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OPTIMIZATION OF EXTRACTION CONDITIONS OF NATURAL DYE FROM *EUCLEA DIVINORIUM* USING RESPONSE SURFACE METHODOLOGY

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Abstract: *Dyes acquired from diverse natural sources are biodegradable and require minimal use of chemicals during extraction which makes them appropriate alternatives to synthetic dyes which are non-biodegradable and toxic to the environment. In this research optimization of extraction conditions of natural dye extract from Euclea divinorium was conducted using Response Surface Methodology (RSM). M:L (material to liquor ratio), temperature and time were optimised using methanol and water as the extraction solvents. The combined effects of the investigated extraction conditions on absorbance at maximum wavelength of 339nm 341nm for aqueous and methanolic extractions, respectively, were studied using Central Composite Design (CCD) of experiment. Analysis of variance (ANOVA) was used to evaluate the efficiency of the statistical model fitted to the experimental design. The optimum values for aqueous dye extraction were found to be 7.5g:100mL, 84℃ and 146 minutes while the optimum values for methanolic dye extraction were found to be 7.5g:100mL, 60℃ and 129 minutes with absorbance values of 1.051 and 1.092, respectively. The theoretical and the experimental optimum absorbance verified experimentally were in agreement hence the statistical model was found to be valid.*

Key words: *Natural dye, E. divinorium, Optimization, Response Surface*.

1. INTRODUCTION

Global environmental awareness has raised concerns on the use of synthetic dyes in various industries such as textile, food, leather etc, due to their eco-toxicological consequences [1]. Most of the synthetic dyes used in textile industries are very soluble, as a result their elimination from the environment is not an easy process [2]. The tremendous shift to natural dyes is attributable to the eco-friendly properties exhibited by the natural dyes [3]. The structures of natural dye molecules are made up of different chromophores and auxochromes and hence have different methods and conditions of extraction [4]. It is important to optimize extraction conditions in order to maximize the yield of the extracted dye and enhance the color strength of the dye on the fabric. Single factor design (one factor at a time) of optimization is simply the process of varying one factor while maintaining the others at constant level [5]. This design does not consider the effects of interactions

among the parameters which can be overcome using Response surface methodology (RSM) [6].

E. divinorium plant has been used traditionally as a source of natural dye however its potential as textile dye has not been exploited. Preliminary studies showed that water and methanol were the most appropriate solvents for the dye extraction. Absence of standard methods for extraction of natural dyes[7] necessitates optimization of extraction conditions so as to maximize the colour strength and fastness of the dye. This study was executed with the aim of determining the optimum temperature, time and material to liquor ratio (M:L) for extraction of a natural dye from *E. divinorium* with methanol and water as the extraction solvents using RSM.

2. MATERIALS AND METHODS

2.1 Materials

Analytical grade methanol was sourced from Py-rex East Africa Ltd. Distilled water was prepared using distiller. UV/Vis spectrophotometer (Model No: DU'720PC, BECKMAN COULTER) was used to obtain absorption spectra and determine absorbance. The root bark of *E. divinorium* was obtained from Nandi County. The samples were washed with water, dried under the shade and ground to powder.

2.2 Dye Extraction

The ground samples of *E. divinorium* plant were used to extract the natural dye. Aqueous and methanol solvents were used for extraction using conical flasks. After extraction, the extracts were allowed to cool and filtered using filter paper.

2.3 Spectroscopic Analysis

The dye extracts were analyzed using UV/Vis spectrophotometer in the spectral range of 300-700 nm so as to determine the characteristic absorption spectra. Extracts of experimental runs were filtered and 1mL was diluted to 100mL using distilled water in a volumetric flask and its aliquot was analyzed. Absorbances of the diluted extracts were measured at maximum wavelength.

2.4 Single Factor Design

This design was used as a preliminary study to determine the range of every parameter to be used in RSM. Aqueous extraction was carried out at different temperatures of 40, 60, 70, 80 and100 °C while methanol extraction was done at 30, 45, 50, 60 and 65 °C. M:L ratio and time for both solvents were 2,4,6, 8 and 10g:100mL and 60, 90, 120, 150 and 180 minutes, respectively.

2.5 Response Surface Methodology Design

The design of the experiment, graphical designs, regression and other statistical analysis were carried out using Central Composite Design (CCD) of RSM using Minitab 17 software. Three factors at different five levels were studied as shown in Table 1.

Table1: *Experimental levels of independent process variables*.

Variables	Factors		Levels				
			$-\alpha$	-1	0	+1	$-\alpha$
M:L (g:100mL)	A		0.8	2	5	8	9.2
Temperature (°C)	B	Aqueous	28	40	70	100	112
		Methanol	35	40	52	64	69
Time	C		36	60	120	180	204

2.6 Statistical Analysis

The experimental data was fitted to a statistical model stated by the general polynomial equation **(1)**

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \quad (1)$$

Where Y is the response; β_0 is the intercept; β_1, β_2, and β_3 are linear; β_{11}, β_{22}, and β_{33} are squared; β_{12}, β_{13}, and β_{23} are interaction coefficients and M: L ratio (A), temperature (B) and time (C).

3 RESULTS AND DISCUSSION

3.1 Spectroscopic Analysis

The UV-Vis spectra for aqueous and methanolic (MeOH) extracts indicated that the dye extracts were made of a mixture of compounds (**Fig. 1**) with maximum absorbance of 339nm and 341nm.

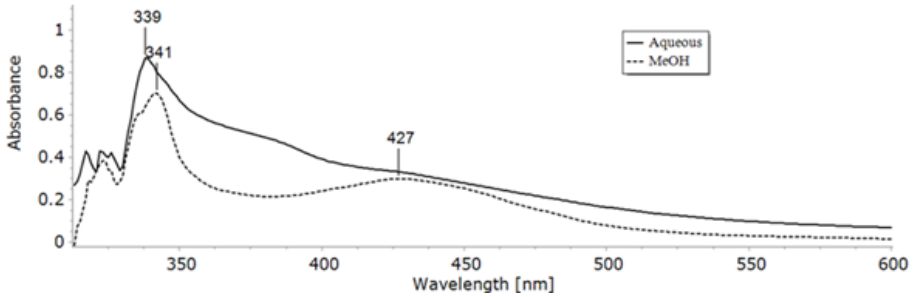

Fig. 1*: UV-Vis absorbance spectra of the aqueous and methanolic extracts of E. divinorum plant*

3.2 Analysis of Single-Factor Design

In both aqueous and methanolic extracts there was fast increase in color concentration in terms of absorbance as the time increased from 60 to 120 minutes (**Fig. 2A**) due to increased contact duration between the sample and the solvent allowing more dye to dissolve [8]. **Fig. 2B** shows that the concentration increased to the optimum then dropped due to sample congestion. Increase temperature led to increased dye concentration up to the optimum due to increased kinetic energy for both aqueous (**Fig. 2C**) and methanolic (**Fig. 2D**) extraction.

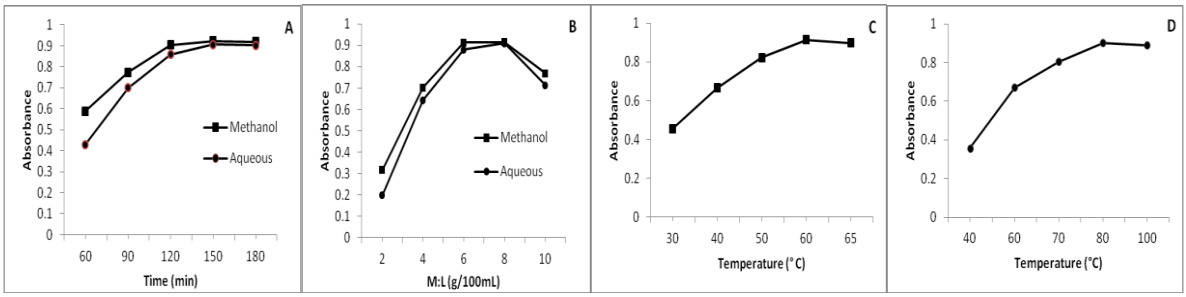

Fig. 2*: Effects of (A)time, (B)M:L, (C)temperature for methanolic dye and (D) for aqueous dye on absorbance*

3.3 Response Surface Methodology

The RSM design was set up and the range for the parameters was based on the single factor design results. The experimental levels of the studied factors: M:L (g of the sample per 100 mL of the solvent), Time (minutes) and Temperature (°C) with their responses (absorbance at λmax) were as shown in Table 2.

Table 2: *Coded CCD design for aqueous and methanolic dye extraction process conditions*

Run order	Aqueous Extraction Design				Methanolic Extraction Design			
	Factors			Absorbance	Factors			Absorbance
	A	B	C		A	B	C	
1	0	0	0	0.872	1	1	-1	0.877
2	1.4	0	0	0.934	-1	-1	-1	0.209
3	0	0	0	0.912	0	0	0	0.961
4	0	-1.4	0	0.123	1	0	-1	0.556
5	0	1.4	0	0.643	1	-1	1	0.605
6	0	0	-1.4	0.701	-1	1	-1	0.303
7	-1.4	0	0	0.180	-1	-1	1	0.313
8	0	0	1.4	0.933	1	1	1	0.954
9	1	-1	1	0.466	-1	1	1	0.379
10	1	1	1	0.932	0	0	0	0.912
11	0	0	0	0.912	0	0	0	0.994
12	1	1	-1	0.924	0	0	0	0.968
13	-1	-1	-1	0.075	1.4	0	0	0.998
14	0	0	0	0.932	0	1.4	0	0.889
15	-1	-1	1	0.194	0	0	0	0.972
16	-1	1	1	0.288	0	0	1.4	0.785
17	0	0	0	0.867	0	-1.4	0	0.527
18	-1	1	-1	0.276	-1.4	0	0	0.248
19	0	0	0	0.921	0	0	-1.4	0.593
20	1	-1	-1	0.372	0	0	0	0.906

3.4. Adequacy of the Models

The residual values in both methods were small (-2 > residual < 2) and the distribution of the data points (**Fig. 3**) was nearer to the straight line with little scattering which is anticipated with normal data [9], therefore the data was found to be normally distributed.

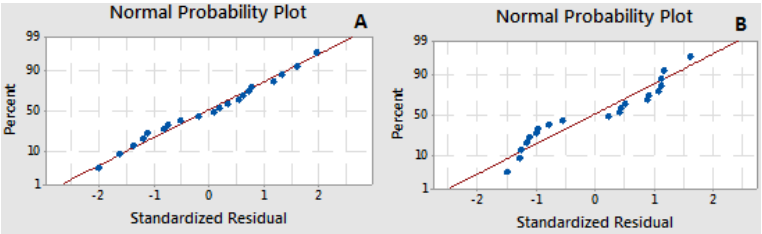

Fig. 3: *Normal probability plots: A is for aqueous and B is for methanolic extraction*

The analyses of the variation of the statistical models lead to regression equations **(2)** and **(3)** for aqueous and methanolic extraction, respectively. The coded factors A, B, C and absorbance refers to M:L, temperature, time and response, respectively.

$$\text{Absorbance} = -1.81 + 0.203\,A + 0.043\,B + 0.004\,C - 0.019\,A^2 - 0.0003\,B^2 - 0.00001\,C^2 + 0.001\,AB - 0.00002\,AC - 0.000013\,BC \quad \textbf{(2)}$$

With $R^2 = 99.26\%$, R^2 (adj) = 98.43%, R^2 (pred) = 93.90%

Absorbance = -2.91 + 0.1797 A + 0.0892 B + 0.01002 C - 0.0188 A^2 - 0.00085 B^2 - 0.000038 C^2 + 0.001771 AB- 0.000038 AC+0.00001 BC **(3)**
With R^2 = 98.98%, R^2 (adj) = 97.85%, R^2 (pred) = 93.04%

The coefficient of determination R^2 shows that the fitted model can explain 99.26% and 98.98% of the variation in the response for aqueous and methanolic dye extraction, respectively, therefore the statistical model used fitted the actual data [10].

3.5 Statistical Analysis

The significance of the model and terms to the response were evaluated using the p-value of ANOVA (Table 3). From ANOVA results the whole model (Equations 2 and 3), linear and squared model were very significant because of the p = 0.000 for both aqueous and methanolic extraction. The two way factor interaction model are also significant with p = 0.001 and 0.014 for aqueous and methanolic extraction, respectively since p < 0.05 is significant [11]. All the linear and squared factors had significant effects on the absorbance but for two way factor interaction terms only the interaction between M:L*temperature for both extractions was significant

Table 3: *Analysis of variance for aqueous extraction*

		Aqueous				Methanol			
Source	DF	Adj SS	Adj MS	F-Value	P-Value	Adj SS	Adj MS	F-Value	P-Value
Model	10	2.0934	0.20934	120.34	0.000*	1.5264	0.15264	87.46	0.000*
Linear	3	1.0892	0.36307	208.71	0.000*	0.8535	0.28450	163.01	0.000*
A	1	0.7136	0.71363	410.23	0.000*	0.6756	0.67569	387.15	0.000*
B	1	0.3494	0.34947	200.89	0.000*	0.1500	0.15009	86.00	0.000*
C	1	0.0261	0.02610	15.00	0.004*	0.0277	0.02771	15.88	0.003*
Square	3	0.9134	0.30448	175.03	0.000*	0.6162	0.20540	117.69	0.000*
A^2	1	0.2413	0.24136	138.74	0.000*	0.2387	0.23876	136.80	0.000*
B^2	1	0.5582	0.55825	320.91	0.000*	0.1303	0.13030	74.66	0.000*
C^2	1	0.0118	0.01186	6.82	0.028*	0.1532	0.15329	87.83	0.000*
Interaction	3	0.0701	0.02336	13.43	0.001*	0.0328	0.01095	6.28	0.014*
A*B	1	0.0653	0.06534	37.56	0.000*	0.0325	0.03251	18.63	0.002*
A*C	1	0.0001	0.00010	0.06	0.811	0.0003	0.00036	0.21	0.659
B*C	1	0.0046	0.00465	2.68	0.136	0.0000	0.00000	0.00	1.000
Error	9	0.0156	0.00174			0.0157	0.00174		
Lack-of-Fit	5	0.0124	0.00248	3.06	0.150	0.0100	0.00200	1.41	0.382
Pure Error	4	0.0032	0.00081			0.0057	0.00142		
Total	19	2.1091				1.5421			

DF-degrees of freedom, SS-sum of squares MS- mean square F- Fischer test, p- probability and *-significant

3.6 Optimization and Validation of the Optimized Conditions

The obtained optimum conditions for aqueous and methanolic extraction were: 7.5g:100mL, 84°C and 146.3 minutes and 7.5g:100mL, 60°C and 129.3 minutes, respectively. The statistical model was validated experimentally. The experimental absorbance was 1.039 for aqueous and 1.088 for methanolic extraction. The theoretical absorbance under optimum conditions for aqueous extraction was 1.051 and 1.092 for methanolic extraction. The experimental and the theoretical values were in agreement hence the used statistical model was confirmed to be adequate.

CONCLUSION

Optimization studies on extraction conditions for aqueous and methanolic extraction of a natural dye from *E. divinorum* using CCD of RSM was carried to determine the optimal extraction condition. The fitted statistical model demonstrated adequacy in predicting the optimum absorbance at the optimum conditions. The optimum conditions for aqueous extraction were found to be 7.5g:100mL, 84°C and 146.3 minutes while the optimum conditions for methanolic extraction were found to be 7.5g: 100mL, 60°C and 129.3 minutes with absorbance values of 1.051 and 1.092, respectively. Interaction effects were observed between temperature and M: L in both methanolic extractions. The theoretical and experimental absorbance were in agreement

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