20	50	35.33
50	20	60	35.99
80	30	70	36.11
65	20	50	35.53
70	70	70	36.24
50	70	60	35.62
65	50	80	37.29

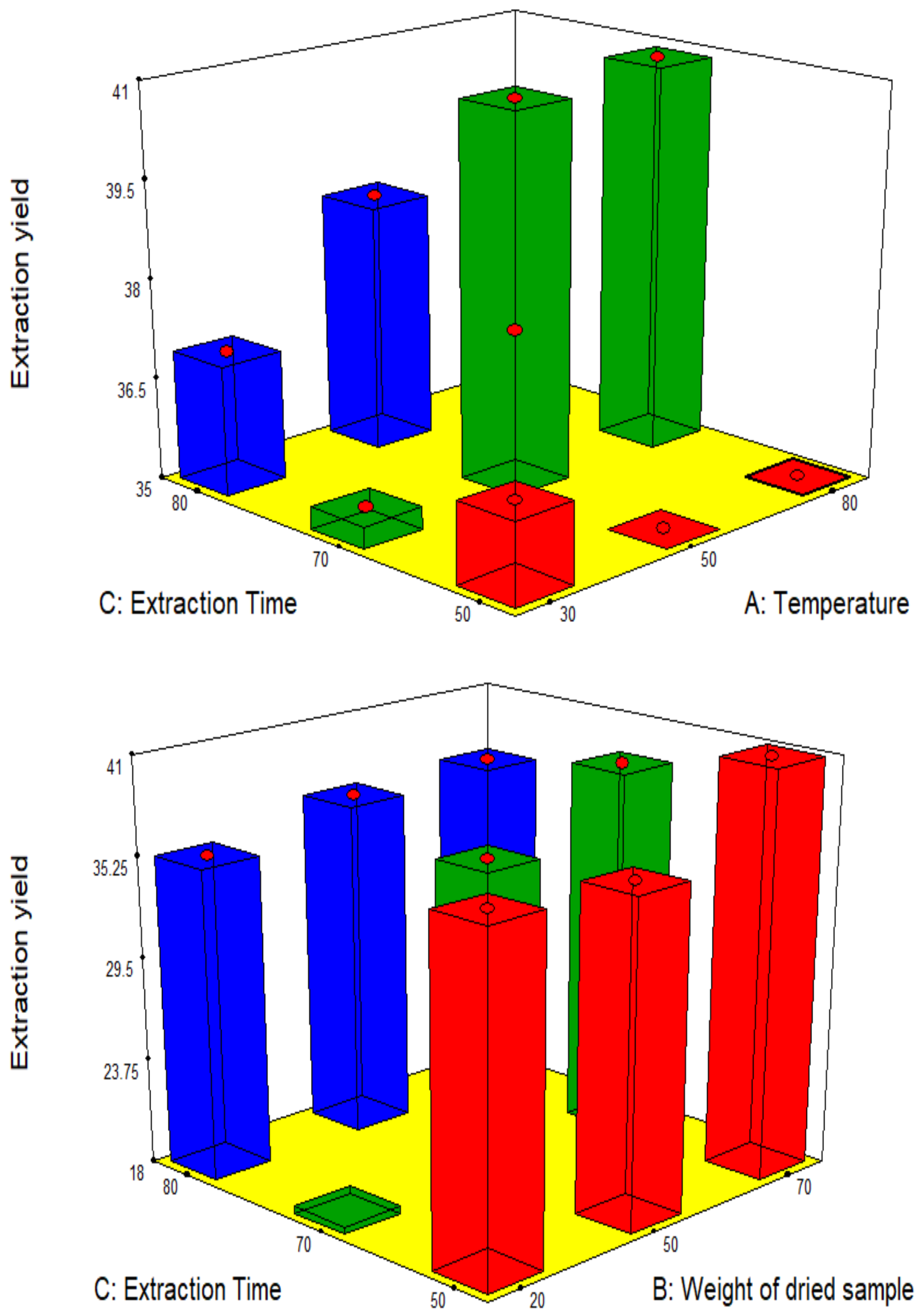

Figure 1: Interaction effect of (a) extraction time and temperature (b) extraction time and weight of dried sample on the extraction yield

Table 3: Analysis of Variance of the D-Optimal design optimization

Source	Sum of Squares	Df	Mean Square	F-Value	P-valueProb > F
Model	94.73485	23	4.118906522	176.87	<0.0001
A-Temperature	8.272506667	2	4.136253333	278.21	<0.0001
B-Weight of dried sample	0.912557658	2	0.456278829	1261.22	<0.0001
C-Extraction Time	1.122026667	2	0.561013333	22.65	<0.0001
AB	33.89302372	4	8.473255929	11.89	0.0180
AC	18.59411705	4	4.648529263	25.21	<0.0001
BC	46.70602372	4	11.67650593	27.56	<0.0001
ABC	9.019284615	5	1.803856923		0.0598
Pure Error	0	0		2.56	0.4583
Cor Total	94.73485	23		156.87	<0.0001
R-Squared	0.999				

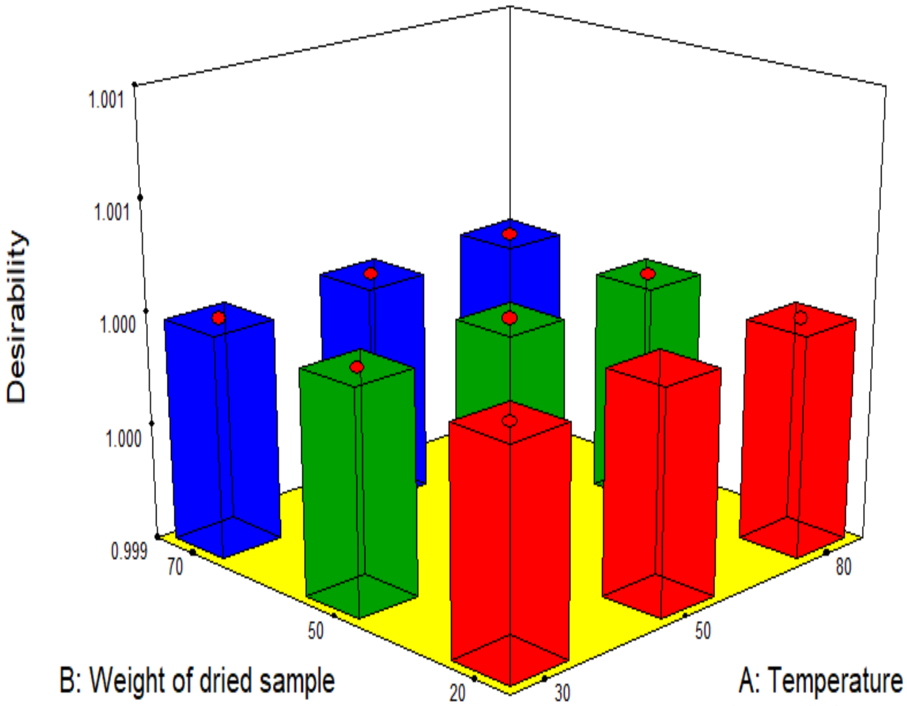

Figure 2: Desirability analysis of the D-optimal design

Table 4: Desirability analysis to obtain the optimum conditions

Number	Temperature (°C)	Weight of dried sample (g)	Extraction Time (min)	Desirability	
1	80	20	50	1	Selected
2	50	70	70	1	
3	80	70	50	1	
4	30	50	70	1	
5	30	70	80	1	
6	30	20	50	1	
7	50	20	80	1	
8	50	50	80	1	
9	80	20	70	1	
10	80	70	70	1	
11	50	70	50	1	
12	30	70	70	1	

3.2 GC analysis of the *Buchholzia coriacea* seed

The GC-MS analysis of the *Buchholzia coriacea* seed extracts based on the chromatogram is depicted in Figure 3. The chromatogram displays the evidence of 16 various peaks representing different bioactive compounds summarized in Table 5. Some of the bioactive compounds identified from the *Buchholzia coriacea* seed extract include Beta-vinyl acrylic acid, 2 methyl perolidine-2-carboxylic acid, hexadecenoic acid, and so on. These bioactive compounds have been reported to possess various medicinal properties [1, 2, 6].

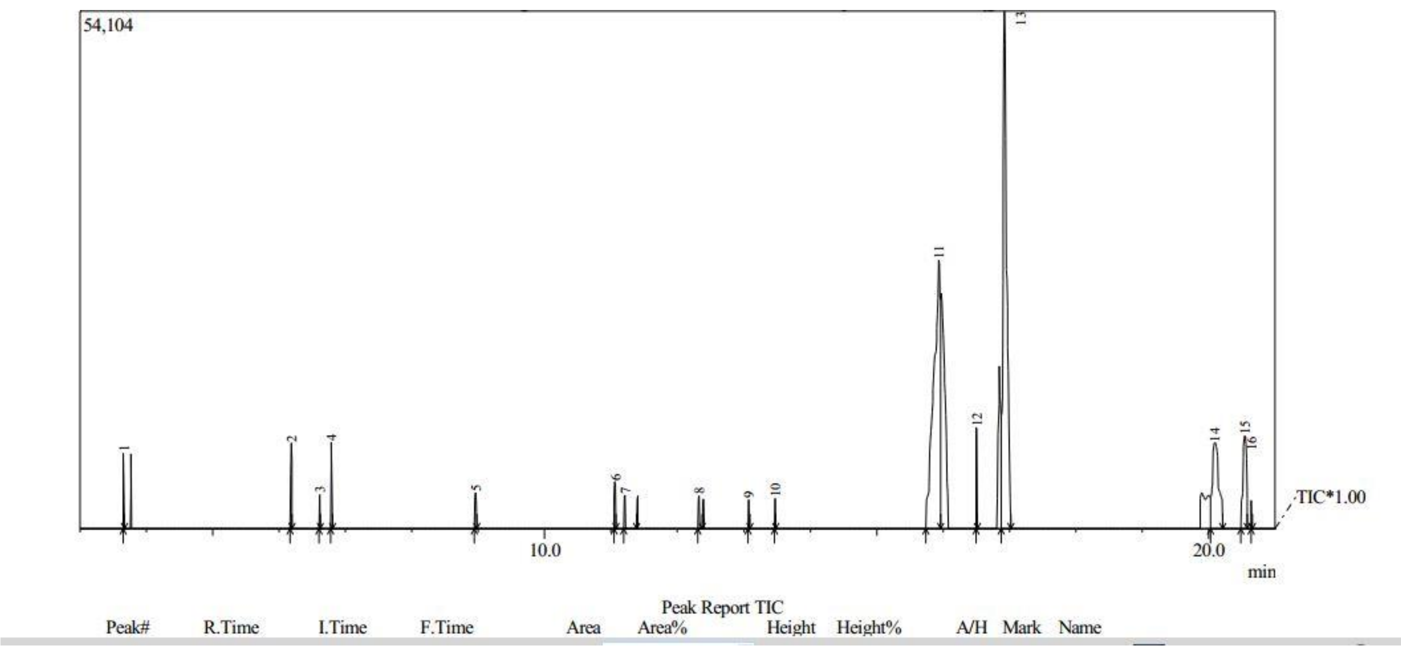

Figure 3: GC chromatogram of the bioactive compounds extract from the *Buchholzia coriacea* seed

Table 5: Details of the GC analysis of the bioactive compounds in the *Buchholzia coriacea* seed. extract

S/No	Name of Compound	Retention time	Peak Area (%)	Molecular weight	Molecular formula	Bioactivity
1	Beta-vinyl acrylic acid	3.389	10.62	98.09	$C_5H_6O_2$	Antiamyloid-Beta, Beta-2-Receptor-Agonist, Beta-Glucuronidase-Inhibitor
2	2-Methyl-pyrrolidine-2-carboxylic acid	6.059	33.60	129.15	$C_6H_{11}NO_2$	Methyl-Guanidine-Inhibitor
3	1,2-Benzenedicarboxylic acid, diethyl ester	11.952	0.89	222.23	$C_{12}H_{14}O_4$	Acidifier
4	14-ketopentadecanoic acid	15.449	0.68	256.38	$C_{15}H_{28}O_3$	Acidulant
5	Methyl 14-methylpentadecanoate	15.890	1.10	270.45	$C_{17}H_{34}O_2$	Catechol-O-Methyltransferase-Inhibitor
6	Norlinolenic acid	16.317	4.26	264.40	$C_{17}H_{28}O_2$	Increase Aromatic Amino Acid Decarboxylase Activity
7	Hexadecanoic acid	16.544	13.26	256.42	$C_{16}H_{32}O_2$	Arachidonic acid-Inhibitor
8	9,12-Octadecadienoic acid	18.251	5.64	294.47	$C_{19}H_{34}O_2$	Inhibit Production of Uric Acid
9	9-Octadecynoic acid	19.258	9.84	280.44	$C_{18}H_{32}O_2$	Urine-Acidifier
10	Linoelaidic acid	19.406	12.87	280.44	$C_{18}H_{32}O_2$	Acidifier
11	Micropine	22.040	1.02	265.39	$C_{16}H_{27}NO_2$	Not found
12	*Anandamide*	24.783	6.22	323.28	$C_{20}H_{37}NO_2$	Neurotransmitter

4. Conclusion

The use of response surface methodology and D-optimal design for optimization of extraction yield of bioactive compounds in *Buchholzia coriacea* seed has been investigated. Based on the analysis of 23 experimental runs generated using the D-Optimal design, temperature, weight of the dried samples, and extraction time were found to have significant individual effects on the extraction yield. These parameters were also observed to have interaction effects on the extraction yield. The ANOVA results revealed that all the effects were significant since the p-values were less than 0.05. The desirability analysis of the response surface model revealed that a maximum extraction yield of 41% can be obtained at optimum conditions of 80°C, 20 g, and 50 min representing temperature, the weight of the dried sample, and extraction time, respectively.

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