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Extraction of natural dye from petals of Flame of forest (*Butea monosperma*) flower: Process optimization using response surface methodology (RSM)

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ABSTRACT

The uncontrolled discharge of synthetic dyes into the aquatic ecosystem is a global environmental concern due to their negative ecotoxicological effects. Dyes obtained from different natural sources have emerged as an important alternative to synthetic dyes. In this study, natural colorant from the petals of the Flame of forest (*Butea monosperma*) flower was extracted under different operating conditions such as extraction time (45–120 min), temperature (60–90 °C) and mass of the petals (0.5–2 g) by conventional extraction technique. Response surface methodology (RSM) with the help of Design Expert Version 7.1.6 (STAT-EASE Inc., USA) was used for optimization of the extraction process and evaluation of interaction effects of different operating parameters. The optimum conditions for dye extraction were found to be 153.65 min, 73.53 °C and 1.47 g for extraction time, temperature, and mass of the flower respectively. The efficiency of extraction under these optimum conditions was found to be 8813.67 mg L^{-1}. Further, Fourier Transform Infrared Spectroscopy was used to identify the major chemical groups in the extracted dye.

1. Introduction

Since time immemorial, natural dyes have been an integral part of the human life and society. The age old art of dyeing with natural dyes was common in India, China, Egypt and Central Asia. Nature provides us with a wealth of plants which yield color for the purpose of dyeing; many have been used since antiquity [1–4]. In ancient times, man used natural resources like stem, bark, leaves, roots and flowers to extract different colors – yellow, orange, blue, red, green, brown, gray etc. for dyeing clothes [5–7]. However, with the development of synthetic dyes, the rich heritage of natural dyes was gradually lost.

Synthetic dyes are extensively used in textile industries for dyeing nylon, wool, silk, leather, and cotton [8,9]. However, research has shown that most of the synthetic dyes are highly cytotoxic and carcinogenic to mammalian cells and acts as a liver tumor promoter. They can also decrease food intake capacity, growth and fertility rates; causes damage to liver, spleen, kidney and heart; inflicts lesions on skin, eyes, lungs and bones [10]. Due to the toxic and harmful effects associated with these synthetic dyes, today craftsmen in various parts of the world want to revive the old tradition of using natural dyes as these dyes are eco-friendly and produce beautiful attractive shades. The use of natural dyes can replace and significantly minimize the volume of toxic effluent resulting from the conventional dyeing process.

Natural colorants are dyes and pigmentary molecules that are obtained from plant, animal or mineral sources with or without chemical treatments [11,12]. They are organic compounds, having the hydroxyl group in their nucleus and are sparingly soluble in water. Some of the natural colorants do not have a solubilising group [13]. Instead, a temporary solubility group is generated at the time of application. Carbohydrates, minerals, mucilage, vitamins, and pigments, namely flavonol, crocin, anthrocianin, carotene, lycopene, zigzantin etc. are commonly present in the petal of flowers. Extensive investigation is now being done by researchers worldwide for extraction of colorants from different plant parts. Most commonly available raw materials for natural dyes are different parts of the plants which are indispensable for the survival of plants. In India, huge amount of herbal materials are unutilized and dispose off daily which can be used for extraction of natural dyes for application in textile industry as a substitute of synthetic dyes. Besides application in textile industry, these dyes can also be used for coloration purpose in food industry, preparation of herbal gulal and manufacture of colorful candles. The primary advantage of natural dyes is that they are cheap, renewable, non-carcinogenic, non-toxic and no associated disposal problems. Some natural

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sources of dyes can produce truly exquisite shades that are economical to purchase when compared to synthetic dyes. Till date, most of the natural dyed textiles are imported from Third World Countries and India is still a major producer of it.

The present research was performed with the explicit objective of extracting natural dye from the petals of Flame of forest flower by aqueous extraction method and to investigate the combined effect of different parameters such as extraction time, temperature and mass of petals used on the dye extraction process using Central Composite Design in Response Surface Methodology (RSM).

2. Materials and methods

2.1. Flower

Common name: Tesu, Palas
English name: Flame of forest
Botanical name: *Butea monosperma* [14]
Kingdom: Plantae
Order: Fabales
Family: Fabaceae
Used part: Flower

The Flame of forest is a medium sized dry season-deciduous tree, growing to 15 m tall. The leaves are pinnate, with an 8–16 cm petiole and three leaflets, each leaflets 10–20 cm long. The flowers are 2.5 cm long, bright orange–red in color, and produced in racemes up to 15 cm long. The fruit is a pod; about 15–20 cm long and 4–5 cm broad [15]. In the Indian state of West Bengal, its bloom marks the onset of spring (March–April). The flowers of this tree have been used traditionally for dyeing turbans and preparation of *abir* – a colored powder used during the Indian festival Holi.

The main coloring pigment of the flower is butrin. Besides, butein and isobutrin are also present in trace amounts. It also contains flavonoids and steroids [16]. Other than these, coreopsin, isocoreopsin, sulphurein (glycoside) and two other compounds with monospermoside and isomonospermoside structures have also been identified [17]. Studies have shown that isobutrin slowly changes to butrin on drying [18]. The bright orange red color of the flower is attributed to the presence of chalcones [19], pterocarpans [20] and aurones but other anthochlor pigments are also present.

2.2. Aqueous extraction of color from fresh flame of forest flowers [21]

The freshly collected flower petals with average size of 1 cm were used for the extraction. Different mass of floral petals (0.5 g, 1 g, 1.5 g and 2 g) was taken in 250 mL Erlenmeyer flasks. 50 mL distilled water was added to each flask in order to keep the plant materials fully immersed in the solvent. The flasks were then incubated at different temperatures (room temperature, 60 °C, 70 °C, 80 °C, 90 °C). Extract samples were taken at different time intervals (45 min, 60 min, 90 min, 120 min). Thus dye from the petals was extracted by aqueous extraction procedure. The extraction process was carried out at different temperature, different extraction time and with different mass of flower petals in order to obtain the optimal extraction conditions. After the complete extraction of dye, the flowers were taken out from the liquor and used for further extraction of dye.

2.3. Analytical method

The optical density was determined with the help of UV–VIS spectrometer (U-2800, Hitachi, Japan) after suitably diluting the extracted dye with distilled water. At the end of the extraction process, the samples taken from extracts were filtered and placed in clean, dried and pre-weighed glass dishes. The extracts were then dried in a hot-air oven until all the water has evaporated and only the extract was left. The dishes were then cooled in a desiccator and weighed. The drying, cooling and weighing procedure was repeated to get a constant weight and thus the weight of the extract was determined. The weight of the colorant extract obtained per gram of the flame of forest used was then calculated.

2.4. Optimization of extraction conditions

The present work involves optimization of different parameters governing the extraction process. The general practice of determining these optima is by varying one parameter while keeping the others at an unspecified constant level. The major disadvantage of this single variable optimization is that it does not take into consideration the interactive effects among the variables; thus it does not depict the net effects of various parameters on the reaction rate. In order to overcome this problem, optimization studies have been done using Response surface methodology (RSM) [22]. RSM is an effective statistical technique for optimizing a complex processes. RSM reduces the number of experimental trials required to evaluate multiple parameters and their interactions. It is less laborious and less time-consuming than other approaches.

In this study, the optimization of dye extraction process was carried out by three chosen independent process variables including extraction time, temperature and mass of the petals used in the extraction. The ranges and levels of variables investigated in the research are given in Table 1. The total amount of dye extracted was taken as response of the system. The quadratic equation model for predicting the optimal point was expressed according to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i=1} \sum_{j=1+1} \beta_{ij} x_i x_j + \varepsilon$$

where

Y response (dependent variable)
β_0 constant coefficient
β_i, β_{ii}, β_{ij} coefficients for the linear, quadratic and interaction effect
x_i, x_j factors (independent variables)
ε error

Three factors were studied and their low and high levels are given in Table 1. Total amount of dye extracted was studied with a standard RSM design called the Central Composite Design (CCD). Twenty experiments were conducted in duplicate according to the scheme mentioned in Table 2. Design Expert Version 7.1.6 (Stat Ease, USA) was used for regression and graphical analysis of the data obtained. The optimum values of the selected variables were obtained by solving the regression equation and by analyzing the response surface contour plots. The variability in dependent

Table 1
Experimental range and levels of independent process variables.

Independent variable	Range and levels (coded)				
	$-\alpha$	-1	0	$+1$	$+\alpha$
Time, min (A)	5.68	50.00	115.00	180.00	224.32
Temperature, °C (B)	43.07	55.00	72.50	90.00	101.93
Mass of petals, g (C)	0.12	0.60	1.30	2.00	2.48

Table 2
Central composite design for three independent variables used in this study.

Run no.	Coded values			Real values		
	A	*B*	*C*	*A*	*B*	*C*
1	−1	−1	−1	50.00	55.00	0.60
2	+1	−1	−1	180.00	55.00	0.60
3	−1	+1	−1	50.00	90.00	0.60
4	+1	+1	−1	180.00	90.00	0.60
5	−1	−1	+1	50.00	55.00	2.00
6	+1	−1	+1	180.00	55.00	2.00
7	−1	+1	+1	50.00	90.00	2.00
8	+1	+1	+1	180.00	90.00	2.00
9	$-\alpha$	0	0	5.68	72.50	1.30
10	$+\alpha$	0	0	224.32	72.50	1.30
11	0	$-\alpha$	0	115.00	43.07	1.30
12	0	$+\alpha$	0	115.00	101.93	1.30
13	0	0	$-\alpha$	115.00	72.50	0.12
14	0	0	$+\alpha$	115.00	72.50	2.48
15	0	0	0	115.00	72.50	1.30
16	0	0	0	115.00	72.50	1.30
17	0	0	0	115.00	72.50	1.30
18	0	0	0	115.00	72.50	1.30
19	0	0	0	115.00	72.50	1.30
20	0	0	0	115.00	72.50	1.30

variables was explained by the multiple coefficient of determination, R^2 and the model equation was used to predict the optimum value and subsequently to elucidate the interaction between the factors within the specified range.

3. Results and discussions

Natural dye from Flame of Forest flowers was extracted by following aqueous extraction procedure and the extraction process was carried out at different temperatures, different extraction times and with different mass of flower petals with an explicit objective of determining the optimum extraction conditions. Dye extraction from flower depends on both the amount of flower used and the extraction time. With increase in the mass of flower used for extraction (0.5 g to 2 g) as well as the extraction time (45 min–120 min), the total amount of dye extracted also increase. Temperature also significantly influenced the extraction process. As shown in Fig. 1, at 70 °C the dye extraction was maximum compared to that obtained at other temperatures (60 °C 80 °C, 90 °C). The reason behind the phenomenon was that at 70 °C the chemical groups and pigments of the petals of flame of forest was more efficiently disrupted and the dye present in the petals was efficiently extracted in the solution. At higher temperatures (above 90 °C), there was charring and a blackish shade was obtained. So the optimum temperature for dye extraction from flower was taken as 70 °C. With increasing time, extraction of dye in aqueous solution increases, but time duration of 2 h was taken as the optimum time for dye extraction in order to make the technology more cost effective.

3.1. Experimental design and statistical analysis

The results obtained from the 2^3 factorial central composite design experiments were evaluated for total extraction of dye from

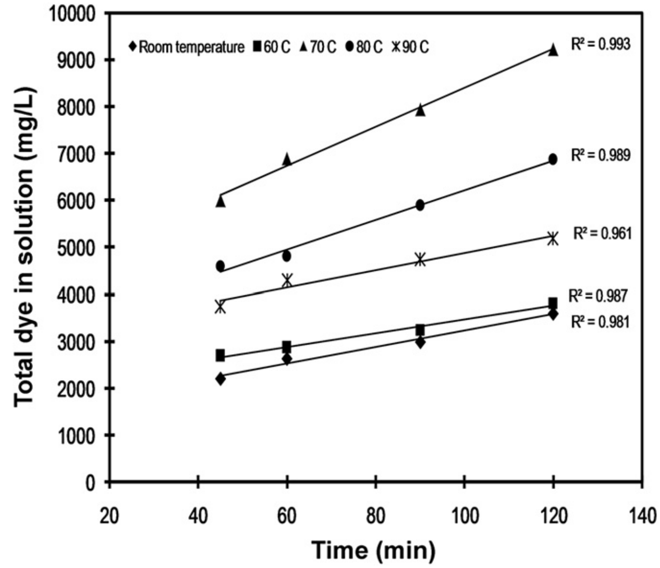

Fig. 1. Effect of time on extraction of dye from flame of forest petals (2 g) at different temperatures.

Flame of forest. An empirical relationship between the response and the independent variables has been expressed by the following quadratic model.

$$\begin{aligned}\text{Total Dye in solution}\left(\text{mgL}^{-1}\right) = & -30279.61324+700.766A \\ & +78.436B+8977.70C \\ & -0.0258AB-0.6837AC \\ & +1.5687BC-4.9857A^2 \\ & -0.24256B^2-2304.337C^2 \end{aligned} \quad (1)$$

The results of second-order response surface model in the form of analysis of variance (ANOVA) are shown in Table 3. The statistical significance of the model equation was evaluated by the *F*-test ANOVA. The significance of each coefficient was determined by *F*-values and *P*-values. The *F*-value (948.91) with a low probability value ($P < 0.0001$) demonstrates a high significance for the regression model. The goodness of fit of the model was also checked by the multiple correlation coefficient (R^2). In this case, the value of the multiple correlation coefficient was 0.9988, which revealed that this regression is statistically significant and only 0.0012% of the total variations is not explained by the model. The value of predicted multiple correlation coefficient (pred. $R^2 = 0.9911$) is in reasonable agreement with the value of the adjusted multiple correlation coefficient (adj. $R^2 = 0.9978$). The non-significant value of lack of fit (more than 0.05) showed that the quadratic model was valid for the present study.

Table 3
Analysis of variance (ANOVA) of the response surface quadratic model for dye extraction.

Source	Sum of squares	Degree of freedom (df)	Mean square	*F*-value	Probability > *F*
Model	1.545×10^8	9	1.716×10^7	948.91	<0.0001
Residual	1.809×10^5	10	18089.10		
Lack of fit	1.809×10^5	5	36178.20	0.29	0.9027
Pure error	0.000	5	0.000		
Total	1.547×10^8	19			

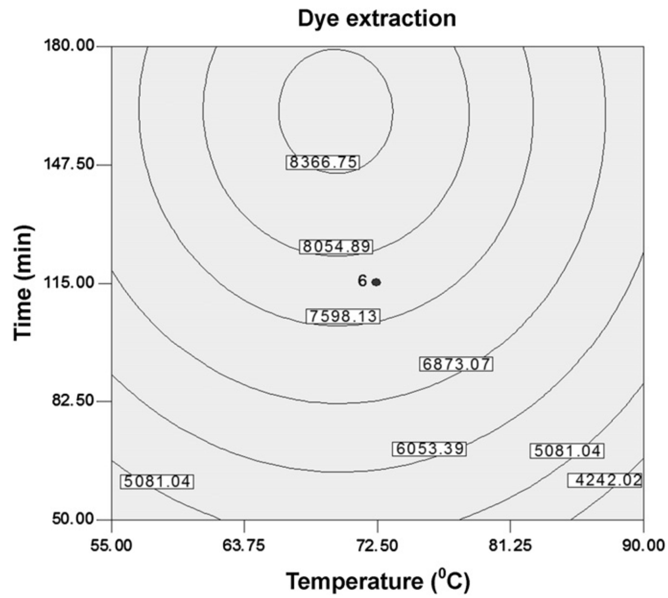

Fig. 2. Contour plot showing the interaction effect of extraction temperature and extraction time on dye extraction.

3.2. Effect of extraction temperature and extraction time on dye extraction

The combined effect of extraction time and temperature on dye extraction from flame of forest is shown in the contour plot of Fig. 2. It is observed that extraction of dye increased with decreasing temperature as well as with increasing extraction time.

3.3. Effect of extraction temperature and mass of petal on dye extraction

The contour plot of Fig. 3 demonstrates the interaction effect of the independent variables (extraction temperature and mass of petals) on the dye extraction process. Both the parameters have a strong effect on the extraction efficiency. The total amount of dye extracted increased with increase in both the temperature as well as the mass of the petals used for extraction.

3.4. Effect of extraction time and mass of petal on dye extraction

The contour plot in Fig. 4 shows the combined effect of mass of petals used and extraction time on the total amount of dye extracted. Evidently, both the independent variables had a strong effect on the extraction process.

3.5. Validation of the model equation

1st order derivative of Eq. (1) gives:

$$\frac{\partial Y}{\partial A} = 700.766 - 0.0258B - 0.6837C - 9.9714A \tag{2}$$

$$\frac{\partial Y}{\partial B} = 78.436 - 0.0258B + 1.5687C - 0.4851B \tag{3}$$

$$\frac{\partial Y}{\partial C} = 8977.70 - 0.6837A + 1.5687B - 4608.67C \tag{4}$$

2nd order derivative of Eqs. (2)–(4) yield:

$$\frac{\partial^2 Y}{\partial A^2} = -9.9714 \tag{5}$$

$$\frac{\partial^2 Y}{\partial B^2} = -0.4851 \tag{6}$$

$$\frac{\partial^2 Y}{\partial C^2} = -4608.67 \tag{7}$$

Since all the second order derivatives showed the negative values, it signifies the absence of local maximum and applicability of maximization. Thus Eqs. (2)–(4) were equated with zero and solved for A, B, C to get the maximum values:

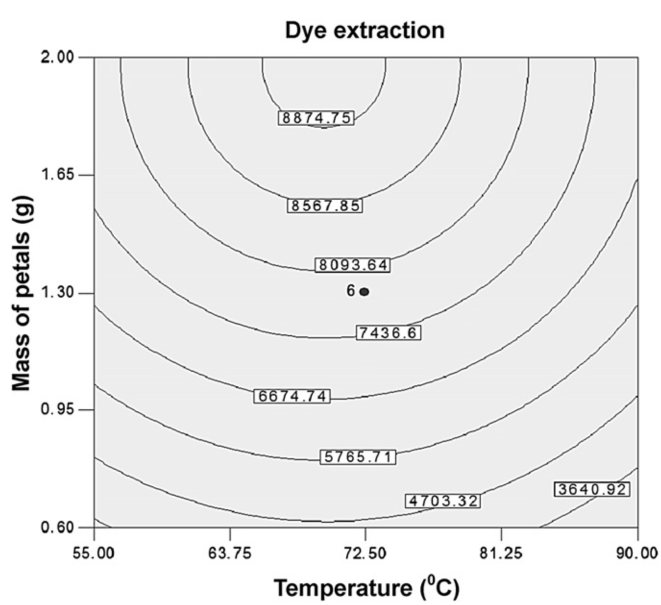

Fig. 3. Contour plot showing the interaction effect of extraction temperature and mass of petals on dye extraction.

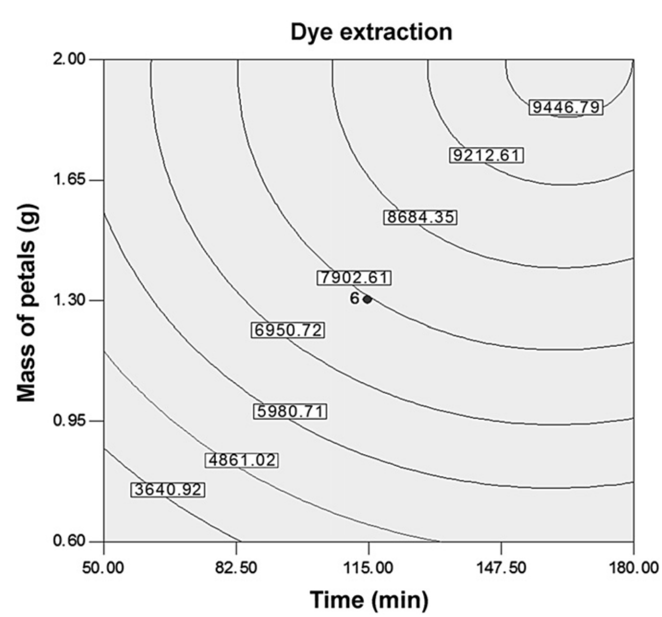

Fig. 4. Contour plot showing combined effect of extraction time and mass of petals on dye extraction.

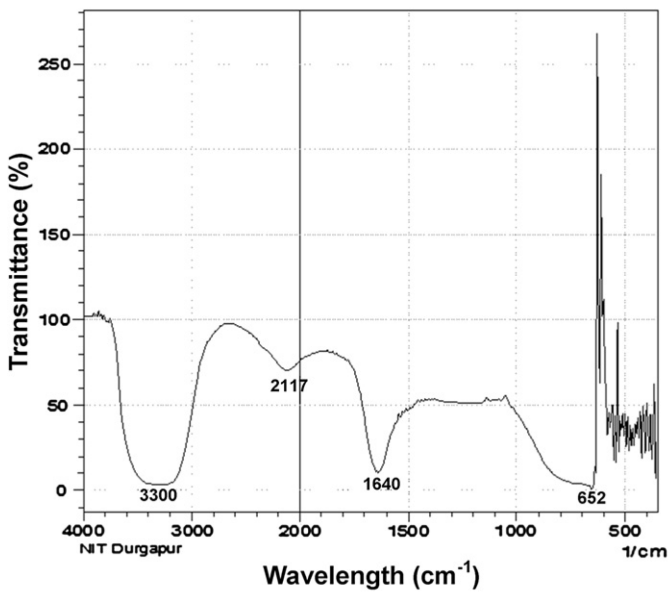

Fig. 5. FTIR spectra of the dye extracted from the flame of forest flower.

$$700.766 - 0.0258B - 0.6837C - 9.9714A = 0 \quad (8)$$

$$78.436 - 0.0258A + 1.5687C - 0.4851B = 0 \quad (9)$$

$$8977.70 - 0.6837A + 1.5687B - 4608.67C = 0 \quad (10)$$

Algebraic solution to the above three equations gave: $A = 69.72$ °C, $B = 164.46$ min, $C = 1.994$ g. Under the optimal conditions, the total yield of dye as calculated using the RSM model equation was 9544.7 mg L^{-1} while the experimental yield was around 9654.5 mg L^{-1}. The relatively low percentage error between the experimental and theoretical values (1.13%) confirmed the validity of the response surface quadratic model.

3.6. Characterization of the extracted dye

The FTIR spectral analysis is important to identify the characteristic functional groups of the extracted dye. The FTIR spectral analysis of the extracted dye show distinct peaks at 3300, 2117, 1640 and 652 cm^{-1} respectively (Fig. 5). The broad and strong band at 3300 cm^{-1} can be attributed to bonded −OH groups. The peak at 2117 cm^{-1} is indicative of C≡C symmetry stretching vibration. The strong peak at 1640 cm^{-1} represents −C=O stretching in carboxyl or amide groups. Finally, the peak at 652 cm^{-1} can be ascribed to CH=CH stretching vibration. Hence FTIR spectral analysis show the presence of different chemical functional groups like −OH, C≡C, −C=O, CH=CH in the extracted dye.

4. Conclusion

The present work showed that natural dye can be successfully extracted from the petals of Flame of forest flower. Maximum dye extraction was observed at 70 °C using 2 g of flower petals. With increasing the extraction time, the dye extraction efficiency increased, but an optimum time of 2 h was considered for extraction of the dye in order to make the technology more cost effective. Central composite design in Response surface methodology was used to investigate the combined effect of various process parameters on extraction of dye. Extraction temperature, time and mass of the petal used markedly influenced the dye extraction efficiency from Flame of forest. The optimum conditions were found to be — extraction temperature: 69.72 °C, extraction time: 164.46 min and mass of the petals: 1.994 g. Under these conditions, the total amount of dye extracted from the Flame of forest petals was estimated to be 9654.5 mg L^{-1}.

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