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Research Article

Evaluating the Effects of Process Parameters on the Yield of Bioactive Compounds from Tetrapluera Tetraptera Using Full Factorial Design

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Abstract

A three variables full factorial design was use to optimize the yield of bioactive components of Tetrapluera tetraptera fruit extract. This study using extraction temperature, particle size, extraction time, considered for experimental design. During the optimization of the extraction process parameters, the yield was optimized to obtain 30.545% which correlated the experimental observed yield of 31.17%. The optimized of temperature, particle size and time were obtained: 90°C, 3mm and 50 mins respectively. Gas chromatography Mass spectrometer (GC-MS) analysis of Tetrapluera tetraptera fruit showed pharmaceuticals and bioactive compounds such as Acetic acid, Glyceraldhyde, Glycidol, D-Fructose etc. with D-fructose was most abundant, about 49.6% area of the sample.

INTRODUCTION

Plants are just as significant as the sun is in the solar system [1]. They remain the fundamental of all life here on earth and essential Resources for human's well-being [2]. 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) [3]. Ranked as one of the fastest growing economy in Africa, agriculture over the years has been the economy's main stay in Nigeria [4,5]. In the early 60'sagriculture lead to an increased GDP of the country to about 69 to 80% [6,7]. In a sector where plant cultivation and production plays a major role, the significance of plants and its derivatives are endless [8,9]. 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 [10,11]. 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 [12,13].

Nigeria, being one of the foremost producers of Tetrapleura tetraptera in the West Africa coast disposes this fruit's parts such as seedlings [14,15], 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 [16,17]. 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 [18]. The Inefficiency of traditional experimental methods of changing one factor at a time to determine the effect, or the interactions between experimental parameters and how they affect the optimization of the extraction process was improved upon using the full factorial design this helped in enhancing the extraction process [19]. To optimize the effects of process parameters required to maximize the bioactive compound yield from Tetrapleura tetraptera fruit using full factorial design and the work was aimed also at determining the major bioactive compounds been extracted from the fruits using GC-MS (Gas chromatography and Mass spectrometry) [20], and using a special extraction technique i.e. Soxhlet extraction method as a means of carrying out the extraction process [21]. Nigeria, a well-known world producer of Tetrapleura tetraptera has not attained maximum satisfaction from this produce 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 Tetetraptera [22]. This study will provide the necessary experimental data to pilot a commercial production of D- Fructose and Flavonoids as the major bioactive compounds from Tetrapleura tetraptera fruit [23,24].

MATERIALS AND METHODS

Materials

The materials used to carry out this study include the following:

Samples: Tetrapluera tetraptera blended sample, Lubricants, Ethanol, Distilled water.

Apparatus: Soxhlet apparatus, Beaker, Erlenmeyer flask, Round bottom flask, Stop watch, Digital weigh balance, Thermometer, sieves.

Sample collection

Tetrapleura tetraptera fruits samples used was obtained from the Oba Market here in Oredo local government, Benin city, Edo state. The Ethanol was bought from JHD chemicals branch office at Effurun, Delta State, Nigeria. The blending and sizing of feedstock was carried out a Uselu market, Ugbowo, Benin city, Edo state.

Extraction method principle

In Soxhlet Extraction, Heat was supplied through a mantle into the round bottom flask containing the extracting solvent. Above this flask is the extraction apparatus being held by a retort stand. The solute to be extracted is held by a thimble (paper, cloth, cellulose etc.) which seats in the apparatus [25]. The condenser was placed above the extracting apparatus with cooling water into the lower inlet and leaving through upper outlet [17]. The mantle also was continuously adjusted to ensure the solvent boils adequately. The condensed solvent falls into the thimble from the distillation path slowly extracting the solute substance present there [26]. When the extraction chamber is filled, the solvent siphons back into the flask as reflux. The process is then allowed to continue for as long as necessary [27] (Figure 1 and Table 1).

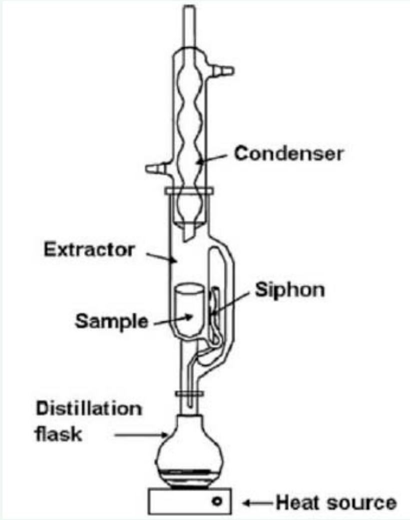

Figure 1 Experimental kit of Soxhlet apparatus.

Table 1: Variables and their levels for full factorial design.

Independent Variables	Symbols	Coded and actual levels		
		H	Aug	L
		-	0	+
Extraction time (mins)	X_1	10	30	50
Particle size (mm)	X_2	1	3	5
Temperature (°C)	X_3	78	84	90

Methods

20g of dried grounded ginger was measured and weighed in the weighing balance and placed in a small transparent sample sack and was placed in the extractor section of the soxhlet apparatus. 200ml of ethanol (extracting solvent) was measured in the distillation flask for the first run. The soxhlet apparatus was set as shown in Figure 3 with the condenser connected to the water outlet at the top and a water inlet at the bottom. This mechanism helps in the cooling and therefore makes condensation in the condenser possible. The heating mantle was set to a temperature of 90°C for 90mins (for the first run). After heating for a while the extracting solvent (C_2H_3OH), begins to evaporate because of the boiling-point which is 78.37°C. The solvent vapour travels up the distillation arm and meet with the cold water in the condenser which condenses the vapour to liquid and back to the extractor to extract the Tetrapluera tetraptera sample in the sack. The extractor containing the ginger sample is slowly filled with warm ethanol solvent and some of the desired component from the Tetrapluera tetraptera is then dissolved in warm ethanol solvent and some of the desired component from the Tetrapluera tetraptera is then dissolved in the warm solvent. When the soxthlet chamber (extractor), is almost full, it is automatically emptied by the siphon side arm with the solvent running back down to the distillation flask. After 50 mins the process is stopped and the ethanol in the extractor is poured back into the distillation flask and the one contained in the ginger sample is pressed with hand into the distillation flask. Next is to evaporate the quantity of ethanol from the ginger component extracted. The mixture containing ethanol and the ginger component is then heated at 78°C, the ethanol starts to escape as vapour and is condensed back to the extractor but this time, caution is taken not to allow reflux occur as this bring back the ethanol been removed from the mixture, to avoid this when it close to reflux level the process is stopped and the ethanol liquid is removed and poured into a container [28]. This process is repeated till the ethanol in the mixture is almost completely removed and all we have left is the ginger extract. The extract is collected from the distillation flask and stored in a co can, and weighted to get the mass of extract [29]. Also the ethanol evaporated from the ginger extract is collected and weighted to get the volume of ethanol after the extraction process. This method was repeated for all the runs, keeping the mass of 20g constant but varying the volume of solvent and temperature.

Let:

Mass of beaker = Ml (g)

Mass of beaker + oil = M2 (g)

Mass of oil = M2 -Ml = M3 (g)

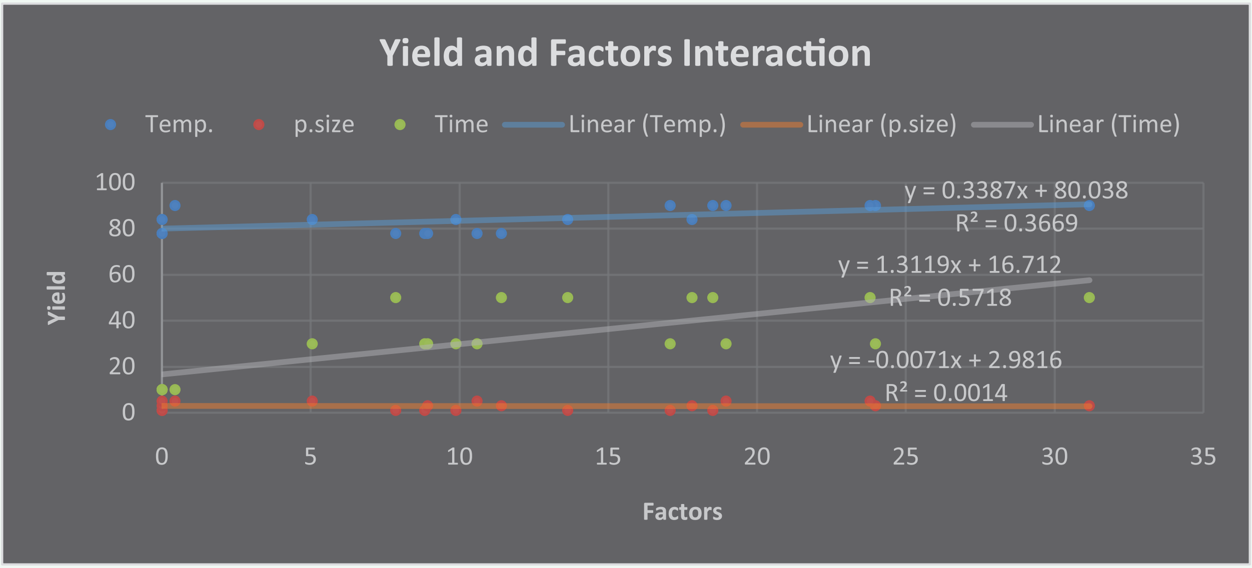

Figure 2 Yield and Process Parameters Interaction.

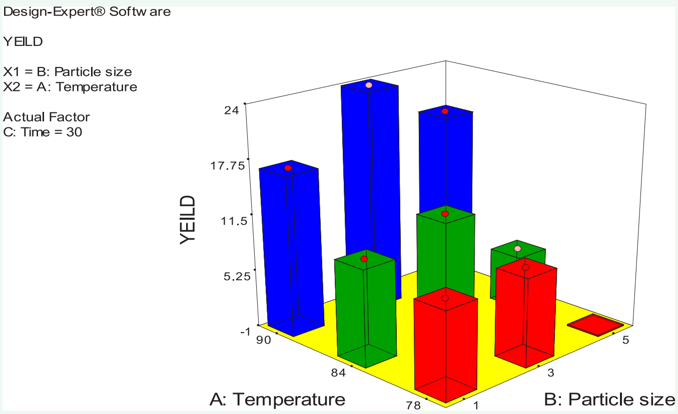

Figure 3 3D bar chart Response of temperature (°C) and particle size(mm).

Mass of sample (ground bitter kola seeds) = M (g)

$$\frac{mass\ of\ oil}{mass\ of\ sample} \times \frac{100}{1} = \frac{M3}{m} \times \frac{100}{1}$$

The values of volume of solvent, weight of sample and extracting time were used according to the experimental design and a total of 11 experimental runs were recorded. The experimental set-up of the soxhlet assembly in the laboratory is shown in Figure 3.

RESULTS AND DISCUSSIONS

Statistical analysis

The results of the 21 experimental runs carried out according to the full factorial design are shown in Table 2. The response variable was chosen as the yield of the tetrapluera tetraptera extract. Equation 1 is the statistical model in term of actual variables that was obtained after applying multiple regression analysis to the experimental data presented in Table 2.

Actual factors

$$Yield = 29.414 + 4.2611E - 0.03X_1 - 0.0109X_2 - 0.1637X_3 - 6.7278E - 0.05X_1X_2 - 4.778E - 0.05X_1X_3 + 3.6E - 0.05X_2X_3 + 1.3389E - 0.06X_1X_2X_3$$

From the equation of the model, it was noted that the effects of the extraction temperature is positive, we can affirm that X_1 has a positive effect on the response, which is important for the X_1. This result means that the yield increases when two factors changes from low level to high level. On the other hand, the Particle size and Extraction time have negative and positive effect on the response respectively. This means that, the yield falls when the factor particle size is passed from the low level to the high level. While the extraction time tends to have similar effect on the responses just like the extraction temperature.

Investigation was carried out on main effects, two-factor interaction (2FI), and three-factor interaction (3FI), models

Table 2: Three variable full factorial design for Tetrapluera tetraptera Extraction.						
Std	**Run No.**	**Block**	**Temperature °C**	**Particle size (mm)**	**Extraction time (mins)**	**Response YIELD %**
11	1	Block 1	90	1	30	17.08
13	2	Block 1	90	1	50	18.51
9	3	Block 1	78	1	10	0.00
10	4	Block 1	78	1	30	8.83
19	5	Block 1	78	1	50	7.86
2	6	Block 1	84	1	10	0.00
5	7	Block 1	84	1	30	9.88
4	8	Block 1	84	1	50	13.63
15	9	Block 1	78	3	10	0.00
18	10	Block 1	78	3	30	8.93
21	11	Block 1	78	3	50	11.41
12	12	Block 1	84	3	50	17.82
8	13	Block 1	90	3	30	23.98
3	14	Block 1	90	3	50	31.17
20	15	Block 1	78	5	10	0.00
22	16	Block 1	84	5	10	0.00
7	17	Block 1	84	5	30	5.05
14	18	Block 1	90	5	10	0.44
6	19	Block 1	90	5	30	18.96
1	20	Block 1	90	5	50	23.80
17	21	Block 1	78	5	30	10.59

Table 3: Model summary statistics.					
Source	**Standard Deviation**	**R-squared value**	**Adjusted R-squared value**	**Predicted R-squared value**	**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	0.050	Significant
A-Temperature	340.65	2	1510.33	41.36	0.0236	
B-Particle size	29.40	2	14.70	3.57	0.2188	
C-Time	801.21	2	400.61	97.27	<0.0102	
AB	55.67	4	13.92	3.38	0.3412	
AC	185.55	4	46.39	11.26	0.5432	
BC	58.99	4	14.75	3.58	0.2300	
Residual	8.24	2	4.12			
Cor Total	1626.72	20				

to determine the best model that is statistically significant and best describes the relationship between response and the inputs (independent variables). From the sequential model sum of squares (Table 4), it can be seen that the p value is less than 0.05 and has a F value (16.92), for main effects order. Also, lack of fit was found to be not significant with a p value of 0.032.

While model sum of squares for 2FI was found to have a F value of (21.83), with a p value less than 0.05 the fit becomes less or not significant.

From the model summary statistics show in Table 3, it was observed that the 2FI order has the lowest value for standard deviation (which is the Square root of the residual mean square. It is the standard deviation associated with the experimental error). Although 3FI order model has the highest R^2 value it was not able to ascertain the predicted R^2 value (This is the measure of how good model predicts a response value) and PRESS (The Predicted Residual Sum for the model. measure of how well a particular model fits each point in the design).

Thus, it can be concluded that 2FI order best describes the relationship between response and independent variables. Since the model gave us an R^2 value of 0.9949 and was also able to provide us with a predicted R^2 value of 0.6701 which effectively described the predictive nature of the model and this was not presented by the 3FI Model.

The three factors and yield interaction

It has been established from previous plots the individual factor relationship with the response i.e, yield. 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 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.

Response surface effects between factors

Figure 3 and 4 shows the response surface interaction from design expert 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 time in minutes, extraction temperature in Celsius and Particle size in mm were analyzed and the response shows that yield increases with a corresponding increase in extraction time as well extraction temperature but the particle size effect was not critically felt but at particle size 3mm the experimental optimal yield was reached and thus also producing a geometric increase in yield with decrease in particle size.

Validity 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. Table 4 presents the variation of the observed yield while the software generates its own predicted yield based on the R^2 value of the model. It is found that there is a correlation between the two performances with a coefficient of about 0.9949 (Table 3). That is to say that 90% of the results are explained by the model.

The "Pred R-Squared" of 0.6701 is in reasonable agreement with the "Adj R-Squared" of 0.8397 having a difference less than 0.2 makes the model with effective and predictive.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. (design expert 7.0.0) The ratio of 10.510 indicates an adequate signal. Therefore, this model 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.

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 [26]:

$$D = \left(\Pi_{i=1}^{n} M_i\right)\frac{1}{\Sigma^{ri}}$$

where (D), varies in range of, $0 \le D \le 1$ 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 in SFME is whenever extraction was at high level of all three factors. In other words, the best combination of the condition to extract the bioactive component from the Tetrapluera tetraptera fruit. At an Extraction temperature of 90°C, Extraction Time of 50 minutes and Particle Size of 3mm the highest yield of 31.17% were attained. From Figure 5 and 6 it can be observed that an Increase in extraction temperature, and extraction time the Yield Also Increased alongside though the Particle Size has no significant effect on the Yield but at 3mm the optimal extraction yield was obtained. Another important Information gathered was that at elevated temperature the bioactive component becomes more volatile and can be destroyed during extraction, such components are called thermolabile components: this are components subject to destruction, decomposition or change in response to heat.

In Summary, the optimal conditions to attain the possible maximum Yield of the Tetrapluera tetraptera Fruits were: Time = 50 mins; Extraction Temperature = 90°C and Particle Size = 3mm. Desirability = 0.98. But since we are optimizing that is the optimal yield with the desirability above from design expert was 30.5405% while that from the experimental procedure was 31.17%.

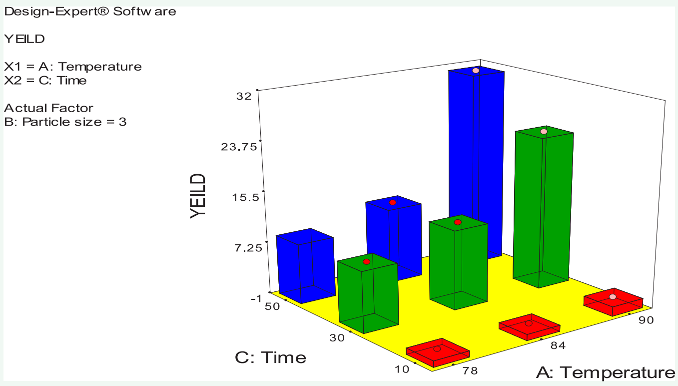

Figure 4 3D bar chart Response of Time (minutes) and Temperature (°C).

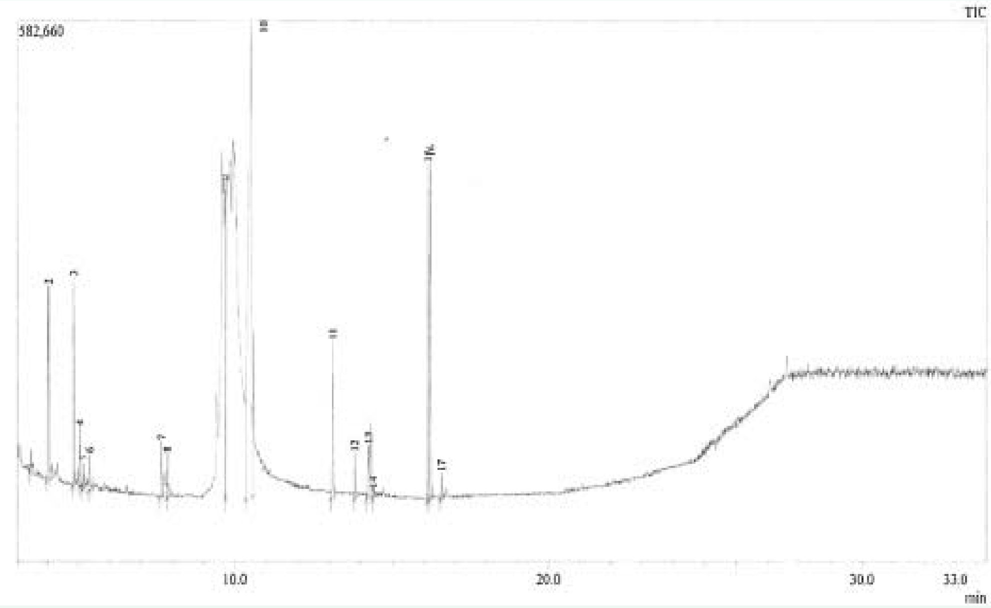

Figure 5 GC-MS chromatogram of Tetrapleura tetraptera peels ethanoic extract.

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 [26]. We can also say that the GC-MS instrument is made up of two parts. The gas chromatography (GC), separates the chemical mixtures into pulses of pure chemicals and the mass spectrometer (MS), identifies and quantifies the chemicals. Tetrapluera tetraptera samples obtained from the extraction processes was sent to B.O.B Global Resources limited, Lagos to be analysed and the following reports were ascertained. From extensive and intensive research on the report attained, 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 depicts the preliminary phytochemical screening and 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 (23.87mg/100g), phenol (21.70 mg/100g), and flavonoids (20.48mg/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.

From Table 5 above 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%. The scope of this study is gear 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 [26].

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 [26]

CONCLUSIONS

This study discusses the performance of full factorial designs on the modelling 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 established and the following conclusions have been drawn.

The yield of Tetrapluera tetraptera fruit extract obtained from a solvent extraction could be significantly increased to its maximum value by the optimization of the process parameters.

Optimization of the yield of Tetrapluera tetraptera fruit using full factorial design allowed us to determine the optimal conditions to have a better yield of Tetrapluera tetraptera fruit extract.

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

Peak No.	Compound	Retention Time.	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
11	1,3-Dioxolane-4-methanol n-Decanoic acid	13.81	1.12
12	9,12-Octadecenoic	14.25	1.87
13	Acid	16.14	14.05
14	6-Octadecenoic acid	16.22	8.75
15	1,3-Dioxolane-4-metano	16.57	0.67

According to this study, we find that 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 excellent correlation between the predicted and observed yields of Tetrapluera tetraptera fruit was high and significant. R^2 = 0.9949 and R^2 adjusted = 0.9494 values, giving good accordance between the model and experimental data which confirmed the validity and practicality of the adopted model.

Optimization using the full factorial design gave an extraction temperature of 90°C, extraction time of 50mins and particle size of 3mm as the optimal process parameters to obtain a maximum yield of 30.54 wt.%.

Reason for the low yield outcome (approximately 31%), of the bioactive and pharmaceutical compounds may be from the conditions associated with experimental constraints or extracting time.

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