Ultrasonic-assisted rapid extraction of *Cassia sieberiana* D.C.: a Box-Behnken design process optimisation

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Abstract: Ultrasonic-assisted extraction (UAE) on the *Cassia sieberiana* root was optimised by a three-factor-three-level Box-Behnken design (BBD). A single factor experiment revealed the logic ranges for the UAE parameters for extraction time, temperature and solvent to sample ratio were 10–60 min, 30–60°C and 10–30 mL/g, respectively. The BBD showed that a second-order polynomial model produced a satisfactory fitting of the experimental data (R^2 = 0.9917, $P < 0.001$) with regards to extraction yield. The optimal UAE conditions were 29 min, 45°C and 16.79 mL/g. Under the optimised conditions, the UAE yielded 1.28 ± 0.7% of *C. sieberiana* root extract as to 0.69 ± 1.1% obtained by maceration and 0.82 ± 0.5% obtained by Soxhlet extraction. The results affirmed that the BBD was suitable in establishing the optimum UAE conditions for a maximum extraction yield while being more efficient and rapid than conventional methods (maceration and Soxhlet extraction).

Keywords: Box-Behnken; *Cassia sieberiana*; UAE; ultrasonic-assisted extraction; Soxhlet extraction; optimisation; temperature; design; solvent; polynomial.

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1 Introduction

Cassia sieberiana D.C. (Fabaceae) commonly known as 'drumstick' in English, is a plant that is widely distributed across the African continent. The plant is typically used by traditional herbalists to treat a plethora of ailments such as diabetes, malaria, ulcer, fever, stomach ache, gonorrhoea and piles, as well as for wound healing (Toma et al., 2009). Specifically, extracts of the *C. sieberiana* roots have been found to have therapeutic and bioactive properties which include antioxidant, antimalarial and laxative effect (Nartey et al., 2012; Jibril et al., 2017a), originating from the presence of an array of secondary metabolites *viz.* flavonoids, stilbene and anthraquinones (Jibril et al., 2017b). For these reasons, the *C. sieberiana* root extracts have found potential applications in the biomedical field for treatment of diabetes, cardiovascular, inflammatory and several cancer diseases (Jibril et al., 2017b; Khala et al., 2014). The root extracts are also valuable as a bioactive component in formulations for cosmetics and agriculture preparations, as well as food additives (Mavituna, 1992).

Nonetheless, bioactivity of plant-based extracts are largely influenced by the chemical and physical characteristics, in particular, the quantity and quality of certain chemical groups in secondary metabolites of the extracted crude suspension (Majeed et al., 2016). Retention of bioactivity in these plant components may be impacted by the techniques used for their extraction. Given these circumstances, selection of an appropriate extraction method in consort with an optimised extraction protocol may prove useful in ensuring retention of quality (high bioactivity) of the plant-based extract (Sarker and Nahar, 2012), i.e., the extract of *C. sieberiana* root. In this study, an extraction protocol which explores the use of ultrasonic-assisted extraction (UAE) that would maximise the extraction yield of *C. sieberiana* root while minimising duration and cost of the process was developed.

The different conventional techniques reported, so far, to extract bioactive compounds in the root of *C. sieberiana* have mainly been techniques by maceration and Soxhlet extraction (Abdullahi et al., 2013; Kpegba et al., 2011). The problems when using such conventional methods is that such physical treatments, require lengthy extraction times, lasting between 24–72 h and consume large quantities of organic solvents, thus the large-scale extraction of *C. sieberiana* root will be laborious and cost-prohibitive (Wang and Weller, 2006). Aside to the high energy cost, an extraction process performed under an elevated temperature especially for an extended duration may degrade the extracted bioactive components (Cacace and Mazza, 2003). These major challenges must first be overcome before the root extracts of *C. sieberiana* can be considered for extended applications in medical and pharmaceutical industry. The study believes the UAE technique can potentially be used to improve as well as complement current extraction techniques to acquire high extraction yield in *C. sieberiana* root. To the best of our knowledge, studies focusing on the use of UAE for obtaining extract from

C. sieberiana root remain unreported. Furthermore, feasibility of this technique and the establishment of the best protocol for efficacious extraction yield in *C. sieberiana* root would constitute a useful study.

UAE technique is gaining popularity among researchers for extracting bioactive substances in plant materials, since the technique is regarded amongst the simplest and inexpensive extraction systems. The technique is also operable in a broad range of solvents for large-scale preparations. UAE relies on the use of ultrasonic sound waves that create cavitation in the liquid medium which consequently cause the collapse of the cavitation bubbles close to the plant material surface. This results in the build-up of pressure and temperature in the surrounding liquid that disrupt the cell walls of the plant matrix, thus releasing the extractive compounds (Kuo et al., 2014). The ultrasonic-assisted cell-wall disruption process enhances penetration of the extraction solvents into plant cells, as well as increases solubility of the plant constituents, resulting in improved extraction yield along with a higher yield in bioactive components of the extracted plant sample (Wang and Weller, 2006). Nonetheless, UAE conditions for maximum extraction yield may vary greatly from one plant to another (Hussain et al., 2012). In our study, key UAE parameters considered for efficient extraction of *C. sieberiana* root included extraction temperature, extraction time and solvent to sample ratio, following several suggestions in a previous literature (Kuo et al., 2014; Ebrahimi-Najafabadi et al., 2014; Esclapez et al., 2011; Prado et al., 2017).

The traditional way in dealing with optimisation of extraction parameters can be quite unreliable, prone to misinterpretation of response and time consuming (Ebrahimi-Najafabadi et al., 2014). In this regard, the study resorted to using the method of response surface methodology (RSM) as an optimisation tool. RSM is a mathematical and statistical technique that has been successfully applied to assess the possible interactions and to optimise experimental factors in various processes (Kuo et al., 2014; Manan et al., 2016; Mohamad et al., 2015). The technique statistically provides a more effective way of simultaneously identifying the individual parameter and their interactions, as well as establishing the optimum conditions for the extraction process while reducing the process time and cost (Silva et al., 2007) as compared to the one-variable-at-a-time design that is frequently adopted in many optimisation studies (Manan et al., 2016). The present work employed a Box Behnken design (BBD) to examine interactions between the extraction factors (time, temperature and solvent to sample ratio) for a maximal singular response i.e., extraction yield (%) in the UAE of *C. sieberiana* root. The work also investigated the efficiency in extraction between the UAE and the conventional methods (maceration and Soxhlet extraction) that are typically employed in the extraction of medicinal plants.

2 Materials and methods

2.1 Materials and reagents

The root part of *C. sieberiana* was collected in Bauchi State in Nigeria during the month of January 2016. The plant sample was identified and a voucher specimen (BUKHAN 0065) has been deposited by Mr. Baha'uddeen Said Adam at the Herbarium of the Department of Plant Biology, Bayero University Kano, Nigeria. The plant sample was air-dried and ground into powder. The powdered sample was passed through a standard 12 μm sieve and stored in an air-tight bag until further use. Methanol was purchased from

Sigma Aldrich (St. Louis, USA). Ultrasonic cleaning bath (Elmasonic S70 H, Elma Schmidhauer GmbH, Germany) with a power of 750 W and 60 Hz frequency, equipped with time and temperature controller was used.

2.2 Selection of UAE conditions

It is important to mention here, our earlier preliminary screening experiments revealed three influential factors that can significantly affect the extraction yield of the UAE of *C. sieberiana* root. This was carried out by first, assessing the influence of time on the extraction yield for durations between 10–60 min while keeping the extraction temperature and solvent to sample ratio constant at 50°C and 12 mL/g, respectively. An extraction time of 30 min that gave the best extraction yield was chosen for the second experiment. Next, the extraction temperature was assessed between 30–60°C while holding the extraction time and solvent to sample ratio constant at 30 min and 12 mL/g. Finally, the extraction time and temperature were kept constant at 30 min and 50°C, respectively. The solvent to sample ratio was inspected between 10–30 mL/g, as observed from the first and second experiments. Based on preliminary data, the logic ranges for the three levels (low, middle and upper) of each UAE parameter in the RSM assessment were ascertained.

2.3 Experimental design

The effect of the independent factors; time, *A*, temperature, *B*, and solvent to sample ratio, *C*, at three levels (–1, 0, +1) were evaluated for maximum extraction yield of the *C. sieberiana* root extract (Table 1). A three-variable Box-Behnken design (BBD) (software; Design Expert V 7.1.6) consisting of 17 experimental runs with three replicates at the design centre point were carried out. All assessments were randomised and performed in triplicate. Table 1 shows the rank of the independent factors, levels and experimental design in coded and decoded terms.

Table 1 BBD matrix depicting the independent factors rank, levels and experimental data for a three-factor-3-level response surface analysis, represented in both coded and decoded terms

		Levels		
Independent factors	***Symbols***	*–1*	*0*	*+1*
Time	A	20	30	40
Temperature	B	30	45	60
Solvent-sample ratio	C	12	21	30

The runs were performed in random order.

2.4 Data analysis

For the OVAT study, statistical analysis used the IBM SPSS version 20.0 software, and the level of significance of $P < 0.05$ was used. Analysis of variance (ANOVA) with Tukey's test was utilised to determine significance difference between the means. Normality of the data was first checked using the Kolmogorov-Smirnov and Shapiro-

Wilk tests. Data for the preliminary one-variable-at-a-time (OVAT) study were expressed as mean ± standard deviation of three replicated determinations. For the RSM experiment, the response obtained from each experimental data were fitted to a quadratic equation polynomial model and regression coefficient obtained. The generalised second-order polynomial model for predicting the optimal point in the response surface analysis is illustrated below:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{3}\beta_{ii} X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij} X_i X_j \quad (1)$$

where Y is the estimated response, β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively, while X_i and X_j are the independent coded factors. Contour and response surface plots were generated by varying two independent factors within the experimental range and one variable was kept constant at the central point.

The statistical significance of terms in the regression equations were examined using ANOVA for each response and only factors that are significant ($P \leq 0.05$) were fitted. The optimised conditions as per the Derringer's desirability prediction tool of RSM for the factors were used to validate the model. The fitness of the second-order polynomial model was expressed by the value of coefficient of determination, R^2. The main indicators to represent the significance and adequacy of the generated model in this study cover the model probability value (*Prob* > *P*), *F*-value, lack of fit, Adjusted coefficient of determination (*Adj,* R^2) and Adequate precision.

2.5 Verification of model

The optimum conditions predicted by the BBD were validated using the results obtained under specific experimental conditions proposed by the Design Expert software, to verify the prediction power and adequacy of the model. The experimental and predicted data were then compared in order to determine the validity of the model (Table 2).

2.6 Conventional methods of C. sieberiana root extraction

Two conventional methods, maceration and Soxhlet extraction were performed. The dried powdered *C. sieberiana* sample was macerated in MeOH at room temperature for 30 min, 1 h, 2 h, 3 h and 24 h. Soxhlet extraction was conducted on the plant sample at 65°C for 30 min, 1 h and 3 h using MeOH. Extracts of *C. sieberiana* root obtained from both extraction techniques were filtered and then concentrated on a rotary evaporator at 40°C. The ratio of solvent to sample used in both methods was kept constant at 16.67 mL/g and the procedures were performed in triplicate. Data on percentage yield (Y%; equation (2)) of the extracts are expressed as mean ± standard deviation (SD). For calculating improvement in extraction yield, the lowest yield among the three extraction techniques was taken as 100%. Relative improvement was defined as the ratio of extraction yield to the yield obtained in the reference method (lowest extraction yield amongst the assessed methods).

$$\text{Y (\%)} = \frac{\text{weight of dried crude extract}}{\text{weight of } \textit{C. sieberiana} \text{ powder}} \times 100 \quad (2)$$

Table 2 BBD matrix along with the experimental and the predicted values for the extraction yield from the root of *C. Sieberiana*

Run*	***A: time* (min)**	***B: temperature* (°C)**	***C: sample to solvent ratio (mL/g)***	***Yield (%)***	
				Actual	*Predicted*
1	30 (*0*)	60 (+*1*)	12 (–*1*)	1.41	1.41
2	40 (+*1*)	45 (*0*)	12 (–*1*)	1.11	1.11
3	30 (*0*)	45 (*0*)	21 (*0*)	1.38	1.40
4	30 (*0*)	45 (*0*)	21 (*0*)	1.37	1.40
5	20 (–*1*)	45 (*0*)	30 (+*1*)	0.88	0.90
6	30 (*0*)	30 (–*1*)	12 (–*1*)	0.86	0.84
7	40 (+*1*)	60 (+*1*)	21 (*0*)	1.13	1.15
8	30 (*0*)	45 (*0*)	21 (*0*)	1.43	1.41
9	40 (+*1*)	30 (–*1*)	21 (*0*)	0.71	0.71
10	30 (*0*)	45 (*0*)	21 (*0*)	1.42	1.41
11	30 (*0*)	45 (*0*)	21 (*0*)	1.40	1.40
12	20 (–*1*)	60 (+*1*)	21 (*0*)	1.17	1.19
13	30 (*0*)	60 (+*1*)	30 (+*1*)	1.06	1.09
14	40 (+*1*)	45 (*0*)	30 (+*1*)	0.77	0.79
15	20 (–*1*)	45 (*0*)	12 (–*1*)	1.20	1.18
16	20 (–*1*)	30 (–*1*)	21 (*0*)	0.86	0.86
17	30 (*0*)	30 (–*1*)	30 (+*1*)	0.71	0.71

*The runs were carried out in random order.

3 Results and discussion

3.1 Selection of ranges for UAE conditions

In general, several parameters such as time, temperature and solvent to sample ratio are rated higher in significance as compared to others in terms of influencing efficacy of an UAE technique (Wang and Weller, 2006). The logic ranges for UAE parameters employed for the RSM was obtained from the preliminary experiments carried out using the OVAT method. According to review of the literature, longer contact time between the sample and solvent can favourably improve the absorption of solvent by plant materials and promote the softening of plant tissue. However, the use of an extensive extraction time can lead to extraction of unwanted constituents and inefficient use of time (Liu et al., 2016).

As shown in Figure 1, the extraction yield was elevated with the concomitant increase in UAE time from 10 min to 40 min and reached its peak at 40 min, before a slight decrease was observed. The statistical data also indicate that extraction time has a significant ($P < 0.05$) effect on the extraction yield of the root of *C. sieberiana*, hence the parameter may be incorporated into the subsequent RSM assessment.

Figure 1 Effect of UAE time on the extraction yield of *C. Sieberiana* root under constant temperature (50°C) and solvent to sample ratio (12 mL/g) (see online version for colours)

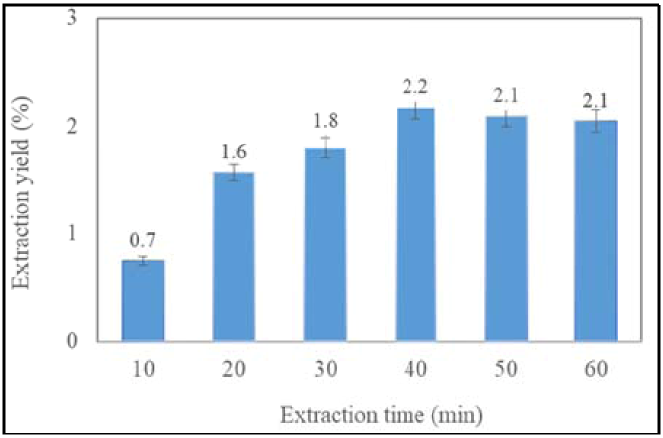
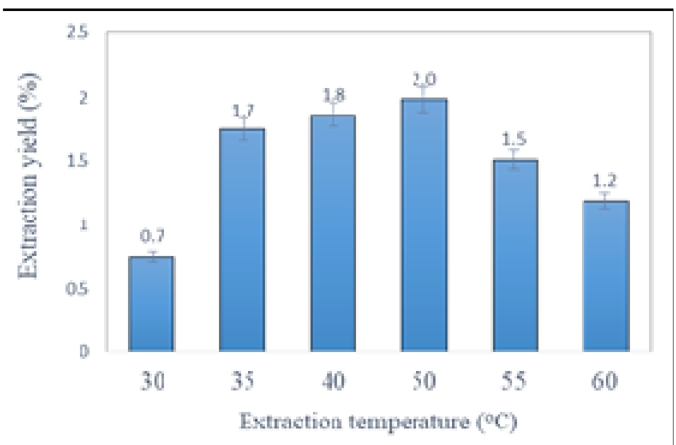


Figure 2 Effect of UAE temperature on the extraction yield of *C. Sieberiana* root under constant UAE time (30 min) and solvent to sample ratio (12 mL/g) (see online version for colours)



The temperature of extraction is also another key factor that can affect solubility of the active constituents and enhance their transfer from inside the plant material to the solvent used in the extraction process (Spigno et al., 2007). The study found the UAE temperature ($P < 0.05$) has a significant impact on the extraction yield of *C. sieberiana* root (Figure 2). Steady increase in extraction yields of *C. sieberiana* root was recorded as the extraction temperature was elevated from 30–50°C. Conversely, the extraction yields showed a decline trend as the UAE temperature was increased beyond 50°C, indicating that 50°C is possibly the mid-level point in the BBD. The use of an appropriate solvent to sample ratio is crucial to ensure availability of a sufficient amount of solvent to immerse the plant material completely during extraction. It is noted that the extraction yield remained low when solvent to sample ratios between 7.5–10 mL/g were used. Extraction yields steadily increased as the ratio of solvent to sample ratio was elevated from

10 mL/g to 20 mL/g and, decreased again when the ratios exceeded 30 mL/g (Figure 3). Correspondingly, ANOVA of the ratio of solvent to sample corroborates the factor being a significant ($P < 0.05$) influence on the extraction yield. Based on preliminary experiments, the chosen logic ranges for the BBD of UAE of *C. sieberiana* root for factors, extraction time, extraction temperature and solvent to sample ratio were 20–40 min, 30–60°C and 12–30 mL/g, respectively.

3.2 Fitting the model

It is important to highlight that a *P*-value is an indicator for significance and interaction ability of each variable (Manan et al., 2018). Therefore, factors showing lower *P*-values than others exhibit greater significance. A very large F-value implies the magnitude of a given term to influence the response, i.e., yield of (%) *C. sieberiana* root. Hence, comparing the F-values of the terms can facilitate the ranking order of the assessed factors which may influence the response of the study. Consequently, BBD data on the UAE of *C. sieberiana* root were regressed to fit the second-order polynomial equation which revealed the process was well described by a quadratic polynomial model. This was inferred from the highly significant *P*-value (<0.0001), a high value of determination coefficient, R^2 (0.9917) and the insignificant lack of fit (*P* value 0.1288). This means that over 99% of the variability is accountable and only 0.0083% of the total variation in the response could not be explained by the model. The model *F*-value of 93.40 indicates that the model is highly significant and imply a well correlated and a satisfactory model is attained, that can sufficiently represent the relationship between the response and independent factors in this study. Moreover, the close agreement between the R^2 (0.9917) and adjusted R^2 ($R^2 adj = 0.9811$) as well as a high index of signal to noise ratio (*Adeq precision* = 25.63) further confirm the validity of the obtained model (Table 3). Hence, the BBD model obtained in this study can be used to navigate the design space.

Figure 3 Effect of solvent to sample ratio on the extraction yield of *C. Sieberiana* root under constant UAE temperature (50°C) and time (30 min) (see online version for colours)

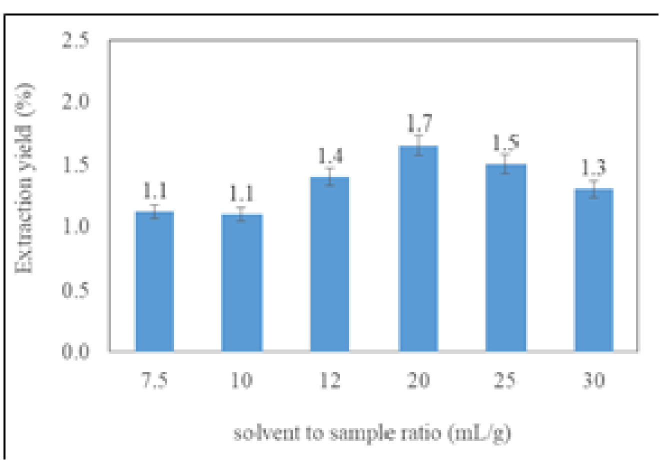


Table 3 ANOVA of the fitted quadratic polynomial model of the BBD

Source of variation	*Sum of squares*	*Mean squares*	*Degree of freedom*	*F value*	*P value*
Model	1.13	0.13	9	93.40	< 0.0001***
Residual	9.425E-003	1.346E-003	7		
Lack of fit	6.825E-003	2.275E-003	3	3.50	0.1288
Pure error	2.600E-003	1.14	16		

R^2 = 0.9917, Adjusted R^2 = 0.9811, Adequate Precision = 25.63; ***highly significant, missing asterisk: Not significant.

3.3 Analysis of the regression coefficient and the response surface

ANOVA of the regression parameters of the predicted second order polynomial models for the extraction yield of *C. sieberiana* is shown in Table 4. All the linear terms (*A*, *B*, *C*), as well as the quadratic terms (A^2, B^2, C^2) are either significant ($P < 0.005$) or highly significant ($P < 0.0001$). However, the interaction terms, *AB* and *AC*, were observed to be insignificant $P > 0.05$), implying their insignificance in affecting extraction yields of *C. sieberiana* root. The term *BC*, is significant as seen by its small *P* value ($P < 0.05$). The predicted second-order polynomial model is as shown below:

$$\text{Yield (\%)} = 1.40 - 0.049\text{A} + 0.20\text{B} - 0.15\text{C} + 0.027\text{AB} - 5.000\text{E}.003\text{AC} - 0.050\text{BC} - 0.23\text{A}^2 - 0.21\text{B}^2 - 0.18\text{C}^2 \quad (3)$$

where *Y* is the predicted extraction yield of *C. sieberiana* (%), and *A*, *B*, *C* are the coded factors for the UAE time, temperature and solvent to sample ratio, respectively.

Table 4 ANOVA for the second-order polynomial models and coefficient values for percentage extraction yield obtained from the root of *C. Sieberiana*

Factors	*Regression coefficient*	*Sum of squares*	*Mean square*	*F-values*	*p-value*
Linear					
A	–0.049	0.019	0.019	14.12	0.0071**
B	+0.20	0.33	0.33	246.66	<0.0001**
C	–0.15	0.17	0.17	124.92	<0.0001**
Interaction					
AB	+0.027	3.025E-003	3.025E-003	2.25	0.1776
AC	–5.000E.003	1.000E-003	1.000E-003	0.074	0.7931
BC	–0.050	0.010	0.010	7.43	0.0295*
Quadratic					
A^2	–0.23	0.22	0.22	160.08	< 0.0001**
B^2	–0.21	0.18	0.18	133.03	< 0.0001**
C^2	–0.18	0.14	0.14	105.59	< 0.0001**

**indicates that the effect is highly significant, * indicate that the effect is significant; no asterisk indicates that the effect is not significant

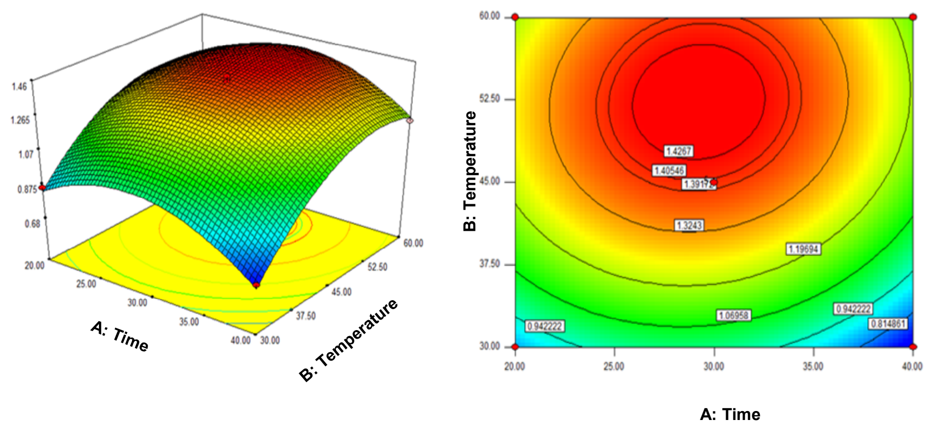
The positive sign in front of the terms implies a synergistic effect, while the negative sign is indicative of an antagonistic effect between the assessed interaction factors on the response. A positive coefficient detected for the linear term temperature (*B*) indicates a positive effect of the variable on extraction yield, implying that an increase in extraction temperature may improve the extraction conditions for a faster extraction rate (Cacace and Mazza, 2003). Conversely, the negative coefficients for the linear effects of time (*A*) and solvent to sample ratio (*C*) indicate that there is a maximum in the extraction yield at a particular sonication time and solvent to sample ratio, above which the extraction yield will start to decrease. The constructed three-dimensional response surface and contour plots are important graphical representations of regression equation that shows the interaction effect of the factors on the response (Zou et al., 2015). Since the plots generated in this study showed presence of a saddle point, the ridge maximum and canonical analyses are then employed to detect the critical levels of the design factors where the maximum extraction yield can be found.

3.4 Mutual interaction of UAE factors on the extraction yield of C. sieberiana root

The associations between the factors and the extraction yield of *C. sieberiana* root can be better understood by holding the variable for solvent to sample ratio (*C*) constant at the central value (21 mL/g), and observing the interaction between the extraction time (*A*) and temperature (*B*). The result for this assessment is depicted in Figure 4. Under an optimised condition, it is evident a maximum extraction yield, as high as ~1.43% was achievable when both factors, extraction time and temperature are near their central values. The extraction yield increases as the temperature is elevated from 40°C to 60°C within the sonication time of 22 to 37 min as observed in Figures 4(a) and 4b, respectively. The relatively pointed arc of the surface plot which (Figure 4(a)) signifies the surface limited within the smallest ellipse in the contour plot (Figure 4(b)). As indicated by the ANOVA of linear factors (Table 4), the yields of *C. sieberiana* root using the UAE technique are largely influenced by the extraction temperature (*B*) (F-value 246.66) as compared to time (*A*) (F-value 14.12). The data clearly illustrates a maximum yield (~1.43%) of the plant extract is possible when extraction temperatures of the UAE are maintained between 47–52.5°C and extraction time is at any values between 27–33 min (Figure 4(b)). Correspondingly, the interaction is synergistic (+0.027AB) (equation (3)), inferring that a concurrent increase of both factors beyond their central values can render the UAE process yielding higher percentages of the *C. sieberiana* root extracts. Most importantly, the data also conveyed that, elevating the temperature (*B*) of the UAE process can progressively invoke reduced viscosity alongside an increase in solubility, and diffusion coefficient of plant constituents into the extraction solvent. These promote a higher rate of extraction as higher concentrations of the extractive components are present in the extraction solvent, hence increasing extraction yield of the UAE on *C. sieberiana* root.

A report by Cacaze and Mazza (2003) corroborates our observation, in which the rate of diffusion can be enhanced as temperature of the UAE system is elevated. This invariably increases the mass transfer of constituents from the plant material into the solvent, as described by Fick's second law (Cacaze and Mazza, 2003). As can be seen, a concurrent increase in reaction time also results in an increasing trend in the percent of extraction yield *C. sieberiana* root. The reason for this sort of behaviour is due to a longer contact time which increases the solubility and transfer of extracted bioactive constituents into the surrounding liquid medium. However, extraction yields declined when the extraction temperature is increased > 60°C and exposure time > 34 min. As an elevated temperature of extraction is applied over an extended span of time, so does the tendency of increased denaturation of certain heat-sensitive constituents in the *C. sieberiana* root extract. Similar observations have been reported for earlier works (Silva et al., 2007; Altemimi et al., 2015).

Figure 4 (a) 3D response surface and (b) contour plot showing the effect of time and temperature, and their mutual interaction on the UAE of *C. Sieberiana* root at constant solvent to sample ratio (21 mL/g) (see online version for colours)

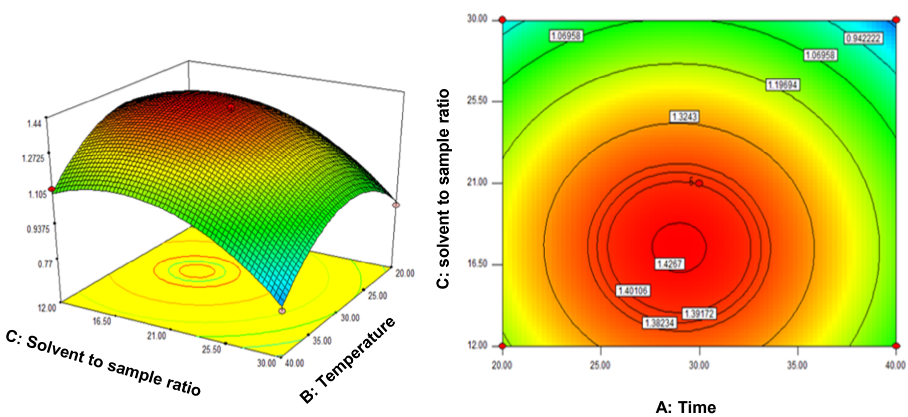


(a) (b)

The contribution of solvent to sample ratio is also another main factor for consideration in any UAE processes. To assert a favourable condition that renders a high extraction yield would require increasing the proportion of solvent over the mass of *C. sieberiana* root sample in the UAE system. This is based on the fact that more bioactive components in the plant cells may be readily solubilised in the presence of sufficient solvent in the surrounding solid sample (Cacaze and Mazza, 2003). The mutual interaction, for the effects of solvent to sample ratio (*C*) and extraction time (*A*) was investigated at a fixed central point for temperature (45°C). The impact of solvent to sample ratio (*C*) (F-value 124.92) is greater than that of time (*A*) (F-value 14.12) (Table 4), implying the former has a more dominant influence on the response. The surface (Figure 5(a)) and contour (Figure 5(b)) plots at the lower and upper levels for the effect of solvent to sample ratio (*C*) is observed to affect the response in a quadratic manner, as described in the generated equation (equation (3)). Maximum extraction yield that reached ~1.43% was obtained when the range of the UAE time (*A*) is set between 25–34 min with values of solvent to sample ratio (*C*) ranging between 13–21 mL/g. Beyond these ranges, lower extraction yields of *C. sieberiana* root are observed. The data seen here is consistent with the negative coefficient of *AC* (–5.000E.003AC) (equation (3)) which implies an antagonistic interaction between both factors. The results thus indicate that higher extraction yields of the *C. sieberiana* root extract is possible when the settings of each factor are inversed, meaning that a high extraction yield is possible when the solvent to sample ratio is set to low while the extraction time is maintained at a higher setting, or *vice versa*. Notably, the significant increase in extraction yield with the increase of the solvent to sample ratio from 13 to 21 mL/g is consistent with the principle mass of transfer (Cacaze and Mazza, 2003). This outcome can be explained by the fact that concentration gradient is the driving force for the higher mass transfer of extracted bioactive constituents within the plant sample, as more bioactive components within the ruptured plant cells may be dissolved by the presence of excess extraction solvent. The data proves that increasing the solvent to sample ratio from 13 to 21 mL/g positively improved the diffusion rate, hence the extraction yield (Figure 5).

Previous studies also described improved extractions yields when elevated solvent to sample ratios are used (Li et al., 2011; Ahamad et al., 2015). In contrary, the significantly lower extraction yields at solvent to sample ratios <13 mL/g and extraction time <25 min are symptomatic of a slower diffusion rate of the extracted bioactive constituents from the ultrasonic-assisted disrupted plant material into the liquid medium. A matter of fact, a similar trend is also reported in literature (Kuo et al., 2014). Additionally, a short UAE contact time means insufficient time for the bioactive components in *C. sieberiana* root to dissolve into the extraction solvent. Hence, resulting in their lower concentrations in the extractive solution and subsequently lower extraction yields. This phenomenon is supported by the low yields of the *C. sieberiana* root when lower solvent to sample ratios are used in the UAE technique (Figure 5).

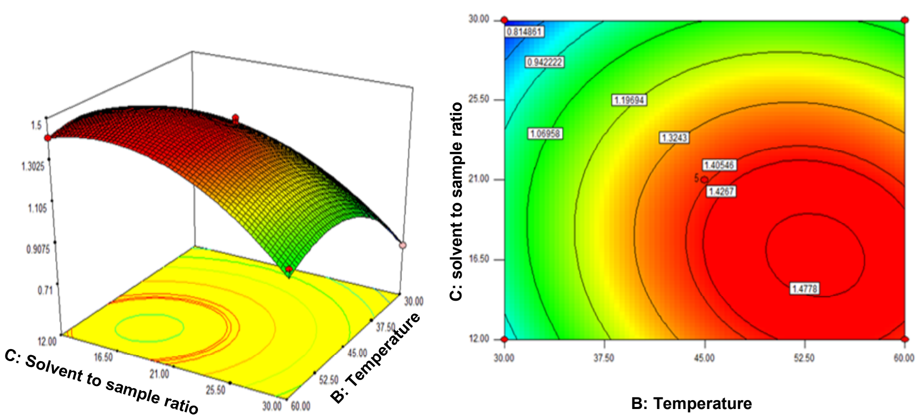
Figure 5 (a) 3D response surface and (b) contour plot showing the effect of time and solvent to sample ratio, and their mutual interaction on the UAE of *C. Sieberiana* root at constant extraction temperature (45°C) (see online version for colours)



(a) (b)

Figure 6 shows the effect of extraction temperature (*B*) and solvent to sample ratio (*C*) on the extraction yield at fixed extraction time (30 min). It can be seen that the UAE temperature (*B*) is crucial in attaining high extraction yields as, reflected in its very large F-value (246.66) as compared to solvent to sample ratio (*C*) (F-value 124.42) (Table 4). A maximum extraction yield of ~1.48% was attainable when the UAE of *C. sieberiana* root employs an extraction temperature and solvent to sample ratio between 48 – 56°C and 12.5–20.5 mL/g, respectively. The mutual interaction of *BC* is seen as antagonistic due to the negative coefficient (–0.050BC) (equation (3)), signifying that the model dictates the conditions for temperature to be inversed, to a certain extent, to that of solvent to sample ratio (*C*) in the UAE system, to guarantee a high extraction yield of the *C. sieberiana* root. Based on the data, it is clear that further increasing the solvent to sample ratio beyond 20.5 mL/g along with a decrease in UAE temperature < 45°C only was counterproductive and resulted lower extraction yields. The possible reasoning for this outcome can be attributed to the equilibrium distribution constant or partition coefficient which is greatly affected by the temperature within the UAE system (Cacaze and Mazza, 2003). On one hand, the raising the extraction temperature from 45°C to 60°C may aid in the attainment of equilibrium concentration of constituents in the solvent, which increases solubility of the constituents in the plant sample. On the other hand, the use of higher ratios of solvent to sample that exceeded 21 mL/g would advantageously dilute the bioactive components *C. sieberiana* root and consequently facilitates their mass transfer in the UAE system, hence improving extraction yield. Essentially, the study also proves that the use of solvent to sample ratio < 12 mL/g is unsuitable for this investigation, largely due to inadequate amount of the extractive solvent in the surrounding medium to solubilise and extract the bioactive components from the ruptured matrix of *C. sieberiana* root. Although the bioactive components are more concentrated in the extractive solvent when a low ratio of solvent to sample is used, the phenomenon of reduced mass transfer that can potentially decrease the extraction yield, should not be ignored. It was concluded that the extraction temperature (*B*) and solvent to sample ratio (*C*) are essential parameters governing the extraction yield of *C. sieberiana* root and can be regarded as indicators of UAE effectiveness.

Figure 6 (a) 3D response surface and (b) contour plot showing the effect of temperature and solvent to sample ratio, and their mutual interaction on the UAE of *C. Sieberiana* root at constant extraction time (30 min) (see online version for colours)



(a) (b)

3.5 *Optimisation of UAE parameters and validation of the predicted models*

To verify the predictive capacity of the model, the optimum UAE condition as suggested by the BBD model with the highest desirability was selected. The validation experiment was performed within the design space and the result was compared to the predicted value generated by Design Expert software. The experiment was carried out using the predicted optimum UAE conditions; extraction time of 29 min, temperature of 45°C and solvent to sample ratio of 16.79 mL/g. The study found that the predicted results corresponded well with the attained experimental results based on the suggested optimum extraction conditions, hence verifying a good correlation of the RSM model. The validation experiment showed that a maximum extraction yield of ~1.40 ± 0.1% is in good agreement with the predicted extraction yield of 1.43% (deviation < 5%). Thus, the results affirmed that the BBD model can be employed to optimise the UAE parameters for high extraction yield from the root of *C. sieberiana*.

3.6 Ultrasonic-assisted extraction (UAE) and conventional techniques for extraction of C. sieberiana root

The efficiency of extraction using UAE method was studied with two other conventional methods (maceration and Soxhlet extraction) and the results are tabulated in Table 5. It is important to indicate here that the results presented for the conventional techniques of extraction of *C. sieberiana* root were performed concurrently as that of UAE in our lab. The extraction yield obtained at 30 min, 1 h for maceration and soxhlet extraction, and at 3 h for maceration technique was negligible (results not shown). According to the tabulated data, the extraction yield from the root of *C. sieberiana* (1.43 ± 0.7%) obtained under an optimised UAE condition [29 min, 45°C, solvent to sample ratio 16.67 mL/g] resulted in a 207.2% improvement over the Soxhlet extraction (0.82 ± 0.5%) [65°C, 3 h] and improved by 118.8% over the maceration technique (0.69 ± 0.9%) [room temperature, 24 h]. Based on the findings, the application of UAE to effectively extract components from the root of *C. sieberiana* appears feasible. Hence, our results affirmed that the technique of UAE on *C. sieberiana* root is more effective than the two conventional extraction techniques in giving higher percentage yield of extract from *C. sieberiana* root (Table 5).

Table 5 Comparison of UAE with conventional extraction techniques

Extraction technique	*Extraction time*	*Extraction temperature*	*Extraction yield (%)*	*Relative improvement (%)*
UAE	29 min	45°C	1.43 ± 0.7%	207.2
Maceration	24 h	Room temperature	0.69 ± 0.1%	100.0
Soxhlet	3 h	65°C	0.82 ± 0.5%	118.8

16.67 mL/g proportion solvent to sample ratio was use in all three techniques. Data collected were calculated from experiments carried out in triplicates. Improvement in extraction yield was calculated by taking extraction yield of the maceration technique as 100%.

4 Conclusion

In the present study, the BBD was successfully used to optimise the extraction yield of *C. sieberiana* root by UAE technique. The statistical data showed that the developed model was accurate, precise and adequate to predict the optimum condition that favour a high extraction yield. This was mirrored in the very high coefficient of determination value

($R^2 = 0.9917$) of the second-order polynomial regression model. The optimum conditions using Derringer's desirability prediction tool were found to be 29 min, 45°C and 16.79 mL/g solvent to sample ratio. Most importantly, carrying out of UAE technique alongside with two conventional methods (maceration and Soxhlet extraction) showed that the former is more rapid and efficient in obtaining high yields of the root extract. In a nutshell, it is evident that UAE can be used to facilitate rapid and more efficient extraction of components and subsequently isolation of bioactive constituents from the root of *C. sieberiana.* Additionally, the same technique may be suited to improve extraction yield for other plant sources.

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