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Response Surface Optimization of Extraction of Bioactive Constituents and Antioxidant Activity of *Codiaeum variegatum* Leaves

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ABSTRACT

The study aimed at optimizing the yield of extraction of flavonoids, phenolics, and DPPH (2, 2- diphenyl-1-picrylhydrazyl free radical scavenging activity) of *Codiaeum variegatum* leaves as a function of drying temperature (60-100 °C) and ethanol concentration (50-80 v/v %) at constant drying time (80 minutes) using Response Surface Methodology (RSM). FT-IR (Fourier Transform Infrared Spectrophotometer) and proximate analyses of the leaves were also carried out using standard methods. From the results, linear term was significant for phenolic content, while quadratic term was significant for both flavonoid content and DPPH. The coefficient of determination (R^2) for flavonoids, phenolics and DPPH were 0.9116, 0.9155, and 0.9612 respectively, showing a good fit model. The model terms were significant ($P<0.05$) for all the responses which provided its suitability for prediction purposes. The coefficients of variation (CV) were less than 10 %. Using desirability function, the optimum operating conditions to obtain higher extraction of flavonoids, phenolic and DPPH was found to be 72.72 °C and 75.61 v/v ethanol concentrations. The FT-IR results of the optimum processing conditions confirmed the presence of alkanes, alkenes, alcohol, amines, phenol, nitro compounds, and sulfoxides in the ethanol extracts of *Codiaeum variegatum* The study revealed that *Codiaeum variegatum* leaves have significant antioxidant potential and can therefore be used in various medicinal applications.

Keywords: RSM, flavonoid, phenolic, DPPH, optimization, FT-IR

INTRODUCTION

Codiaeum variegatum (L.) universally known as crotons; are among the most popular ornamental foliage plants cultivated for either landscaping or interior scaping. Currently, more than 300 cultivars are obtainable and each of them has their distinct phenotype, particularly in leaf morphology [1]. The genus *Codiaeum A.* variegatum (L.) Juss. belongs to the family

Euphorbiaceae and encompasses 17 species native to tropical forests from Indonesia and the Philippines to New Guinea and Australia [2]. It is traditionally used in the treatment of amoebic dysentery, bacterial infections, skin infections, gastric ulcer [3], bacterial infections and fever [4].

The research conducted on the comparative study of leaf morphology, phytochemical, mineral and proximate analysis of Codiaeum variegatum (L.) A. Juss. (Malpighiales: Euphorbiaceae) and its stable mutant revealed the presence of important phytochemicals such as terpenoids, cardiac glycosides, tannins, phenolic compounds, and saponins [5]. They also investigated that the plants contained substantial amount of ash, protein, fat, fiber and carbohydrate in ovalifolium and its mutant. Phytochemicals are bioactive compounds that are very vital in the knowledge of the therapeutic properties of plants. Some of this bioactive compounds which are regularly been analyzed for, include alkaloids, tannins, anthraquinones, cardiac glycosides, flavonoids, saponins, phenols, and phenolic compounds, phlobatannins, terpenes, and essential oils [6]. Phytochemicals are the biologically active substances in plants that are responsible for giving them color, flavor, and natural disease resistance. They are very powerful ammunition in the fight against many health disorders such as cancer, inflammatory, cardiovascular, and infectious diseases. For example, phytochemicals have been found active to suppress cancer by interfering with one or more carcinogenic pathway [7]. Flavonoids consist of a common basic structure comprising two aromatic rings linked by three carbons that form an oxygenated heterocyclic compound, and phenol compounds are the main antioxidant substance which can promote human health through reducing oxidative damage [8,9]

Antioxidants have been recognized as neutralizing chemicals that minimize oxidative damaging to biological processes. They do these by giving free radicals electrons and passing them off as harmless [10]. Free radicals are mostly associated with oxidative stress. When oxygen combines with specific chemicals, there will be formation of free radicals. When the free radicals are generated, they become potential threat causing damage when they are in combination with important cellular elements such as Deoxyribonucleic Acid (DNA) and proteins, as well as the cell membrane [11]. Antioxidant activities of plants can been successfully optimized through the use of varying processing methods in combination with modelling tools such as response surface methodology.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques that are useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response [12]. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions [13]. Therefore, it is less laborious and time-consuming. RSM has been widely used for different purposes in chemical, biochemical, engineering processes, and industrial

research. The extractions of biologically active compounds from *Codiaeum variegatum* using Response Surface Methodology (RSM) have not been explored. Therefore, the aim of this present study was to investigate proximate composition, optimize the process variables for the quantification of flavonoids, phenolics and DPPH free radical scavenging activity using RSM, and to investigate the functional groups present in *Codiaeum variegatum* leaves extract.

MATERIAL AND METHOD

Experimental Design

Response surface methodology was used to optimize the extraction parameters of *Codiaeum variegatum* leaf. Central Composite Design (CCD) was employed to identify the relationship between the response functions and process variables. The independent variables in this study were drying temperature (X_1: 60–100 °C) and ethanol concentration (X_2: 50–80 % v/v ethanol/water). The dependent variables (responses) measured were flavonoid (FC), phenolic content (PC) and (2, 2- diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH). The experimental data were evaluated using the response surface methodology. The generation of response surface plots and statistical analysis were performed using Design expert software (STAT-EASE, MINNEAPOLIS, MN, USA). The regression analysis was performed on the data of response variables obtained as affected by the process variables and was fitted into a second-order regression equation as shown in the following equation;

$$Y_k = bk_0 + \sum_{i=1}^{m} bk_i x_i + \sum_{i=1}^{m} bk_{ii} X_i^2 + \sum_{i=1}^{m} bk_{ij} X_i X_j \ + € \qquad (1)$$

Where Y represents the response variables to be modeled, bk_o is the value of the fitted response at the center point of the design, and bk_i, bkii, and bk_{ij} are the linear, quadratic, and interaction regression terms, respectively. k is the number of variables and € is the random error of the model.

Collection of Plant Sample

Matured fresh leaves of *Codiaeum variegatum* were collected by hand-plucking from parent plants within Babcock University, Ilishan-Remo, Ogun State, Nigeria.

Preparation and Extraction of Plant Material

The freshly collected leaves of *Codiaeum variegatum* were thoroughly washed with tap water followed by distilled water. The leaves were dried in the hot air oven at different temperatures based on the experimental design. They were allowed to cool and then pulverized with the use of a laboratory grinder (USHA MG 3473). Extractions of the leaves were carried out by maceration in ethanol with different weight-to-volume ratios according to the process design. The mixtures were vigorously shaken and allowed to stand for 48 hr. at room temperature. The mixture was thereafter filtered with a Whatman No. 1 filter paper and the residue was macerated again in an equal volume of ethanol for 24 hr to obtain more quantities of the extract. The mixtures were combined and then evaporated to dryness. This was done under reduced pressure at about 40 °C with the use of an Eyela N-1001 vacuum rotary evaporator.

Flavonoid Content

The flavonoid content of the samples (crude extract) was analyzed following the spectrophotometric method of [14]. Quercetin served as the standard substance. 1 ml of the sample (containing 100 µg/ml), prepared in ethanol was mixed with distilled water (4 ml) in a 10 ml volumetric flask. Then, 5 % $NaNO_2$ solution (0.3 mL) was added to the flask. After 5 mins, 10 % $AlCl_3$ (0.3 mL) was added and at the 6th minute, 1.0 M NaOH (2 mL) was added. Distilled water (2.4 mL) was added to the reaction flask and thoroughly shaken. The absorbance of the resulting reaction mixture was then taken at 510 nm on a spectrophotometer (JENWAY 6305, Staffordshire, UK). Reagent blank; containing 1 ml ethanol (instead of the extract) was concomitantly prepared and treated in the same manner as the samples. A calibration curve was prepared by repeating the same procedure for standard solutions of Quercetin (2 to 10 µg/ml, R^2 = 0.986). From the measured absorbance of the samples, the total flavonoid content was estimated from Quercetin calibration curve and results were expressed as mg Quercetin Equivalent per gram

(mgQE/g) of the sample on a dry weight basis. The test was carried out in triplicates.

Phenolic Content

The phenolic content of the sample was assayed by the method of [15]. The assay is based on the reduction of Folin-Ciocalteu reagents (Phosphomolybdate and phosphotungstate) by the phenolic compounds present in the extract. The reaction mixture was made by mixing 0.5 mL of ethanolic solution of the sample (containing 100 μg/mL), 2.5 mL of 10 % aqueous solution of Folin-Ciocalteu reagent, and 2.5 mL of 7.5 % $NaHCO_3$ solution. Blank was concomitantly prepared by mixing 0.5 mL ethanol, 2.5 mL of 10 % aqueous solution of Folin-Ciocalteu reagent, and 2.5 mL of $NaHCO_3$ solution. The sample was incubated in a Uniscope SM801A laboratory water bath at 45 °C for 45 min and thereafter the absorbance was measured with a spectrophotometer (JENWAY 6305, Staffordshire, UK) at 765 nm. Standard solutions of gallic acid were taken through the same procedure and the absorbance values obtained were used to construct a standard calibration curve. The measured absorbance of a sample was used to extrapolate its phenolic content from the standard calibration curve. The phenolic content was then expressed as gallic acid equivalent (mg of GA/g) of the sample. Each sample was analyzed in triplicate.

DPPH Radical Scavenging Activity

The free radical scavenging capacity of *Codiaeum variegatum* extract was determined following the assay suggested by Ramadan *et al.* [16] and modified by [17]. The extract (0.8 mL) was mixed firstly with 4 mL of ethanolic solution of DPPH (0.1 mM) and filled up to 5 mL with ethanol solution. The mixture was kept for 30 min in dark at room temperature, and then the absorbance was read at 517 nm and expressed as percent (%) inhibition of DPPH. % inhibition of DPPH was calculated based on the following equation:

$$\% \text{ inhibition of DPPH} = (A_o - A) \times 100 / A_o \quad (2)$$

Where A_o and A were the absorbance of the control (without the plant extract) and sample solution, respectively. Where Abs control is the absorbance of the control; and abs sample is the absorbance of the sample.

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Fourier Transform Infrared Spectrophotometer (FT-IR) analysis

Dried powder of the leaf extract of *Codiaeum variegatum* was used for FT-IR analysis. 10 mg of the powder was encapsulated in 100 mg of KBr pellet, to prepare translucent sample discs. The powdered sample of the extract was loaded in an FT-IR spectroscope, with a scan range from 400 to 4000 cm-1 with a resolution of 4 cm^{-1} [18].

Proximate Analysis

The samples were analyzed for moisture, ash, crude fibre, crude protein (N*6.25), crude fat and the carbohydrate was determined by difference according to the method described by [19].

Table 1: Design Matrix with Factors and Levels

Factor	Unit	Low	High
X_1- Drying temperature	°C	60	100
X_2- Ethanol Concentration	% v/v	50	80

Statistical Analyses

The results of the experiment were subjected to analysis of variance (ANOVA) using Statistical package for the Social sciences (SPSS), version 23.0. Significance was accepted at 0.05 level of probability ($P < 0.05$).

RESULTS AND DISCUSSION

Table 2: Central Composite Design Arrangement and Responses

Run	Factor 1 Drying Temperature X_1 (°C)	Factor 2 Ethanol Concentration X_2 (v/v)	Response 1 Flavonoid content (FC) (mg/g GAE)	Response 2 Phenolic content (PC) (mg QE/g)	Response 3 % inhibition DPPH (%)
1	80.00	50.00	32.27±0.04	81.91±0.01	52.14±0.06
2	80.00	65.00	46.36±0.02	84.00±0.00	55.66±0.18
3	65.86	75.61	47.54±0.02	89.55±0.00	62.14±9.24
4	80.00	65.00	46.36±0.02	84.00±0.00	55.66±0.18
5	60.00	65.00	46.72±0.02	85.78±0.03	58.93±0.01
6	80.00	65.00	46.36±0.02	84.00±0.00	55.66±0.18
7	65.86	54.39	36.45±0.03	83.01±0.02	53.89±0.06

8	94.14	75.61	37.82±0.00	83.99±0.04	57.70±0.00
9	80.00	65.00	46.36±0.02	84.00±0.00	55.66±0.18
10	100.00	65.00	43.36±0.03	80.22±0.01	58.25±0.15
11	80.00	80.00	45.09±0.01	86.68±0.00	60.06±0.01
12	94.14	54.39	31.80±0.02	77.67±0.04	52.95±0.03
13	80.00	65.00	46.36±0.02	84.00±0.00	55.66±0.18

All values are means of triplicate determination ± standard deviation (SD)
FC- Flavonoid content, Phenolic content (PC), DPPH (2, 2- diphenyl-1-picrylhydrazyl)

Effect of processing variables (drying temperature and ethanol concentration) on the FC, PC and DPPH

The experimental design and the corresponding three response variables are presented in Table 2. The result of extracted FC, PC and DPPH from *Codiaeum variegatum* leaves ranged from 31.80 -47.54, 77.67 – 89.55 mg/g GAE/g and 52.14-62.14 %. The highest content of the responses was observed in experimental run no. 3. Total flavonoid content of *Codiaeum variegatum* leaves ranged from 31.80–47.54 mg QE/g of extract. There were variations in the responses that were analyzed. Run 3 had the highest concentration of flavonoids, phenolic, and DPPH. Run 5 with flavonoid content of 46.72 mg/g GAE was next to run 3 and run 11 with phenolic content of 86. 68 mg QE/g was next to run 3. The quadratic model was selected for total flavonoids and DPPH while the linear model was selected for total phenolic. The independent variables and responses fitted well as suggested by the software. The final empirical regression model of the relationship between responses and the three tested variables for phenolic, flavonoid contents, and DPPH in terms of actual factors could be expressed by the following equation:

Flavonoid $=-236.57699+1.60054X_1+6.52863X_2-7.62688E\text{-}003X_1^2-0.041826X_2^2-8.45000E\text{-}003X_1X_2$ (3)

Phenolic $=+82.04186-0.16584X1+0.23106X_2$ (4)

DPPH $= +54.67720-0.71389X1+0.69265X2+6.48125E\text{-}0.03X_1^2+4.55556E\text{-}0.04X_2^2-5.83333E\text{-}003X_1X_2$ (5)

Where X_1 is the drying temperature, X_2 is the ethanol concentration.
A negative sign in each equation represents an antagonistic effect of the variables and a positive sign represents a synergistic effect of the variables.

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Table 3: Analysis of Variance (ANOVA) for Flavonoid based on quadratic model

Sources Flavonoid Content (mg/g GAE)	Sum of square	Degree of freedom	Mean of square	F-value	P-value
Model	367.27	5	73.45	14.44	0.0014
X_1-Drying temperature	45.71	1	45.71	8.99	0.0200
X_2-Ethanol concentration	158.23	1	155.23	30.52	0.0009
X_1^2	16.19	1	16.19	3.18	0.1176
X_2^2	154.02	1	154.02	30.28	0.0009
X_1X_2	6.43	1	6.43	1.26	0.2981
Residual	35.60	7	5.09		
Lack of Fit	35.60	3	11.875-934E+005		<0.0001
Pure Error	8.000E-0.05	4	2.000E-0.05		
Cor Total	402.87	12			
R^2 =0.9116					
Adj R^2= 0.8485					
Adeq precision =11.404					
CV= 5.30					

Table 4: Analysis of Variance (ANOVA) for Phenolic Content based on linear model

Phenolic content (mg QE/g)	**Sum of square**	**Degree of Freedom**	**Mean square**	**F-Value**	**P-value**
Model	92.05	2	46.03	54.17	<0.0001
X_1Drying Temperature	44.01	1	44.01	51.79	<0.0001
X_2-Ethanol concentration	48.05	1	48.05	56.54	<0.0001
Residual	8.50	10	0.85		
Lack of Fit	8.30	6	1.38	27.66	0.0032
Pure error	0.20	4	0.050		
Cor Total	100.55	12			

Table 5: Analysis of variance (ANOVA) DPPH Free Radical Scavenging Activity based on Quadratic Model

DPPH (%)	Sum of square	DF	Mean square	F-value	P-value
Model	93.09	5	18.62	34.69	< 0.0001
X_1-Drying Temperature	5.03	1	5.03	9.37	0.0183
X_2-Ethanol concentration	73.21	1	73.21	136.43	<0.0001
X_1^2	11.69	1	11.69	21.78	0.0023
X_2^2	0.018	1	0.018	0.034	0.8588
X_1X_2	3.06	1	3.06	5.71	0.0482
Residual	3.76	7	0.54		
Lack of fit	3.76	3	1.25		
Pure error	0.000	4	0.000		
Cor Total	96.84	12			
R^2 = 0.9612					
Adj R^2 = 0.9335					
Pred R^2 = 0.7242					
Adeq Precision =20.528					
CV = 1.30					

Table 3, 4, and 5 showed the ANOVA for flavonoid, phenolic and DPPH free radical scavenging activity respectively. The ANOVA results were calculated based on 95 % confidence intervals and the analysis was crucial to determine the best fitted model for the two independent variables. It was observed that the models were significant ($P < 0.05$) indicating a well-fitted model in all the responses. The linear variables X_1 and X_2 had a significant influence ($P < 0.05$) on the flavonoid yield, the quadratic variable X_2^2 was statistically very significant ($P < 0.01$), while the quadratic variable X_1^2 was insignificant. The performance of the models was also checked by calculating the determination coefficients R^2, adjusted R^2, regression (*p-value*), regression (*F-value*),

lack of fit (*p-value*), coefficient of variation (C.V %), and probability values related to the effect of the two independent variables. Lack of fit for phenolic content was significant. Flavonoid, phenolic, and DPPH had R^2 = 0.9116, 0.9155 and 0.9612 respectively showing a good fit model. The model with $R^2 > 0.75$ was considered acceptable according to [20]. The closer the R^2 value to unity, the better and more significant an empirical model fits the actual data. The smaller R^2 is, the less important the dependent variables in the model have in explaining the behavior of variation [21]. Furthermore, the calculated adjusted R^2 values for studied responses variables were higher than 0.80, hence there is a close agreement between the observed values and the theoretical values predicted by the proposed model. The calculated adjusted R^2 values for studied flavonoid, phenolic and DPPH were R^2= 0.8485, R^2 = 0.8986, and 0.9612 hence there is a close agreement between the experimental results and the theoretical values predicted by the proposed models. Moreover, the lack of fit is significant for the three responses, indicating a good model. Meanwhile, the coefficients of variation (*CV*) of FC, PC and DPPH were 5.30, 1.10, and 1.30 respectively. The small value of *CV* implies that variation in the mean value is low and can suitably develop an adequate response model [22].

Table 6: Differences between Actual and Predicted Values

Standard order	FCA(mg/g GAE)	FCP(mg/g GAE)	PCA(mg QE/g)	PCP (mg QE/g)	DPPHA (%)	DPPHP (%)
1	32.27	30.72	81.91	80.33	52.14	51.08
2	46.36	46.36	84.00	83.79	55.66	55.66
3	47.54	48.19	89.55	88.59	62.14	61.70
4	46.36	46.36	84.00	83.79	55.66	55.66
5	46.72	46.69	85.78	87.11	58.93	59.37
6	46.36	46.36	84.00	83.79	55.66	55.66
7	36.45	36.85	83.01	87.11	53.89	53.90
8	37.82	40.88	83.99	80.33	57.70	58.36
9	46.36	46.36	84.00	83.79	55.66	55.66
10	43.36	39.93	80.22	80.48	58.25	57.13
11	45.09	43.18	86.68	87.26	60.06	60.04
12	31.80	34.60	77.67	79.00	52.95	54.06
13	46.36	46.36	84.00	83.79	55.66	55.66

FCA –Flavonoid content Actual value, FCP- Flavonoid content predicted value, PCA- phenlic content actual value, PCP-Phenolic content predicted value, DPPHA- DPPH actual value, DPPHP-DPPH predicted value

Table 6 showed the differences between actual and predicted values. The difference in the actual and the predicted values was also used to test the suitability of the model developed for prediction purpose by comparing the actual values and predicted values. Predicted values are realistically closed to the actual or experimental values. The results confirmed that the model is valid and that predicted model is adequate.

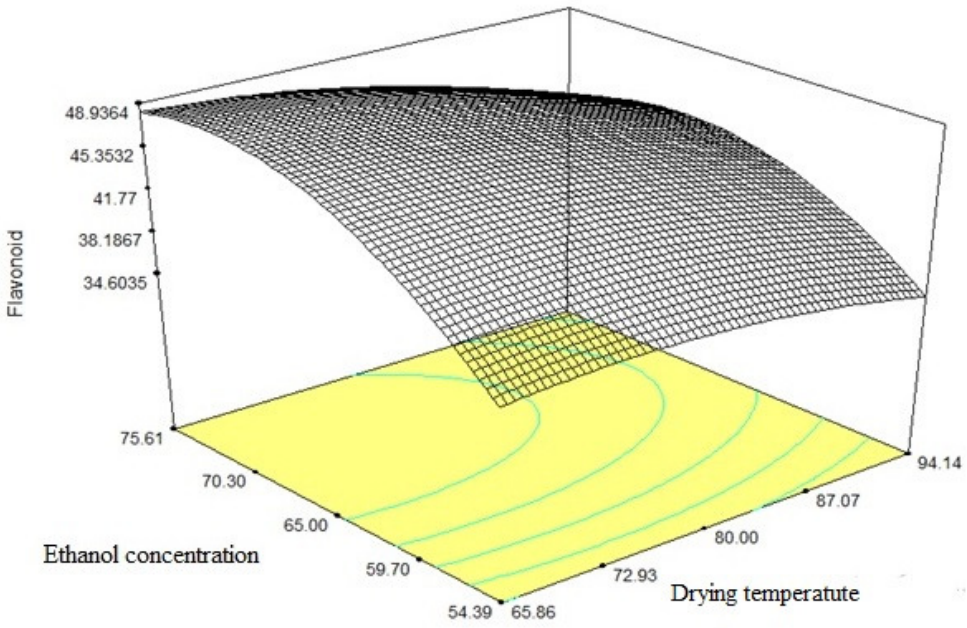

Figure 1. Random surface plot depicting the effect of temperature and ethanol concentration on flavonoid levels

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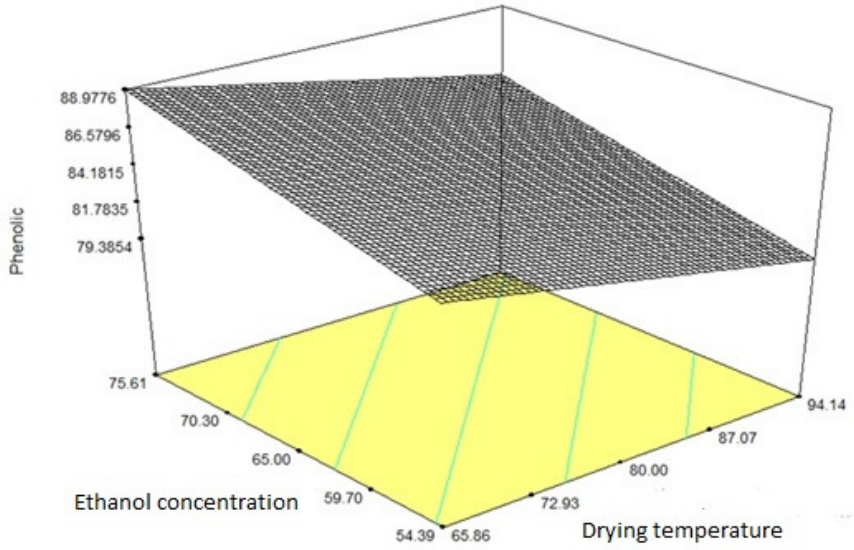

Figure 2: Random surface plot depicting the effect of temperature and ethanol concentration on phenolic levels

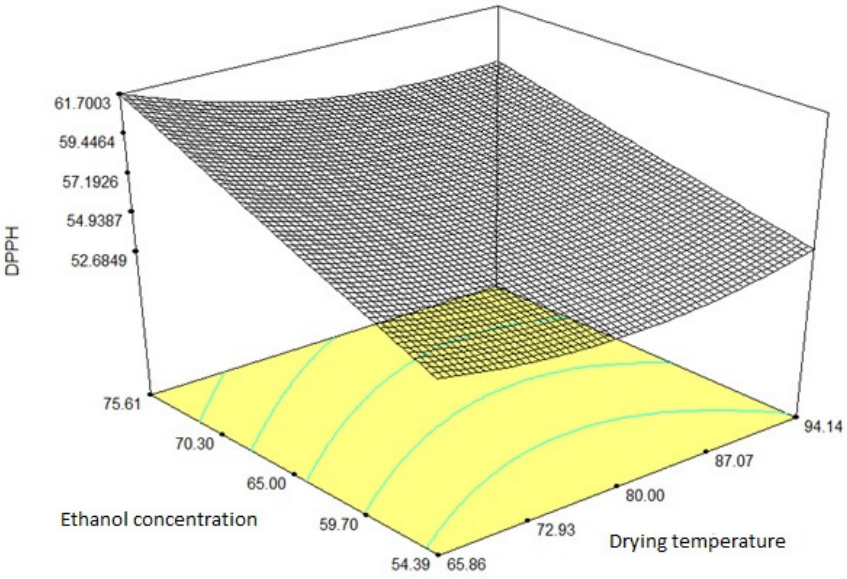

Figure 3: Random surface plot depicting the effect of drying temperature and ethanol concentration on DPPH levels

Figure 1, 2 and 3 showed the random surface plot of the effect of drying temperature and ethanol concentration on flavonoids, phenolic and DPPH. The independent variables were created with ethanol concentration and drying temperature as X and Y coordinate,

respectively, and the responses (FC, PC, and DPPH) [23]. It could be observed in figure 1a that the value of FC increased with increased ethanol concentration and low temperature. The same progression was also observed in figure 1b and 1c for phenolic and DPPH. In contrast, higher drying temperature and lower ethanol concentrations resulted in decreased FC, PC and DPPH values. According to [24], ethanol solvent favors the efficient extraction of flavonoids and their glycosides from plant materials. This was attributed to the diffusion of the particles that caused the rupturing of plant tissue and therefore brought about higher solubility of the solvent until it started to degrade to a lower value as it has achieved the stable state [25].

Table 7: Optimization of process parameters and validation of the model

X_1	X_2	FCA (mg/g GAE)	FCP (mg/gGAE)	PCA (mg QE/g)	PCP (mg QE/g)	DPPHA (%)	DPPHP (%)
72.72 °C	75.61 v/v %	46.98±0.08	47.42±0.12	88.32±0.03	87.79±0.03	60.86±0.06	60.18±0.11

FCA –Flavonoid content Actual value, FCP- Flavonoid content predicted value, PCA- phenolic content actual value, PCP-Phenolic content predicted value, DPPHA- DPPH actual value, DPPHP-DPPH predicted value

Table 7 showed the result of the optimization of process parameter from the optimized conditions. The aim of this study was to find the processing conditions that resulted in the maximum yield of the responses. The final results for the simultaneous optimization using the desirability function method suggested that the optimal extraction conditions for *Codiaeum variegatum* leaves extract were at 72.72 °C and 75.61 v/v % of ethanol concentration to achieve the best combination for highest phenolic, flavonoids, and DPPH. In order to verify the optimum conditions, the *Codiaeum variegatum* leaves were subjected to analysis using these optimal conditions and the results were compared statistically to the predicted values given by the design expert 6.0.8 software of the RSM model. Based on the results, the predicted values of flavonoid, phenolic, and DPPH were found to be reasonably comparable with experimental values at 95% confidence level as shown in Table 7.

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Table 8: FT-IR analysis data interpretation of the ethanolic extract at optimum condition

S/N	PEAK	FUNCTIONAL GROUP
1	3632, 3519, 3171	O-H stretching- alcohol
2	3335	N-H stretching – aliphatic primary amine
3	3057	C-H stretching – alkene
4	2928	C-H stretching alkane
5	2360	N-H/C-O stretching
6	1591	NO_2 stretching
7	1370	O-H bending- phenol
8	1040	S=O stretching – sulfoxide

Figure 4 shows the FTIR spectrum of the extract at the optimal condition. The *Codiaeum variegatum* leaf spectra showed the identity of O-H stretching of intermolecular bonded alcohol at 3632, 3519, and 3171 cm^{-1}. The peak at 3335 cm^{-1} indicated the presence of N-H stretching – aliphatic primary amine. The peak at 3057 cm^{-1} and 2928 cm^{-1} indicate C-H stretching of alkenes and alkane, respectively. The peak at 2360 cm^{-1} elucidated the occurrence of N-H/C-O stretching. The peak at a frequency of 1591 cm^{-1} showed the occurrence of NO_2 stretching. The absorbance peak of 1370 cm^{-1} and 1040 cm^{-1} indicate the O-H bending-phenol and S=O stretching – sulfoxide. The FT-IR results established the presence of alkanes, alkenes, amines, alcohol, phenol, nitro compounds, and sulfoxides in the ethanol extracts of *Codiaeum variegatum* leaves. This is in consonant with previous studies conducted on other plants [26, 27]. Fourier Transform Infrared Spectrophotometer (FT-IR) is perhaps the most potent tool for identifying the types of chemical bonds/functional groups present in phytochemicals.

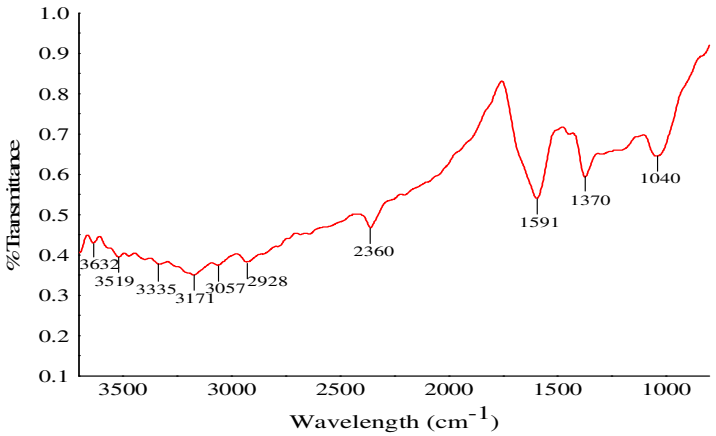

Figure 4: FTIR spectrum at the optimized condition

Table 9: Proximate Composition of *Codiaeum variegatum*

Proximate Parameter	Composition
Moisture (%)	8.87±0.01
Ash (%)	9.69±0.04
Crude fibre (%)	14.50±0.01
Crude Fat (%)	5.50±0.12
Crude Protein (%)	2.82±0.04
Carbohydrate (%)	58.62±0.02

Table 9 showed the results of the proximate composition of *Codiaeum variegatum*. The result revealed the presence of moisture (8.87±0.01), ash (9.69±0.04), crude fibre (14.50±0.01), crude fat (5.50±0.12), crude protein (2.82±0.04), and carbohydrate (58.62±0.02). The moisture and carbohydrate values in this research were more than the values obtained from findings of [5] while the ash (9.69±0.04), crude fibre (14.50±0.01), crude fat (5.50±0.12) and crude protein (2.82±0.04) were lower than the values obtained by [5]. This means that this plant is nutrient dense and can therefore be harnessed to meet some nutritional demands.

CONCLUSION

The Response Surface Methodology (RSM) was used to optimize the extraction process variables of flavonoids, phenolic, and DPPH free radical scavenging activity of *Codiaeum variegatum* leaves using a central composite experimental design. The optimum extraction condition for maximum flavonoid, phenolic contents, and DPPH was an

extraction temperature of 72.72 °C and an ethanol concentration of 75.61 v/v %. Based on the R^2 value of total flavonoid, total phenolic contents and DPPH which were 0.9116, 0.9155, and 0.9612 respectively, the model suggests a good fit model. From the FTIR analysis of the optimizes sample revealed the presence of functional groups including OH, C-H, N-H, N-H/C-O, S=O, and NO_2; suggesting the presence of pharmacologically important compounds such as alkanes, alkenes, amines, phenols, nitro compounds, sulfoxide and alcohols in the ethanol extracts of *Codiaeum variegatum.* This study also revealed that *Codiaeum variegatum* leaves have significant antioxidant activity and can therefore offer great therapeutic advantage. The proximate analysis revealed that *Codiaeum variegatum* can help in meeting some nutritional demands if it is well harnessed.

Conflicts of Interest: The authors declare no conflict of interest.

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