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Optimization of Anthocyanin Extraction from Cockspur Coral (*Erythrina Crista-Galli L.*) Petals with Microwave-Assisted Extraction (MAE) using Response Surface Methodology

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Abstract. Cockspur coral (*Erythrina crista-galli L*) petals are flowers that contain anthocyanins and active compounds of flavonoids and tannins. This study aims to determine the optimum conditions for the anthocyanin extraction process of cockspur coral petals using Microwave-Assisted Extraction (MAE), analyzed through the Response Surface Method (RSM). This process uses ethanol-hydrochloric acid solvents and a Box-Behnken experimental design involving three factors, namely the solvent ratios (w/v) (1:5, 1:15, and 1:25), microwave powers (300, 450, and 600 watts), and extraction times (3, 9, and 15 minutes). As a result, the second-order polynomial model was enhanced and sufficient to explain the variation of the data that denoted the significant correlation with the independent variables and the response. Derringer's desired function methodology was used for optimizing studies and generated ideal conditions for each or combined an independent variable. The optimum anthocyanin extract of 5.82 mg/L was obtained at a power condition of 325,5 Watts, an extraction time of 3.05 minutes, and a solvent ratio of 20.5. Meanwhile, the operating conditions at a power of 310.8 Watts, a time of 14.94 minutes, and a solvent ratio of 24.96 resulted in the optimum color intensity (IC) of 1040.26. In the meantime, the optimum antioxidant activity was obtained at a power of 585.97 Watts, a time of 4.93 minutes, and a solvent ratio of 5.43 with IC_{50} of 0.115.

Keywords: Anthocyanins, Cockspur coral, Microwave-Assisted Extraction, Optimization

INTRODUCTION

Natural dyes (bio pigments) from plants are widely used in various fields of technology, mainly food and color-sensitive *solar cells* (DSSC) (Enciso et al., 2017) with the commonly used pigments including anthocyanins, chlorophyll, carotenoids, and betalain (Cortez et al. 2017, and Richhariya et al. 2017). The advantages of using natural dyes are renewable, low production cost, and environmentally more friendly than synthetic dyes (Khoo et al. 2017).

One of the plant pigments abundant in

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Indonesia is anthocyanin (Saati 2015) in cockspur coral petals. Anthocyanins are flavonoid compounds that contain phytochemicals and contribute to the antioxidant activity that functions to overcome various diseases (Benvenuti et al. 2016, Adaku et al. 2020).

The extraction technique widely used for active compounds from plants, particularly anthocyanins, is Microwave-Assisted Extraction (MAE) (Chan et al. 2014a, Sommer and Cohen 2018, Cassol et al. 2019, and Mahardika and Roanisca, 2019) due to the use of less solvent and short extraction time (Cassol et al. 2019), and the yield produced that is more optimum than the conventional techniques (Farzaneh and Carvalho, 2017). MAE is an innovative tool that makes use of microwaves that associate electromagnetic energy with a wavelength range of 300 MHz to 300 GHz (Sadeghi et al. 2017, Akhtar et al. 2020, Xiaokang et al. 2020). The energy is transmitted as waves, able to penetrate the biomaterial and to interact with polar molecules containing the dielectric constant to produce heat by ionic conduction and dipole rotation (Al-dhabi and Ponmurugan 2020, Sadeghi et al. 2017, Ramos et al. 2019). The MAE parameters, essentially the extraction operating condition such as temperature, time, and pressure, the types of solvents, microwave powers, and solvent ratios, have a significant effect on the extraction process. Thus, determining the optimal conditions is important to obtain maximum anthocyanin yields (Chan et al. 2014a, David et al. 2017, Arroy et al. 2017).

The extraction of cockspur coral petals anthocyanins using acidified ethanol has been examined by Enciso et al. 2017. The use of ethanol acidified with 1% HCl demonstrated the highest solubility of anthocyanin pigments from the concentrations of 2% and 3% (Sofyan 2018) and 1% acetic acid (Ayelaw 2016).

The factors influencing the extraction process using MAE are the types of solvents, the solvent ratio which indicates the solute-to-solvent ratio, the extraction time, and the microwave power extraction (Jafari et al. 2019).

Research on anthocyanins from cockspur coral petals using MAE on a variety of acidified ethanol (1% HCl, 4% citric acid, and 4% tartaric acid), the solvent ratios (1:5, 1:15, and 1:25), the extraction times (3, 6, 9, 12 and 15 minutes) and the powers (300, 450, and 600 W) obtained the highest anthocyanin content of 28.522 mg/L at 1% HCl-ethanol, a 12-minute extraction time, a 1:25 of the solvent ratios, and a 600 W power (Damayanti et al. 2020). The temperature of 70°C was used to prevent the anthocyanins undergo degradation process while using ethanol as a solvent (Ekici et al. 2014)

The operating conditions can be improved by optimizing various parameters to be efficient and effective (Benvenuti et al. 2016). The Response Surface Methodology (RSM) is useful for optimizing the process to determine the best conditions (Farzaneh and Carvalho 2017). Therefore, this study aimed to optimize the MAE parameters of the independent variables (the HCl solvent ratios, extraction times, and microwave powers) and responses (anthocyanin yields) using RSM with Box-Behnken design experiment (Hutabarat et al. 2019).

MATERIALS AND METHODS

Plant materials and reagents

Cockspur coral petals were obtained from Semarang, Central Java, Indonesia. Perfect cockspur coral petals were selected and were then washed clean with water in a bucket

until the dirt that is stuck to the cockspur coral petals was gone. The clean and drained cockspur coral petals were cut into small pieces using scissors to ease the drying process. The cockspur coral petals were dried using an oven and an incubator at a temperature of 50°C until the mass was constant. The cockspur coral petals were weighed in a plastic container using an analytical scale hourly. The dried cockspur coral petals were crushed using a blender until a powder form. The cockspur coral petal powder was sieved with a 35 mesh sieve. The results of the sieved cockspur coral petals were stored in a tightly closed jar, and there were silica gel, ethanol (96%,) and hydrochloric acid (HCl) (37%) outside the jar in advance of the experiments. The solvents and chemicals for this study were purchased from Sigma Aldrich, Semarang, Central Java, Indonesia. The solvent was acidified ethanol with the solvent (w/v) ratios of 1:5, 1:15, and 1:25. The preparation of 1:5 solvent ratio was carried out by weighing out 10 grams of dried powder of cockspur coral petals dissolved in a 50 mL solvent consisting of 0.5 mL of 1% HCl and 49.5 mL of ethanol. The same proportion for the solvent ratio is 1:15 and 1:25.

Extraction of anthocyanins from dried cockspur coral petals using MAE

MAE of anthocyanins was performed in accordance with the methods carried out by Damayanti et al. 2020 in an ordinary household microwave oven (Samsung MS23K3515AS) at a working frequency of 2450 MHz with adjustable microwave powers and times. A flow chart for the extraction of anthocyanins is depicted in Figure 1.

About 10 g of dried cockspur coral petal powder were weighed and put into a 250 ml two-neck flask. The extraction was carried out by using the Microwave-Assisted Extraction method with a Box-Behnken experimental design involving acidified ethanol, solvent (w/v) ratios (1:5, 1:15, and 1:25), microwave powers (300, 450, and 600 watts), and extraction times (3, 9, and 15 minutes).

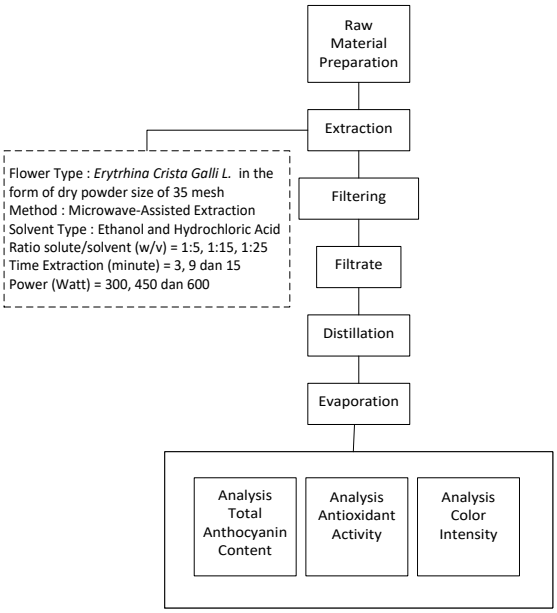


Fig. 1: Flowchart for anthocyanin extraction from cockspur coral petals

The extracted sample was then filtered using Whatman paper and a Buchner funnel with a volume of 500 ml and a vacuum pump to separate the filtrate and residue. The residue in the form of impurities was disposed of in the trash. While the filtrate was continued to the distillation process. The filtrate was put into a glass bottle using a glass funnel, then the glass bottle was coated with aluminum foil from the outside. The evaporation used oil in a 100 mL beaker and was heated with a hotplate at a temperature of 70°C measured by a thermometer in the oil. Every hour, the filtrate was weighed using an analytical balance. The weighing was done in glass bottles without the aluminum foil (the aluminum foil was removed). The evaporation was carried out until the filtrate mass was

constant. The evaporated extract was left in a glass bottle until the temperature dropped. It was then covered and coated with aluminum foil and then was placed in the refrigerator at a temperature of 5-6°C.

Determination of Total Anthocyanin Content (TAC)

A slightly modified pH determination method (see Maran et al., 2017) was used for determining the TAC of cockspur coral petal extract. The 10mL volumetric flask was filled with 1 mL of crude anthocyanin extract and 9 mL of 0.025 M potassium chloride buffer (pH 1.0). Another volumetric flask was filled with 1 ml of cockspur coral petal extract and 9 ml of 0.4 M sodium acetate buffer (pH 4.5). The sample was diluted with pH 1 and pH 4.5 buffers correspondingly. Finding the absorbance of each sample at 510 nm and 700 nm using a UV-visible spectrophotometer (UV/Vis Genesys 10 Spectrophotometer) and distilled water as a blank. The absorbance difference between wavelengths and pH values was calculated by Eq. (1).

$$A = (A_{515} - A_{700})pH_{1.0} - (A_{515} - A_{700})pH_{4.5} \quad (1)$$

The result of TAC was reported as milligram of cyanidin-3-glucoside per 100 g of a dry sample (TAC, mg/g). It was calculated by Eq. (2) (Chen et al. 2018):

$$\mathrm{TAC(mg/g)} = \frac{\mathrm{A} \times \mathrm{MW} \times \mathrm{DF} \times 1000}{\varepsilon \times \mathrm{L}} \quad (2)$$

where L (the cell path length) is 1 cm; ε (molar absorbance of cyanindin-3-glucoside) is 26,900 L mol^{-1} cm^{-1}; DF is the dilution factor; MW (the molecular weight of anthocyanin) is 449.2; and A (Absorbance) is [(A_{510}-A_{700}) pH1.0]-[(A_{510}-A_{700}) pH4.5].

Determination of the antioxidant activity by DPPH radical scavenging assay

The free radical scavenging activity of cockspur coral petal extract and standard solutions (ascorbic acid) was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay (Alhakmani et al. 2013).

1.9716 mg DPPH was put in a 10 mL volumetric flask, then, ethanol was added and shaken. A total of 10 mL of a DPPH solution was put into a volumetric flask and then added to 50 mL of ethanol. Then, 5 mL of the blank solution was taken and homogenized, and left for 30 minutes then its absorption wavelength was measured at 517 nm. In another volumetric flask, 10 mg cockspur coral petal extract was put and ethanol was added up to 10 mL. Therefore, a concentration of 1000 ppm was obtained. Furthermore, the solution was diluted by 0.5; 0.4; 0.3; 0.2; 0.1, and 0.05 mL of the sample mother liquor. Accordingly, each was put into a 10 mL volumetric flask and added to ethanol p.a. in order to obtain a concentration of 100, 80, 60, 40, 20, and 10 ppm. Furthermore, the solution was piped as much as 2.5 mL of each concentration and was put into a test tube and added with 2.5 mL of a blank DPPH solution. The solution was left to stand for 30 minutes and its absorbance was measured at a wavelength of 517 nm. The percentage of the radical scavenging activity was calculated by Eq. (3).

$$\text{radical scavenging activity (\%)} = \frac{A_0 - A_1}{A_0} x100 \quad (3)$$

where A_0 constitutes the absorbance of control at 517 nm, and A_1 constitutes the absorbance of the sample.

Determination of Color Intensity

200 mL of the alkaline citric acid-sodium phosphate buffer solution pH 3 was prepared from a mixture of 2.1% citric acid solution (159 mL) and 0.16% alkaline sodium phosphate solution (41 mL). Then, the expected pH 3 was obtained by adding a solution of citric acid or a solution of alkaline sodium phosphate. The extract of 20 mg was diluted in 25 mL of buffered citric acid-dibasic sodium phosphate with pH 3. The sample absorbance was then measured at 515 nm. The determination of the color intensity was measured by Eq. (4).

$$\text{Color intensity} = \frac{Ax25}{sample\ weight} \quad (4)$$

Experimental design

RSM was employed to specify the supreme conditions for the anthocyanin extraction of cockspur coral petals. Box-Behnken Design (BBD) matrix was performed in the RSM experimental design. BBD with a 3x3 factorial design was used to perform the optimization process and to gain the influence of independent variables in this study. The selected independent variables comprised the Power (A), the extraction time (B), and the solvent ratios (C) (see Table 1). Single-factor experimental analysis is used to determine each process variables and range. The experiments were conducted based on the BBD and the complete design of 15 experiments. Three center points were used to estimate statistical experiment error. Design-Expert version trial 11 (USA) was used to encode and integrate each level of each factor. The number of detailed experiment variables was calculated through Eq. (5) (Maran et al. 2013).

$$N = 2k(k-1) + C_0 \quad (5)$$

where k is the number of factors and C_0 is the number of central points.

For analysis purposes, the independent variables were coded within three levels (-1, 0, and +1) and the coding was calculated through Eq. (6) (Maran et al. 2013).

$$X = \frac{(X_i - X_0)}{\Delta X_i} \quad (6)$$

where X constitutes the dimensionless value of an independent variable; X_i constitutes the real value for an independent variable; X_0 constitutes the real value of an independent variable at the center point; ΔX_i constitutes the step change of the real value of variable i.

After the data were associated with the models, the models were used for the construction of three-dimensional (3D) response surface plots to predict the relationships between independent and dependent variables.

Table 1. Box–Behnken Design of Various Factors and Their Coding Levels

Factor	Coding level		
	–1	0	1
(A) power (watt)	300	450	600
(B) extraction time (minute)	3	9	15
(C) solvent ratios (–)	5	15	25

In addition, second-order polynomial equations are applied to obtain the most optimal modeling for predicting response results. Four types of polynomial models such as linear, quadratic, cubic, and interactive effects of the process variables are used.

The general second-order polynomial model was used for the response surface analysis in Eq. (7):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ii} X_i X_j \quad (7)$$

where the response function (Y, TAC) constitutes partitioned into linear, quadratic, and interactive components; β_0 is defined as the constant; β_i is defined as a linear coefficient; β_{ii} is defined as a quadratic coefficient; β_{ii} is defined as a cross-product coefficient; X_i, X_j, and X_{ii} are the levels of the independent variables.

In this regard, analysis of variance (ANOVA) tables was done, and the impact and regression coefficients of the individual linear, quadratic, and interaction terms were determined.

Statistical analysis of optimal conditions

The various statistical analyses calculations such as the determination coefficient (R^2), the adjusted determination of coefficient (R_a^2), the sum of squares (SS), the predicted determination of coefficient (R_p^2), and the coefficient of variation (CV) were used for experimental data to evaluate the acceptability of various models (linear, interactive (2FI)). The regression coefficients of each polynomial model were analyzed by the Pareto analysis of variance (ANOVA). All the terms in the model were tested by the student's F-test, and the significance of the F-values at probability levels ($p \leq 0.05$) were analyzed.

After the effect of independent variables on the responses was depicted by analyzing the polynomial equation, the optimization process was carried out by Derringer's desired function methodology (Maran et al. 2013). This numerical optimization technique will optimize the combination of one or more objectives, both in the form of process and response variables. The possible goals that result from this technique are: *minimize, maximize, target, within reach, set to the exact value* (*factor only*), and *none* (*response only*). In this study, the response objective was selected as maximal, and the objective of the process variable was selected as *within the range*. A weight factor of 1 was chosen for the response, used to suit the shape of its particular desired function. A given value of 1 makes the ramp function linear between a low value and a goal or a high value and a goal. In addition, a given significance value of 3 was selected for the response so that the objective becomes equally important.

RESULTS AND DISCUSSION

Optimization of the Extraction Process and Verification of the Optimal Process

To study the random repetition of the software on input independent variables (power, time, and solvent ratio) with anthocyanin extraction results, color intensity and antioxidant activity were calculated statistically with the experimental design shown in Table 2.

The observed model fit was performed on the experimental data to determine the most suitable models for calculating response outcomes. Four types of polynomial models (linear, interactive (2FI), quadratic, and cubic) were used to predict response variables for the experimental data. Some parameters such as sequential p-value, lack of fit p-value, adjusted R^2, dan predicted R^2 (Table 3) were used to deduce the most appropriate model for optimizing the yields of anthocyanins and color intensity.

Table 3 shows that the justified quadratic model is the most suitable model for optimizing anthocyanin extracts. Meanwhile, the 2FI model is suggested for being a model for optimizing the color intensity. The two models are further justified through ANOVA.

Table 2. Experimental Design of The Box-Behnken Design (BBD) Method with experimental and Predictive Data

Run	Power (Watt, A)	Time (Minute, B)	Solvent Ratio (C)	Anthocyanin Extract (AE) (mg/L)		Color Intensity (CI)		Antioxidant Activity (AA)		%error AE	%error CI	%error AA
				Experiment	Prediction	Experiment	Prediction	Experiment	Prediction			
1	300	15	15	2.087	3.026	625	732.216	236.998	246.792	44.270	17.140	4.480
2	600	3	15	3.339	2.399	525	516.964	71.869	62.075	30.940	1.550	12.320
3	300	9	5	2.505	1.566	400.170	348.581	233.202	245.29	37.940	12.910	5.370
4	450	9	15	3.339	4.592	408.750	554.590	215.713	205.841	36.140	35.660	4.083
5	300	3	15	5.427	5.532	537.920	566.279	201.702	188.302	1.360	5.260	6.510
6	450	9	15	5.427	4.592	488.750	554.590	215	205.841	16.240	13.450	3.760
7	600	9	5	1.669	1.773	556.250	541.056	21.856	30.338	2.670	2.750	43.490
8	600	9	25	0.835	1.774	359.170	378.809	173.279	161.191	100.200	5.440	5.890
9	450	9	15	5.009	4.592	692	554.590	186.810	205.841	9.250	19.870	10.760
10	300	9	25	3.757	3.653	966.670	949.914	175.470	166.988	3.840	1.740	4.580
11	450	15	25	3.757	2.922	821.670	712.643	184.538	183.226	23.690	13.280	0.150
12	450	3	5	1.252	2.087	462.500	467.165	81.297	82.609	63.340	0.990	1.910
13	600	15	15	4.592	4.488	332.080	402.901	138.870	152.270	3.660	21.290	11.230
14	450	15	5	2.922	2.922	496.670	422.475	204.042	182.160	0.660	14.960	10.170
15	450	3	25	4.175	4.175	646.250	616.080	112.211	134.093	1.870	4.680	20.100
					Average Error					25.070	11.400	9,650

Table 3. Determination of The Model for The Optimization of Anthocyanin Extracts, Color Intensity, and Antioxidant Activity

Model	*sequential p-value*			*lack of fit p-value*			*adjusted R^2*			*predicted R^2*			Remarks
	AE	CI	AA	AE	CI	AA	AE	CI	AA	AE	CI	AA	
Linear	0.6833	0.1189	0.0099	0.3547	0.5625	0.1083	-0.1172	0.2364	0.5290	-0.6431	-0.1805	0.3080	
2FI	0.4207	0.0249	0.0827	0.3401	0.8818	0.1574	-0.1012	0.6540	0.7065	-1.3554	0.3857	0.5299	Recommended for CI
Quadratic	0.1667	0.6144	0,0601	0.4510	0.8429	0.3087	0.3081	0.6023	0.8806	-1.8342	0.1118	0.4455	Recommended for AE and AA
cubic	0.4510	0.8429	0,3087				0.4300	0.2951	0.9349				Aliased

Equation Model for Anthocyanin Extract, Color Intensity, and Antioxidant Activity

The empirical relationship was expressed by using the quadratic model and the 2FI model with the interactions obtained from the experimental results based on BBD (the variable responses) and input variables will be converted into the second-order polynomial equation. The final equation for optimizing anthocyanin extracts is presented in Eq. (8).

$$\text{Anthocyanin Extract (mg/L)} = -3.702 + 0.02226\,A - 0.48717\,B + 0.7722\,C + 0.00128\,AB - 0.00035\,AC - 0.0087\,BC - 0.000035\,A^2 + 0.001453\,B^2 - 0.01618\,C^2 \quad (8)$$

Meanwhile, the optimization equation for the color intensity is presented in Eq. (9). Moreover, the optimization equation for the antioxidant activity is presented in Eq. (10).

$$\text{Color Intensity} = -440.122 + 1.978\ A + 28.33\ B + 62.9488\ C - 0.0778\ AB - 0.1273\ AC + 0.589\ BC \quad (9)$$

$$\text{Antioxidant Activity} = 231.5984 - 2.0904\ A + 17.6109\ B - 1.7232\ C + 0.0088\ AB + 0.034859\ AC - 0.210073\ BC - 0.000846\ A^2 - 0.679317\ B^2 - 0.358635\ C^2 \quad (10)$$

Statistical Analysis for Anthocyanin Extract and the Color Intensity

ANOVA regression models for the prediction of anthocyanin extract, the color intensity, and the antioxidant activity are presented in Tables 4, 5, and 6.

The experimental data were analyzed by using ANOVA and the significance of the regression coefficients was analyzed with the *p-value* as shown in Table 4. This model has an F-value of 1.69 with a p-value of 0.2918, which indicates that there is a lot of noise in the data. There is a 29.18% probability that it will make the F-value relatively large due to interference. In a situation where there is no p-value lower than 0.05, the p-value higher than 0.1 is considered insignificant to the model. In this case, only the variable C^2 is considered significant to the model.

Table 4. ANOVA Regression Model for Prediction of Anthocyanin Extracts

Source	Estimated Coefficient	Sum of square	Degree of Freedom	Mean square	F value	p-value
Model	4.59	22.57	9	2.51	1.69	0.2918
A	-0.4176	1.40	1	1.40	0.9417	0.3764
B	-0.1044	0.0872	1	0.0872	0.0588	0.8180
C	0.5220	2.18	1	2.18	1.47	0.2793
AB	1.15	5.27	1	5.27	3.56	0.1179
AC	-0.5215	1.09	1	1.09	0.7342	0.4307
BC	-0.5220	1.09	1	1.09	0.7356	0.4302
A^2	-0.7827	2.26	1	2.26	1.53	0.2715
B^2	0.0523	0.0101	1	0.0101	0.0068	0.9374
C^2	-1.62	9.66	1	9.66	6.52	0.0511
Residual		7.41	5	1.48		
Lack of fit		4.97	3	1.66	1.36	0.4510
Pure error		2.44	2	1.22		
Cor Total		29.98	14			
Adeq prec	3.9904					

Table 5. ANOVA Regression Model for Predicting Color Intensity

Source	Estimated Coefficient	Sum of square	Degree of Freedom	Mean square	F value	p-value
Model	554.59	3.398E+05	6	56629.19	5.41	0.0163
A	-94.66	71680.34	1	71680.34	6.85	0.0308
B	12.97	1345.51	1	1345.51	0.1285	0.7292
C	109.77	96397.82	1	96397.82	9.21	0.0162
AB	-70.00	19600.00	1	19600.00	1.87	0.2084
AC	-190.90	1.458E+05	1	1.458E+05	13.92	0.0058
BC	35.31	4987.89	1	4987.89	0.4765	0.5096
Residual		83748.06	8	10468.51		
Lack of fit		41101.01	6	6850.17	0.3212	0.8818
Pure error		42647.04	2	21323.52		
Cor Total		4.235E+05	14			
Adeq prec	8.6034					

Table 6. ANOVA regression model for prediction of Antioxidant Activity

Source	Estimated Coefficient	Sum of square	Degree of Freedom	Mean square	F value	p-value
Model	205.84	55928.87	9	6214.32	12.47	0.0063
A	-55.19	24365.11	1	24365.11	48.89	0.0009
B	37.17	11053.57	1	11053.57	22.18	0.0053
C	13.14	1380.78	1	1380.78	2.77	0.1569
AB	7.93	251.30	1	251.30	0.5043	0.5094
AC	52.29	10936.50	1	10936.50	21.95	0.0054
BC	-12.60	635.48	1	635.48	1.28	0.3100
A^2	-19.03	1336.54	1	1336.54	2.68	0.1624
B^2	-24.46	2208.25	1	2208.25	4.43	0.0892
C^2	-35.86	4749.02	1	4749.02	9.53	0.0273
Residual		2491.73	5	498.35		
Lack of fit		1948.20	3	649.40	2.39	0.3087
Pure error		543.53	2	271.77		
Cor Total		58420.61	14			
Adeq prec	11.8753					

Table 5 shows that the F-value and p-value of the model are 5.41 and 0.0163, indicating that the model is significant. There is only a 1.63% chance that there is
a disruption in the data affecting the model. At a p-value lower than 0.05, the value will indicate that the model is significant.

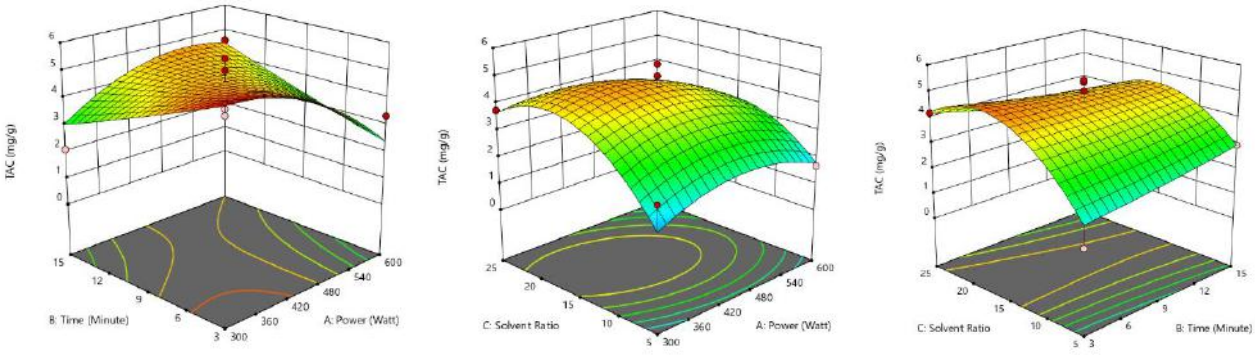
Table 6 shows that the F-value and p-value of the model are 12.47 and 0.0063 indicating that the model is significant. There is only a 0.63% chance that there is a disruption in the data affecting the model. A p-value lower than 0.05 indicates that the model is significant.

There is only a 1.63% chance that there is a disruption in the data affecting the model. A p-value lower than 0.05 will indicate that the model is significant.

Effects of Process Variables for Anthocyanin Extract and Color Intensity

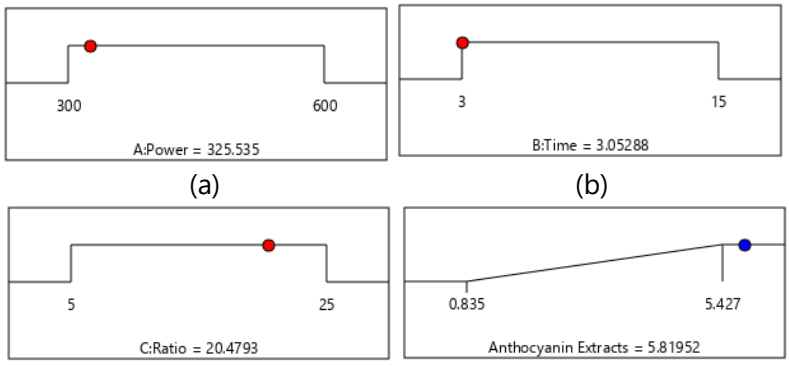
Three factors with three levels of BBD were used in this study to investigate the effect of process variables such as powers, extraction times, and solvent ratios on the anthocyanin extraction process from cockspur coral petals. From the model that has been developed, a 3-dimensional response surface response will be synthesized, and a contour plot illustrates the main and interactive effects of the process variable (input) on the response variable. The resulting graph comes from one constant variable (derived from its midpoint) and differs from the other two variables. This is done to determine the effect of each process variable on the response variable for anthocyanin extract (Figure 2), the color intensity (Figure 4), and the antioxidant activity (Figure 6).

Powers did not have any significant effect on the anthocyanin extract result. This is in accordance with the study results of Kazan et al. (2016), which was probably due to the extremely microwave heating rate. Figure 2(a) shows that the highest anthocyanin yield concentration has the potential to occur at a 300 Watt power. The increase of power did not give a large effect on the anthocyanin extract produced. It is confirmed in Figure 2(b) that when the power increased to a temperature of 600 Watts, it does not have any significant impact on the anthocyanin extract.



(a) (b) (c)

Fig. 2: Response surface for the effect of process conditions on the resulting anthocyanin extract at (a) solvent ratio = 15 at an interaction between power and time; (b) time = 9 minutes at an interaction between power and solvent ratio; and (c) power = 450 Watt at an interaction between time and solvent ratio



(a) (b) (c) (d)

Fig. 3: Optimization results for anthocyanin extract (a) power; (b) time; (c) solvent ratio; and (d) anthocyanin extracts

From Figure 2(a), it can also be seen that there is an improvement of anthocyanin extract in the span of 3-6 minutes but it decreases slowly if the time is added. This phenomenon is related to the research results conducted by Pap et al. 2012 that the maximum anthocyanin yields occur when the extraction time is lower, and the microwave power is higher. This may occur because most of the anthocyanin substances have been extracted at the beginning of the extraction time. The longer extraction time has the potential to degrade the anthocyanin content in cockspur coral petals. The solvent ratio is the main factor affecting the anthocyanin extraction in cockspur coral petals. Figure 2(a) shows that the highest peak occurs at low power, and the extraction time is relatively short, justifying that the increase in power and the increase in time are not significant. Figure 3(c) shows that there was an increase in the resulting anthocyanin extract in the solvent ratio ranging from 5 to 20. The addition of solvent concentration tends to decrease the yields of anthocyanin extract.

This might be due to an occurrence that

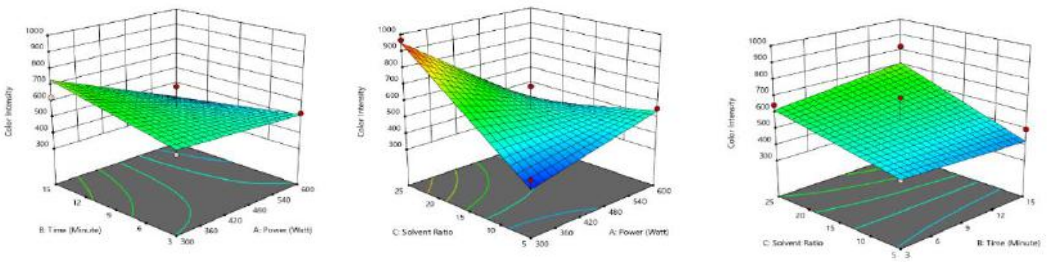
the increase in the microwave power causes a steep increase in temperature. As a result, there is excessive heat and evaporation of the solvents which cause anthocyanins to decrease as they are sensitive to heat (Chan et al. 2014b). Hence, the higher the solvent concentration is, the higher the generated heat is.

Figure 3 illustrates that the Derringer method is used to optimize the extraction process conditions to produce optimum anthocyanin extract in the extraction process of cockspur coral petals. The results were the anthocyanin extract of 5.82 mg/L obtained at a power condition of 325,5 Watts, an extraction time of 3.05 minutes, and a solvent ratio of 20.5. In this condition, the value of the desirability ramp result is 1.

Figures 4(a) and 4(b) show that the increase in power causes the color intensity of the extract to decrease. From Figure 4(b), it can also be seen that the higher the solvent ratio is, the maximum color intensity is produced. It can also be justified in Figure 4(c) that the solvent ratio is an important determinant of the color intensity results. Figure 4(a) shows that the color intensity tends to increase over the span of 3-12 minutes but decreases if the extraction time is continued. This occurs because the extension of the extraction time has a consequence that the higher energy received by the sample has the potential to change the pigment which leads to a decrease of the color intensity.

Figure 5 illustrates that Derringer methodology is applied to optimize the operating conditions in the extraction process to obtain the highest color intensity. The operating conditions resulting from this method are at a power of 310.8 Watts, a time of 14.94 minutes, and a solvent ratio of 24.96 with a color intensity of 1040.26. The desirability-ramp result is 1 which is generated from the optimal point using numerical optimization techniques.

The antioxidant activity is indicated by the IC_{50} value, which means the smaller the IC_{50} value is, the greater the antioxidant activity is. Figures 6(a) and 6(b) show that the power shows a significant value where the higher the power is, the smaller the IC_{50} value is, denoting a greater antioxidant activity. This is justified by Figure 6(c) where the lowest IC_{50} value indicating the greatest antioxidant activity was obtained at a ratio of 5 and 3 minutes



(a) (b) (c)

Fig. 4: Response surface for the effect of process conditions on the resulting color intensity at (a) solvent ratio = 15 at an interaction between power and time; (b) time = 9 minutes at an interaction between power and solvent ratio; and (c) power = 450 Watt at an interaction between time and solvent ratio

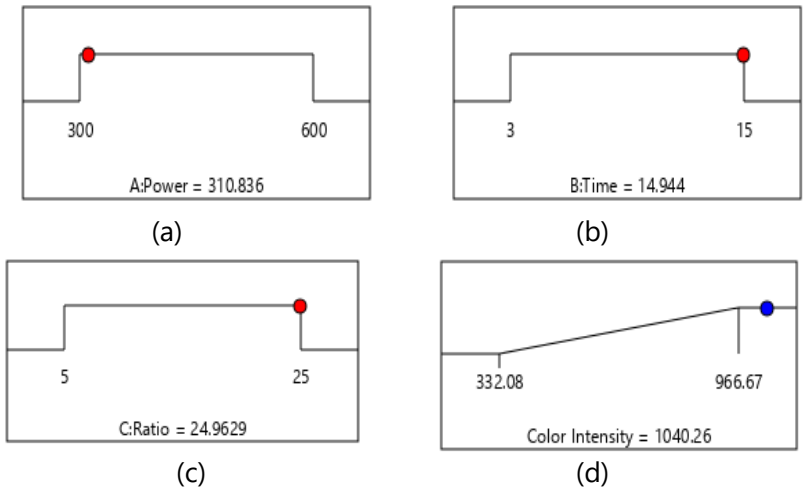


Fig. 5: Optimization results for color intensity (a) power; (b) time; (c) ratio; and (d) color intensity

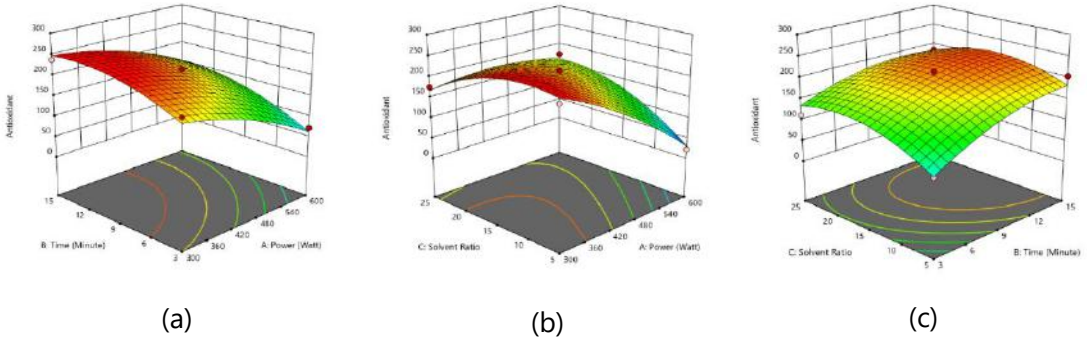


Fig. 6: Response surface for the effect of process conditions on the resulting Antioxidant Activity at (a) solvent ratio = 15 at an interaction between power and time; (b) time = 9 minutes at an interaction between power and solvent ratio; and (c) power = 450 Watt at an interaction between time and solvent ratio.

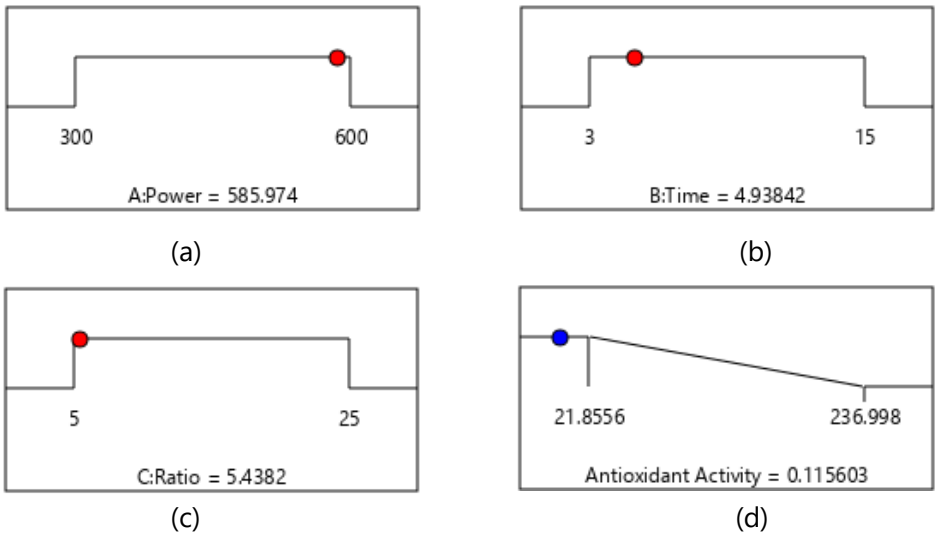


Fig. 7: Optimization results for antioxidant activity (a) power; (b) pime; (c) solvent ratio; and (d) antioxidant activity

Figure 7 illustrates that Derringer methodology is applied to optimize the operating conditions in the extraction process to obtain the highest antioxidant activity, where the greatest antioxidant value is obtained when the IC_{50} value is minimum. The operating conditions resulting from this method are at a power of 585.97 Watts, a time of 4.93 minutes, and a solvent ratio of 5.43 with an IC50 of 0.115. The desirability-ramp result is 1 which is generated from the optimal point using numerical optimization techniques.

CONCLUSIONS

The BBD method is used to optimize the response surface of the anthocyanin extraction of cockspur coral petals. The variables reviewed include power (A), time (B), and solvent ratio (C) which will be optimized, and their effects were studied on the process both individually and interactively. The results indicate that the solvent ratio (C) is the most significant factor in both the anthocyanin extract level and the resulting color intensity, alongside other factors such as power (A) and the power-to-solvent interaction (AC). The optimum anthocyanin extract of 5.82 mg/L was obtained at a power condition of 325,5 Watts, an extraction time of 3.05 minutes, and a solvent ratio of 20.5. Whereas, the operating conditions at a power of 310.8 Watts, time of 14.94 minutes, and a solvent ratio of 24.96 resulted in the optimum color intensity of 1040.26. The optimum antioxidant activity is obtained at a power of 585.97 Watts, a time of 4.93 minutes, and a solvent ratio of 5.43 with an IC_{50} of 0.115.

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