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Butea Frondosa Flower Dye Extraction Optimization through Taguchi Design

P. D. Patil *[1], A. I. Wasif [2] , S. V. Anekar[3] and J. R. Nagla[4]

[1.] Assistant Professor, Department of Technology, Shivaji University, Kolhapur, P. O. Box:416 004, Maharashtra, India.

[2.] Professor, Department of Textiles, Textile and Engineering Institute, DKTES, Ichalkaranji, P. O. Box: 416 115, Maharashtra, India.

[3.] Professor, Department of Chemical Engineering T.K.I.E.T Warananagar, P. O. Box: 416 113, Maharashtra, India.

[4.] Associate Professor, Department of Textiles, Textile and Engineering Institute, DKTES, Ichalkaranji, P. O. Box: 416 115, Maharashtra, India.

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ABSTRACT

In the past few decades, there is increasing trend to use products from natural resources. Dyes obtained from natural sources are among such products. The present research mainly focuses on optimization of extraction conditions during a natural dye extraction from the flower petals of a tree 'Flame of Forest'. Taguchi technique was employed for optimization of the parameters namely raw dyestuff particle size, solid-liquid ratio, time of extraction and method of extraction assistance. The results indicated that 150 µ particle size, 1:20 solid-solvent ratio, two hours for extraction and enzyme addition as assistance for extraction were the optimum conditions for extraction. The optimum and control extractions were compared for their absorbance values and the subsequent dye yields. The respective absorbance values were 1.144 and 1.036. It was encouraging to observe 10.58% yield increment in the extraction with optimized parameters. Prog. Color Colorants Coat. 8(2015), 25-35 © Institute for Color Science and Technology.

1. Introduction

In the ancient times, natural dyes were used for various purposes like coloration of clothing and food, leather tanning, painting etc. Natural dyes are defined as the colorants extracted from vegetative matter, animal residues or mineral origin. Vegetative sources for dyes include the plant roots, leaves, barks, trunks, fruits and flowers. These are also derived from animal sources such as cochineal and shellfish or sometimes from soils or clay. However by the mid-1800's chemists and others began producing synthetic substitutes for them. By the early part of this century, only a small percentage of textile dyes were extracted from plants.

*Corresponding author: *pdpchem@gmail.com*

Today, the market is dominated by synthetic dyes because of their varied colors, vast, easy production and their good fastness properties [1]. Natural dyes had been in use for thousands of years while their synthetic substitutes have history of mere 150 years. Some serious drawbacks of synthetic dyes have been realized within such short time span. Ecology and environment problems related to synthetic dyes have made people pretty aware. Therefore natural dyes have once again started gaining good popularity [2]. Such dyes offer variation of dyestuff sources. They too have a far superior aesthetic quality which is pleasing to the eye due to unique natural color. Better biodegradability, non-toxic and eco-friendly properties are among the major advantages of these dyes. The other benefit of natural dyes is significant reduction in the amount of toxic effluent resulting from the synthetic dye processes [3]. As against synthetic dyes derived from non renewable resources, natural dyes are obtainable from renewable resources.

In spite of these benefits, the present international consumption of natural dyes is just 1% of the synthetic dyes. The major difficulty in up-bringing natural dyes is their existing limitations and technical drawbacks. Some of these drawbacks are color yield, complexity of dyeing process, limited shades and inadequate fastness properties [4]. There are certain technical drawbacks and limitations of the natural dyes. It is customary to solve all these drawbacks through adequate scientific research efforts. The greatest challenge is to increase yield of these dyes. Present research has been carried out to address this particular issue. The plant part selected for study was flower petal of a tree called 'Flame of Forest'. The main objective of the work was to optimize extraction process to get maximum dye from the flower petals.

2. Experimental

2.1. Chemicals and equipments used

Cellulase and pectinase enzymes (HIMEDIA Make): These were used in 1:2 proportions and mixed with the raw dyestuff in case of enzyme addition as the assistance for extraction. Buffer tablets pH 9.5 (Merck Make): The buffer tablets were used to maintain the pH constant. Weighing balance: Shimadzu AUX220 was used to weigh the raw material and the enzymes as well. Essential Glassware (Borosil): The required glassware was used for optimization runs. Domestic microwave oven: Samsung DE68 02233G, oven was used for microwave heating as the assistance for extraction. Orbital Shaker-incubator (Nanolab India Model NLSIC-23#25/50): The set up was used to conduct 18 extraction runs at once. HIMEDIA Syringe and holder assembly with nylon membrane filters (0.2 μ porosity): These were used for microfiltration of the extracted color. Electronic pH meter (HANNA Instruments Make): It was used to ensure the pH of the solutions. Borosil glass double distillation unit. In the extraction runs the solvent was double distilled water obtained from the unit. UV-Vis Spectrophotometer (Shimadzu, Germany, Model UV1800): The instrument was used to check the absorbance values of the filtered extracts.

2.2. Raw material and its preparation for dye extraction

Butea monosperma is a species of Butea native to tropical and sub-tropical parts of the Indian Subcontinent and Southeast Asia. Common names of the tree include Palash, Dhak, Flame of Forest, Bastard Teak and Parrot Tree. It is a medium sized dry season-deciduous tree, growing to 15 m tall [5]. The flowers (Figure 1) of the tree were used as the actual raw dyestuff for extraction. Scientific classification of the tree is as below:

- Scientific name: *Butea monosperma*
- Kingdom:Plantae
- Order:Fabales
- Family:Fabaceae
- Genus:Butea
- Species:B. monosperma
- Synonym: Butea frondosa

Flowers were collected from local area trees. Petals separated from their buds, were dried in a tray dryer. The dry petal mass was ground in a domestic mixer-grinder to get mixed particle sizes. The powder was screened to get three different sizes (150, 300 and 425 μ) of the dyestuff required for experimental work.

Figure 1: An individual flower with bud.

Table 1: The control factors and levels.

Factor↓ Level→	0	1	2
Particle size, μ	150	300	425
Solid: liquid ratio, g: ml	1:20	1:30	1:40
Time, min	60	120	180
Method	Microwave assistance	Enzyme assistance	Microwave + Enzyme assistance

2.3. Taguchi design of experiment

Design of experiment is a powerful statistical technique for improving product or process designs and solving production problems. Its standardized version forwarded by Dr. Genichi Taguchi, allows us to easily learn and apply the technique for product design optimization and production problem investigation. Since its introduction in the U.S.A. in early 1980's, the approach has been popular as 'product and process improvement tool' in the hands of engineering and scientific professionals [6, 7]. It was primarily used to achieve efficient extraction of dye and to significantly reduce the number of experimental trials. The investigation parameters shown in Table 1 include: raw dyestuff particle size, solid-solvent ratio, extraction time and method of assistance for extraction.

L9 orthogonal arrays were selected for the Experiment. There were nine experimental runs with 4 factors and 3 levels [8, 9]. Each of the nine runs was conducted in duplicate. The other parameters ascertained from the literature review included extraction temperature 60 ºC, Speed of the orbital shaker 150 rpm, microwave power and irradiation time 300Watt and 40 second respectively, enzymes proportion 2% of cellulase and 1% pectinase both on weight of raw dyestuff basis [10, 11]. It is important to notice that water was used as a solvent. The orthogonal array has been depicted in Table 2 with absorbance values obtained as the response variable.

The control experiment was also conducted simultaneously with the array runs. Based on the statistical analysis of the experiment, the optimum set of parameters was chosen. Using the optimized set of extraction conditions, the optimized extraction was also carried out. It was aimed to compare control extraction performance with that of the optimized extraction. The absorbance values obtained from the two experiments were 1.036 and 1.144 respectively. The Figure 2 shows the respective UV absorption spectra.

Table 2: Taguchi Orthogonal Array Design.

Factor →	Levels				Response variable
Experimental Run↓	Particle Size	Solid-Liquid Ratio	Time	Method of Assistance	Absorbance value At λ_{max} 273 nm
1	0	0	0	0	1.136
2	0	1	1	1	1.134
3	0	2	2	2	0.800
4	1	0	1	2	0.982
5	1	1	2	0	0.691
6	1	2	0	1	0.630
7	2	0	2	1	1.142
8	2	1	0	2	0.764
9	2	2	1	0	0.715
10	0	0	0	0	1.135
11	0	1	1	1	1.137
12	0	2	2	2	0.790
13	1	0	1	2	0.979
14	1	1	2	0	0.692
15	1	2	0	1	0.629
16	2	0	2	1	1.139
17	2	1	0	2	0.762
18	2	2	1	0	0.714
Control	Coarse	1:20	3 hours at boil	No any assistance	1.036

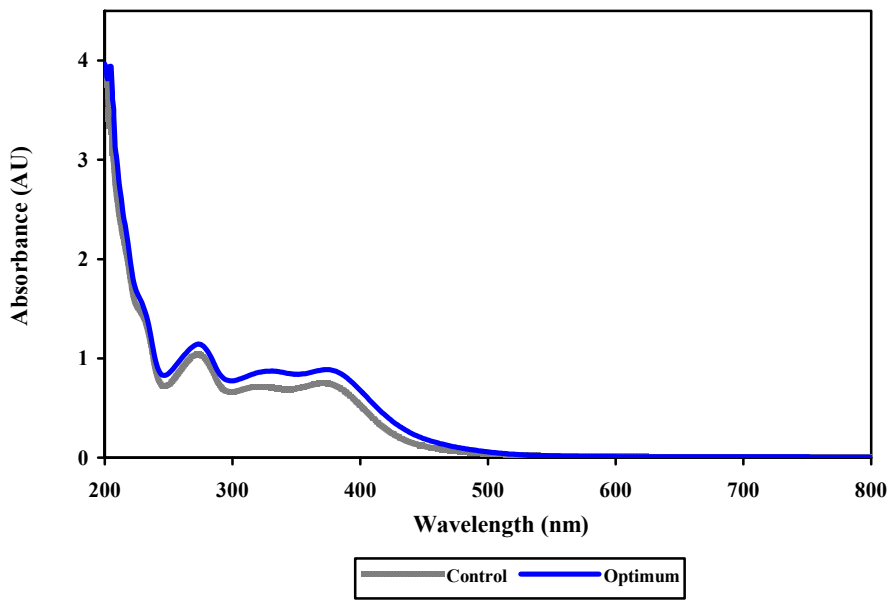

Figure 2: UV-Vis Spectrum Comparison.

2.4. Statistical analysis of data in extraction optimization experiments

The General Linear Model (GLM) was used to perform analysis of variance with balanced and unbalanced designs, analysis of covariance, for the response variable. Calculations were performed using a regression approach. A full rank design matrix was formed from the factors and covariates and the response variable was regressed on the columns of the design matrix [12]. Using Minitab software, analysis was conducted for the response variable namely absorbance. The analysis is shown in Tables 3 and 4.

2.4.1. Determination of dye yield

To find out the concentration of the dye extracted, the dye powder obtained from the spray drier was used to prepare solutions of different concentrations. These solutions were tested for their respective absorbance values using UV- Vis spectrophotometer.

The dye concentration was plotted against the respective absorbance values. The graph showed the BEST-FIT line (Table 5 and Figure 3). The slope of this line is the product of path length and molar extinction coefficient. Using slope and absorbance, the concentration and finally the yields were determined and compared for the control and optimized extraction runs.

Table 3: Analysis of Variance for Absorbance, using Adjusted SS for Tests.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Replica	1	0.00003	0.00003	0.00003	5.64	0.045
Particle Size	2	0.25069	0.25069	0.12535	2.60E+04	0.000
Solid-Liquid ratio	2	0.59733	0.59733	0.29866	6.30E+04	0.000
Time of extraction	2	0.01401	0.01401	0.00701	1470.83	0.000
Method of assistance	2	0.08119	0.08119	0.0406	8521.55	0.000
Error	8	0.00004	0.00004	0.0000	-	-
Total	17	0.94329	-	-	-	-

Table 4: Least Squares Means for Absorbance.

Parameter	Level	Mean	SE Mean
Particle Size	0	1.0562	0.00089
	1	0.7672	0.00089
	2	0.906	0.00089
Solid-Liquid Ratio	0	1.1522	0.00089
	1	0.8642	0.00089
	2	0.713	0.00089
Time of Extraction	0	0.876	0.00089
	1	0.9443	0.00089
	2	0.9090	0.00089
Method of Assistance	0	0.8805	0.00089
	1	1.0027	0.00089
	2	0.8462	0.00089

Table 5: Absorbance vs Concentration

Absorbance	Concentration g/lit
0.13	0.1
0.26	0.2
0.39	0.3
0.49	0.4

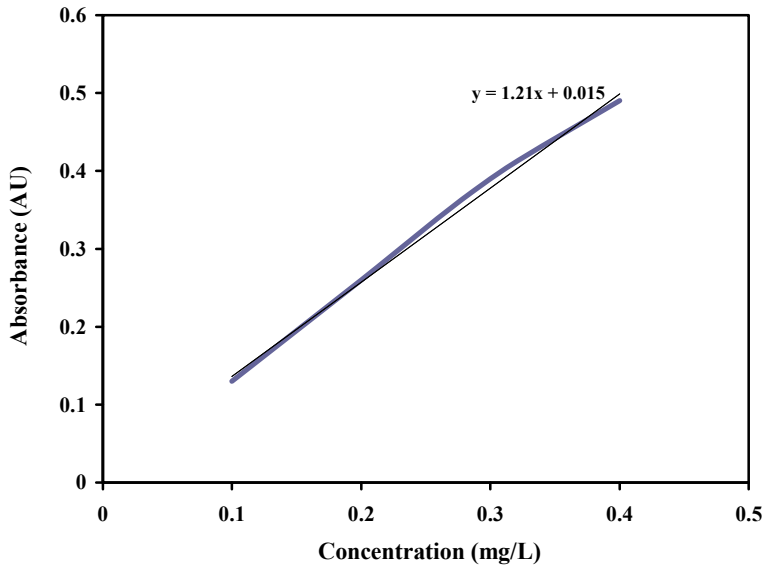

Figure 3: Absorbance vs Concentration.

Beer-Lambert law was used to determine concentration of an absorbing species in solution.

$$A = \mathrm{Log}_{10}\left(\frac{I_0}{I}\right) = \{\varepsilon \times L\} \times c = \mathrm{slpoe} \times c \qquad (1)$$

Concentration= absorbance / slope (2)

The equation of the trend line obtained is given below.

y=1.21x+0.015 (3)

Molecular mass of predicted colorants and concentration obtained were used to calculate dye yields.

2.4.1.1. Yield in control extraction

$$c = \frac{\text{Absorbance}}{\text{slope}} = \frac{1.036}{1.21} = 0.8561 \frac{\text{moles}}{L} \qquad (4)$$

0.5 mL of the original extract was diluted to 100 mL (200 times). Therefore the original concentration was 0.8561 multiplied by 200 which is equal to 171.23 moles per lit.

Molecular weight of the predicted colorant was 272. Therefore the colorant concentration in the extract was 171.23 divided by 272 which is equal to 0.6295 gm per lit which comes to be 0.06295 gm per 100 mL.

However, source of the colorant was 3 gm of the raw dyestuff used for extraction.

$$\%\text{yield(control)} = \frac{[\text{color extracted}]}{[\text{quantity of rawd dyestuff}]} \times 100$$

$$\%\text{yield(control)} = [\frac{0.06295}{3}] \times 100 = 2.098 \quad (5)$$

2.4.1.2. Yield in optimized extraction

$$C = \frac{\text{Absorbance}}{\text{Slope}} = \frac{1.144}{1.21} = 0.9454 \text{ moles/litre} \quad (6)$$

0.5 mL of the original extract was diluted to 100 mL (200 times). Therefore the original concentration was 0.9454 multiplied by 200 which is equal to 189.09 moles per lit.

So, the presence of colorant in the extract was 189.09 divided by 272 which is equal to 0.6952 g per lit which comes to be 0.06952 g per 100 mL. However, source of the colorant was 3 g of the raw dyestuff used for extraction.

$$\%\text{yield (optimized)} = \frac{[\text{color extracted}]}{\text{quantity of raw dyestuff}]} \times 100$$

$$\%\text{yield (optimum)} = [\frac{0.06952}{3}] \times 100 = 2.32 \quad (7)$$

2.5. Extraction efficiency enhancement calculations

When control extraction was compared against optimum, the improvement in extraction efficiency was as follows:

Extraction efficiency enhancement
= [yield (optimized-control)]/yield (control)
= [2.32-2.098] ÷ 2.098=10.58% (8)

3. Results and discussion

3.1. Optimization results

Variance analysis showed statistical significance of chosen conditions. The F-value for each parameter indicated which parameter has significant effect on extraction and is simply a ratio of the squared deviation to the mean of squared error. Usually, larger F-value shows greater effect on extraction value due to the change of the process parameters (Table 3). Optimal combination of process parameters was predicted using ANOVA (Figure 4).

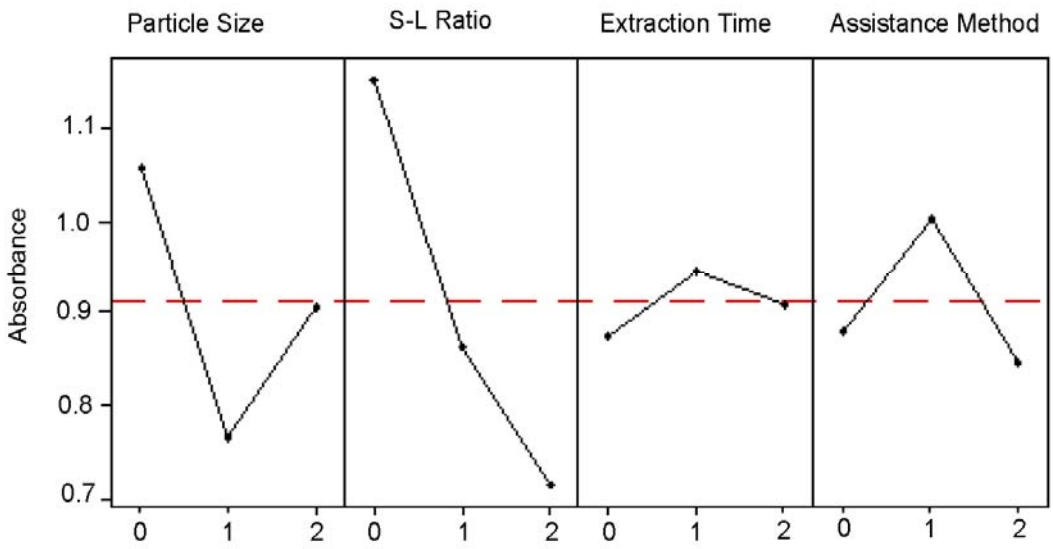

Figure 4: The Main effect plots.

3.1.1. Effect of particle size

Amongst four selected conditions, raw dyestuff particle size was found as the second significant extraction parameter. The particle sizes 150, 300 and 425 μ were coded as 0, 1 and 2 respectively. Results indicated the highest absorbance (1.0562) for the finest particle size (150 μ). This value is well above mean absorbance value. Hence the optimum particle size is 150 μ. The finest particle size provides greater surface area of the raw material accessible for extraction. Therefore it has reflected in the highest absorbance value indicating greater amount of dye extraction. Thus, the reason for this may be the highest contact surface area of the finest size thereby increasing the extraction efficiency.

3.1.2. Effect of solid-solvent ratio

Solid-solvent ratio was found as the most significant extraction parameter. Three ratios under investigation were 1:20, 1:30 and 1:40 coded as 0, 1, and 2, respectively. Results indicated the maximum absorbance (1.1522) for 1:20 ratio. Hence 1:20 was the optimum ratio to accommodate the colorant from the source. 20 parts of the solvent were adequate enough for complete transfer of dye from one part of the solid dyestuff. Dynamic dye transfer equilibrium might have reached with this ratio. Volume greater than 20mL was therefore showing significantly low absorbance values. The volume greater than 20 mL must have diluted the solution showing the absorbance values lower than the first one.

The lesser solvent would have been insufficient for complete transfer of dye to the solvent. The volume lesser than 20 mL say 10 or 15 mL would have been insufficient to completely extract the dye from the source. The literatures also support 20 mL as the optimum value in most of the natural dye extraction cases.

3.1.3. Effect of Extraction time

Figure 4 shows that extraction time is of less significance. For one hour and 2 hours of extraction, absorbance values were above mean absorbance while absorbance for three hours of extraction was below the mean. The absorbance values for 0, 1 and 2 levels were 0.8760, 0.9443 and 0.9090 respectively. Only for 2 hours of extraction, the absorbance was above mean absorbance while absorbance for three hours of extraction was almost equal to the mean absorbance and that of one hour extraction was below the mean. It also indicates that there is no point in searching the optimum time beyond three hours. Therefore the optimum time for higher extraction was two hours.

3.1.4. Effect of assistance method

Microwave pretreatment, enzyme addition and combination of both microwave and enzyme assistance were the three assistance methods coded as 0, 1 and 2, respectively. Results showed that the aspect of providing assistance is also significant for efficient extraction. Table 5 and Figure 4 indicated that the absorbance for respective assistance methods were 0.8805, 1.0027 and 0.8462. Absorbance in case of enzyme assistance was very well above mean while other two methods showed their absorbance below the mean. Therefore use of enzyme was the optimum method of assistance for extraction.

Extraction enhancement of the colorant was seen through the highest absorbance in the enzyme method. Recent studies have also shown that use of enzymes is beneficial for efficient extraction. Enzymes loosen the structural integrity of the plant material making their cells easily breakable. This might be the reason releasing maximum solute from the plant material, increasing the extraction yield. In our work also, the enzyme assistance has shown maximum absorbance value leading to enhancement in color extraction.

3.2. Colorant: chemical nature

The results of both UV-Vis spectroscopy and GCMS support the presence of colorant, chalcone, a type of flavonoids. The molecular formula and molecular weight of the colorant are $C_{15}H_{12}O_5$ and 272 respectively. Figure 5 shows the structure of butin.

3.3. Absorbance, dye yields comparison and extraction efficiency enhancement

Control and optimized extraction experiments were run to study the comparative performance of the two. Figure 6 shows the comparison of absorbance values. These values 1.036 and 1.144 were further used for yield calculations. Taguchi design with its subsequent ANOVA showed increase in absorbance to 16.66% for optimum experiment. Figure 7 shows the yield comparison of the control and the optimum extractions.

Significant improvement in the extraction efficiency to about 10.58% was noted in the optimized extraction. In the optimized extraction, all four parameters values were chosen from the respective levels which had given the highest response values of absorbance. Thus the reason behind the highest value of absorbance in case of optimized extraction may be due to the collective effect of optimum conditions employed during extraction operation. Hence, it is proposed that the optimization process employed here has achieved enhancement in extraction efficiency.

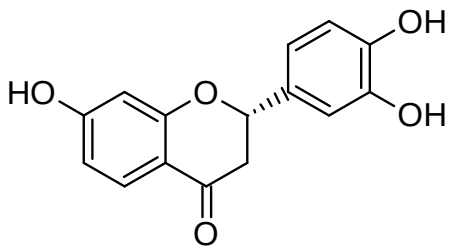

Figure 5: Structure of butin.

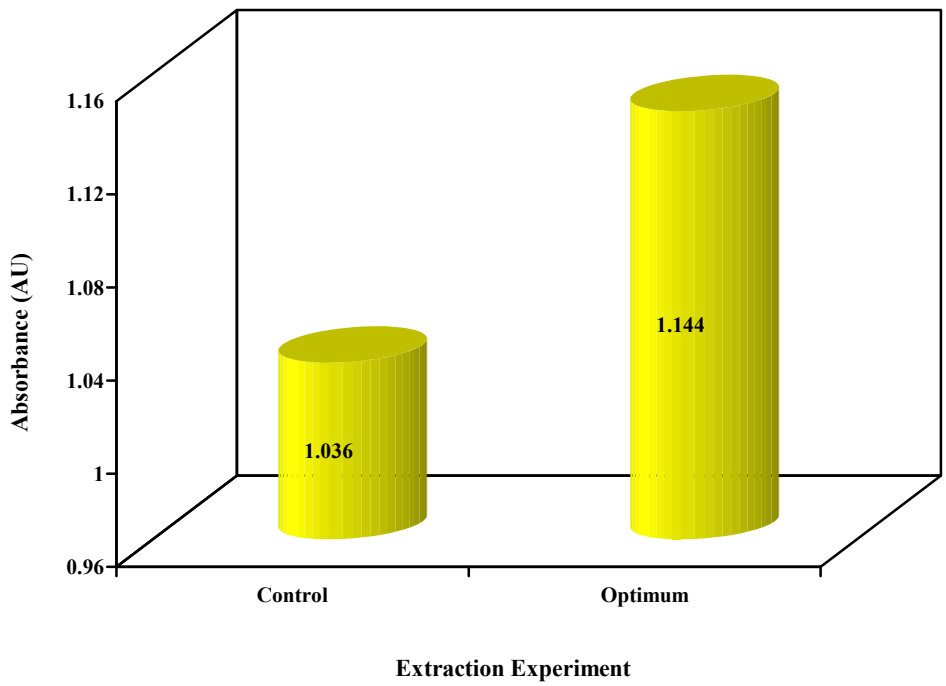

Figure 6: Absorbance Comparison.

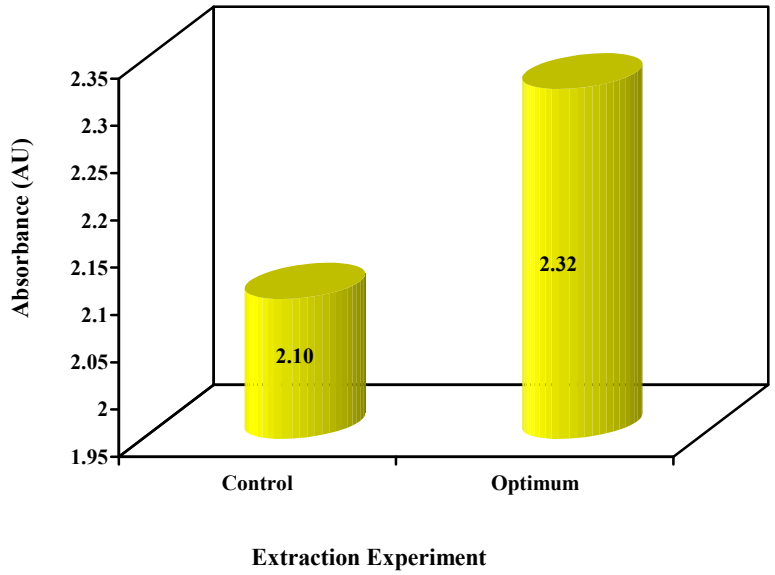

Figure 7: Yield comparison.

4. Conclusions

Taguchi method was successfully applied to determine the optimal extraction conditions. It was validated by conducting extraction operation with the optimum conditions suggested by the analysis. 150 µ particle sizes, 1:20 solid-liquid ratio, two hours extraction time, enzyme assistance for extraction were the optimum conditions. It was very much encouraging that the absorbance with the optimum conditions was significantly higher than the control conditions. Outcome of the research effort includes efficient extraction leading to 10.58% increase in dye yield. Besides there is extraction time saving also. This research can certainly contribute to the future scale-up of Butea frondosa flower dye production useful for textile, food, pharmaceutical and cosmetic industries.

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