MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)

http://mjbmb.org

STATISTICAL OPTIMIZATION OF ALKALOIDS EXTRACTION FROM *Ficus sycomorus* LEAVES USING RESPONSE SURFACE METHODOLOGY

Abba Babandi[1,2]*, Chioma A Anosike[1], Hafeez Muhd. Yakasai[2], Salihu Ibrahim[3] and Lawrence U S Ezeanyika[1]

[1] Department of Biochemistry, University of Nigeria, Nsukka, 410001, Enugu-Nigeria
[2] Department of Biochemistry, Bayero University, P.M.B. 3011, Kano-Nigeria
[3] Centre for Biotechnology Research, Bayero University, P.M.B. 3011, Kano-Nigeria

*Corresponding Author: ababandi.bch@buk.edu.ng

Keywords:

Alkaloids, central composite design (CCD), Extraction, Ficus sycomorus, OFAT, Response Surface Methodology

Abstract

Numerous plant bioactive metabolites such as alkaloids have an exceptional role in medicine as drugs or as chemical sculpt for the design and synthesis/semi-synthesis of new drug agents by academia and industries. Extraction process is very crucial and initial step in discovering potential bioactive agents from medicinal plant such as *Ficus sycomorus*. This study is therefore, preliminarily focused on maximizing the extraction yields of total alkaloids from *F. sycomorus* leaves as a function of independent variables: time (70-100 min), solid: solvent ratio (60-100; w/v; g/mL) and methanol concentration (70–100 v/v %) using Response Surface Methodology (RSM), after one-factor-at-a-time (OFAT) investigation. The optimum operating conditions for OFAT to attain a higher extraction yield of alkaloids were found to be 1: 80 g/mL solid: solvent ratio; 100 (v/v) % methanol concentration and 80 min of extraction time, which was determined using desirability function. Optimization of the extraction conditions using RSM resulted in an increase in percentage alkaloids yield of 4.34 %, when extracted with 95.83 g/mL solid: solvent ratio; 82.67 (v/v) % methanol concentration and 81.07 min of extraction time. The second-order polynomial models developed were satisfactory in describing and predicting the extraction of the alkaloids from *F. sycomous*. This study indicates that RSM can predict the best experimental design and identify the relationship between extraction variables for alkaloids extraction from *F. sycomorus* leaves for maximum yield. From the industrial and environmental viewpoint, the RSM is a much more economical and safe method of alkaloids extraction.

INTRODUCTION

Ficus sycomorus (L) (fig or the fig *Mulberry*) belongs to the mulberry plant family which is native to Africa and grows along South of Sahel and North of the tropic of Capricorn [1][2]. The plant mostly grows well in tropical countries [1] such as Nigeria. It is used in local treatment of many ailments such as diabetes, epilepsy, cancer, cough, diarrhea, skin infections, stomach disorders, liver diseases, tuberculosis, lactation disorders, helminthiasis, human infertility, antimicrobials, antioxidants, anti-plasmodium properties [2][3][4][5]. The *F. sycomorus* possibly possesses good insect repellent properties and hence reduces the contact of the vector with humans, for example, mosquitoes thereby minimizing the incidence of malaria transmission in the local communities [6]. Also, this plant was previously investigated and reported for insecticidal and pesticidal activities [7].

Many plant bioactive compounds such as alkaloids have an outstanding roles in medicine as drugs or as chemical model for the design and synthesis (or semi-synthesis) of new drug molecules such as the opiates, aspirin, or etoposide (semi-synthetic anti-neoplastic agent; a derivative of *Podophyllum peltatum*) [8][9][10]. The alkaloids (Alkali-like) are generally low-molecular-weight, secondary plant metabolites, nitrogen-containing (Nitrogen in a negative oxidation state) biomolecules and due to the presence of a heterocyclic ring having a nitrogen atom, it is typically have alkaline properties. This class of phytochemicals is commonly distributed in plant kingdom, making up approximetly 20 % of identified plant phytochemicals [11]. It is produced by plant due to plant-insect co-habitation [12].The alkaloids have been generally reported as the most important group of natural substances [13], which play an important role in the ecology of organisms synthesizing them. For instance, it has been suggested that they constitute part of the plant defenses against phytophagous animals and regulate plants growth [14]. These compounds have high bioactivity, distinctive mechanism of action, and are of different classification [15].

The therapeutic roles of plant alkaloids were known as cardioprotective, anaethetics and anti-inflammatory compounds. These alkaloidal agents used in clinical applications include quinine, morphine, nicotine and cocaine [16]. The alkaloidal compounds were also reported to have broad biological impact on human health such as antibacterial, anti-malarial, cytotoxic and anti-cancerous properties [17]. It is also has strong natural insect antifeedants [18]. The alkaloids can be used as a lead compounds for the design and development of new alkaloid-based drugs and insecticides by industries. The availability of alkaloidal compounds in *F. sycomorus* plant was previously reported [3][4][5]. However, the economical feasibility of an industrial process which also requires working in such a way that high extraction efficiency of the alkaloids is attained is a general objective in present day economy.

The phytochemical extraction method is a crucial step for the discovery of bioactive constituents from plants [19]. These compounds are widely used in nutraceuticals, pharmaceuticals, and cosmetic products as well as functional food ingredients and food additives [20][21]. Extraction times, temperatures, solid to solvent ratios, solvent concentrations, and volume of solvent, pH, pressure, and the particle size of the plant matters have been identified from the previous studies as important factors affecting the extraction efficiency of the plant bioactive compounds [22][23][24][25][26]. Other natural factors such as genetic content variation, plant growth stage, harvesting time, the soil structure, geographical location, the drying method used, etc., may also affect the quality and quantity of phytochemicals to be extracted from the plant matters [27][28][29].

One-factor-at-a-time (OFAT) is a traditional or classical method used in optimizing specific extraction parameters, playing with only one factor (at a time) or variable while keeping constant all other variables or factors during extraction process [30]. Conversely, the OFAT is less reliable due to non-inclusiveness of interactive effects among the participating factors or variables, tedious to perform, time-consuming, and industrially or economically expensive. Other experimental drawbacks of the OFAT were ambiguous conclusion of the findings and false optimizing conditions might occur and probably ignoring the true significant factors during the extraction process [21]. To overcome some of these problems, a Response Surface Methodology (RSM) is adopted and widely used by many researchers nowadays for optimizing the extraction conditions.

The RSM, a statistical and optimization tool has been practically introduced to overcome the weakness and limitations associated with classical (OFAT) method of plant metabolites extraction [31]. It composes of factorial design and regression analysis that facilitate in measuring the significant factors, categorizes the relationship between factors, choosing the optimal extraction conditions among the factors used or the suitable responses [32]. It is also a mixture of statistical and mathematical techniques which is important for modeling and analyzing problems or responses that influenced by some experimental factors or variables. Thus, RSM is an effective technique for optimization of complex process such as extraction of bioactive compounds from medicinal plants, due to its characteristic efficiency and easy interpretation of experimental conditions [33][34]. The RSM also evaluates the effects between the multiple factors and their interactions towards one or more response variables simultaneously, producing lesser number of experimental procedures, leading to the most fitting experimental conditions and eventual assessment of the experimental response(s)[30][35][36][37][38]. It also helps to locate the region where the extraction is optimized by using clearer image or graphic support [39]. The information of the interaction between the variables or factors is vital in order to find the output-input relationship which is hardly determined using classical approach (OFAT) [40]. The most commonly used form of RSM is central composite design (CCD) [41]. Several studies employed the RSM for the optimization of different elements or factors in extraction process in order to maximize yield of phytochemicals after the process [42][43][44][45].

To the best of our knowledge, no study has been done to optimize the alkaloids extraction conditions from the *F. sycomorus leaves* for large scale production. Due to huge medicinal values of the *F. sycomorus* and specifically, the important of alkaloids in medicinal and industrial applications, and after the initial One-factor-at-a-time (OFAT) investigation, a preliminary investigation study on the optimization of alkaloids extraction yield is required. This study is therefore, aimed to optimize the extraction condition variables (time 70-100 min; solid: solvent ratio 60-100; w/v; mg/mL and methanol concentration 70–100 v/v % for total alkaloids yield) of the alkaloids from the *F. sycomorus* leaves using response surface methodology (The

CDD form) in order to boast high medicinal and pharmaceutical values of the plant.

MATERIALS AND METHODS

Chemicals

Methanol 99.7 v/v % was purchased from Sigma, Aldrich USA. Other chemicals used are analytical grade that were obtained from recognized chemicals suppliers, Fisher (Malaysia) and Merck (Darmstadt, Germany).

Plant Collection and Identification

F. sycomorus leaves were collected from a botanical garden in Bayero University Kano (BUK), old campus (11°98′14″N, 8°48′02″E). The sample was identified by a certified botanist at the Department of Plant Science and given an accession number of: Bayero University, Kano herbarium Accession Number *BUKHAN 109.*

Extraction of Alkaloids Content

F. sycomorus fresh leaves sample was shade-dried for two (2) weeks; it was then powdered and subsequently used for extraction of alkaloids. The process was carried out according to the method described by Harbone [46] and Li [47]. The *F. sycomorus* powder (5 g) was weighed. The extraction was carried out by macerating the powder in aqueous acid solution for 2 h, followed by degreasing with n-hexane and extraction with chloroform until Mayer's test was negative. The methanol extract was evaporated in water bath at 35°C, and the residue reconstituted with 200 mL 10 % hydrochloric acid (HCl) for 1 h under shake up (orbital shaker) and allowed to stand for 12 h at 10 °C prior to filtering. The filtrate was washed with chloroform, $CHCl_3$ (3 x 50 mL). The aqueous phase was adjusted to pH 10 with (NH_4OH) and extracted with $CHCl_3$, (3 x 50 mL). Finally, the solvent was evaporated under reduce pressure for obtaining dried extract containing alkaloids. The solvent was evaporated for obtaining crude extract containing alkaloids. Extraction solvent, extraction time and solid: solvent ratio was considered as single factors and their effects on the extraction of alkaloids were studied on the basis of single factor experiments and response surface methodology (RSM). The weight of the solid residues were recorded and taken as yield of crude extracts. The percentage yield was calculated as follows:

Percentage alkaloids yield = (weigh of extract/weigh of sample) X 100………[46]

Experimental Design

The experimental design for this study was divided into two parts: i) single factor experiments were conducted to establish the suitable range of conditions for the alkaloids extraction by varying one independent variable at a time while keeping the remaining constant. ii) The optimization of alkaloids extraction from *F. sycomorus* using the RSM and a second order polynomial model was developed.

Design of Single Factor Experiments

Effect of Solid:Solvent Ratio on Alkaloid Yield

The *F. sycomorus* sample was extracted as above with solid:solvent (w/v; g/mL) ranging 1:20 to1:100 g/mL by fixing constants, the solvent (Methanol) concentration v/v (%) and extraction time (min) at 80 % and 80 minutes respectively. The optimum solid:solvent ratio was then chosen based on percentage alkaloids yield (%). All the experiment was conducted in triplicate.

Effect of Extraction Time on Percentage Alkaloids

The suitable extraction time for alkaloids from *F. sycomorus* leaves was determined by fixing constants, the solid solvent ratio and solvent concentration at 1: 80 g/mL and 80 v/v % respectively. The best time was selected base on percentage alkaloids yield (%). The experiment was repeated three (3) times.

Effect of Solvent Concentration (Methanol v/v %) on Percentage Alkaloids

The *F. sycomorus* leaves sample was extracted with solvent concentrations ranging 10-100 v/v %, after setting constants, the solid: solvent ratio (g/mL) and extraction time (min) at 1: 80 and 80 minutes respectively. The optimum solvent concentration was then chosen based on percentage alkaloids yield (%). The experiment was conducted in triplicate.

The results of the single factor experiments above, provided the ranges of three factors (solid: solvent ratio, solvent concentration, and extraction time) used for the RSM study.

Optimization using Response Surface Methodology

The design experiments contained three response parameters, which are solid:liquids ratio (A), methanol concentration (B) and the extraction time (C), with five levels (-α, -1, 0, 1, and + α) was carried out, involving 20 experimental points and 5 centre points. The software, design-Expert 6.0.8 (Stat-Ease, Inc. USA) using central composite design (CCD) was used to regressively analyze the experimental data and calculate the mathematical model, so as to confirm the optimizing process conditions for extraction of alkaloids from the plant. Therefore, data were fitted to second-order polynomial equation for each dependent variable *Y* (Equation 1). All experiments were conducted in triplicates and the mean result was used as a response variable, *Y*.

$$Y = \beta_0 + \Sigma\, \beta_i x_i + \Sigma\, \beta_{ii} x_i^2 + \Sigma\, \beta_{ij} x_i x_j \qquad (1)$$

where y is the dependent variable (Percentage alkaloids yield), Xi and Xj are independent variables, k is a number of independent variables, β_0, β_i, β_{ii}, β_{ij} are regression coefficients for equation intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are coded value of the independent variables while k equals to the number of the tested factors (k=3). The ANOVA tables were generated and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The statistical significance of all the terms in the polynomial equation was analyzed by calculating the F-value at a probability (p) of 0.001, 0.01 or 0.05. The important criteria for choosing an adequacy model for this design were regression analysis (R^2), adjusted R^2, Predicted versus Actual graph and ANOVA analysis ($p<0.05$).

Validation of Experiments

Validation was carried out by conducting an experiment based on the optimal conditions setting through a mathematical model generated from RSM-FCCD. The Design-Expert version 6.06 (Stat-Ease Inc., Minneapolis, USA) statistical software package was used for the regression analysis of experimental data and to plot response surface. Subsequently, the real values obtained from the experiments were compared with the predicted value of response generated by CCD.

Test for Alkaloids

Dragendroff's Test

The *F. sycomorus* extract was treated with Dragendroff's reagent (Potassium Bismuth Iodide solution). The red precipitate formation confirms the alkaloids presence.

Wagner's Test

Two (2) ml of Wagners solution (Dilute iodine solution) was added to 0.5mLof the *F. sycomorus* extract solution. Observation of reddish-brown precipitate indicated the presence of alkaloids.

Mayer's Test

The *F. sycomorus* extract (0.5 g) was heated with 5 mL of Hydrochloric acid (HCl) in water bath and the mixture was stirred for 10 minutes, after cooling; the extract was filtered into test tube. A few drops of Mayer's reagent were added. A slight turbidity or heavy cream precipitate is presumptive indication for the presence of alkaloids [48].

Statistical Analysis

All experiments were performed in triplicate and analyzed using SPSS statistics V.24 software package (SPSS Inc., Chicago, Illinois, USA). The data is presented in figures were the means ± SD. One-way Analysis of variance (ANOVA) was used to compare between groups of data. *Post hoc* and Tukey's tests were run to identify the significance of the data. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Medicinal plants extraction process is crucial to decide the quantity, quality and class of the phytochemicals, each with different biological properties and health benefits. Globally, the interest in bioactive plant metabolites is receiving more attention in several different fields such as food and beverages, pharmaceutical and cosmetic industries [49] clinical and possibly academia. Other than the extraction methods, the quality and quantity of these phytochemicals depends on the extraction time, the solvent, the temperature, the pH, the agitation rate, ratios and particle size [31]. The preliminary investigation and the optimization of alkaloids extraction process from *F. sycomorus* leaves using OFAT and RSM were carried out in this study and the results were presented.

Single Factor Experiments

Effect of Solid:Solvent Ratio on Alkaloids Extraction from *F. sycomorus*

The solid:solvent ratio is critical variable in the extraction process. The effect of solid-solvent ratio was evaluated. The data obtained indicated optimum alkaloids extraction at 80 (Figure 1). One-way ANOVA showed significant differences between the solid-solvent ratio extracted ($F (4, 10) = 116.35$, $p < 0.001$). *Post hoc* test showed no significant differences between the mean values for 80 and 100 ($p = 0.890$) but there was a significant difference between the optimum 80 and all other solid:solvent ratios ($p < 0.05$). As shown from the Figure 1, the percentage alkaloids from *F. sycomorus* increases with solid:solvent ratio increase when solvent concentration and time were constant (80% v/v and 80 min). The solid:solvent ratio was found to affect the percentage alkaloids yield (%) in comparison with other variables. This might be due to the increase of the driving force for the mass transfer of the alkaloids from the plant matter. This was in line with the mass transfer principle observed by Campos *et al*. [50]. An optimum of 1: 80 g/mL was obtained from this study (Figure 1). Previously, a solvent to solid ratio of 10:1 was suggested to be enough to obtain an optimum yield during extraction process [51], while other studies [27] [26] [37] showed a quite different solid to solvent ratio.

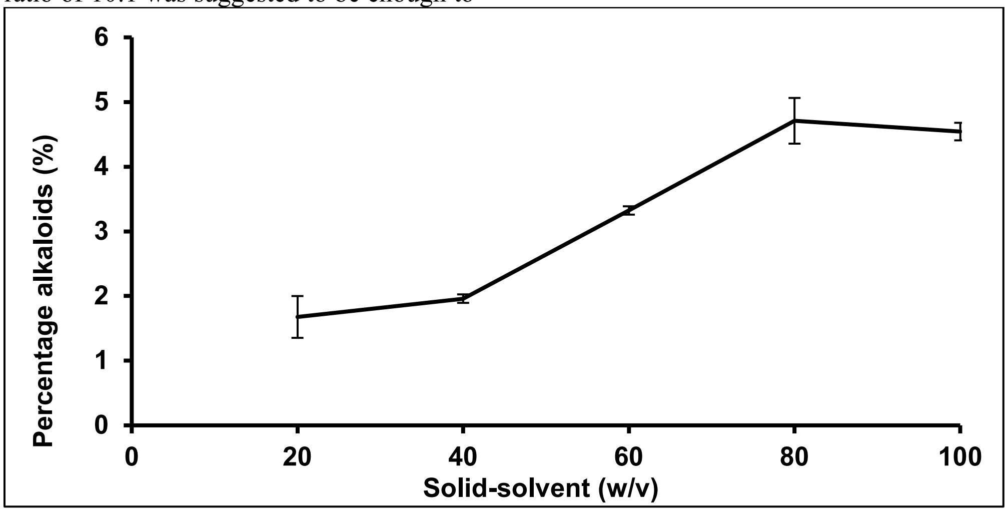

Figure 1. Effect of solid:solvent ratio on percentage alkaloids yield, the other constant extraction conditions were 80 v/v % methanol and 80 min extraction time. Error bars represent mean ± standard deviation, n =3

Effect of Solvent Concentration on Alkaloids Extraction from *F. sycomorus*

The solvent type and its concentration used are critical tool in the extraction process of bioactive metabolites from plant matters. Aqueous alcohols particularly acetone, ethanol and methanol are most commonly employed for botanical materials especially alkaloids [52][53][54]. Most alkaloids are generally polar compounds that have to be extracted with polar reagents or solvents. Methanol is the most polar reagent after water [54], thus, suitable for alkaloids extraction. Figure 2 shows that percentage alkaloids from *F. sycomorus* increases with methanol concentration (v/v %) increase when solid: solvent and time were constant (1:80 g/mL; 80 min). An optimum of 80% methanol was obtained from this OFAT study (Figure 2). This finding corroborated high alkaloids extractions yield using methanol previously reported [53][54]. One-way ANOVA identified a significant overall difference between the methanol percentages in the extraction ($F (4, 10) = 18.55$, $p < 0.001$). *Post hoc* comparisons suggested that there was no significant difference between the alkaloids extracted at 70 % and 100 % ($p = 0.089$), but there was a significant difference between 100 % and all other percentages ($p < 0.05$).

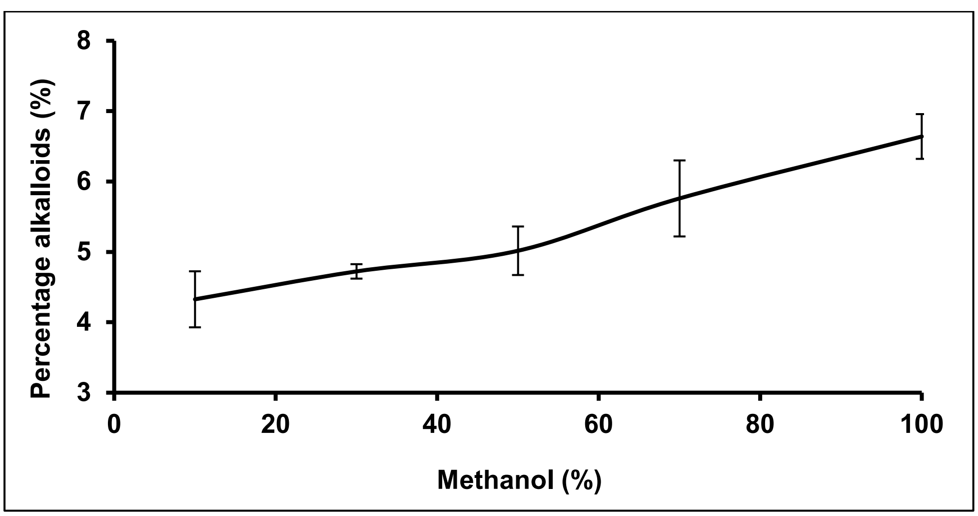

Figure 2. Effect of methanol concentration (v/v %) on percentage alkaloids yield, the other extraction conditions were 1: 80 g/mL solid liquid ratio and 80 min extraction time. Error bars represent mean ± standard deviation, n=3

Effect of Extraction Time on Alkaloids Extraction from *F. sycomorus*

The extraction time was also another main independent variable in the alkaloids extraction procedure. The time for extraction can be small as few minutes or as lengthy as 24 hours [55][56]. In this study, the extraction time range was considered based on the practical and economic benefits (to enhance large scale extraction yield within a shortage period of time possible). Figure 2 showed that an increase in extraction time increased from 60 to 80 min was accompanied by increase of percentage alkaloids yield and further increase in this process duration led to small decrease of the percentage alkaloids. An optimum extraction time of 80 min was obtained from this study as suitable time for high percentage alkaloids yield (Figure 2). Analysis of variance (ANOVA) identified significant differences between different time tested ($F(4, 10) = 33.98$, $p < 0.001$). However, in *post hoc* tests the extraction achieved at the optimum time 80 minutes did not differ significantly with 100 minutes ($p = 0.609$) but there significant difference between 8 and other minutes ($p < 0.05$).

This result of the OFAT study was fully explained by Fick's second law of diffusion, which declared that final equilibrium will be achieved between the solute concentrations in the plant matter (solid matrix) and in the solvent at certain or specific extraction time, hence, an excessive extraction time will not be valuable for extraction of more alkaloids from the *F. sycomorus* [30]. The relative increase of the extraction yield with increase of extraction time has been suggested by several studies [57][58][59][60]. Conversely, the high extraction time (more than 80 min) in this study, present a snag in respect of percentage alkaloids content from *F. sycomorus* leaves (Figure 3).

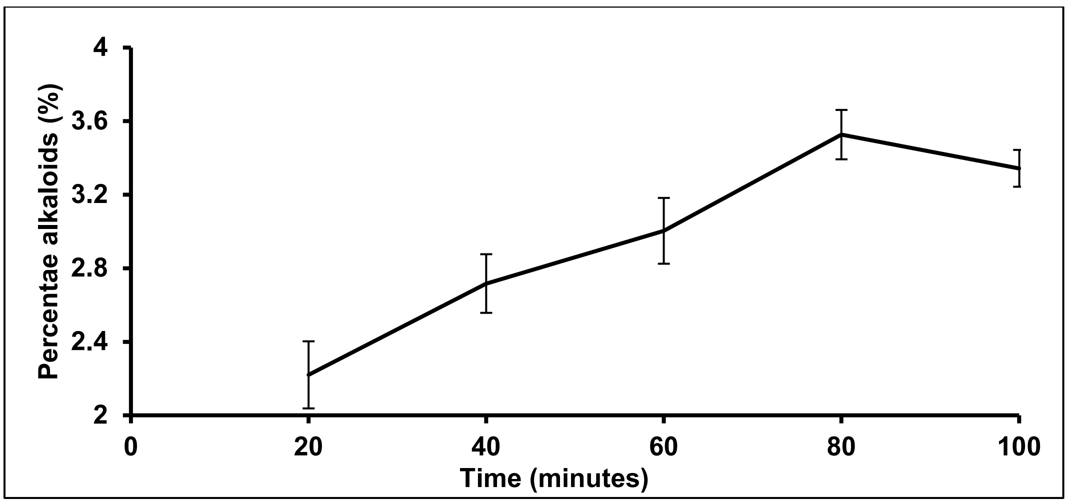

Figure 3. Effect of extraction time on percentage alkaloids yield, the other extraction conditions were 1: 80 g/mL solid liquid ratio and 100 v/v % methanol concentration

Response Surface Experiment

The influence of the extraction time (min), solid:solvent ratio (g/mL) and solvent concentration (v/v %) on the extraction yield of alkaloids (%) from *F. sycomorus* leaves, was investigated using RSM. The experiments were designed according to CCD, and results are presented in Table 1. The twenty (20) randomized analyses were determined and presented based on the CCD workflow. The highest experimental and predicted values of alkaloids extraction were 4.87 and 5.14 % respectively at 100 (w/v) solid-solvent ratio, 100 v/v % methanol and time of 80 minutes. The lowest extraction for experimented value was found in run 5 while for predicted value was found in run 14.

Fitting the Model

Normally, the significant of model variables are determined based on *p*-value or *F*-value ("Prob > F" value). The larger the size of *F*-value and correspondingly smaller the "prob > F" value, the more important is the corresponding coefficient [32]. The results of second-order response surface model in the form ANOVA for the maximum percentage alkaloids yields are summarized in Table 2. From the table, the model was highly significant when the computed *F*-values were greater than the tabulated *F*-value and the probability values were low ($p < 0.001$), indicating that the individual terms in each response model were significant on the variables interaction effect. The *p*-value for the lack of fit from this

Table 1. Central composite design (CCD) experimental matrices with observed and predicted values for dependent responses of percentage alkaloids yield (%)

Std	Run	Solid-solvent ratio (w/v)	Methanol Concentration (v/v %)	Time (mins)	Actual value (%)	Predicted value (%)
2	1	100.00	70.00	80.00	3.36	3.39
5	2	80.00	70.00	100.00	3.00	3.04
19	3	90.00	85.00	90.00	4.27	4.39
16	4	90.00	85.00	90.00	4.26	4.39
6	5	100.00	70.00	100.00	2.42	2.71
15	6	90.00	85.00	90.00	4.78	4.39
1	7	80.00	70.00	80.00	4.12	4.42
10	8	106.81	85.00	90.00	3.68	3.47
17	9	90.00	85.00	90.00	4.42	4.39
9	10	73.18	85.00	90.00	4.82	4.59
18	11	90.00	85.00	90.00	4.39	4.39
13	12	90.00	85.00	73.18	6.12	5.90
12	13	90.00	110.23	90.00	5.20	5.00
11	14	90.00	59.77	90.00	2.54	2.30
14	15	90.00	85.00	106.82	4.16	3.94
4	16	100.00	100.00	80.00	4.87	5.14
8	17	100.00	100.00	100.00	4.18	4.19
20	18	90.00	85.00	90.00	4.20	4.39
3	19	80.00	100.00	80.00	6.12	6.14
7	20	80.00	100.0	100.00	4.22	4.50

study was identified as 0.1442, highlighting that the lack of fit of the model used was not significant at $p > 0.05$. Ibrahim *et al*. [32] and Ibrahim *et al*. [61] reported a non-significant lack of fit and described that the model was an excellent fit for the data. The determination coefficient R^2 value of yield was obtained to be 0.954, explaining about 95.4 % of variations in the percentage alkaloids yield (Table 2). The R^2 which was defined as the ratio of the explained variation to the total variation was a measure of the degree of the model fitness [62]. The closer the R^2 value is to the unity (1), the better the empirical model used fits the actual data [63][64]. While if the values of R^2 are lower, then the independent variables used are not predicting the values of the experimental responses correctly [64]. All determination coefficients of responses from this study were more than 0.9, proving good representation of the variability of the factors by the model. It is important to notice that the model is considered accurate and reliable as the R^2 is more than the threshold of 0.75 [65]. Even though, a larger R^2 value does not always show a sound regression model [66]. But, the R^2 value should be compared to adjusted R^2 value for a good model. The obtained R^2 values from this study were not differed greatly from adjusted R^2 (Table 2). In addition, the value of adjusted R^2 value (0.912) was also very high, advocating for a high significance of the model (Table 2). The adjusted R^2 value was a corrected value for R^2 after the removal of the unnecessary model terms. If there were numerous non-significant terms been incorporated in the model, the adjusted R^2 value would be remarkably smaller than the R^2 [67]. In this study, the adjusted R^2 (0.912) was very close to the R^2 value (0.954), indicating a reasonable relationship between the independent variables used (solid: solvent ratio, solvent concentration and time) and the response (percentage alkaloids yield) (Table 2). The fitness and adequacy of the model used was generally judged by the coefficient of determination (R^2) and the significance of lack-of-fit. The coefficient of variation (CV) describes the degree to which the generated data dispersed. Thus, the CV (6.77) for percentage alkaloids yield from *F. sycomorus* leaves were lower which showed a better reproducibility, high degree of precision and reliability of the experimental values [31]. The model adequate precision was used to measure the signal to noise ratio. In this study, adequate precision of 18.85 was found to be greater than 4, thus indicating an adequate signal (Table 2).

Table 2. ANOVA for quadratic polynomial model developed for extraction yield of alkaloids

Source	Sum of Squares	DF	Mean Square	F Value	Prob > F	Significant
Model	17.09048	9	1.898943	22.8796	< 0.0001	
A	1.514069	1	1.514069	18.24246	0.0016	
B	8.80143	1	8.80143	106.0452	< 0.0001	
C	4.623605	1	4.623605	55.7081	< 0.0001	
A^2	0.242526	1	0.242526	2.922103	0.1182	
B^2	1.004991	1	1.004991	12.10876	0.0059	
C^2	0.492887	1	0.492887	5.938615	0.0350	
AB	0.000312	1	0.000312	0.003765	0.9523	
AC	0.241513	1	0.241513	2.909894	0.1188	
BC	0.035113	1	0.035113	0.423057	0.5301	
Residual	0.82997	10	0.082997			
Lack of fit	0.609637	5	0.121927	2.766884	0.1442	
Pure error	0.220333	5	0.044067			
Cor total	17.92046	19				
Std. Dev.	0.288092		R-Squared	0.9537		
Mean	4.2565		Adj R^2	0.9120		
C.V.	6.768284		Pred R^2	0.7136		
PRESS	5.133117		Adeq Precision	18.85		

The generated data fit the second-order of polynomial models, which was confirmed based on coefficients of determination (R^2) (>0.9) [68] and that the response surface analysis can be applied to optimize the extraction of the alkaloids from *F. sycomorus* leaves. All determination coefficients of responses are greater than 0.9, proving good representation of the variability of the factors by the model. Thus, the models explain more than 90% of the response variation. This suggested that the predicted second order polynomial models defined soundly, the actual behavior of the percentage alkaloids extraction system from the leaves of *F. sycomorus*.

The similarities between the predicted and the corresponding actual values of the response were closely related as obtained by the CCD (Figure 4). There was a strong correlation between the actual and the predicted model values. The majority of the points in the figure was closed to the centre line, indicating the best possible conditions for achieving high percentage alkaloids yield and determines the predictability of the designed model (Figure 4). This indicates that the proposed percentage alkaloids extraction model is suitable for predicting the percentage alkaloids yield, violating the independent and consistent variation hypothesis. Hence, the developed predicted model is appropriate because it satisfies all adequacy tests. The model analyses, lack of fit test, coefficient of determination (R^2), adjusted R^2 and Predicted versus Actual graph were determined for the model adequacy [69]. The equations describing this in terms of coded and actual factors are Equation 2 and 3 for percentage alkaloids extraction.

$$Y = 4.399\text{-}0.33A+0.80B\text{-}0.58C\text{-}0.13A^2\ \text{-}0.26B^2+0.18C^2+0.006AB+0.17AC\text{-}0.07BC \text{ .... } (2)$$

$$Y = 15.09+0.04A+0.29B\text{-}051C\text{-}0.013A^2\ \text{-}0.001B^2+0.002C^2+4.17E\text{-}05AB+0.002AC\text{-}0.0004BC \text{ ...... } (3)$$

A negative sign (-) in each of the above equations (Eqns 2 and 3) represents an antagonistic effect of the variables, and a positive sign (+) represents a synergistic effect of the variables used for alkaloids extraction [39]. The independent variables with negative coefficients from this study were solid: solvent ratio (A) and the extraction time (C) which generally depict a decreased response (percentage alkaloids yield, Y), while increase in response (Y) was due to positive coefficient of the solvent, methanol (variable B) (equation 2). The positive coefficient of methanol (variable B) is indicative of increase of percentage alkaloids yield (linear effects) while in contrast the negative coefficients of solid: solvent ratio (A) and extraction time (C) depicted a decrease in percentage alkaloids yield with increase in solid: solvent ratio and extraction time respectively. This might be related to the fact that the solubility of molecules present in plant matter depends on the polarity and chemical properties of solvent (B) used.

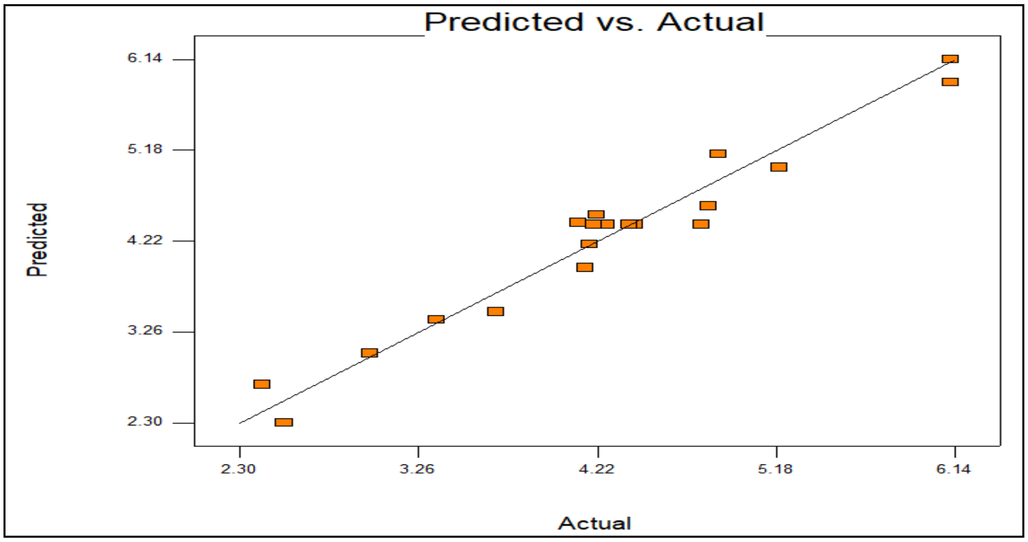

Figure 4. Correlation plot between predicted and actual values of extraction yield

A positive, non-significant ($p > 0.05$) quadratic effect was only observed for extraction time (C) while the solid:solvent ratio (A) and solvent concentration shows negative quadratic effects on alkaloids yield (Table 3). All the variables show non-significant interactions ($p > 0.05$) (Table 3).

Table 3. Regression coefficients of the second-order polynomial model for *F. sycomorus* alkaloids content

Regression coefficients	Percentage alkaloids yield (%)	*p* value (ρ < 0.05)
Intercept		
X_O	4.399	
Linear		
A	-0.33	0.0016
B	0.80	0.0001
C	-0.58	0.0001
Quadratic		
A^2	-0.13	0.1182
B^2	-0.26	0.0059
C^2	0.18	0.0350
Interaction		
AB	0.006	0.9523
AC	0.17	0.1188
BC	-0.007	0.5301

In optimization process of alkaloids extraction from *Rhizome coptidis*, the CCD form was used by the researchers to investigate the effect of ethanol concentration, extraction time and extraction temperature on alkaloids yield from the rhizome [45]. The design and the results partly corresponds to the findings of this study, which shows all the independent variables especially solvent concentration (variable B), positively affects the efficacy of the alkaloids extraction process. The CCD form can generally assess a single variable (one factor) or the collective effect of the variables on the response (percentage alkaloids yield). This optimization study also corresponds to the work of Sahin *et al*. [70]. Others researchers such as Sharma *et al*. [71], studied the combined effects of different components on the biomass growth of *Aspergillus lentulus* via RSM technique. Recently, the independent variables; the steaming time and soaking temperature on the quality properties of parboiled rice were optimized using the RSM tool [72]. Also, Danbaba *et al*. [73] used RSM techniques for optimization of parboiling conditions including drying time, steaming time, and soaking temperature.

Analysis of Response Surface Plot

Figure 5 illustrates the three-dimensional response surfaces plots (3D) by presenting the response in function of two factors and keeping the other constant at its middle level. The X- and Y-axes of the three-dimensional response surfaces plots represent two variables. Each of the figures revealed the effects of the selected variables (solid: solvent ratio, extraction time and solvent concentration) on percentage alkaloids yield. Correlation between the response and independent variables could be found out from 3D response surface contour plots, which are simultaneously representing the interaction of three (3) variables on the response and finding the location of optimum experimental variables [74].

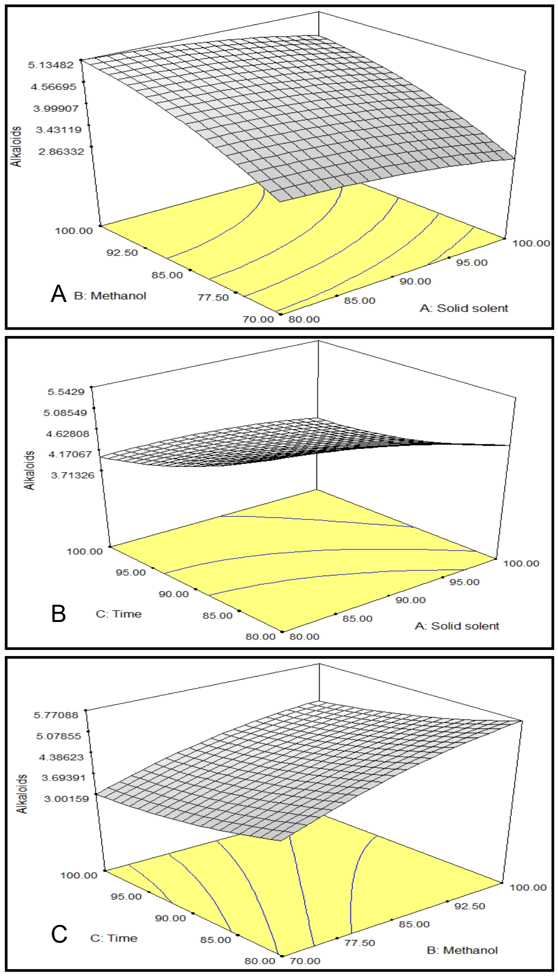

Figure 5. Response surface 3D plot corresponding to alkaloids as a function of (A) Methanol concentration (v/v %) and solid:solvent ratio (g/mL); (B) solid:solvent ratio and extraction time (min); and (C) solvent concentration (v/v %) and extraction time (min)

The interaction of two variables is shown in the contour of the plot. The rounded contour line depicts a weak interaction of the two variables and an indistinct contour line reflects a significant interaction of two variables [75[. The predicted response surface showing the effect of methanol concentration and solid: solvent ratio on the percentage alkaloids yield was depicted in Figure 5A. The figure shows linear effects of methanol concentration (%) for percentage alkaloids yield (%) with maximum yield of 5.52% while solid: solvent ratio shows slight increase of alkaloids (4.68%) at 1:100 g/mL. Furthermore, extraction of alkaloids was observed to be positively influenced by the synergism between methanol concentration and extraction time (p<0.05). From the industrial point of view, low methanol concentration with shorter extraction time would be more adequately required, as a long extraction period and large solvent concentration rendered the extraction procedure time consuming and practically uneconomical respectively. Figures 5B shows the three-dimensional response surface plots for the influence of extraction time, and solid-to-liquid ratio on percentage alkaloids yield. The percentage alkaloids extraction yields decrease as the extraction time increased from 80 to 100 min, while keeping the solid-to-liquid ratio constant. This effect of time is due to the increasing contact time of solvent with the plant matter to improve the diffusion of the alkaloidal compounds [76][77]. Figure 5C displays the effect of extraction time and methanol concentration on percentage alkaloids. The yield decreased when the extraction time increased. As shown in the figure, as the ratio of solid-to-liquid increased from 1: 80 to 1: 100 g/mL, the yield non significantly decrease.

Validation of the Predicted Model

In order to verify the reliability of the derived model, the predicted response must be compared with the experimental result at fixed variables conditions. The results were found to be not significantly different from predicted values at $p > 0.05$ using a paired t-test. The close agreement between predicted and experimental data confirmed the validity of the generated models (Table 4). This implied that there was a high fit degree between the values observed in the validated experiment and the value predicted from the regression model [78]. Hence, the response surface modeling could be applied effectively to predict extraction of alkaloids from *F. sycomorus* leaves. The extracted alkaloids from *F. sycomorus* leaves under the optimized conditions were qualitatively tested for the presence of alkaloids using standard methods and the results were presented in Table 5.

Table 4. Optimum conditions, predicted and experimental values of response under the model selected condition

Factors	Name	Predicted levels	Experimental level
A	Solid solvent (w/v)	95.83	95.83
B	Methanol (%)	82.67	82.67
C	Time (min)	81.07	81.07
		Prediction	**Experimental**
Responses (%)		4.339	4.756

Table 5. The qualitative analysis of Alkaloids from *F. sycomorus* leaves

S/N	Alkaloids Test	Inference
1	Dragendorff's	+
2	Wagner's test	+
3	Mayers	+

Key: +-present

CONCLUSION

From the results of this study, it can be concluded that the RSM can be used to optimize variables needed for alkaloids extraction from *F. sycomorus* leaves. RSM predicted 4.339 % alkaloids yield with optimum medium conditions of: 95.83 g/mL solid: solvent ratio; 82.67 % (v/v) methanol concentration and 81.07 min of extraction time which was determined using desirability function. The model also showed the Fisher value (*F* - value) of 22.88 representing a significant model. The R^2 of the model was 0.954 predicting 95.40 % model accuracy. The R^2 also implies statistical correlation that exists between the predicted and actual values, showing a good correlation (0.95) between the variables. This study indicates that RSM can predict the best experimental design and identify the relationship between extraction variables for alkaloids extraction from *F. sycomorus* leaves for maximum yield. From the industrial and environmental points of view, the RSM is more economical and safer extraction method for alkaloids extraction.

ACKNOWLEDGMENT

The authors acknowledged the support granted by *Dr SS Ibrahim*, Fellow, Welcome Trust, UK, LSTM, *Dr Aminu Ibrahim, Abdullahi Muhd*, *Mr Muhd. Mahe Mukhtar, Kamaludeen Babagana, Haruna Tsoho Bala* and other staff from Biochemistry department, BUK for technically supporting the course of this study. The authors were also grateful to TETFUND for sponsoring the project.

REFERENCES

1. Maryam, R., Negisa, S.T., Solmaz, M.D. (2015) Pharmacological and medicinal aspects of the verses containing fig (at-tin) in Holy Quran. Health, Spirituality Med. Ethics, 2(3):30–36.
2. Braide, W., Dokubo, K.O., Adeleye, S.A., Uzoh, C.V. and Akobundu, C.I. (2018) Phytochemical properties, toxicological screening and antibacterial qualities of various parts extracts of *Ficus sycomorus*. J. of Med. Plant and Herbal Ther. Res., 6:1–8.
3. Bello, O.M., Ojediran, O.J., Dada, O.A., Olatunya, A.M. and Awakan, O.J. (2015) *In vivo* toxicity studies and phytochemical screening of stem bark of *Ficus sycomorus* Linn (Moraceae). J. Environm Sci., Toxicol. Food Technol., 9(3):72-74.
4. Sheikha, K.M., Ruqaiya, N.S.W. and Hossain, M.A. (2015) *In vitro* evaluation of the total phenolic and flavonoid contents and the antimicrobial and cytotoxicity activities of crude fruit extracts with different polarities from *Ficus sycomorus*. Pac Sci Rev A: Nat Sci Eng., 17:103–108.

5. Babandi A, Muhammad MA, Anosike CA, Ezeanyika LUS (2019) *In vitro* Anti-malarial Potential of Chloroform Fruit Crude Extract of *Ficus sycomorus* L. against Blood-stage *Plasmodium falciparum.* Nig. Res. J. of Chem. Sci., 6:33-48.

6. Dangarembizi, R., Kennedy H., Erlwanger, D. M. and Chivandi, E. (2013) Phytochemistry, Pharmacology and Ethnomedicinal Uses of *Ficus thonningii* (Blume Moraceae): A Review. African J. Trad. Complement Altern. Med., 10(2):203-212.

7. Romeh, A. A. (2013) Phytochemicals from *Ficus sycomorus* L. leaves act as insecticides and acaricides. African J. of Agric. Res., 8(27):3571-3579.

8. Cassady, J.M. and Douros, J.D. (1980). *Anticancer Agents Based on Natural Product Models*. Academic Press, New York, 1980.

9. Balandrin, M.F., Klocke, J.A., Wurtele, E.S. and Bollinger, W.H. (1985) Natural plant chemicals: sources of industrial and medicinal materials. Science, 228, 1154.

10. Mann, J., Davidson, R.S., Hobbs, J.B., Banthorpe, D.V. and Harborne, J.B. (1994) *Natural Products. Their Chemistry and Biological Significance*, Addison Wesley Longman, Harlow, UK, 1994.

11. Kaur, R. and Arora, S. (2015) Alkaloids—Important Therapeutic Secondary Metabolites of Plant Origin. J. Crit. Rev., 2:1–8.

12. Li, Q.H. (2005) Application of response surface methodology for extraction optimization of germinal pumpkin seeds protein. Food Chem., 92(4):701-706.

13. Bruneton, J. (1999) *Pharmacognosie, Phytochimie, Plantes me´dicinales*, Technique & Documentation, Paris, Pp. 781–800.

14. Chik, S.C.C. Or, T.C.T., Luo, D. Yang, C.L.H., Lau, A.S.Y. (2013) Pharmacological Effects of Active Compounds on Neurodegenerative Disease with Gastrodia and Uncaria Decoction, a Commonly Used Poststroke Decoction. Sci. World J., 1–22.

15. Zhang, Y., Yang, K., Zhang, M.X. (2013) Optimization of vinegar steamed processing of *Schisandrae sphenantherae* Fructus with RSM. Chinese Trad Patent Med, 35(9):1976-1980.

16. Kurek, J. (2019) Introductory Chapter: Alkaloids—Their Importance in Nature and for Human Life. In Alkaloids—Their Importance in Nature and Human Life; IntechOpen: London, UK, 2019.

17. Debnath, B., Singh,W.S., Das, M., Goswami, S., Singh, M.K., Maiti, D., Manna, K. (2018) Role of plant alkaloids on human health: A review of biological activities. Mater. Today Chem., 9:56–72.

18. Sani, U. M. (2014) Phytochemical screening and antifeedant activity of the seed extracts of Parkia biglobosa against cowpea vean (*Vigna unguiculata*) storage pest (*Callosobruchus maculatus*). Int. J. of Innovative Sci. Eng. Technol*, 3*(9):15991-15995.

19. Abarca-Vargas, R., Pena Malacara, C. F., and Petricevich, V. L. (2016) Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougain villea x buttiana holttum* and standl, (Var. rose) extracts. Antioxidants, 5(4). https://doi.org/10.3390/antiox5040045.

20. Gao, L and Mazza, G. (1996) Extraction of anthocyanin pigments from purple sunflower hulls. J. of Food Sci,. 61(3):600–603.

21. Prasad, K.N., K. W. Kong, R. N. Ramanan, A. Azlan, and Ismail, A. (2012) Selection of experimental domain using two-level factorial design to determine extract yield, antioxidant capacity, phenolics, and flavonoids from *Mangifera pajang* Kosterm," Separation Sci. Technol., 47(16):2417–2423.

22. Juntachote, T., Berghofer, E., Bauer, F. and Siebenhandl, S. (2006) The application of response surface methodology to the production of phenolic extracts of lemon grass, galangal, holy basil and rosemary. Int. J. of Food Sci. Technol., 41:121-133.

23. Prasad, K. N., E. Yang, C. Yi, M. Zhao, and Jiang, Y. (2009) Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. Innovative Food Sci. Emerging Technol, 10(2):155–159.

24. Aybastıer, O., E. Isık, S. S¸ahin, and Demir, C. (2013) Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology," Ind. Crops Prod., 44:558–565.

25. Cvetanovic, A., Svarc-Gajic, J., Maskovic, P., Savic, S. and Nikolic, L. J. (2015) Antioxidant and biological activity of chamomile extracts obtained by different techniques: perspective of using superheated water for isolation of biologically active compounds. Ind Crops Prod, 65:582-591.

26. Maskovic, P.Z., Diamanto, L.D, Cvetanovic, A., Radojkovic, M., Spasojevic, M.B., Zengin, G. (2016) Optimization of the Extraction Process of Antioxidants from Orange Using Response Surface Methodology. Food Anal. Methods 9:1436–1443.

27. Ben Jemaa, J.M., Haouel, S., Bouaziz, M., and Khouja, M.L. (2012) Seasonal variations in chemical composition and fumigant activity of five Eucalyptus essential oils against three moth pests of stored dates in Tunisia. J. of Stored Prod. Res., 48:61-67.

28. Sefidkon, F., Assareh, M.H., Abravesh, Z., and Barazandeh, M.M. (2010) Chemical composition of the essential oils of four cultivated Eucalyptus species in Iran as medicinal plants (*E. microtheca*, *E. spathulata*, *E. largiflorens* and *E. torquata*). Iranian J. of Pharm. Res., 6:135-140.

29. Zandi-Sohani, N. and Ramezani, L. (2015). Evaluation of five essential oils as botanical acaricides against the strawberry spider mite *Tetranychus turkestani* Ugarov and Nikolskii. Int. Biodet. Biodeg. 98:101-106.

30. Silva, E. M., Rogez, H. and Larondelle, Y. (2007) Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. Sep. Purification Technol. 55:381-387.

31. Liyana-pathirana, C and Shahidi, F. (2005) Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem. 93:47–56.

32. Ibrahim, S., Shukor, M. Y., Khalil, K. A., Helmi, M. I. E., Syed, M. A., and Ahmad, S. A. (2015) Application of response surface methodology for optimising caffeine-degrading parameters by *Leifsonia* sp. strain SIU. J. Environm Biol, 36(5):1215–1221.

33. Azahar NF, Gani SSA, Mohd Mokhtar N.F. (2017) Optimization of phenolics and flavonoids extraction conditions of *Curcuma zedoaria* leaves using response surface methodology. Chem Cent J., 11(1):96. https://doi.org/10.1186/s13065-017-0324-y.

34. Zekovic Z, Cvetanovic A, Pavlic B, Svarc-Gajic J, Radojkovic M. (2014). Optimization of the polyphenolics extraction from chamomile ligulate flowers using response surface methodology. Int J Plant Res, 2014, 4(2):43–50.

35. Strnad, J., Brinc, M., Spudić, V., Jelnikar, N., Mirnik, L., Carman, B. and Kravanja, Z. (2010) Optimization of cultivation conditions in spin tubes for Chinese hamster ovary cells producing erythropoietin and the comparison of glycosylation patterns in different cultivation vessels. Biotech. Progr. 26: 653-663.

36. Zainol, S., Basri, M., Basri, H. Bin, Shamsuddin, A. F., Abdul-Gani, S. S., Karjiban, R. A., and Abdul-Malek, E. (2012) Formulation optimization of a palm-based nanoemulsion system containing levodopa. Int J. of Mol. Sci, 13(10), 13049–13064. https://doi.org/10.3390/ijms131013049

37. Ilaiyaraja, N., K. R. Likhith, G. R. Sharath Babu, and Khanum, F. (2015) Optimisation of extraction of bioactive compounds from

Feronia limonia (wood apple) fruit using response surface methodology (RSM)," Food Chem., 173:348–354.

38. Melgar, B., Dias, M. I., Barros, L., Ferreira, I. C. F. R., Rodriguez-Lopez, A. D., and Garcia-Castello, E. M. (2019) Ultrasound and microwave assisted extraction of Opuntia fruit peels biocompounds: Optimization and comparison using RSM-CCD. Molecules, 24(19):1–16. https://doi.org/10.3390/molecules24193618.

39. Azahar, N.F., Gani, S.S.A and Mokhtar, N.F.M. (2017) Optimization of phenolics and flavonoids extraction conditions of *Curcuma zedoaria* leaves using response surface methodology. Chem Central J., 11(1):96.

40. Elksibi, I., Haddar, W., Ben Ticha, M., Gharbi, R., Mhenni, M. F. (2014) Development and optimisation of a non conventional extraction process of natural dye from olive solid waste using response surface methodology (RSM)., Food Chem., 161:345–52. doi:10.1016/j.foodchem.2014.03.108.

41. Yasini, P., Shemirani, F. and Khani, R. (2012) Combination of In Situ Surfactant-based Solid Phase Extraction and Central Composite Design for Preconcentration and Determination of Manganese in Food and Water Samples. Food Anal. Method, 5(6):1303-1310.

42. Mane, C., Souquet, J.M., Ollé, D., Verriés, C., Véran, F., Mazerolles, G., Cheynier, V. and Fulcrand, H. (2007) Optimization of simultaneous flavanol, phenolic acid, and anthocyanin extraction from grapes using an experimental design: Application to the characterization of champagne grape varieties. J. Agric. Food Chem. 55,7224-7233.

43. Huang, W., Li, Z., Niu, H., Li, D. and Zhang, J. (2008) Optimization of operating parameters for supercritical carbon dioxide extraction of lycopene by response surface methodology. J. Food Eng., 89:298–302. doi:10.1016/j.jfoodeng.2008.05.006.

44. Wang, H., Liu, Y., Wei, S. and Yan, Z. (2012) Application of response surface methodology to optimise supercritical carbon dioxide extraction of essential oil from *Cyperus rotundus* Linn., Food Chem., 132:582–587. doi:10.1016/j.foodchem.2011.10.075.

45. Teng, H. and Choi, Y.H. (2014) Optimization of ultrasonic-assisted extraction of bioactive alkaloid compounds from rhizoma coptidis (*Coptis chinensis* Franch.) using response surface methodology. Food Chem., 142:299–305. doi:10.1016/j.foodchem.2013.06.136.

46. Harborne, J. (1984) *Phytochemical methods*. 2nd edition. Chapman and Hall, 1984.

47. Li, C. (2002) Phenolic compound in the leaves of *Catalpa ovata*. Foreign Med Sci., 24(6):358-359.

48. Sofowora, A. (1993) *Medicinal Plants and Traditional Medicines in Africa*. Chichester John, Willey & Sons New York, 1993; 256.

49. Chammem,N., Sifaoui, I., Mejri,A., Ben slama, M., Hamdi, M. and Abderabba, M. (2015) Optimization of extraction of phenolic and antioxidant contents from olive leaves using composite central design, GJBB 4:145-152.

50. Campos, D., R. Chirinos, O. Barreto, G. Noratto, and Pedreschi, R. (2013) Optimized methodology for the simultaneous extraction of glucosinolates, phenolic compounds and antioxidant capacity from maca (*Lepidium meyenii*)," Ind. Crops and Prod., 49:747–754.

51. Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J. and Nunez, M.J. (2005) Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. J. Agric. and Food Chem., 53:2111-2117.

52. Orio, L., Alexandru, L., Cravotto, G., Mantegna, S. and Barge, A. (2012) Ultrasonics Sonochemistry UAE, MAE, SFE-CO2 and classical methods for the extraction of *Mitragyna speciosa* leaves. Ultrasonics -Sonochemistry, 19(3):591–595. https://doi.org/10.1016/j.ultsonch.2011.10.001

53. Begashaw, B., Bharat, M., Asegedech, T. and Zewdneh, S. (2017) Methanol leaves extract *Hibiscus micranthus* Linn exhibited antibacterial and wound healing activities. BMC Compl. Altern Med., 17:337. doi 10.1186/s12906-017-1841-x.

54. Andayani, D.G.S., Anggraeni, S.R., Liviawaty, E., Chrisentia, R.M. and Srikandace, Y. (2018) Isolation, identification of alkaloid from *Rhizophora mucronata* and the activity of its methanol extract against barnacles. IOP Conf. Series: Earth Environm Sci. 160:012005 doi :10.1088/1755-1315/160/1/012005.

55. Lapornik, B., Prosek, M. and Wondra, A. G. (2005) Comparison of extracts prepared from plant by-products using different solvents and extraction time. J. Food Eng., 71: 214–222.

56. Lee, B. K., Jung, J. E. and Choi, Y. H. (2005) Optimization of microwave-assisted extraction process of Rehmannia Radix preparata by response surface methodology. Food Eng. Progr., 9(4):283-290.

57. Silva, L.V., Nelson,D.L., Drummond, M. F. B., Dufossé, L. and Gloria, M. B. A. (2005) Comparison of Hydrodistillation Methods for the Deodorization of Turmeric', Food Res. Int., 38 (8–9):1087–1096.

58. Spigno, G. and De Faveri, D. M. '(2007) Antioxidants from Grape Stalks and Marc: Influence of Extraction Procedure on Yield, Purity and Antioxidant power of the extracts', J. Food Eng., 78:793–801.

59. Telli, A., Mahboub, N., Boudjeneh, S., Siboukeur, O. E. K. and Moulti-Mati, F. (2010) Optimisation Des Conditions d'extraction des Polyphenols De Dattes Lyophilisees (*Phoenix dactylifera* L) Variete Ghars', Annales Des Sciences et Technologie, 2, 2.

60. Chavan, Y. and Singhal, R.S. (2013) Ultrasound-assisted extraction (UAE) of bioactives from areca nut (*Areca catechu* L.) and optimization study using response surface methodology', Innov. Food Sci. Emerg. Tech., 17:106–113, 2013.

61. Ibrahim, S., Zahri, K. N. M., Convey, P., Khalil, K. A., Gomez-fuentes, C., Zulkarnain, A., Alias, A. S., González-rocha, G., and Ahmad, S. A. (2020) Optimisation of biodegradation conditions for waste canola oil by cold-adapted *Rhodococcus* sp. AQ5-07 from Antarctica. Elect. J. of Biotechnol., 48: 1–12. https://doi.org/10.1016/j.ejbt.2020.07.005.

62. Wang, L. H., Yang, B., Du, X. Q. and Yi, C. (2008) Optimisation of supercritical fluid extraction of flavonoids from *Pueraria lobata*. Food Chem. 108: 737-741.

63. Fan, G. J., Han, Y. B., Gu, Z. X. and Chen, D. M. (2007) Optimizing conditions for anthocyanins extraction from purple sweet potato using response surface methodology (RSM). LWT 41:155–160.

64. Myers, R.H.; Montgomery, D.C.; Anderson-Cook, C.M. (2016) *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*; John Wiley & Sons: Hoboken, NJ, USA, 2016.

65. Ghazouani, N., Abderrabba M. and Bouajila, J. (2016) *Teucrium ramosissimum* (Lamiaceae): Volatile Composition, Seasonal Variation, and Pharmaceutical Activity. Bioanalytical. 49:1258-1271.

66. Dahmoune, F., Nayak, B., Moussi, K., Remini, H. and Madani, K. (2015) Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chem. 166:585–595.

67. Myers, R. H. and Montgomery, D. C. (2002) *Response surface methodology: Process and product optimization using designed experiments,* P. 32. New York, USA: Wiley.

68. Prasad, K.N., Hassan, F.A., Yang, B., Kong, K.W., Ramanan, R.N., Azlan, A. and Ismail, A. (2011) Response surface optimisation for the extraction of phenolic compounds and antioxidant capacities of

underutilised *Mangifera pajang* Kosterm. peels. Food Chem., 128:1121-1127.

69. Yolmeh, M., Habibi Najafi, M.B. and Farhoosh, R. (2014) Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM)., Food Chem., 155:319–24. doi:10.1016/j.foodchem.2014.01.059.

70. Şahin, S., C. Demir, C. and Malyer, H. (2011). Determination of total phenolic content of Prunella L. by immobilized enzyme bioreactor, Anal. Methods. 3, 944. doi:10.1039/c0ay00732c.

71. Sharma, S., Malik, A. and Satya, S. (2009). Application of response surface methodology (RSM) for optimization of nutrient supplementation for Cr (VI) removal by *Aspergillus lentulus* AML05. J Hazard Mat., 164:1198–204.

72. Taghinezhad, E. and Brenner, T. (2017). Mathematical modeling of starch gelatinization and some quality properties of parboiled rice based on parboiling indicators using RSM. J. Food Process Eng.,40(3), e12483.

73. Danbaba, N., Nkama, I., Badau, M.H., Ukwungwu, M.N., Maji, A.T., Abo M.E, et al. (2014) Optimization of rice parboiling process for optimum head rice yield: a response surface methodology (RSM) approach. Int J Agric Forestry, 4:154–65.

74. Heydari, M.M., Rajaei, A., Salar, Bashi, D., Mortazavi, S.A. and Bolourian, S. (2014) Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (*Phlomidoschema parviflorum*) leaves using response surface methodology. Ind Crop Prod., 57:195-202.

75. Holetz, F.B., Ueda-Nakamura, T., Filho, B.P.D., Cortez, D.A.G., Morgado-Diaz, J.A., Nakamura, C.V. (2003) Effect of essential oil of *Ocimum gratissimum* on the trypanosomatid *Herpetomonas samuelpessoai*. *Acta Protozoologica*, 42(4):269–276. doi: 10.13140/RG.2.1.1605.5208.

76. Corrales, M., García, A.F., Butz, P., Tauscher, B. (2009) Extraction of anthocyanins from grape skins assisted by high hydrostatic pressure. J. Food Eng., 90:415–421.

77. Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., Ju, Y.-H. (2014) Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. J. Food Drug Anal., 22:296–302.

78. Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A. and Madani, K. (2014) *Pistacia lentiscus* leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Ind. Crops Prod. 61:31–40.