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Optimization of Extract Yield and Total Phenolic Content of *Pavetta crassipes* leaves using Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AJS, KDN did the Conceptualization. Authors KDN, AAF performed the methodology. Author AAF did the Software and formal analysis. Authors the KDN and AAF wrote original draft of the manuscript. Authors KDN, AAF, IJK, AJS wrote, reviewed and edited the manuscript. Authors IJK and AJS supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

The necessity to increase the utilization of some neglected but valuable plant leaves in the forest of African countries and use them to the advantage of man motivated the design of this work. The leaves of *Pavetta crassipes* plant were harvested and processed into fine powder of moisture content 8.15%. While the resulting dried *Pavetta crassipes* leaves extract obtained, had moisture

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content of 13.14%. The extract was obtained using central composite design. The design involved varying the extraction time (30 -50 min), extraction temperature (30 to 70^{o}C) and ratio of material to extracting solvent (5 to 10 %). The design resulted into seventeen experimental runs. The responses such as the yield and the total phenolic content were measured. The results showed that the yield of the extract and the phenolic content ranged between 3.52 to 8.84 % and 30.53 to 55.57 mgGAE/100 g. Based on the degree of reliability, the system predicted the optimum extraction conditions as 60.94 ^{o}C, 57.70 min and 5 % for temperature, time and concentration respectively with the corresponding yield and total phenolic content as 8.84 % and 59.87 mgGAE/ 100g respectively. The optimum conditions were found to agree reasonably with the experimental conditions. The study concluded that response surface methology and the developed models could be used to produce the leaves extract of *Pavetta crassipes* plant on a large sclae with enhanced yeild and phenolic content.

Keywords: Pavetta crassipes; central composite designs; phenolic content; response surface methology.

1. INTRODUCTION

The forests of some West African countries are homes of many potent herbs. There is a growing interest in the use of herbs in recent times globally as a result of the unaffordability of the orthodox medicine, perceived toxic effect as well as the native believe that these drugs were originally isolated from these herbs [1]. Some schools of thought are also of the opinions that herbs contain more phytomedicine and phytochemicals than the orthodox medicine and the understanding that a single herb preparation could remedy numerous illnesses simultaneously [2].

One of the herbs that is usually been relied on by locals in Nigeria; is the *Pavetta crassipes* K. Schum (member of the family Rubiacaea). It is a low glabrous shrub of the savannah with stout sub-quadrangular branches covered with pale corky bark which splits and falls off [3]. The plant grows in the savanna regions of the West and Central Africa. The leaves are used for various medicinal purposes, including the treatment of fever, mental ailment, pains, convulsion, hookworms and even schistosomiasis [4]. In other parts of Africa like Tanzania, the leaves are used to treat gonorrhoeae. In Central Africa, the leaves of *Pavetta crassipes* are infused in water and the extract are used to cure cough. In Nigeria, it is known for its crucial therapeutic qualities as the leaves of this plant are used medicinally in the management of respiratory infections and abdominal disorders. Locally; in most parts of Benue State, Nigeria, the plant is generally called "*Ato a ikpain*" in Tiv, "*Ruba tari*" or "*Gadu*" in Hausa and "*Ado mnbio*" in Etilo [5]. The leaves could also be fermented and the steep water consumed together with pap. Furthermore, the leaves are used as part of ingredients in soup preparation [6]. In all of these usage, the phytochemical and bioactive properties as a result of the presence of phenolics in the leaves are motivating factors [7]. Phenolic compounds are a large and diverse group of plant-based secondary metabolites characterized by the presence of one or more aromatic rings with hydroxyl groups attached. They contribute to several important functions in the body and offer potential benefits when consumed. Examples include flavonoids, tannins, phenolic acids, and anthocyanins [8]. They are known for their ability to scavenge free radicals, and higher concentrations suggests greater potential for this activity. Phenolic compounds are known for their strong antioxidant properties, meaning they can neutralize free radicals that damage cells and contribute to various diseases. Research suggests various health benefits associated with consuming plants rich in phenolics [9].

Traditionally, the juice of the *Pavetta crassipes* leaves is extracted by macerating the leaves soaking in water and then blend to make the juice extraction easier. Many of these processes are done without recourse to the appropriate extraction time and temperature, the quantity of the extracting solvent (water) and even the general extraction variables. As such, the required amount of phytochemicals that should be present in the extract obtained would be absent [10]. A look through the literature showed a dearth of information on maintaining adequate conditions to obtain the extracts of the leaves for optimal utilization [11].

According to Saelee, et al. [12], one good method to ensure adequate extraction of the

important component in the extract is the optimum extracting conditions through response surface methodology (RSM). Response surface methodology has been known as one of the most utilized methods to optimize the extraction processes [13]. It describes the statistical approach that is used to optimize a number of complex processes that involve various experimental trials [14]. It brings about the level of interaction between different variables and the extent of the effects of these interactions on the outcome of the experiments [15]. There are several methods that define the response surface methodologies, which include the Central Composite Designs (CCD), Box Benkehn Methods (BBM), factorial designs, mixture designs etc. However, CCD is known as the most commonly used design in RSM. In CCD, the center points are augmented with a group of axial points called star points. Using central composite designs, it is easy and fast to estimate the first and second order terms [16].

RMS has been used to obtain a reasonable amount of extract, called the yield from various plant materials. Previous studies have reported successful use of this method to obtain plants' extracts that are rich in bioactive compounds [17]. Noha, *et al*., [18] had produced phenolic rich extracts rich from *Leontodon hispidulus*, Azahar, *et al*., [19] produced flavonoid and phenolic rich plant extract from *Curcuma Zedoaria* leaves, and Koraqi, *et al*,. [20] established the optimum conditions of extraction to extract antioxidant phenolic rich extract from strawberry fruits. Establishing optimum conditions of extraction, such as the ratio of material to water, time and temperature ranges are important to obtain extract of high phytochemical and bioactive potentials [21]. However, these type of information has not been reported in literature for *Pavetta crassipes.* This informed the designs of this study.

2. MATERIALS AND METHODS

2.1 Plant Material (*Pavetta crassipes* leaves)

Fresh leaves of *Pavetta crassipes* plant were harvested from Ukum Local Government area (*N 7° 38' 59.8848", E 9° 34' 22.4796*") of Benue state, Nigeria, in the month of August, 2022. The *P. crassipes* leaves samples were authenticated by an experienced botanist - J. I. Waya from the Department of Biological Sciences, Benue State University where the *P. crassipes* leaves were stored.

2.2 Methods (Production of Pavetta crassipes leaves powder)

The Fresh leaves of *Pavetta crisprices* were converted to powder as described by Famuwagun, et al. [22] with slight modification. The leaves were washed, drained, oven (Genlab Widnes England. Model B 65) dried at 40 ^{o}C for 10hours. The dry leaves crushed in a hammer mill and the fractions sieved through the laboratory test sieve (Serial No-12041261, B.S.ISO3310, ELE International) with aperture size of 425 microns to obtain fine leaves powder and this was packed in air-tight container until needed for extraction. The flow chart for the production of *P. crassipes* leaves powder was as shown in Fig. 1.

2.3 Experimental Design for the Extract

A central composite design [20] was used to obtain the poly-phenolic rich extract from the leaves of *P. crassipes.* Three level factors were used and this resulted in seventeen experimental runs. The variables selected to produce the extracts were Leaf powder: Water ratio (A), Temperature (B) and Time (C). The coded levels for the independent variables are shown in Table 1. Randomized experiments were carefully employed to reduce the effect of external factors on the observed responses.

2.4 Production of the Leaf Extract

20 g of *Pavetta crassipes* leaves powder was weighed and dispersed in 200, 400 and 600 ml of distilled water that had been pre-heated to the required temperature. The mixture was placed in the 1-liter glass beaker to form suspension at different selected times. The ration resulted in 1:10, 1:20 and 1: 30 weight per volume of the extracting solvent. The glass beakers were then placed on thermostatic magnetic stirrer for the extraction. On reaching the specified and the desired length of time, the suspension was first passed through s muslin cloth, and then centrifuged (3500 ×g for 30min) to obtain a clear filtrate. The liquid extract obtained were concentrated in a rotary evaporator (Rotary Evaporator RE-52A, Union Laboratories, England) at 50°C and dried by pouring the concentrates in pre-weighed vacuum oven (Shell Lab, ILMAC Vacuum). Drying in the vacuum

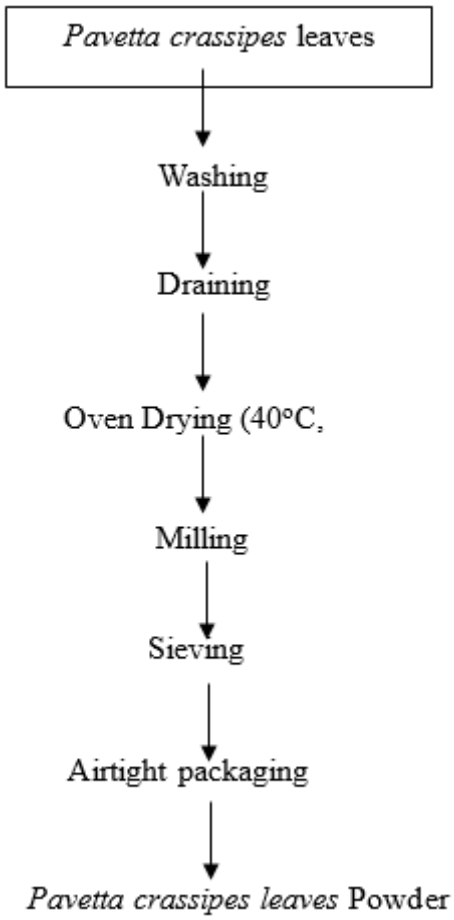

Fig. 1. Flow chart for production *of Pavetta crassipes* leaves powder
Source: Famuwagun, et al., [22]

Table 1. Experimental range and levels of the dependent variables for the production of the extracts of *Pavetta crassipes*

	Code	Range of Values				
Independent Variables		-α	-1	0	+1	+α
Leaf powder concentration in water (%)	A	3.30	5.00	10.0	15.00	11.70
Extraction temperture (℃)	B	23.18	30.00	50.00	70.00	56.82
Extraction time (min)	C	19.77	30.00	45.00	60.00	70.23

Key: Leaf powder:Water ratio (A), Temperature (B) and Time (C).

oven was done at a pressure of 100mBar for 12 h. The dried product obtained was weighed as a function of the starting leaf powder to determine the yield of the extracts [22].

2.5 Determination of Total Phenolic Content

The total phenolic content (TPC) of the dried extract was determined using the Folin-Ciocalteu's phenol reagent as described by Saroj, & Prasad [23]. The calibration curve solutions for the standard were prepared by pipetting 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0mL of gallic acid standard solution (1.0mg/mL gallic acid) in triplicate into clean dried test tubes. The samples were prepared as 1 mg/mL dispersions in distilled water. From the bulk preparation, 0.2mL each of 1mg/mL solution was pipetted into clean dry test tubes in triplicate. Each test tube

(containing standard and samples) was made up to 1.0mL with distilled water. To each of the test tube was added 1.5mL of diluted (1: 4v/v) Folin-Ciocalteu's reagent. The mixture was incubated at room temperature for 5min followed by the addition of 1.5mL of 10% (w/v) NaHCO3 solution to give a total volume of 4.0 mL. The reaction mixtures were further incubated for additional 90min and the absorbance was read at 725nm in a spectrophotometer (INESA, 752N UV-VIS Spectrophotometer) against a blank that contained all reagents except the standard gallic acid which was replaced with distilled water. The standard curve was obtained by plotting absorbance against the concentration of each diluted gallic acid solution. The concentrations of the phenolic compounds in the extract were extrapolated from standard curve and expressed as mg gallic acid equivalent per g (mg GAE/g).

2.6 Determination of Moisture Content

Moisture content of both the *Pavetta crassipes* leaves powder and the dried *Pavetta crassipes* leaves extract were determined using the standard method of AOAC [24-26].

3. STATISTICAL ANALYSIS

The data obtained were analyzed using response surface methodology to fit the quadratic polynomial equation generated by the Design Expert software version 7.0.3.1 (Stat-Ease Inc., Minneapolis, MN, USA). The quality of the fit of the model was evaluated using a test of significance and analysis of variance (ANOVA). The fitted quadratic response model in the present study is described by:

$$Y = \beta_0 + \sum_{j=1}^{k=3} \beta jXj + \sum_{j=1}^{k=3} \beta jjX^2j + \sum_{i>j=1}^{k=3} \beta ijXiXj + e \quad (1)$$

Where "Y" is the response variable, $\beta 0$, βj, βjj, βij are the regression coefficients of variables for the intercept value, linear, quadratic, and interaction effect terms, respectively; Xi and Xj stand for the independent variables, k is the number of them and e is the random error.

4. RESULTS AND DISCUSSION

4.1 Experimental Runs and Responses

The data presented in Table 2 showed the experimental runs for the three variables, such as the extraction temperature, extraction time and the amount of leaf powder in the extracting solvent. It also showed the yield of the extract as well as the phenolic content of the extracts. The highest yield of extract was obtained in the experimental 3, which contained extraction temperature, extraction time and concentrations of 50 ^{o}C, 60 min and 5% respectively, while the least yield of the extract was obtained in the experimental run 4, which was made up of 30 ^{o}C, 30 min and 10 % respectively. The results showed that extract that was obtained using a higher level of temperature, high extraction time and low concentration of leaf powder in water had the highest yield, while the lowest yield was obtained where a low temperature and high leaf powder concentration was used. The lowest phenolic content was obtained in the experimental run 16 (70 ^{o}C, 45 min and 15 %) while the highest phenolic content was observed in the experimental run 3 (50 ^{o}C, 60 min and 5 %). The result also revealed that the experimental run 3 which had the highest yield of the extract also resulted in the highest phenolic content of the extract.

4.2 Analysis of Variance

Tables 3 and 4 showed the analyses of variance for the yield of the extract and the total phenolic content of the samples respectively. The result showed that the lack of fits for all the variables is significant to the pure error. Also, the model was also found to be significant for both the yield and phenolic content. All the linear variables (A, B and C) were found to be significant for the yield of extract but variable A was not significant for the phenolic content. This suggests that only variables B (extraction temperature) and C (extraction time/) are important to determine the trend of phenolic contents. The mixed variables were not significant for the yield of the extract and only AB was also significant for the phenolic content. Only A^2 was not significant for both the yield and the phenolic contents of the extract, while other squared variables (B^2 and C^2) were found to be significant. Famuwagun et al. [22] showed that a non-significant variable does not have influence on predicting the trend of the responses. This implies that only the variables that are significant would affect the behavior of the responses. By implication, the trend of responses in this study would be strongly determined by the extraction temperature and time. The R-squared value, adjusted R-squared value and predicted R-squared value for the yield and phenolic contents ranged between 0.823 to 0.836 and 0.700 to 0.898 respectively. The result

indicated that the R-squared values are in reasonable agreement with the predicted and adjusted values, this suggest that the model is fit to correctly navigate the system. Ohale, et al. [27] reported that R-values had great implication on the fitness and suitability of the model to predict the process and the closeness of the R-squared values to the adjusted and predicted is required for the fitness of the model. In this study, the different R-values are in reasonably close agreement with one another, which suggests that the model is fit to predict the variables. The regression equations to predict the yield and the total phenolic contents are shown in equations 2 and 3.

Yield of Extract = +5.67+0.99A+0.75B -1.13C-0.038AB-0.27 AC-0.14BC-0.39 A2

+0.71B2+0.22C2 (2)

Total Phenolic content = +43.99+1.55A+4.66B-5.31C+4.48AB-3.22AC-2.28BC-5.49A2+1.42B2+0.21C2 (3)

As shown in the equation (2), the co-efficient of the two out of the three linear terms (A & B) are positive while the variable C was negative. All the interactive terms were negative while all the squared terms were positive. On the total phenolic contents (equation 3), the coefficient of the linear terms (A and B) were positive while C was negative. The interactive terms (AC and BC) were negative while AB is positive. The squared terms (B2 and C2) were positive while only A2 was negative. The observed positive and negative coefficient terms of some variables in the equation suggest that the regression equation has degrees of fitness to predict the optimum values of the responses.

4.3 Analyses of the 3-Dimensional Plots

The three dimensional plots are representations that showed the pictorial imitation of the regression equation. Figs. 2a, b and c showed the representation of the regression equation for the yield of the extract obtained from the leaves of *P. crassipes.* Fig. 2a showed the interaction between the extraction temperature and time on the yield of the extract. The result showed that increase in the extraction time and temperature resulted in a progressive increase in the yield of extract. This aligns with Guglielmetti et al. [28]. who observed a positive relationship between an increase of temperature and total phenolic content (TPC) for predictable solvent extraction. They established that temperature was the most active process variable on extraction procedures.

The high yield of extracts might have resulted from continuous homogenization that enhanced the widening of the surface area of the sample. The trend aligns with the position of De Faveri et al. [29] who reported the increase in temperature and time of extraction allowed the solvent to percolate into the matrix of material to support the migration of the extract to the surface. Fig. 2b showed the interaction between the extraction temperature and ratio of material to extracting solvent. The result showed that the interaction between these two factors resulted in a decrease in the yield of extract. By implication, the higher the ration of material to solvent, the lower the quantity of extract obtained and vice-versa. Even when the temperature is raised in the extracting chamber, high material to solvent concentration would not allow the increase to produce high volume of extract. The low amount of extract obtained in this case might be attributed to reduction in the kinetic energy in the chamber. The more of material in the extracting chamber, the homogenizer finds it difficult to speed up the mixing, even at high temperature, few amount of extract was still produced.

Fig. 2c showed the interaction between the ratio of the material to extracting solvent and the extraction time. The interaction between these two variables resulted in reduced amount of extract. This might have been possible as a result of the reduction in the speed of homogenizer when more of the material was present in the extracting chamber. As shown in the Fig. 2c, increasing the time of extraction could not increase the amount of extract obtained, because the solvent had no room to percolate the material to expand its surface area.

Similarly, Figs. 3(a-c) showed the pictorial representation of the interaction between extraction temperature, time and material to water concentration. As shown in Fig. 3a, increase in the extraction time and temperature resulted in a proportionate increase in the phenolic content of the samples. This trend might be related to the increase in the kinetic energy of the mixture in the extraction chamber for a lengthy amount of time, thereby enhancing the release of the extracts from the material. In the

same manner, Fig. 3b showed the interaction between the extraction temperature and the ratio of material to extracting medium. The interaction between the two factors resulted a marginal increase in phenolic content of the samples. However, it was noticed here that an increase in the temperature above 60 ^{o}C was seen to have lower phenolic content. In Fig. 3c, which depicted the interaction between extracting time and ratio of material to extracting solvents, increase in the extraction time produced lower phenolic content of the samples. This trend could be explained on the basis that neither the material concentration in extracting solvent nor the extraction time could increase the kinetic energy of the mixture in the chamber. However, a low amounts of material to extracting solvent coupled with longer extraction time resulted to maintaining a relatively high phenolic content of the samples.

Table 2. Effect of processing variables on the experimental yield and phenolic content of the extract of *Pavetta crassipes* leaves

Experimental run	Extraction temp (^{o}C)	Extraction time (min)	Leaf powder to water (%)	Extract yield (%)	Phenolic content (mg GAE/100 g)
1	30	45	5	5.32	40.43
2	50	30	5	6.54	45.43
3	50	60	5	8.84	55.57
4	30	30	10	4.56	35.55
5	30	60	10	5.62	39.65
6	50	45	10	5.01	43.99
7	50	45	10	5.04	40.54
8	50	30	15	4.65	40.21
9	50	45	10	5.85	49.54
10	70	60	10	7.34	53.24
11	70	45	5	8.02	48.43
12	50	45	10	6.43	42.65
13	50	60	15	6.38	41.24
14	50	45	10	6.01	43.22
15	70	30	10	6.34	31.23
16	70	45	15	5.12	30.54
17	30	45	15	3.52	35.43

Table 3. Analysis of variance (ANOVA) yield of extract from *Pavetta crassipes*

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value	Prob>F
Model	25.83	9	2.87	9.27	0.0039	Significant
A	7.78	1	7.78	25.14	0.0015	Significant
B	4.50	1	4.50	14.54	0.0066	Significant
C	10.24	1	10.24	33.07	0.0007	Significant
AB	0.00562	1	0.00562	0.018	0.8966	Not Significant
AC	0.30	1	0.30	0.98	0.3558	Not Significant
BC	0.081	1	0.081	0.26	0.6242	Not Significant
A^2	0.65	1	0.65	2.11	0.1895	Not Significant
B^2	2.14	1	2.14	6.92	0.0338	Significant
C^2	0.21	1	0.21	0.66	0.4419	Significant
Lack of fit	0.61	7			0.31	Significant
Pure error	1.56	4			0.39	
R-squared value	0.922					
Adjusted R-squared value	0.823					
Predicted R-squared value	0.836					

Table 4. Analysis of variance (ANOVA) phenolic content of polyphenol from the leaf powder

Source	Sum of squares	Degree of freedom	Mean square	F-value	P-value	Prob>F
Model	692.68	9	76.96	6.81	0.0096	Significant
A	**19.16**	**1**	19.16	1.69	0.2342	Not Significant
B	173.72	1	173.72	15.37	0.0057	Significant
C	225.14	1	225.14	19.92	0.0029	Significant
AB	80.19	1	80.19	7.09	0.0323	Significant
AC	41.54	1	41.54	3.67	0.0968	Not significant
BC	20.75	1	20.75	1.84	0.2176	Not significant
A^2	126.80	1	126.80	11.22	0.0123	Significant
B^2	8.46	1	*8.46*	*0.75*	*0.4157*	Not Significant
C^2	0.18	1	0.18	0.016	0.4419	Not signficant
Lack of fit	34.04	3			0.4767	Not sig
Pure error	45.09	4			11.27	
R-squared value	0.898					
Adjusted R-squared value	0.766					
Predicted R-squared value	0.700					

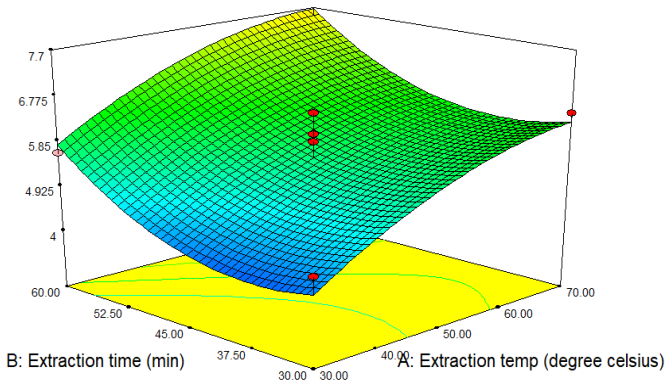

Fig. (2a).

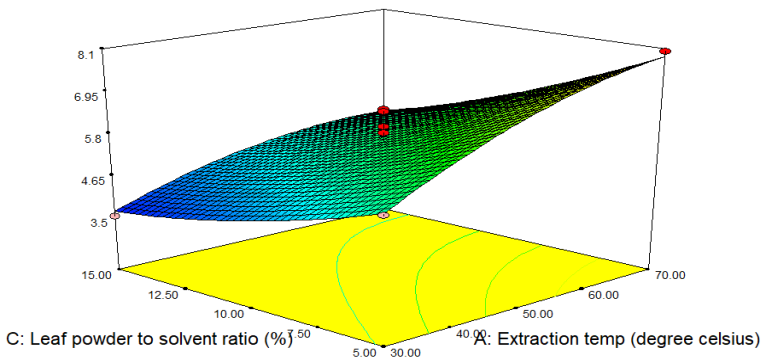

Fig. (2b).

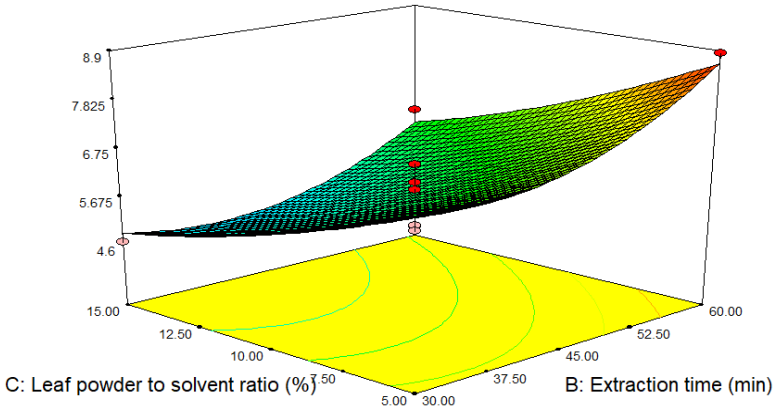

Fig. (2c).

Fig. 2: 3-D showing (a) effect of temperature and time on the yield of extract, keeping the concentration constant (b) effect of temperature and concentration on the yield of extract, keeping the time constant, and; (c) effect of concentration and time on the yield of extract, keeping the tempereature constant

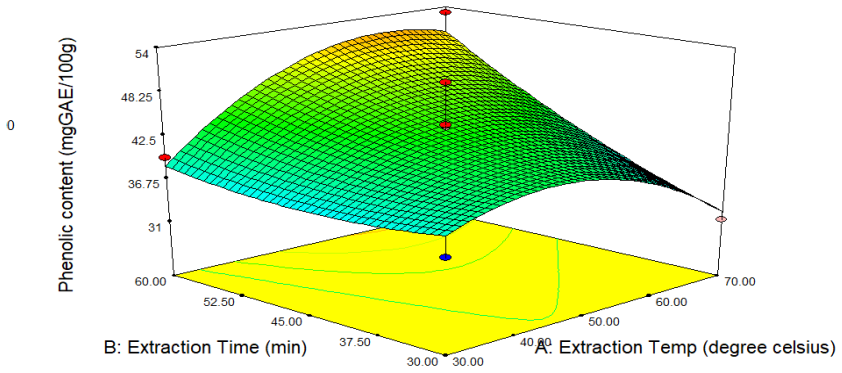

Fig. (3a).

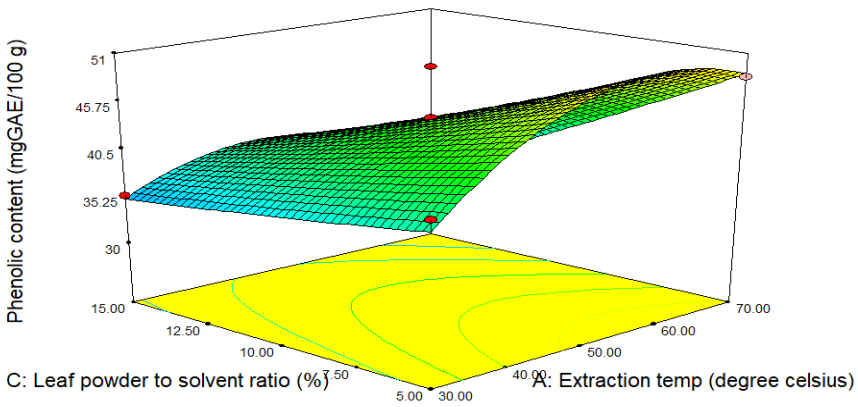

Fig. (3b).

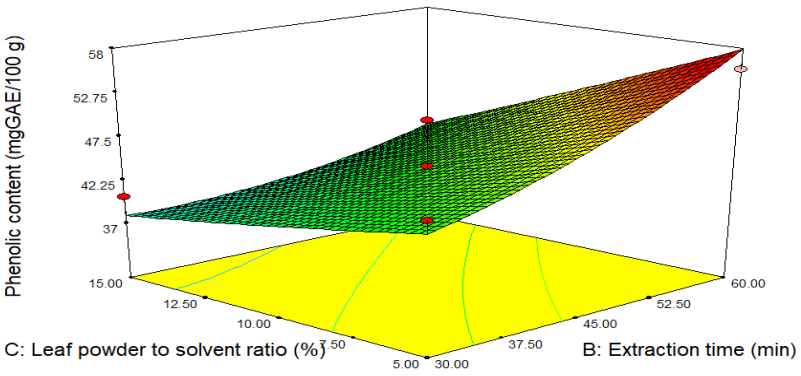

Fig. (3c).

Fig. 3: 3-D showing (a) effect of temperature and time on the phenolic content of extract, keeping the concentration constant (b) effect of temperature and concentration on the phenolic content of extract, keeping the time constant, and; (c) effect of concentration and time on the phenolic content of extract, keeping the tempereature constant

4.4 Process Optimization and Validation

To arrive at the predicted process conditions, it is important to consider the goals of optimization in this study. The intention was to keep the process conditions (temperature, time and ratio of material to solvent) at minimum while having higher yield of extract and phenolic contents. Keeping these goals in mind, it is possible to obtain optimum extraction conditions for this process. Based on the level of desirability of the various possible numerical solutions suggested by the design expert software, the optimum conditions for extracting temperature, predicted time and ratio of material to concentration were 60.94 ^{o}C, 57.70 min and 5 % respectively. With these optimum conditions, the predicted yield was 8.84 % while that of the phenolic content stood at 59.87 mgGAE/ 100g of the sample extract.

On validating the optimum conditions using the establshed process variables, the yield of the extract was found to be 5.03 % while the total phenolic contents were found to be 59.82 mgGAE/100 g. The validated values of the responses were found to be in a close agreement with the predicted values by the soafware, which suggest that the response surface could be used to predict the extracting variables for the process [30].

5. CONCLUSION

The yield of the extract and phenolic content of *Pavetta crassipes* leaves has been optimized using response surface methodology. Good experimental descriptions of the data were given using second order polynomials and predicted optimum conditions for the extraction temperature, time and ratio of water to material. The optimum values obtained for these extraction variables (60.94 ^{o}C, 57.70 min and 5 %) for temperature, time and concentration was found to be in reasonable agreement with the experimental values. The study therefore concludes that using the optimum condition in this study would translate to high extraction yield and phenolic contents which would eventually lead to popularization of the *Pavetta crassipes* leaves extract as a possible source for natural antioxidants in food formulations for the development of new class of functional foods to promote or enhance health benefits.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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