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EVALUATION OF EFFECTS OF PROCESS PARAMETERS ON THE EXTRACTION OF BIOACTIVE COMPOUNDS FROM *TETRAPLUERA TETRAPTERA* FRUIT USING FULL FACTORIAL DESIGN

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Abstract

Three variables Full Factorial Design (FFD) was employed in this work to analyze and optimize the yield of bioactive components from Tetrapluera tetraptera fruit extract. The extraction process variables considered and their range of values was extraction temperature (78-90℃), particle size (1-5 mm) and extraction time (10-50 mins). Gas chromatography/Mass spectrometer (GC-MS) was employed for the identification of the bioactive compounds in the extract of Tetrapluera tetraptera fruit. It was observed that 2FI (two factor interaction) model best described the relationship between response and independent variables. Statistical analysis showed that temperature and time had significant effect on the yield of bioactive components from the fruit. There was reasonable correlation between the predicted yield of extract at optimum condition (30.55%) and the observed yield of 31.17%. The optimal yield of extract was obtained at extraction temperature of 90℃, particle size of 3 mm and extraction time of 50mins. GC-MS analysis of the optimum extract of Tetrapluera tetraptera fruit showed the presence of some pharmaceuticals and bioactive compounds with D-fructose being the most abundant.

***Keywords*:** *Tetrapluera tetraptera*, Extraction variables, Full factorial design, Bioactive compounds

1. INTRODUCTION

Plants are just as significant as the sun is in the solar system. They remain the fundamental of all life here on earth and essential resources for human well-being. Plants provides us with foods, water (by aiding in regulating of the water cycle), air (produces oxygen that we then breathe), habitat (apart from myriad uses, plants also from the backbone of habitats in that fishes and other wild life also depends on plants), climate (plants store carbon which helps reduce carbon (iv) oxide in the atmosphere) and medicine (most drugs come directly or from derivatives of plants, a good percentage of persons around the globe rely on plants for primary health care). Ranked as one of the fastest growing economy in Africa, agriculture over the years has been the economy's main stay in Nigeria. In the early 60'sagriculture lead to an increased GDP of the country to about 69 to 80% [1]. In a sector where plant cultivation and production plays a major role, the significance of plants and its derivatives are endless. The study of medicinal plants used in folklore regime in treatment of diseases has attracted the attention of many scientists as possible and reliable alternatives to existing drugs. The use of plants in traditional medical practice has a long drawn history, and remains the main stay of primary health care in most of the third world countries [2].

Nigeria, being one of the foremost producers of *Tetrapleura tetraptera* in the West Africa coast disposes this fruit's parts such as seedlings, bark and leaves down to the root into the environment without having a full knowledge of how useful the plant could be leading to environmental pollution and wastage of natural resources [3]. The shortcomings of technologies in isolating these bioactive components in *Tetrapleura tetraptera* to meet demands in pharmaceutical industries also possesses a problem which a careful follow up of this study has been able to meet that identified knowledge gap. Nigeria, a well-known world producer of *Tetrapleura tetraptera* has not attained maximum satisfaction from this product just yet in that there is no optimum isolation of the required bioactive compound as this could go a long way in reducing cost to achieve more out of *T. tetraptera*. This study is aimed at providing the necessary experimental data to pilot a commercial production of D-Fructose and flavonoids as the major bioactive compounds from *Tetrapleura tetraptera* fruit [4].

Full factorial design improves on the inefficiency of traditional experimental methods of changing one factor at a time to determine the effect of factors on processes by employing a suitable statistical technique which considers not only the effects of the main factors but also the effects of interaction between process parameters and their effects on the peak performance of a process. In this work, the full factorial design was employed for the design, analysis and optimization of the process parameters involved in the extraction of bioactive compounds from *Tetrapleura tetraptera* fruit.

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2. MATERIALS AND METHODS

2.1 MATERIALS

Tetrapleura tetraptera fruits were obtained from Oba Market in Benin City, Edo State. Analytical grade ethanol used for the extraction and other chemicals/reagents were purchased and used without further purification.

2.2 METHODOLOGY

2.2.1 Preparation of sample

The fruit was prepared using method described in [5]. The fruit were chopped into tiny pieces to increase the surface area needed for the sample to dry quickly. Fresh *Tetrapleura tetraptera* fruit was cleaned with fresh water to discard dust and other impurities. It was sun-dried for three weeks to reduce the moisture content after which the peels were handpicked and blown away remaining the chopped seeds of the fruit. The seed was ground to fine powder with a view to increasing the surface area so as to facilitate the extraction process and was sieved into particle sizes of 1, 3 and 5 mm.

2.2.2 Extraction of bioactive compounds

The extraction process was done using method described in [6] with modification of the precursor material. The fine *Tetrapleura tetraptera* of the different particle sizes were subjected to solvent extraction process using ethanol as solvent. The extraction process was carried out using Soxhlet apparatus placed in a heating mantle. 20 g of each size was used for the extraction process as given by Full Factorial Design of Table 1. After the attainment of the desired extraction time, the heating mantle is switch off and the filter paper thimble (holding the fine *Tetrapleura tetraptera* saturated with the heated solvent) is subjected to squeezing so as to get as much liquid component as possible. This liquid component was transferred back into the distillation unit to separate the *extract* from the extraction solvent and the mass of the *extract* was obtained. The yield of extract was determined using Equation (1).

$$Yield\ of\ extract\ (\%) = \frac{mass\ of\ extract}{mass\ of\ precursor\ material} \times 100\% \quad (1)$$

Table 1:Coded and actual levels for extraction process

Variables	Symbols	Coded and actual levels		
		-1	**0**	**+1**
Extraction temperature (℃)	X_1	78	84	90
Particle size (mm)	X_2	1	3	5
Extraction time (mins)	X_3	10	30	50

2.2.3 Identification of bioactive components

The extract obtained at the optimum condition was characterized using a Gas Chromatograph Mass Spectrophotometer (GC-MS). The conditions of the equipment are as follows: GCMS 7890A, Detector: mass spectrometer (MS 5975C), Column thickness: 0.25µm, Length: 30m, Internal diameter: 0.32mm, Carrier gas: Helium, Flow rate: 1ml/min.

3. RESULTS AND DISCUSSIONS

3.1 Analysis of yield of extract

The percentage yield of bioactive compounds obtained from the 21 experimental trials carried out according to the full factorial design is given in Table 2.

Table 2: Yield of *Tetrapluera tetraptera* fruit extracts

Std. order	Run order	Temperature (℃)	Particle size (mm)	Extraction time (mins)	Yield of extract (%)
11	1	90	1	30	17.08
13	2	90	1	50	18.51
9	3	78	1	10	0.00
10	4	78	1	30	8.83
19	5	78	1	50	7.86
2	6	84	1	10	0.00
5	7	84	1	30	9.88
4	8	84	1	50	13.63
15	9	78	3	10	0.00
18	10	78	3	30	8.93
21	11	78	3	50	11.41
12	12	84	3	50	17.82
8	13	90	3	30	23.98
3	14	90	3	50	31.17
20	15	78	5	10	0.00
22	16	84	5	10	0.00
7	17	84	5	30	5.05
14	18	90	5	10	0.44
6	19	90	5	30	18.96
1	20	90	5	50	23.80
17	21	78	5	30	10.59

Equation (2) is the regression model in terms of coded units showing the relationship between the yield of extract and extraction process parameters considered.

$$Y = 29.41 + 4.26 \times 10^{-3}X_1 - 0.0109X_2 - 0.1637X_3 + 6.73 \times 10^{-5}X_1X_2 - 4.78 \times 10^{-5}X_1X_3 + 3.6 \times 10^{-5}X_2X_3 + 1.33 \times 10^{-6}X_1X_2X_3 \quad (2)$$

From the equation of the model, it was observed that the effect of the extraction temperature (X_1) is positive; we can affirm that X_1 has a positive effect on the yield of extract (Y) which is important. This result means that the yield increases when two factors changes from low level to high level. On the other hand, the particle size (X_2) and extraction time (X_3) had negative and positive effects on the yield of extract respectively. This means that, the yield falls when the particle size is varied from the low to high level while the extraction time tends to have similar effect on the responses just like the extraction temperature. From the model summary statistics shown in Table 3, it was observed that the 2FI (two factor interaction) model had greater R^2 values than the main effects model. Although 3FI model had the highest R^2 value, it is normally aliased from the model based on the assumption that the effects of three factors are highly insignificant. Thus, it can be concluded that 2FI model best describes the relationship between response and independent variables. Analysis of variance (ANOVA) was used to determine the factor(s) that has significant effect(s) on the yield of extract at 5% significant level i.e. the probability value of 0.05 ($p - value = 0.05$).Factors/terms with p-values greater than the significant level are considered insignificant. From Table 4, it can be seen that the p-value of the model of 0.05 and a large F-value of 21.83 shows model is significant for predicting the yield of extract..Among the main factors, extraction time and extraction temperature with p-values less than 0.05 had significant effects on the yield of extract while particle size was found to be insignificant.

Table 3: Model summary statistics

Source	Standard deviation	R-squared	Adjusted R-squared	Predicted R-squared	PRESS
Main Effects	4.41	0.8088	0.7610	0.6626	548.88
2FI	3.61	0.9949	0.8397	0.6701	536.71
3FI	3.80	1.0000	0.9724	N/A	N/A

Table 4: Analysis of variance for response surface 2FI order

Source	Sum of Squares	df	Mean Square	F- Value	P-Value Prob>F
Model	1618.48	18	89.92	21.83	0.0500*
X_1-Temperature	340.65	2	1510.33	41.36	0.0236*
X_2-Particle size	29.40	2	14.70	3.57	0.2188
X_3-Time	801.21	2	400.61	97.27	<0.0102*
X_1X_2	55.67	4	13.92	3.38	0.3412
X_1X_3	185.55	4	46.39	11.26	0.5432
X_2X_3	58.99	4	14.75	3.58	0.2300
Residual	8.24	2	4.12		
Cor. Total	1626.72	20			

*=significant

3.2 Effects of interaction of process parameters on yield of extract

A further research was carried out to evaluate the significance of the various factors as it affects the response and this was done using the factor plot interaction (Figure 1) with the R^2 value to determine the most significant factor among other factors and it was concluded that the extraction time with a R^2 value of 0.5718 is the most significant factor to consider if process optimization is of paramount interest. Extraction temperature had an R^2 value of 0.3387 and the particle size had an R^2 value of about 0.0014 and the above values distinctively gave the relevance of the various factors to the extraction process.

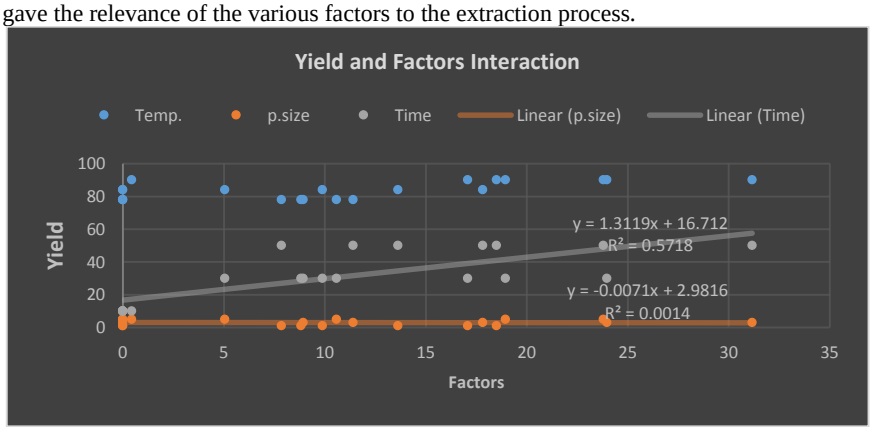

Figure 1: Yield and process parameters interaction.

3.3 Responses surface effects between factors

Figure 2 and 3 shows the response surface interaction from Design Expert (version 7.0) software under full factorial design of experiment and the response expressly explains the effects of the factors on the yield obtained from the extraction process. The factors which includes extraction temperature, particle size and extraction time were analyzed and the response shows that yield increases with a corresponding increase in extraction time as well as extraction temperature but the particle size effect was not significant on yield of extract.

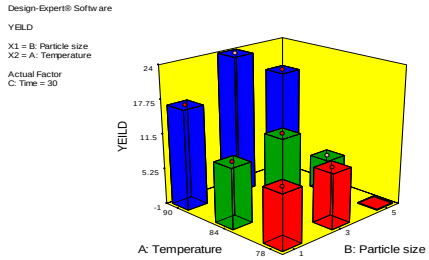

Figure 2: 3D bar chart Response of temperature and particle size.

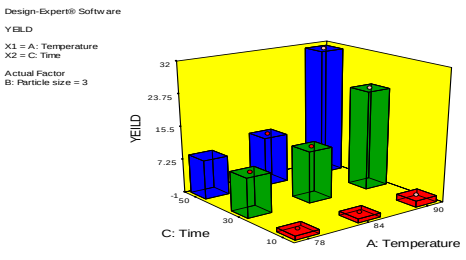

Figure 3: 3D bar chart Response of time and temperature.

3.4 Validation of the model

The fit of the model was further checked by the coefficient of determination R^2. The R^2 value is always between 0 and 1. The closer the R^2 is to 1, the better the model predicts the response [7].It was found that there is a strong correlation between the two performances with a coefficient of about 0.9949(Table 3). That is to say that about 99.5% of the variability in the yield of extract can be explained by the model. The predicted R^2 of 0.6701 is in reasonable agreement with the adjusted R^2 of 0.8397. Having a difference less than 0.2 makes the model effective and predictive. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 10.510 indicates an adequate signal. Therefore, the model (Equation2) can be used to navigate the design space. In addition, according to Table 4, the F- value of the model is significant, so the model adopted in this study (full factorial design) is acceptable and validated.

3.4 Process optimization

In order to optimize the conditions or process parameters of the extraction experiment, a desirability function (D) for the simultaneous optimization of multiple responses was used. This function can be described as follows [8]:

$$D = (\prod_{i=1}^{n} M_i)^{\frac{1}{\sum ri}} \quad (3)$$

Where D varies in range of $0 \leq D \leq 1$ and N, r_i and m represent the number of responses, importance of a particular responses, and partial desirability function for specific responses, respectively. Design expert software has inbuilt optimization tool which uses the best combination to give the highest yield. This study aimed at extract the highest amount of extract from the fruit or sample. The optimum condition is whenever extraction was at high level of all three factors. In other words, the best combination of the conditions that gives maximum extract of the bioactive component from the *Tetrapluera tetraptera* fruit. The maximum yield of extract of 31.17% was obtained at an extraction temperature of 90℃, particle size of 3mm and extraction time of 50 mins.

3.6 Gas Chromatography/Mass Spectrometry (GC-MS) analysis

The gas chromatography/mass spectrometry works on the principle that a given mixture would separate into its component substances or individual substances when heat is applied. The heated gases are carried through a column with an inert gas (such as helium). The separated substances then emerge from the column opening the flow into the MS for identification of the individual substances [9]. About 31% of the total compounds quantified from the samples *Tetrapluera tetraptera* showed pharmaceutical properties or can be used for the production of our modern day drugs for treatment and prevention of various ailment. Table 5 gives the composition of ethanolic extract from *T.tetraptera* peels. The table revealed that the extract has appreciable significant ($p<0.05$) amount of cardiac glycoside (24.5 mg/100g), tannins (23.87 mg/100g), phenol (21.70 mg/100g) and flavonoids (20.48 mg/100g) and low concentration of alkaloids (1.43%). Terpeneoids, steroids and phelebotanin were also detected in the extract but were not determined. GC-MS analysis revealed the presence of D-fructose, 2-hydroxy-gamma-butyrolacetone, acetic acid, glyceraldehydes, piperazine, octodrine, glycidol, and n-decanoic as shown in Table 5.The highest reducing activity was observed at the highest concentration for the ethanoic extract and standards used in this study.

Table 5: Identified Bioactive Compounds of *Tetrapleura tetraptera* peels ethanoic extract.

Peak No.	Compound	Retention time (mins)	Area (%)
1	*Acetic acid*	3.41	0.04
2	Glyceraldehyde	4.01	5.18
3	Piperazine	4.83	5.12
4	DL-Alanine, N-Acetyl	5.03	1.4
5	Octodrine	5.15	0.67
6	Glycidol	5.35	1.03
7	*2-Hydroxy-gamma-Butyrolacetone*	7.67	1.92
8	4H-Pyran-4-one	7.85	1.3
10	D-fructose	10.49	49.6
12	1,3-Dioxolane-4-methanol	13.81	1.12
13	n-Decanoic acid	14.25	1.87
15	9,12-Octadecenoic acid	16.14	14.05
16	6-Octadecenoic acid	16.22	8.75
17	1,3-Dioxolane-4-methanol	16.57	0.67

From Table 5, it can be concluded that *Tetrapluera tetraptera* ethanoic extract contains the above bioactive compound and according to the screening result it can be said that D-fructose is the most active bioactive compound in the fruit haven occupied the largest % Area of the entire Fruit i.e. 49.6% (Figure 4). The scope of this study is geared towards optimizing the production of this most active compound of the fruit by varying extraction parameters as evaluated in this study previously. It has also been discovered that the bioactive compound of this fruit demonstrates good health benefits and prevent the risk of chronic diseases such as diabetes, cancer, obesity, neurodegenerative and cardiovascular diseases [10]. Phytochemicals such as flavonoids, phenols, cardiac glycosides, and terpenoids which were detected in the ethanoic extract have been reported to possess various pharmacological effects such as antioxidants, antidiabetic, antihypertensive, and anti-Alzhemic activities [11].

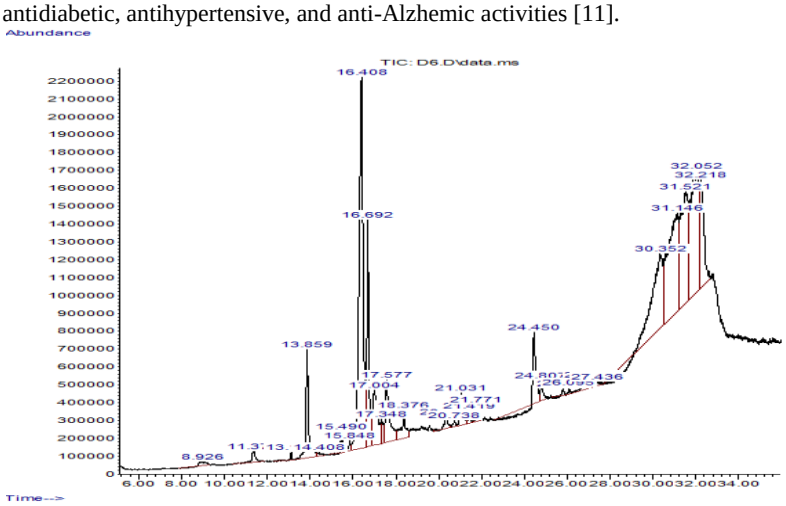

Figure 4: GC-MS chromatogram of Tetrapleura tetraptera peels ethanoic extract.

4. CONCLUSION

This study discusses the performance of full factorial designs on the modeling and optimization of the effects of extraction temperature, extraction time and particle size on *Tetrapluera tetraptera* fruit yield using ethanol. The effects of the three variables (temperature, time and particle size) and their interactions on the yield of *Tetrapluera tetraptera* fruit were investigated. From the study, the main parameters influencing the extraction process are extraction temperature and extraction time. The interaction between extraction time and extraction temperature and the interactions between the extraction time and particle size were the most important interactions. The reasonable correlation between the predicted and observed yields of *Tetrapluera tetraptera* fruit was high and significant with R^2 of 0.9949 and 0.9494 respectively which confirmed the validity and practicality of the adopted model. Optimization using the full factorial design gave an extraction temperature of 90℃, particle size and extraction time of 50mins of 3mm as the optimal process parameters to obtain a maximum yield of 30.54% yield of extract.

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