Comparative Study of Response Surface Methodology and Artificial Neural Network for Modeling and Optimization of Extraction Process Parameters on *Tetrapleura Tetraptera*

*OYEDOH, EA; ERUMI, GA; AKHABUE, CE

Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Nigeria
Corresponding Author Email: egheoyedoh@uniben.edu

ABSTRACT: Bioactive compounds in the fruits of *Tetrapleura tetraptera* is widely used in food as a flavouring agent and for spices. In this study, bioactive compounds were extracted by solid-liquid extraction process and the yield was optimized by response surface methodology (RSM) and artificial neural network (ANN). The process parameters optimized were the extraction temperature, particle size and extraction time. Box-Behnken Design was used to study the effect of the process parameters on the extract yield. A quadratic model was obtained by RSM which was used to predict the extract yield. While for ANN, Bayesian Regularization learning algorithm with hyperbolic function (Tanh) for both hidden and output layers was the best model for predicting the extract yield. The performance of both models was established based on their R^2 and RMSE values. (R^2 and RMSE values were 0.9391 and 3.10 for RSM and 0.9637 and 0.8193 for ANN respectively). ANN gave the maximum extract yield of 29.15 % higher than that of RSM which evaluated a yield of 27.70 % with optimum conditions at extraction temperature of 90℃, particle size of 3.26 mm and extraction time of 50 mins. It was therefore concluded that ANN is better than RSM in the modeling and optimization of the extraction process parameters.

In the ancient past, man has been able to harness the remedial effects of plants even before microorganisms and the diseases they cause became known. With this, there has been a growing interest in the discovery and research of these plants. Medicinal plants such as *Tetrapleura tetraptera* is a deciduous forest plant that belongs to the Mimosaceae family and is mostly found in the West and Central African rainforest zone (Adesina *et al.*, 2016). Commonly called "Aridan/Aidan" in Yoruba, "Imiminje" in Etsako and "Ighimiakia" in Bini, the plant is known for its use in traditional medicine (Adesina *et al.*, 2016). In West Africa, the fruits find their application in drug preparation and production for ailments like fever, arthritis, constipation, inflammation, epilepsy, hypertension, jaundice, post-partum contraction, schistosomiasis, asthma, malaria, microbial infections and pain. The fruits contribute to the diets and well-being of indigenous Nigerians in the Southern and Eastern part as it is being used as ingredients for "pepper soup" (Adesina *et al.*, 2016). Researchers have discovered the fruit possess bioactive compounds or phytochemicals, minerals and nutrient contents. These bioactive components which have a physiological action on humans, animals and even micro-organisms include tannins, alkaloids, polyphenols, flavonoids, steroids, saponins, reducing compound, anthraquinones, phlobatannins, glycosides and hydroxynmethyl anthraquinones (Edet *et al.*, 2016). With these bioactive components present, *Tetrapleura tetraptera* is fit for being used in food and drug production. A bioactive compound is simply defined as a substance having a biological activity on living organism. This biological activity could either be positive or negative depending on the quantity (Azmir *et al.*, 2013). These bioactive compounds from plant materials go through qualitative and quantitative studies that depend mostly on the selection of the right extraction method (Azmir *et al.*, 2013).

In the study of medicinal plant, extraction is the first step which is important in determining the results and outcome of the study. Extraction technique is used in separating the different types of bioactive compounds from plant materials for characterization. The classical techniques used to obtain bioactive compounds from plants include solvent extraction, maceration, and percolation. Solvent extraction is widely being used for extracting variable and desired bioactive compounds from various natural sources (Azmir *et al.*,

Corresponding Author Email: egheoyedoh@uniben.edu

2013). The aqueous and ethanol extract of *Tetrapleura tetraptera* fruits has been proven to contain the aforementioned bioactive compounds such as 9, 12-Octadecenoic acid, 6-Octadecenoic acid and *n*-Decanoic acid. However, these extracts are analyzed using GC-MS (gas chromatography with mass spectrometry) analysis to separate and identify the various bioactive compounds desired. Factors such as temperature, particle size, time and agitation speed determine the extract yield. To maximize extract yield, the extract factors are optimized using statistically designed experiments. In the last two decades, Artificial Neural Networks (ANN) has proven to be much more powerful in modeling and simulation in the various engineering fields, and for predicting the behaviour of non-linear functions. ANN works like the human brain. Hence it is formed by a series of "neurons" (or "nodes") that are organized in layers (Amato *et al.*, 2013; Ding *et al.*, 2013; Zhang, 2014). While comparing optimization using RSM and ANN, M.Mourabet *et al.*, (2014) reported ANN to be a better optimization method.

MATERIALS AND METHODS
Collection of plant material: Tetrapleura tetraptera fruit was obtained from a local market in Egor Local Government Area, Benin City, Edo state. The fruit was then transported in a polythene bag to the Department of Plant Biology and Biotechnology, University of Benin, Benin City for identification.

Preparation of Tetrapleura tetraptera plant powder and extract: Fresh fruits of the plant were sun-dried for 5 days and chopped into small chips before the extraction process. After chopping the sample into small chips, the sample was further dried under the sun for two weeks so as to reduce the moisture content as low as possible. The dried sample was then ground into different particle sizes and later sieved to obtain 1 mm, 3 mm and 5 mm as the various particle sizes, before being used as feed for the extraction process. Twenty grams (20g) of the dried sample was subjected to extraction with 200 ml of ethanol being used as the solvent by Soxhlet apparatus. The extract obtained for different runs were stored in sample bottles for further analysis.

$$\%YE = \frac{mass\ of\ crude\ extract}{mass\ of\ sample} \ X \ \frac{100}{1}\% \qquad (1)$$

Where %YE = percent yield extract

Identification of the components using GC-MS: Computer searches on a NIST Version – Year 2014 were used as mass spectrum data library and the mass spectrum of the unknown compound as compared with

the spectrum of known compound. The name of the compound present in the extract yield were identified. Also, the relative percentage of each of the extract constituents was expressed as percentage with peak area.

Fig 1: *Tetrapleura tetraptera* fruit

Fig 2: Pulverized *Tetrapleura tetraptera* fruit

Design of Experiments: A three-factor, three-level Box-Behnken experimental design was employed in order to optimize the extract yield from the *Tetrapleura tetraptera* sample. The selected extraction process factors for the extract yield were temperature (°C), particle size (mm) and time of extraction (min). The levels of variables optimized are shown in Table 1.

Table 1: Coded and actual levels of the factors for three factor Box-Behnken design

Variables	Symbols	Coded and Actual levels		
		-1	0	1
Extraction temperature (°C)	X_1	78	84	90
Particle Size (mm)	X_2	1	3	5
Extraction time (mins)	X_3	10	30	50

The Design Expert® 11.1.2.0 (Stat-ease, Inc. Minneapolis, USA), a statistical software was used to develop the experimental design using Box-Behnken

Design in Table 1 above to generate 21 experimental runs for the extraction process. The experiments were therefore performed in a random manner in order to maximize the effects of unexplained variability in the observed responses due to extraneous factors.

Response Surface Methodology (RSM): Response surface methodology (RSM) was used to model and optimize the extraction process. The data obtained from the extracted were analyzed statistically so as to fit the quadratic polynomial equation (model) generated by Design Expert® 11.1.2.0 (Stat-ease, Inc. Minneapolis, USA). To correlate the response variable (extract yield) to the independent variables (extraction time, temperature and particle size), multiple regression was used to fit the coefficient of their polynomial model of the response. The fitted polynomial equation is as follows;

$$Y_i = b_o + \sum b_i x_j + \sum b_{ij} x_i x_j + \sum b_{ii} x_i^2 + e_i \quad (2)$$

Where: Y_i is the dependent variable or predicted response (extract yield); b_o is the offset term/intercept term; b_i (I = 1, 2, 3, …k) is the regression coefficient/first order model coefficient ; b_{ij} is the interaction effect; b_{ii} is the quadratic coefficient of xi; x_i and x_j are independent variables; e_i is the error term/random error

The Design Expert software thereafter develops the regression model in terms of the actual values that describes the extract yield from the extraction process. The optimal values of the independent variables for the extract yield were obtained by solving eqn. (2.1) and the yield of these optimum conditions predicted by the model was also recorded. The quality of fit of the model was evaluated using test of significance and analysis of variance (ANOVA). *Artificial Neural Network Design:* A commercial software MATLAB version R2018b (Mathworks Inc., Natick, MA, USA) was used to model the extraction process. The extract yield was predicted using the Neural Network Fitting Tool. This network was trained by different learning algorithms such as Levenberg-Marquardt, Bayesian Regularization and Scaled Conjugate Gradient. The artificial neural network architecture consisted of an input layer with three neurons, an output layer with one neuron and a hidden layer with nine neurons. Each ANN was trained using a default stopping criteria of 1000 iterations. The Box-Behnken design experimental data were divided into three sets which are training, validation and testing data set. The training set is used for computing the gradient and

updating the network weights and biases. As the network begins to over-fit the data, the error increases, the training is stopped, and the weights and biases of the epoch, with minimum validation errors are returned as the final ANN structure. In this study, a data set of 15 was used for training, 3 data sets were used for validation while the remaining 3 were used for testing.

Comparison of ANN & RSM performance: In order to check the accuracy of the models from ANN and RSM, coefficient of determination, R^2 and Root Mean Squared Error, RMSE was evaluated using Eqn. 3 and 5 below. While Eqn. 4 gives the Mean Squared Error, MSE used to calculate Eqn. 5;

$$R^2 = 1 - \sum_{i=1}^n \left[\frac{(y_{pred} - y_{exp})^2}{(y_{exp} - y_{avg,exp})^2} \right] \quad (3)$$

$$MSE = \frac{1}{n} \sum_{i=1}^n (y_{pred} - y_{exp})^2 \quad (4)$$

$$RMSE = MSE^{\frac{1}{2}} \quad (5)$$

Where; n = number of experimental data; y_{pred} = calculated values from model; y_{exp} = average experimental values; $y_{avg,exp}$ = average experimental values

A high value of R^2 and a low value of RMSE represent a goodness of fit of the model, which suggest that the model proves suitable for the adequate representation of the actual relationship between the dependent/response variable (extract yield) and the independent variables (extraction time, extraction temperature and particle size).

RESULTS AND DISCUSSION

Bioactive Components in *Tetrapleura tetraptera:* In order to identify the bioactive compounds in the fruit and determine their various intensities, GC-MS analysis was carried using ethanol as an extract. GC-MS analysis revealed the presence of Dodecanoic acid, Tetradecanoic acid, 9, 12-Octadecadienoic acid (Z, Z), Linoelaidic acid, and others as shown in Fig. 3 and Table 2. From Table 2 and Fig. 3 it was observed that Linoelaidic acid, 9,17-Octadecadienal, (Z) and 9,12-Octadecadienoic acid (Z,Z) had the largest peak area of 20.26% and retention time of 16.408 minutes which has been reported by Hema R., (2011) to prevent cancer, act as anti-inflammatory, and also help prevent damage to the liver. Erukainure *et al.* (2017) also reported *Tetrapleura tetraptera* fruit peels to have bioactive compounds such as 9, 12-Octadecenoic acid, 6-Octadecenoic acid and *n*-Decanoic acid which was also observed to be discovered in this research study.

Table 2: Bioactive compounds in *Tetrapleura tetraptera*

Peak no.	Compounds	Retention time	Area (%)	Reported Bioactivity
1	Dodecanoic acid	8.926	0.33	Antimicrobial (Anzaku *et al.*, 2017)
2	Tetradecanoic acid	11.372	0.62	Larvicidal and repllent activity (Abubakar and Majinda, 2016)
3	Hexadecanoic acid, methyl ester Pentadecanoic acid, 14-methyl-, methyl ester	13.118	0.11	Antibacterial and antifungal (Abubakar and Majinda, 2016)
4	n-Hexadecanoic acid Tridecanoic acid	13.859	3.72	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, hemolytic, 5-Alpha reductase inhibitor, potent mosquito larvicid (Abubakar and Majinda, 2016)
5	9,12-Octadecadienoic acid (Z,Z) Linoelaidic acid	14.408	0.18	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
6	11-Octadecenoic acid, methyl ester 9-Octadecenoic acid, methyl ester	15.490	0.35	Anti-spasmodic and immune modulators (Gokul and Priya, 2019).
7	9,17-Octadecadienal, (Z) Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)	15.848	0.28	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
8	Linoelaidic acid 9,17-Octadecadienal, (Z) 9,12-Octadecadienoic acid (Z,Z)	16.408	20.26	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
9	Octadecanoic acid	16.692	5.85	Antioxidant, hypoglycemic and thyroid inhibiting properties (Ramasamy, 2019)
10	9,12-Octadecadienoic acid (Z,Z)	17.004	3.44	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
11	Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)	17.348	0.72	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
12	9,12-Octadecadienoic acid (Z,Z)	17.577	3.28	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
13	cis-9-Hexadecenal 13-Tetradecenal Cyclopentadecanone, 2-hydroxy-	18.376	1.54	Antimicrobial, Anticancer, Hepatoprotective, Anti-arthritic, anti-asthama, diuretic (Ramasamy, 2019)
14	9,17-Octadecadienal, (Z) 9,12-Octadecadienoic acid (Z,Z) Linoelaidic acid	20.347	0.61	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
15	Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)	20.738	0.20	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
16	9,12-Octadecadienoyl chloride, (Z,Z) 9,12-Octadecadienoic acid (Z,Z) 9,17-Octadecadienal, (Z)	21.031	1.30	Anticancer (Abubakar and Majinda, 2016)
17	9,12-Octadecadienoic acid (Z,Z) Oleic Acid	21.419	0.32	Antibacterial (Abubakar and Majinda, 2016) 5-Alpha-Reductase-Inhibitor, Allergenic, AlphaReductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), Cancer-Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, Propecic (Hema R., 2011)
18	9,12-Octadecadienoyl chloride, (Z,Z) 9,12-Octadecadienoic acid (Z,Z) Linoelaidic acid	21.771	0.60	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
19	9,17-Octadecadienal, (Z) Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)	24.450	1.25	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase

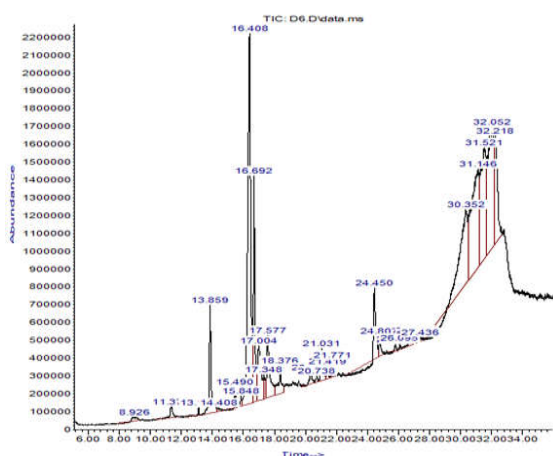|				inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
20	9,12-Octadecadienoic acid (Z,Z) Linoelaidic acid	24.807	0.61	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
21	Linoelaidic acid 9,12-Octadecadienoic acid (Z,Z)	25.803	0.11	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
22	9,12-Octadecadienoyl chloride, (Z,Z) 9,17-Octadecadienal, (Z) 9,12-Octadecadienal	26.095	0.13	Antiinflammatory, hypocholesterolemiccancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
23	9,17-Octadecadienal, (Z) Linoelaidic acid 9,12-Octadecadien-1-ol, (Z,Z)	27.078	1.03	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge (Hema R., 2011)
24	Linoelaidic acid 2-Chloroethyl linoleate 9,17-Octadecadienal, (Z)	27.436	0.17	Flavouring agent (Thomas *et al.*, 2013)
25	Dodecanoic acid, 1,2,3-propanetriyl ester Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	30.352	8.85	Antioxidant Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
26	Dodecanoic acid, 1,2,3-propanetriyl ester Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	31.146	13.95	Antioxidant Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
27	Dodecanoic acid, 1,2,3-propanetriyl ester	31.521	10.72	Antioxidant Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
28	Dodecanoic acid, 1,2,3-propanetriyl ester	32.052	13.50	Antioxidant Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.
29	Dodecanoic acid, 1,2,3-propanetriyl ester Dodecanoic acid, 1-(hydroxymethyl) -1,2-ethanediyl ester	32.218	5.98	Antioxidant Antibacterial, COX-1 & COX-2 inhibitor, Antiviral, Hypocholesterolemic, Candidicide.

Fig 3: GC-MS chromatogram of *Tetrapleura tetraptera* ethanolic extract

Response Surface Methodology: Based on the experimental results of BBD in Table 3, a quadratic polynomial was established to identify the relationship between extract yield and process variables. The resulting RSM model equation is following:

$$Yield = +514.81971 - 12.75541X_1 + 5.89324X_2 - 0.967139X_3 - 0.009207X_1X_2 +$$

$$0.022209X_1X_3 + 0.025278X_2X_3 + 0.077051X_1^2 - 0.970945X_2^2 - 0.010059X_3^2 \quad (6)$$

From Eqn. 6, it can be seen that the extraction temperature and extraction time, have negative effect on the extract yield compared to the particle size which has a positive effect on the extract yield when analyzed linearly, while the quadratic effect of the extraction temperature was positive and negative for both particle size and extraction time (M.Mourabet *et al.*, 2014; Rajkovic *et al.*, 2016). With the positive effects of independent variables revealed that their positive changes can cause an increase in the response value (Rajkovic *et al.*, 2016). The experimental results and the predicted values obtained from the model (Eqn. 6) were compared. According to Fig. 4 it was found that the predicted values were close to matching the experimental values with an R^2 value of 0.9391. This implies that 94% of the variations for extract yield were explained by the independent variables, and this also signifies that the model does not explain about 6% of variation. Also, the value of the adjusted determination of coefficient ($R_a^2 = 0.8892$) was also high showing a significance of the model.

Table 3: Three variable Box-Behnken design for the extraction process optimization by using RSM and ANN

Run order	Factors						Response		
	Coded values			Actual values			Extract Yield (%)		
	X_1	X_2	X_3	X_1	X_2	X_3	Experimental (Actual)	RSM predicted	ANN predicted
1	1	-1	0	90	1	30	17.80	17.70	17.49
2	1	-1	1	90	1	50	18.51	22.74	19.72
3	-1	-1	-1	78	1	10	0	0	1.16
4	-1	-1	0	78	1	30	8.83	7.54	8.55
5	-1	-1	1	78	1	50	7.86	7.26	8.82
6	0	-1	-1	84	1	10	0	0	0.84
7	0	-1	0	84	1	30	9.88	9.85	10.81
8	0	-1	1	84	1	50	13.63	12.23	11.64
9	-1	0	-1	78	3	10	0	2.87	0.070
10	-1	0	0	78	3	30	8.93	11.64	7.94
11	-1	0	1	78	3	50	11.41	12.37	11.91
12	0	0	1	84	3	50	17.82	17.23	18.06
13	1	0	0	90	3	30	23.98	21.58	23.80
14	1	0	1	90	3	50	31.17	27.63	29.15
15	-1	1	-1	78	5	10	0	0	0.023
16	0	1	-1	84	5	10	0	0	0.10
17	0	1	0	84	5	30	5.05	10.06	5.49
18	1	1	-1	90	5	10	0.44	2.57	1.43
19	1	1	0	90	5	30	18.96	17.69	18.83
20	1	1	1	90	5	50	23.8	24.75	30.84
21	-1	1	0	78	5	30	10.59	7.97	2.26

Table 4 gives the results of the quadratic response surface model fitting in the form of analysis of variance (ANOVA). The analysis of variance is important to check for significance and adequacy of the model. It subdivides the total variation of the results in two sources of variation, the model and the experimental error, shows whether the variation from the model is significant when compared to the variation due to residual error (M.Mourabet *et al.*, 2014). The Fisher's F-test value, which is the ratio between the mean square of the model and the residual error, performs this comparison.

Table 4: Analysis of Variance for RSM - Quadratic Model

Source	Sum of Squares	df	Mean Square	F Value	Prob > F	
Model	1625.23	9	180.58	18.84	< 0.0001	significant
X_1-Extraction Temperature	318.53	1	318.53	33.23	0.0001	
X_2-Particle Size	0.1445	1	0.1445	0.0151	0.9045	
X_3-Extraction Time	562.09	1	562.09	58.64	< 0.0001	
X_1X_2	0.0842	1	0.0842	0.0088	0.9270	
X_1X_3	39.74	1	39.74	4.15	0.0666	
X_2X_3	5.74	1	5.74	0.5993	0.4552	
X_1^2	29.24	1	29.24	3.05	0.1085	
X_2^2	53.04	1	53.04	5.53	0.0383	
X_3^2	63.69	1	63.69	6.64	0.0257	
Residual	105.45	11	9.59			
Cor Total	1730.68	20				

Table 5: Statistical information on Box-Behnken Design

Std. Dev.	3.10	R-Squared	0.9391
Mean	10.89	Adj R-Squared	0.8892
C.V. %	8.40	Pred R-Squared	0.7788
PRESS	382.85	Adeq Precision	14.0526

The F-value obtained, 18.84, is greater than the F value (5.75 at 95% significance) obtained from the standard distribution table, confirming the adequacy of the model fits. In addition, the p-value was found to be < 0.0001, which indicated that the model was highly statistically significant. The "Lack of Fit Test" compares the residual error to the pure error from replicated design points. The "Lack of Fit F-value" was not provided by the model. Hence, this model cannot be validated fit. The significance of each term was determined by p-value (Prob>F), which is listed in Table 2. As seen in this table that the terms X_1 and X_3 were significant ($p < 0.05$) while the other term coefficients were not significant ($p > 0.05$).
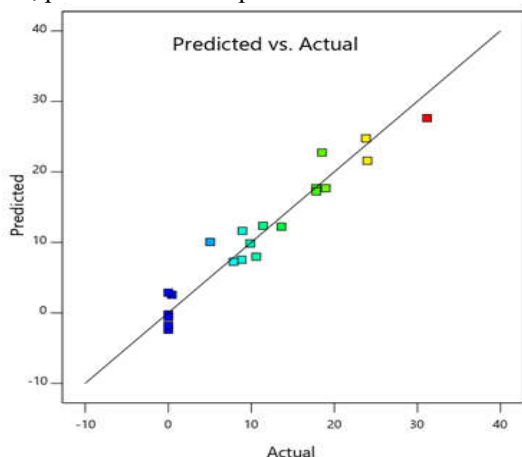

Fig 4: A plot of Predicted against Actual values of the response yield

If the model is a good predictor of the experimental results, F-value should be greater than the tabulated value of F-distribution for a certain number of degrees of freedom in the model at a level of significance (M.Mourabet *et al.*, 2014).

Analysis of Response Surface Plots: 3D response surfaces and the contour plots of the extract yield as a function of extraction temperature, particle size and extraction time are given in Fig. 5 - 7. These plots showed the patterns of the effects of extraction temperature, particle size and extraction time in each individual response.

Effect of extraction temperature and particle size Figure 5 shows the response surface plot and the corresponding contour plot of the combined effect of extraction temperature and particle size on the extract yield. It was observed from the diagram that at low particle size (1 mm), with an increase in extraction temperature, the extract yield increases. Also, it is observed that at moderate particle size (3 mm) and high extraction temperature value (89 °C), the extract yield is seen to be at its maximum.

Fig 5: Response surface plot and corresponding contour plot of the simultaneous effect of extraction temperature and particle size

Effect of extraction time and extraction temperature: From Fig. 6, it was observed that at low temperature of about 78 °C, an increase in the extraction time causes an increase in the extract yield. An increase in temperature was discovered to favour the extract yield at any extraction time which shows how much of an influence temperature has on the extraction process. With this, the optimum extract yield proved to be at a high temperature of about 88 °C to 90 °C and an extraction time of 43 min to 50 min.

Effect of extraction time and particle size: From the Fig. 7, it was observed that at low particle size, the extract yield increases slightly with increase in extraction time. Also, at a high extraction time of 49.06 min, a steady increase of the particle size from 1 mm to 3.21 mm increases the extract yield with the maximum yield at 27.51% after which, the yield decreases with a continuous increase of the particle size.
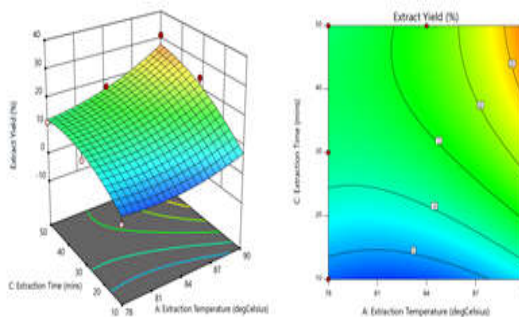

Fig 6: Response surface plot and corresponding contour plot of the simultaneous effect of extraction temperature and extraction time
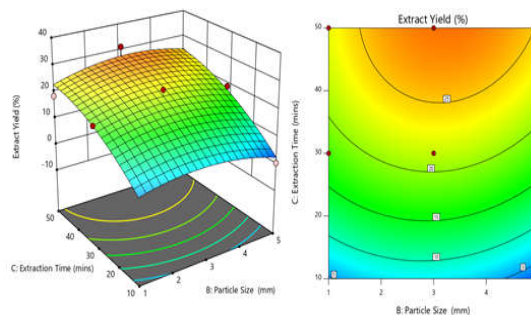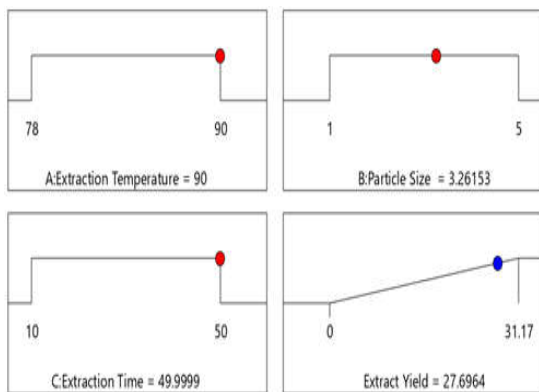

Fig 7: Response surface plot and corresponding contour plot of the simultaneous effect of particle size and extraction time

Optimization using research surface methodology (RSM): To determine the optimal conditions of the independent variables affecting the extract yield obtained from *Tetrapleura tetraptera* fruit, three-dimensional (3D) response surface and contour plots were constructed according to regression model. The optimal condition for this process was established as X_1 = 90 °C X_2 = 3.26 mm X_3 = 49.99 min. The predicted extract yield under this optimal condition was Y = 27.6964 % with a desirability of 0.889 as shown in Fig. 8. It was observed that at a moderate particle size and high extraction temperature had significant effect on the extract yield obtained in this research work.

Artificial Neural Network: A two-layer feed forward network with sigmoid hidden neurons and linear output neurons was used to model the extract. Three training algorithms; Levenberg-Marquadt, Bayesian Regularization and Scaled Conjugate Gradient were used to train the network. Each of these learning algorithms uses 70% of the experimental data for training the network so as to get used to the experimental data, 15% of the experimental data is for validating the model and the remaining 15% is then used for testing the network.

Desirability = 0.889

Fig 8: Numerical optimization of input variables

Table 6: The different learning algorithms for ANN

Learning Algorithm	R^2	RMSE
Levenberg-Marquadt	0.82517	5.2686
Bayesian-Regularization	0.9637*	0.81927*
Scaled Conjugate Gradient	0.70282	10.9044

preferred

From Table 6, the Bayesian Regularization training algorithm was the best to predict the extract yield. The number of neurons used in the hidden layer were two which resulted in a network topology of (3-9-1); three input factors in the input layer, two neurons in the hidden layer and one output layer. The Bayesian Regularization network was chosen since it gave the least RMSE and the highest R^2 value of 0.81927 and 0.9637 respectively. The architecture of the ANN model is shown in the figure 10.

Comparison of RSM and ANN Performance: To determine the best model that accurately optimize the effect of extraction temperature, particle size and extraction time on the extract yield, the R^2 and the RMSE values of both models evaluated and their results are shown in Table 7. The result showed that both models used for optimization produced accurate prediction due to their high R^2 values. However, ANN gave a lower RMSE value when compared to the RSM model. Therefore, ANN was a better modeling tool due to its low RMSE and high extract yield.

Conclusion: The bioactive compounds identified in *Tetrapleura tetraptera* will be useful in the manufacture of drugs to combat certain ailments and beneficial for the production of food spices. RSM and ANN were used to model and determine the optimum process parameters that give the high extract yield. With the ANN model providing a higher yield and R^2 value than RSM, it is suggested that the ANN model be used to optimize the process of bioactive compounds extraction from *Tetrapleura tetraptera* fruit.
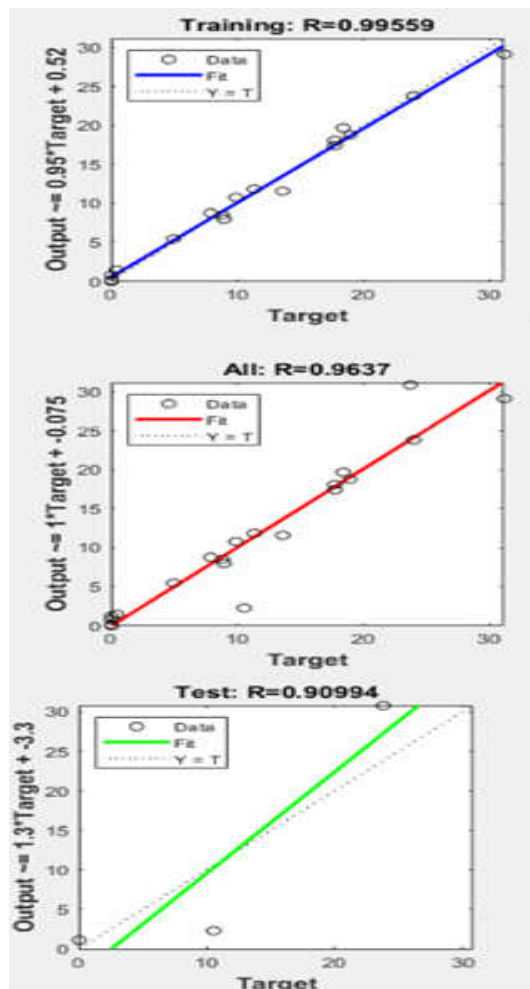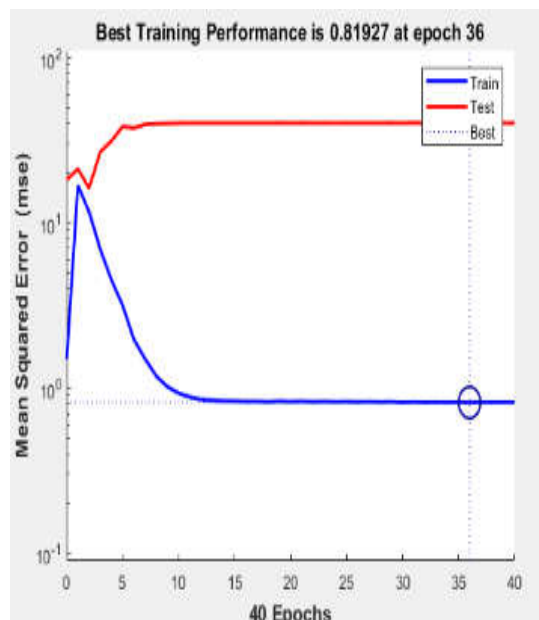

Fig 9: Bayesian Regularization training algorithm
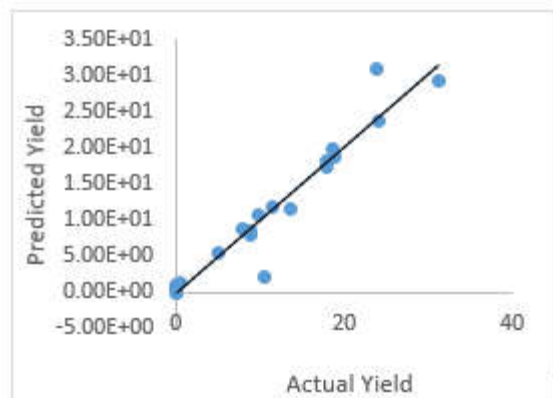

Fig 10: MSE of the Bayesian Regularization algorithm

Fig 11: A plot of Predicted vs Actual values by ANN

Table 7: Comparison of RSM and ANN

Model	Extraction temperature (°C)	Particle size (mm)	Extraction time (mins)	Extract yield (%)	R^2	RMSE
RSM	90	3.26	50	27.696	0.9391	3.10
ANN	90	3	50	29.15	0.9637	0.81927

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