

RESEARCH ARTICLE

Extraction Optimization and Characterization of Pigment from Floral Bracts of *Bougainvillea spectabilis*

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ABSTRACT:

As of late, normal colors and shades acquire significance in nourishment and material ventures as a result of their nontoxic and eco-accommodating qualities. *Bougainvillea spectabilis* flower are wealthy in betanin shades which can be utilized as a color in sharpened sun-oriented cells, restorative and nourishment applications. The point of this examination was to advance the common shade extraction from the botanical blossom by reaction surface philosophy. Central Composite Design (CCD) of Response Surface Method (RSM) was connected to assess the ideal states of three process factors to be specific mass of botanical blossom (g), time (t) and temperature (°C) learned at levels. Mass of blossom furthermore, time were found measurably noteworthy simultaneously and connection coefficient (R²) estimation of 0.96 demonstrated that model was all around fitted with the trial esteems. The ideal procedure conditions were observed to be mass of botanical bracts: 2g, contact time: 205mins and extraction temperature: 30°C with most extreme absorbance. Reaction Surface Method (RSM) was performed well to recognize the ideal levels of extraction process factors and the approval of anticipated model was fitted 98.06% with the exploratory outcomes led at the ideal conditions. Fourier Transform Infrared Spectroscopy was likewise affirmed the nearness of betanin color by distinguishing the major useful gatherings.

KEYWORDS: *Bougainvillea spectabilis*, Betacyanin, Betalanin, Response Surface Methodology (RSM)

INTRODUCTION:

Bougainvillea spectabilis is a class of prickly elaborate vines, shrubberies, and trees with blossom like spring leaves close to its blooms. Distinctive creators acknowledge somewhere in the range of 4 and 18 species in the family. The vine species develop somewhere in the range of 1 to 12m (3 to 40 ft.) tall, scrambling over different plants with their spiky thistles.

The thistles are tipped with a dark, waxy substance. They are evergreen where precipitation happens all year, or deciduous if there is a dry season. The leaves are exchange, basic praise sharpen, 4– 13cm long and 2– 6 cm wide. The genuine blossom of the plant is little and by and large white, yet each bunch of three blooms is encompassed by three or six bracts with the brilliant hues related with the plant, including pink, fuchsia, purple, red, orange, white, or yellow colour is some of the time alluded to as "paper blossom" in light of the fact that the bracts are thin and papery. The organic product is a tight five-lobed achene. *Bougainvillea spectabilis* are generally bother free plants, however they might be defense less to worms, snails and aphids.[1]

Measurable advancement techniques are effectively used to recognize the ideal level of different parameters engaged with the procedure. Streamlining by transforming one factor at any given moment (OFAT) was a typical and very much contemplated technique however it has numerous weaknesses like tedious, costly and so on and it doesn't give insights about the collaboration impact of the factors associated with the procedure (Haaland, 1989). Response Surface Method (RSM) is an accumulation of factual tools used to examine and decide the ideal conditions inside the plan space of the test contemplate (Myers and Montgomery, 1995). The fundamental preferred standpoint of utilizing RSM is to comprehend the association among the procedure factors with fewer exploratory runs and it is used well for different streamlining contemplates (Sharmila et al., 2013; Ramandi et al., 2017; Baboukani et al., 2012). The fundamental target of the investigation was to decide the ideal states of water-solvent color extraction process from *Bougainvillea spectabilis* botanical bracts utilizing Central Composite Design (CCD) of Response Surface Method. Mass of botanical bract (g), contact time (mins) and temperature (°C) were decided for the improvement consider. Auxiliary investigation of removed color was likewise inspected by FTIR [1]

MATERIALS AND METHODS:

Bougainvillea spectabilis were gathered from Gam bang (Pahang). The example was dried under the daylight. At that point, it was ground in a blender to create fine powder test required for the exploratory work and kept in cooler to protect its freshness (Hisami et al., 2011). Water was picked as the dissolvable for the seclusion of betacyanin's from the bracts of *Bougainvillea spectabilis* for the arrangement of common color, to ensure that the color is protected from hurtful synthetic substance

(Kaur, 2014)[6]. One molar of acidic corrosive was utilized to alter the pH to acidic condition. [2]

Collection of flowers:

Fresh floral bracts were collected from plant grown in Vandavasi in local area. Floral bracts were cut approximately into 10 mm in size and used for extraction process.

Pigment extraction:

Ethanol and Water and 12.5:37.5 ratios was used as solvent system for extraction process. 50 mL of the solvent was added to 250 mL Erlenmeyer flask containing known amount of the bracts according to the design (Table 1). Flask was tightly covered by the polyethylene cover to avoid evaporation of the solvent. Flask was kept in a temperature controlled orbital shaker for appropriate time to completely extract the pigment.[2]

Analytical method:

After the extraction process, 10 mL of the solvent (contain soluble pigment) was taken in a falcon tube and centrifuged at 5000 rpm for 10 min. The supernatant was suitably diluted and absorbance was measured at 538 nm by using UV-Vis spectrophotometer. [2]

Statistical optimization using Response Surface Methodology (RSM):

Focal composite structure was utilized to assess the ideal conditions for extraction process. Factual examination was finished by Minitab software. The three parameters selected were mass of bracts (g), extraction time (mins.) and temperature (°c) and were learned at five level(+2, +1,0, -1, -2). Twenty examinations were done by the plan table given(Table1) and reaction colour Optical Density (OD) was classified.[3]

Table No: 1 Central Composite Design for 20 trails

Std Order	Run Order	PT Type	Blocks	Mass	Time	Temp
1	1	1	1	1	120	20
2	2	1	1	3	120	20
3	3	1	1	1	300	20
4	4	1	1	3	300	20
5	5	1	1	1	120	40
6	6	1	1	3	120	40
7	7	1	1	1	300	40
8	8	1	1	3	300	40
9	9	-1	1	0.318207	210	30
10	10	-1	1	3.681793	210	30
11	11	-1	1	2	58.63865	30
12	12	-1	1	2	361.3614	30
13	13	-1	1	2	210	13.18207
14	14	-1	1	2	210	46.81793
15	15	0	1	2	210	30
16	16	0	1	2	210	30
17	17	0	1	2	210	30
18	18	0	1	2	210	30
19	19	0	1	2	210	30
20	20	0	1	2	210	30

Table No: 2 Central Composite Design Represented in Experimental results

Std Order	Run Order	PT Type	Blocks	Mass	Time	Temp	Exp. value
1	1	1	1	1	120	20	1.165
2	2	1	1	3	120	20	3.361
3	3	-1	1	1	300	20	2.145
4	4	1	1	3	300	20	3.075
5	5	1	1	1	120	40	1.748
6	6	1	1	3	120	40	3.267
7	7	1	1	1	300	40	2.519
8	8	1	1	3	300	40	3.447
9	9	-1	1	0.318207	210	30	1.439
10	10	-1	1	3.681793	210	30	3.344
11	11	-1	1	2	58.63865	30	3.143
12	12	-1	1	2	361.3614	30	3.223
13	13	-1	1	2	210	13.18207	3.359
14	14	-1	1	2	210	46.81793	3.322
15	15	0	1	2	210	30	3.627
16	16	0	1	2	210	30	3.346
17	17	0	1	2	210	30	3.222
18	18	0	1	2	210	30	4.000
19	19	0	1	2	210	30	4.000
20	20	0	1	2	210	30	4.000

RESULT AND DISCUSSION:

Optimization by Response Surface Methodology:

Central Composite Design (CCD) of RSM was used to design the experiment programming and Twenty experiments were carried out according to the variables given in the design table and results are given (Table 1). The optimization experiments were conducted by performing 20 sets of experiments using Central Composite Design (CCD)

Regression analysis and model fitting:

The reaction shade Optical Density was changed from 1.165 to 4.00 in the trial runs. The watched trial results in each run were exposed to various relapse investigation to ascertain the regression coefficients of the demonstrate. Determined regression coefficients of the model. Determined relapse coefficients were substituted in to get a model for the extraction of dissolvable colors from the bracts.[4]

The results are relapse examination organized in (Table 3) clarified that direct impact of temperature (°C), Cooperation impact of temperature with mass, time and temperature. The way connection impact of Mass*mass, Time, Mass*Temperture and Time*Temperature. Then square impact of mass*mass, time*time, temperature*temperature were observed to be extraction process. [5][6]

Surface plots and Contour plots:

The two-dimensional contour and three-dimensional surface plot were used to investigate the effect of the individual variable and interaction for the predicted response on the optimization of flower sample. The 3 surface plot spoke to in Fig 1, 2, 3 were utilized to think about the connection among the factors simultaneously (Bas and Boyaci, 2007) [3]. Fig1 demonstrate Surface Plot speaking to the connection between the Mass and Time fixation. The hold value was Temperature 30°C. The clarified the shade absorbance expanded as the time increments and stays steady with mass of botanical bracts. [7] Fig2 demonstrates the Surface Plot speaking to the association between the Mass and Temperature focus. The hold value was Time 210mins. The impact of mass of flower bracts and temperature. The expanded from the low to abnormal state of botanical bracts of mass and consistent with temperature. Fig 3 demonstrates the surface plot speaking to the association between the Time and Temperature fixation. The hold value was Mass 2g. The saw when time and temperature were kept up. [8,9]

The point forecast device of the Minitab programming was utilized to distinguish the ideal estimations of the picked procedure factors [10]. Ideal estimations of Floral Bracts Mass (A), Time (B), Temperature (C) were observed to be 2g, 205mins, 30°C separately with the greatest predicted reaction 4.00.

Table No:3 Analysis of Variance (ANOVA)

SOURCE	DF	ADJSS	ADJMS	F-VALUE	P-VALUE
MODEL	9	11.0931	1.23257	56.21	0
LINEAR	3	6.0006	2.00019	91.22	0
MASS	1	5.7954	5.79544	264.3	0
TIME	1	0.1842	0.18419	8.4	0.016
TEMP	1	0.0209	0.02094	0.95	0.352
SQUARE	3	4.6273	1.54243	70.34	0
MASS*MASS	1	3.97	3.97002	181.05	0
TIME*TIME	1	0.9339	0.93394	42.59	0
TEMP*TEMP	1	0.2615	0.26154	11.93	0.006
2-WAYINTERACTION	3	0.4653	0.15509	7.07	0.008
MASS*TIME	1	0.401	0.40096	18.29	0.002
MASS*TEMP	1	0.025	0.02498	1.14	0.311
TIME*TEMP	1	0.0393	0.03934	1.79	0.21
ERROR	10	0.2193	0.02193		
LACK-OF-FIT	5	0.1141	0.02283	1.09	0.465
PUREERROR	5	0.1051	0.02103		
TOTAL	19	11.3124			

MODEL SUMMARY:

Table No: 4 Model Summary

S	R-sq.	R-sq.(adj)	R-sq.(pred)
0.148079	98.06%	96.32%	91.02%

R² value was found to be 98.06% and the Adjusted R² value was found to be 96.32% and both the values were found to be in good agreement with each other.

Model Validation:

Two grams of floral bract was taken in the 250 ml of conical flask containing 50ml of the solvent (Ethanol: Water:12.5: 37.5). The conical flask was kept at 30°C for 205mins and the optical density of the extracted pigment was taken at 538nm. The optical density was found to be 4.00 which proved the model to be 98.06% when compared with the predicted optical density of 4.000. The results are explained with the predicted values are good results. [11,12].

1. Surface Plot representing the interaction between the Mass(g) and Time(mins) concentration. The hold value was Temperature 30°C.
2. Surface Plot representing the interaction between the Mass(g) and Temperature (°C) concentration. The hold value was Time 210mins.
3. Surface Plot representing the interaction between the Time(mins) and Temperature (°C) concentration. The hold value was Mass 2g.

FTIR Analysis:

The Fourier change infrared phantom examination was done to distinguish the major utilitarian gatherings present in the extricated shade (DeSouza et al; 2003). FTIR range spoke to in Fig.4 indicates unmistakable. [13,14] 3282.84, 2918.30, 2850.79, 1635.64, 1323.17, 1259.52, 1151.50, 1022.27, 947.05, 856.39, 756.10, 572.86, 468.70, 420.48 separately [15].

The expansive and solid band at 3282.84cm recommends (O-H) vibrating mode and band at 2918.30 and 2850.79 demonstrates (C-H) symmetry in stretching mode. The wave number 1635.64 demonstrates (C=O) and 1323.17 shows nitro and (C=C) . The wave number 1259.52 demonstrates non-sweet-smelling amines and 1151.50 to 756.10 shows alkyl halide.

CONCLUSION:

This investigation clarified the usage of *Bougainvillea spectabilis* botanical flower for fruitful extraction of common shade [16]. Nowadays, the pigment (Anthocyanin) play a role in the colors of ripening fruit, food colouring etc.The species also use them as food plants [17]. Mass of the botanical flower and time

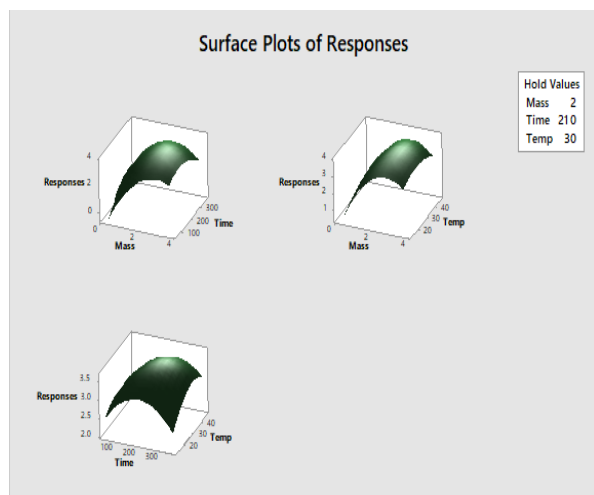


Figure No: 1,2,3 Surface Plots of Response

assume an essential job to build the proficiency of shade extraction at larger amounts [19,20] Exploratory outcomes uncovered that the extraction of colour was exceedingly supported at low than at hoisted levels .The ideal procedure conditions were observed to be mass of botanical blossom: 2g, time: 205mins and temperature 30°C with most extreme optical density 4.000. Approval of the anticipated model was fitted with 98.06% the trial results directed at the ideal conditions.

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