Extraction of Natural Colorant from the Flowers of Flame of Forest Using Ultrasound

Sunny Arora, Meghal A. Desai

***Abstract*—**An impetus towards green consumerism and implementation of sustainable techniques, consumption of natural products and utilization of environment friendly techniques have gained accelerated acceptance. Butein, a natural colorant, has many medicinal properties apart from its use in dyeing industries. Extraction of butein from the flowers of flame of forest was carried out using ultrasonication bath. Solid loading (2-6 g), extraction time (30-50 min), volume of solvent (30-50 mL) and types of solvent (methanol, ethanol and water) have been studied to maximize the yield of butein using the Taguchi method. The highest yield of butein 4.67% (w/w) was obtained using 4 g of plant material, 40 min of extraction time and 30 mL volume of methanol as a solvent. The present method provided a greater reduction in extraction time compared to the conventional method of extraction. Hence, the outcome of the present investigation could further be utilized to develop the method at a higher scale.

***Keywords*—**Butein, flowers of flame of forest, Taguchi method, ultrasonic bath.

I. Introduction

GREEN consumerism and the emphasis on following the principles of green chemistry have attracted the utilization of natural products in various fields. A proliferative use of synthetic colorants (dyes and pigments) in textile industries, food industries, etc. has led to many negative impacts on public health, plants, animals and environment [1]-[4]. Colorants extracted from various natural resources such as plants and animals have the potential to replace the toxic, harmful and persistent artificial colorants. Natural colorants are non-toxic, non-allergic, renewable, biodegradable and do not have detrimental impact on the ecological system [3]-[7]. These colorants possess various medicinal properties making them more suitable for various applications in textile industries, food industries and cosmetic formulations [3]-[9]. Flame of forest (*Butea monosperma*) is one of the important trees grown in India and is a rich source of natural colorants. Every part of this tree has been used extensively in “Ayurveda” for treating various ailments [5], [8]. Flowers of this tree are a good source of natural colorants like butein, butin and isobutrin. Butein, one of the main components in flower, is important in fighting against various pathogens. It is useful in free radical scavenging activity. Butein has shown its efficacy in inhibiting the proliferation of tumor cells, aromatase in breast cancer cells and human colon adenocarcinoma. Further, it was proved useful in apoptotic cell death and protection against cell death against oxidative injuries in hepatic cells [10]-[16]. Extraction of natural dye from the petal of the flower has been performed using conventional aqueous extraction techniques [5].

In order to have sustainable development, there is a need to search for an extraction process which satisfies the principles laid down in green chemistry and green engineering [17]. Driven by these purposes, a number of ecofriendly techniques such as supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), pressurized hot water extraction (PHWE), microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE) have been developed. SFE, PLE and PHWE, though green, are suffering from the issue of high pressure operation which in turn increases the investment and may make the process economically impractical for low cost product. MAE, though rapid, generates too much heat instantaneously which may cause degradation of the product. Further, selectivity may not be achieved in case of MAE due to release of all the compounds from the cell upon cell wall rupture. UAE may lead to cell wall breakage but it is gentle in nature compared to other heat intensive processes.

UAE is a method to effectively extract chemical constituents from plant materials. Due to cavitation, which is growth and collapse of small bubbles, high intensity shock waves are generated leading to have intense pressures, shear forces and temperature gradient within the material producing physical, mechanical and chemical changes forcing the chemical constituents to dissolve in solvent. This method is advantageous as it enhances the movement of molecules, increasing the permeability of solvent, promoting a higher solubility of the plant constituents in lesser time and increasing the extraction yield [18], [19].

In the present study, UAE was employed to develop a “greener” route for extraction of butein from flower petals of flame of forest. The objective of the present work was to optimize the process parameters namely solid loading, extraction time, volume of solvent and nature of solvent to maximize the yield of butein using the Taguchi method, a statistical technique. A quantitative evaluation of each parameter was done to find its impact on the yield using the analysis of variance (ANOVA). Further, the outcome of the present work was compared with the conventional technique also in order to appreciate the importance of sonication. This study will be helpful in developing a sustainable process at a higher scale.

Meghal A. Desai is with the Sardar Vallabhbhai National Institute of Technology, Surat, Gujarat-India (corresponding author; phone: 91-261-2201709; fax: 91-261-2835655; e-mail: mad@ched.svnit.ac.in).

Sunny Arora was with the Sardar Vallabhbhai National Institute of Technology, Surat, Gujarat-India (e-mail: sunny7385arora@gmail.com)

II. Materials and Methods

A. Materials

Powdered flower petals of flame of forest were purchased from M/s Hakim Chichi and Sons, Surat, Gujarat, India. The average size of the particles was found to be 0.152 mm. Ethanol and methanol, both of them having purity > 99%, were procured from Ranbaxy Chemicals Ltd., New Delhi, India. Deionized water was obtained using Elix Essential 10 Water Purification System, Millipore (India) Pvt. Ltd., Banglore, India. Butein (Purity ≥ 98%, Sigma Aldrich, Banglore, India) was purchased for analysis.

B. Ultrasound Assisted Extraction (UAE)

UAE was carried out in an ultrasonic bath (Aqua Scientific Instruments, Surat) with an ultrasound power of 120 W, frequency of 20 KHz, equipped with a digital timer and a temperature controller. Sonication was performed at a fixed power and frequency with various parameters viz. solid loading (2-6 g), volume of solvent (30-50 mL), extraction time (30-50 min), nature of solvent (methanol, ethanol and water). Details of the parameters along-with the levels are shown in Table I.

TABLE I
FACTORS AND THEIR LEVELS

	A (g)	B (min)	C (mL)	D
Level 1	2	30	30	Methanol
Level 2	4	40	40	Ethanol
Level 3	6	50	50	Water

A = Solid loading, B = Extraction time, C = Volume of solvent, D = Nature of solvent

The raw material was kept in a 100 mL beaker and sonicated (Fig. 1) as per the experimental planning suggested by the Taguchi method. Each extraction experiment repeated twice to ensure the accuracy of results.

Fig. 1 Experimental set-up

The extract was filtered with Whatman filter paper grade 1. The absorbance of all the filtrate samples was measured using UV-VIS spectrophotometer (DR-6000, HACH, USA) after suitably diluting the extract. A calibration curve (R^2 = 0.9995) was utilized to calculate the yield from the data obtained. Each analysis was performed three times in order to ensure accuracy.

III. Results and Discussion

For four factors and three levels, L_9 orthogonal array was used and accordingly experiments were performed and yield was measured (Table II). Taguchi method was applied for the optimization followed by performing ANOVA to find out the most significant parameter and percentage contribution of each one of them. Analysis of the data was done with the help of Minitab statistical software (Ver.18, Minitab Inc., PA, USA).

TABLE II
DESIGN MATRIX, YIELD AND S/N RATIO

Exp No.	Factors				Yield, %		S/N Ratio
	A	B	C	D	Y_1	Y_2	
1	1 (2)	1 (30)	1 (30)	1 (Methanol)	4.49	4.77	1.33
2	1	2 (40)	2 (40)	2 (Ethanol)	1.69	2.13	0.55
3	1	3 (50)	3 (50)	3 (Water)	0.60	0.82	-0.33
4	2 (4)	1	2	3	1.42	1.35	0.28
5	2	2	3	1	4.29	4.83	1.31
6	2	3	1	2	2.09	2.08	0.63
7	3 (6)	1	3	2	1.50	1.33	0.30
8	3	2	1	3	1.42	1.59	0.35
9	3	3	2	1	4.02	4.47	1.25

A = Solid loading (g), B = Extraction time (min), C = Volume of solvent (mL), D = Nature of solvent (Values in bracket correspond to physical values of the factors.)

A. Determination of the Optimum Level

The signal to noise ratio (S/N) was found using the yield of the extract. S/N ratio is ordinarily a quality indicator which represents the effect of changing a particular process parameter on the process. Better signal is obtained when the noise is less, so that a larger S/N ratio yields better results. In general, there are three areas of performance characteristics in the analysis of S/N ratio: 'smaller is better', 'nominal is better' and 'higher is better'.

The present study was focused on maximizing the yield of the extract; therefore, S/N ratio for 'higher is better' was chosen. S/N ratio is calculated by (1):

$$S/N = -10 \log \sum_{i=1}^{n} \frac{1}{Y_i^2} \quad (1)$$

where, n is the number of repetitions of a given experiment and Y_i is the yield of i^{th} experiment.

The total S/N ratio for each factor at each level was calculated using single S/N ratio as shown in Table III. For example, the value of 1.55 for factor A at level 1 was calculated by summing the S/N ratio of experiments 1 to 3.

TABLE III
LEVEL TOTAL S/N RATIO FOR EACH FACTOR

		A	B	C	D
S/N Ratio	Level 1	1.55	1.91	2.31	3.89
	Level 2	2.23	2.21	2.08	1.47
	Level 3	1.90	1.56	1.28	0.31
	Total	5.67	5.67	5.67	5.67

A = Solid loading, B = Extraction time, C = Volume of solvent, D = Nature of solvent

The level total S/N ratio can be useful in understanding the impact of variation in each parameter on the yield (Fig. 2). In case of solid loading, the response decreased after 4 g, indicating a lesser impact of sonication at a higher solid loading. With an increase in solid loading, sonication per particle reduces resulting in the weakened impact of ultrasound on cells, thereby reducing the yield of the extract.

While studying extraction time, a decrease in the S/N ratio was observed for the higher value. Degradation of butein might have occurred for extended time resulting in the reduced yield of the product, thereby, decreasing the S/N ratio.

A smaller volume of the solvent has provided the best outcome compared to other volumes. This might be due to poor sonication impact at higher volumes. In case of increased volume of the solvent, since the power applied was kept unchanged, the magnitude of the sonication effect might have reduced, thus, reducing the yield of the solute.

Because of a better solubilization of butein in methanol, the maximum yield was obtained using methanol compared to water and ethanol.

For selecting the optimum set of parameters amongst 3 levels, the level giving the maximum level total S/N ratio for each factor is to be chosen. From Table III and Fig. 2, 4 g solid loading, 40 min extraction time and 30 mL methanol can be selected as the optimum parameters.

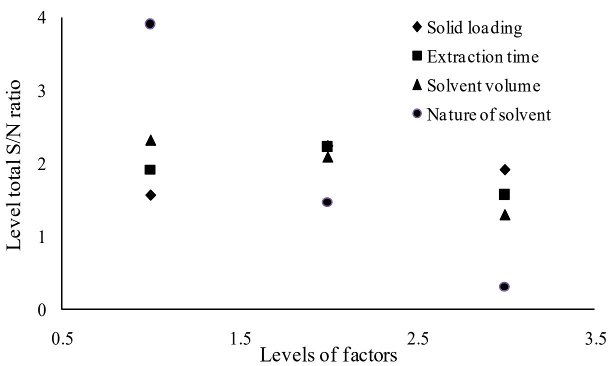

Fig. 2 Effect of various parameters on response

A similar experiment was conducted with same operating conditions except sonication. In this experiment, stirring was provided to ensure suspension of the particles and uniform mixing. The experiment was continued till completion. In case of conventional extraction, it took 180 min to achieve the same yield. Thus, it was possible to reduce the extraction time at a greater extent using UAE.

B. ANOVA

ANOVA was performed to find the importance of various parameters using the *F* test (Table IV). Total variation (S_T) and individual variation (S_A) along-with degree of freedom are used to calculate *F* value. The method is briefly described below. Details of ANOVA are available in various papers [20]-[23].

Total variation focuses on the total magnitude of variations of the experimental data while individual variation signifies the contribution of individual factor towards the final response variable. The equations of these variations can be written as:

$$S_T = \left[\sum_{i=1}^{n} \left(S/N\right)_i^2\right] - \left\{ \left[\sum_{i=1}^{n} \left(S/N\right)_i\right]^2 \Big/ n \right\} \tag{2}$$

where n is the total number of experiments and i denotes value at ith experiment.

$$S_A = \left[\sum_{i=1}^{k} \left(A_i^2 \big/ n_{A,i}\right)\right] - \left\{ \left[\sum_{i=1}^{n} \left(S/N\right)_i\right]^2 \Big/ n \right\} \tag{3}$$

where A is the factor selected for the study, i is the level number of the specified factor A, k is the repetition of each level of the factor A and A_i is the sum of S/N ratio involving this factor and level i.

The relation between total variation and individual variation is given by the equation:

$$S_T = \sum_{A=1}^{n} S_A + S_e \tag{4}$$

where A is the factor for given set of experiment, n denotes number of factors involved and S_e denotes variation due to unaccounted factors.

Degree of freedom for each factor is the number of its levels minus one and the total degrees of freedom is the total number trials minus one. Degree of freedom for the error is the number of the total degrees of freedom minus total of degree of freedom for each factor.

Variance (V) is defined as the individual variation divided by the degree of freedom (D) of that factor.

F-test is a qualitative analytical tool to see which process parameters have a significant effect on the process and can be found by the equation:

$$F_A = \frac{S_A}{S_e} \tag{5}$$

When error is zero, the factor having the lowest variation is selected as the error, termed as 'pooled error', to complete above calculation.

Percentage contribution of each factor (CF) is useful for quantitative evaluation as given by the equation:

$$CF_A = \frac{S_A}{S_T} x100 \tag{6}$$

A higher value of *F* suggests more impact on the experimental results. Nature of the solvent was found to be the most important factor affecting the process compared to the other parameters. However, *F* value provides qualitative information. In order to have fruitful outcome, quantitative analysis can be performed by finding percentage contribution of each factor.

TABLE IV
ANOVA TABLE

	S	DOF	V	*F*	% CF
A	0.08	2	0.04	1.14	3.10
B	0.07	2	0.04	1.00	2.71
C	0.19	2	0.10	2.71	7.36
D	2.23	2	1.12	31.86	86.43
Error	0.00	0	-	-	-
Total	2.58	8	-	-	100
Pooled error	0.07	2	-	-	-

S= individual variation, D= degree of freedom, V= variance, CF= % contribution of each factor. A = Solid loading, B = Extraction time, C = Volume of solvent, D = Nature of solvent.

It can be verified from Table IV that the percentage contribution of nature of solvent was 86.43 compared to other factors. The order of influence of each factor can be written as D (nature of solvent) > C (volume of solvent) > A (solid loading) > B (extraction time).

C. Confirmation Experiment

This is the final step for verifying the results obtained by the Taguchi method. The experiment was conducted twice at the optimum levels (Table V).

TABLE V
CONFIRMATION EXPERIMENT

Factors	Confirmation Experiment	Exp. 5 of L_9 Array
A (Solid loading, g)	4 (Level 2)	4 (Level 2)
B (Extraction time, min)	40 (Level 2)	40 (Level 2)
C (Volume of solvent, mL)	30 (Level 1)	50 (Level 3)
D (Nature of solvent)	Methanol (Level 1)	Methanol (Level 1)
Yield , %	5.15	4.29
	4.91	4.83
S/N Ratio	1.40	1.31

It was found that the yield and S/N ratio obtained at the optimum conditions are higher as compared to the maximum yield and highest S/N ratio of L_9 array. The experimental results thereby confirm validation of the applied technique towards optimization of process parameters of UAE.

IV. CONCLUSION

UAE was employed for the extraction of butein from *Butea monosperma* to obtain higher yield with less extraction time and reduced solvent consumption. The present study showed that various parameters viz. solid loading, extraction time, volume of solvent and nature of solvent could have significant influence on the yield of extract. The process was efficiently optimized using the Taguchi method. The optimum set of parameters was found to be: 4 g plant material, 40 min extraction time, 30 mL volume of methanol as a solvent. Nature of solvent was found out to be the most significant parameter affecting the yield of the process. The order of the influence of the parameter is found to be nature of solvent > volume of solvent > solid loading > extraction time. The yield obtained at the optimum conditions was found to be the highest. Further, UAE has potential to replace the existing time consuming conventional method of extraction (40 min vs. 180 min). Thus, UAE has the potential to replace the existing conventional process.

REFERENCES

[1] W.S. Alencar, E.C. Lima, B. Royer, B.D. dos Santos, T. Calvete, E.A. da Silva, and C.N. Alves, "Application of aqai stalks as biosorbents for the removal of the dye procion blue MX-R from aqueous solution", *Sep. Sci. Technol*., vol. 47, pp. 513–526, 2012.

[2] N. Puvaneswari, J. Muthukrishnan, and P. Gunasekaran, "Toxicity assessment and microbial degradation of azo dyes", *Indian J. Exp. Biol*., vol. 44, pp. 618–626, 2006.

[3] S. Ali, N. Nisar, and T. Hussain, "Dyeing properties of natural dyes extracted from eucalyptus", *J. Text. Inst*., vol. 98, pp. 559–562, 2007.

[4] M. Yusuf, M. Shahid, S.A. Khan, M.I. Khan, S.U. Islam, F. Mohammad, and M.A. Khan, "Eco-Dyeing of wool using aqueous extract of the roots of Indian madder (*Rubia cordifolia*) as natural dye", *J. Nat. Fibers*., vol. 10, pp. 14–28, 2013.

[5] K. Sinha, P. Das Saha, and S. Datta, "Extraction of natural dye from petals of Flame of forest (*Butea monosperma*) flower: process optimization using response surface methodology (RSM)", *Dye. Pigment*., vol. 94, pp. 212–216, 2012.

[6] K. Sinha, P. Das Saha, and S. Datta, "Response surface optimization and artificial neural network modeling of microwave assisted natural dye extraction from pomegranate rind", *Ind. Crops Prod*., vol. 37, pp. 408–414, 2012.

[7] V. Sivakumar, J.L. Anna, J. Vijayeeswarri, and G. Swaminathan, "Ultrasound assisted enhancement in natural dye extraction from beetroot for industrial applications and natural dyeing of leather", *Ultrason. Sonochem*., vol. 16, pp. 782–789, 2009.

[8] T. Michel, E. Destandau, V. Pecher, I. Renimel, L. Pasquier, P. André, and C. Elfakir, "Two-step centrifugal partition chromatography (CPC) fractionation of *Butea monosperma* (Lam.) biomarkers", *Sep. Purif. Technol*., vol. 80, pp. 32–37, 2011.

[9] Y. Caro, L. Anamale, M. Fouillaud, P. Laurent, T. Petit and L. Duffose,"Natural hydroxyanthraquinoid pigments as potent food grade colorants:an overview", *Nat. Prod. Bioprospect.*, vol. 2, pp. 174–193, 2012.

[10] A. Sehrawat, and V. Kumar, "Butein imparts free radical scavenging, anti-oxidative and proapoptotic properties in the flower extracts of *Butea monosperma*", *Biocell*., vol. 36, no. 2, pp. 63–71, 2012.

[11] M.K. Pandey, B. Sung, K.S. Ahn, and B.B. Aggarwal, "Butein suppresses constitutive and inducible signal transducer and activator of transcription (STAT)3 activation and STAT3-regulated gene products through the induction of a protein tyrosine phosphatase SHP-1", *Mol. Pharmacol*., vol. 75, no. 3, pp. 525–533, 2009.

[12] Y. Wang, F.L. Chan, S. Chen, and L.K. Leung, "The plant polyphenol butein inhibits testosterone-induced proliferation in breast cancer cells expressing aromatase", *Life Sci*., vol. 77, pp. 39–51, 2005.

[13] C. C. Yit, and N. P. Das, "Cytotoxic effect of butein on human colon adenocarcinoma cell proliferation", *Cancer Lett.,* vol. 82, pp. 65–72, 1994.

[14] N.Y. Kim, H.O. Pae, G.S. Oh, T.H. Kang, Y.C. Kim, H.Y. Rhew, and H.T. Chung, "Butein, a plant polyphenol, induces apoptosis concomitant with increased caspase-3 activity, decreased BCL-2 expression and increased Bax expression in HL-60 cells", *Pharmacol Toxicol*., vol. 88, pp. 261–266, 2001.

[15] B. Sung, S.G. Cho, M. Liu, B.B. Aggarwal, "Butein, a tetrahydroxychalcone, suppresses cancer-induced osteoclastogenesis through inhibition of receptor activator of nuclear factor-kappaB ligand signaling", *Int. J. Cancer.* vol. 129, pp. 2062–2072, 2011.

[16] Z. Rasheed, N. Akhtar, A. Khan, K. a Khan, and T.M. Haqqi, "Butrin, isobutrin, and butein from medicinal plant *Butea monosperma* selectively inhibit nuclear factor-kappaB in activated human mast cells: suppression of tumor necrosis factor-alpha, interleukin (IL)-6, and IL-8", *J. Pharmacol. Exp. Ther.,* vol. 333, no. 2,pp. 354–363,2010.

[17] F. Chemat, M.A. Vian, G. Cravotto, "Green extraction of natural products: Concept and principles", *Int. J. Mol. Sci*., vol. 13, pp. 8615–8627, 2012.

[18] F. Chemat, N. Rombaut, A.G. Sicaire, A. Meullemiestre, A.S. Fabiano-Tixier, M. Abert-Vian, "Ultrasound assisted extraction of food and natural products. mechanisms, techniques, combinations, protocols and applications. a review", *Ultrason. Sonochem.* vol. 34, pp. 540–560, 2017.

[19] I.A. Saleh, M. Vinatoru, T.J. Mason, N.S. Abdel-Azim, E.A. Aboutabl, F.M. Hammouda, "A possible general mechanism for ultrasound-assisted extraction (UAE) suggested from the results of UAE of chlorogenic acid from *Cynara scolymus* L. (artichoke) leaves", *Ultrason. Sonochem.*, vol. 31, pp. 330-336, 2016.

[20] M.A. Desai, J. Parikh, "Hydrotropic extraction of citral from *Cymbopogon flexuosus* (Steud.) Wats", *Ind. Eng. Chem. Res.*, vol. 51, pp. 3750-3757, 2012.

[21] M.A. Desai, J. Parikh, A.K. De, "Modelling and optimization studies on extraction of lemongrass oil from *Cymbopogon flexuosus* (Steud.) Wats", *Chem. Eng. Res. Des.*, vol. 92, pp. 793-803, 2014.

[22] P.J. Ross, *Taguchi Techniques for Quality Engineering,* McGraw-Hill Book Co: New York, U.S.A., 1989.

[23] G. Taguchi, S. Chowdhury, Y. Wu, *Taguchi's Quality Engineering Handbook*, John Wiley and Sons: New York, U.S.A., 2005.