Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 1700-1705
Received: 27-06-2020
Accepted: 13-08-2020

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Ultrasound assisted extraction of bio-colorant from walnut hull

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Abstract
Walnut hull is considered as agricultural waste which is considered as the rich sources of bio colorant and bio active compounds. Bio colorant can be used as food additive to replace synthetic dyes and colorants. At present, colorant extraction is done by utilizing hot water for a long duration of time which is a time-consuming process. However, it is verified that Ultrasonication lessens the extraction time and maximize yield as compared to the traditional method in use. Identifying the capability of Ultrasound assistance in extraction, this research emphasizes on bio colorant extraction using walnut hull as raw material. Walnut hull converted into the powdered form was utilized for extraction of biocolorant. It was extracted with different combinations of Ultrasound Power (150, 200,250 W), treatment time (10, 20, 30 mins), solvent volume (20, 30, 40 ml/g) and particle size (150, 300, 450 µm).As a result, the highest yield of 49.76% was attained at the Ultrasound power of 200 W Ultrasound Time of 20 Minutes, solvent volume 1:20 and particle size 150µm. The research confirms that utilizing Ultrasound enhanced yield and also saves sufficient amount of time and energy.

Keywords: Walnut hull, ultrasound assisted extraction, RSM

Introduction
Walnut (*Juglans regia)* is one of the most important nut crop of India. The total area under walnut production in India is about 1, 49,502 ha with production and productivity of 2, 84,409 T, 1.90 T/ha respectively (Singh *et al.* 2017) [1]. Walnut's green husk (epicarp) is the by-product of dry fruits (nuts) production. Several works demonstrated that they are rich in natural bioactive compounds. It is found that walnut husk has high phenolic content (Cosmulescu *et al.* 2015) [3]. With the increase demand of bioactive compounds in plants influences the use of novel extraction methods. However, the choice of extraction method is an essential factor which decides the quality and yield of the extraction. Traditionally, the organic colorant was extracted by boiling with water which does not give good amount of colorant as the coloring component is tightly bound to the cell wall, there is a need for novel techniques to improve the major mechanism of natural dye extraction such as rupture of cell wall, release of natural dye and transport of dye into the external medium (Sivakumar *et al.*, 2011) [7].

In the current study, extraction will be carried out by ultrasound assisted extraction. Ultrasound is effective for extraction as its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation (*Toma et al.,* 2001) [9]. Moreover, it is achieved at lower temperatures and hence more suitable for enhancing the extraction of thermally unstable compounds as compared to conventional methods (Wu *et al.*, 2001) [12]. Recently, ultrasound-assisted extraction (UAE) has been widely used in the extraction of phenolics from different vegetable materials (Tiwari *et al.*, 2010; Rostagno *et al.*, 2003; Rodrigues and Pinto, 2007) [6, 5].

Walnut hull is considered as a highly profitable and futuristic candidate for natural dye extraction from an underexplored food waste. Research work reporting extraction of dye from walnut hulls is scarce. However, walnut hulls have many hidden nutritional values which are yet to be utilized for various versatile purposes which include natural food coloring, product development, cosmetic and biomedical research. In this regard, extraction of colorant from black walnut hull could be a good option for improving, extending and advertising its uses for future applications.

Material and Methods
Collection of raw materials
Walnut was procured locally from market and the hull was subsequently separated from the walnut. The hull was kept in tray dryer for drying at 50º C for 24 hours. Then the dried hull

was grinded in grinder to make it in powder form. The hull powder was kept in desiccator for further processing.

Experimental Design

Box-Behnken Design (BBD) of response surface methodology is used for optimization of Ultrasound assisted extraction for extraction of colorant and bioactive compounds. The design consisted of 29 randomized runs (Table 1) with five replicates at the central point. For the designed experiments, four variables having 3 levels of each (Ultrasound power, treatment time, solvent volume and particle size) for Ultrasound assisted extraction were selected for the experiments. Table 2 and Table 3 represent the actual and coded independent variables for extraction.

Table 1: Experimental design BBD for Ultrasound Assisted Extraction

S. No.	Run	Particle size(µm)	Ultrasound Intensity(watt/cm)	Time(min)	Solvent Volume (ml/g)
1	22	300	250	20	20
2	15	300	150	30	30
3	24	300	250	20	40
4	26	300	200	20	30
5	16	300	250	30	30
6	12	450	200	20	40
7	23	300	150	20	40
8	20	450	200	30	30
9	11	150	200	20	40
10	29	300	200	20	30
11	18	450	200	10	30
12	25	300	200	20	30
13	27	300	200	20	30
14	14	300	250	10	30
15	17	150	200	10	30
16	10	450	200	20	20
17	2	450	150	20	30
18	9	150	200	20	20
19	3	150	250	20	30
20	28	300	200	20	30
21	5	300	200	10	20
22	13	300	150	10	30
23	21	300	150	20	20
24	7	300	200	10	40
25	6	300	200	30	20
26	1	150	150	20	30
27	8	300	200	30	40
28	19	150	200	30	30
29	4	450	250	20	30

Table 2: Coded levels for Independent variables in UAE

Independent Variables		Coded Levels		
Name	Code	-1	0	1
		Actual Levels		
Ultrasound power (W)	A	150	200	250
Treatment Time (min)	B	10	20	30
Solvent Volume(ml/g)	C	20	30	40
Particle Size (µm)	D	150	300	450

Bio-colorant extraction

Bio-colorant was extracted with different combinations of Ultrasound Power (150, 200,250 W), treatment time (10, 20, 30 mins), solvent volume (20, 30, 40 ml/g) and paricle size (150, 300, 450 µm). In every experimental run, walnut hull powder (15 g) was mixed with water. It was then sonicated for 10, 20, and 30 minutes at the power 150, 200 and 250 W. After Ultrasound treatment, the mixture was kept at the room temperature for a while then centrifuged at 8000 rpm for 15 min. After that mixture was filtrate using filter paper (whatman no.1) ant the filtrate was kept in hot air oven at 70 º C for overnight. After that sample was stored at room temperature. The yield of bio colrant was calculated according to equation (1).

$$\text{Yield (\%)} = \frac{\text{Weight of extract recovered}}{\text{Weight of dry powder}} \text{ X } 100 \quad (1)$$

The model development was done using response surface methodology through use of Design expert 10.0.1 version. Complete second order model as given in equation was fitted to the data and the model adequacy was tested using R^2 (coefficient of multiple determination) and Fisher's F-test. The parametric effect on various responses was done through the interpretation of developed models. Regarding four independent variables, a second order response function has the following general formula

$$Y = \beta_0 + [\beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4] + [\beta_{11}X_{12} + \beta_{22}X_{22} + \beta_{33}X_{32} + \beta_{44}X_{42}] + [\beta_{12}X_1X_2 + \beta_{23}X_2X_3 + \beta_{34}X_3X_4 + \beta_{41}X_4X_1] \quad (2)$$

The second order polynomial equation was solved using a statistical approach called the method of least square (MLS) which is a multiple regression technique used to fit a mathematical model to a set of experimental data generating the lowest residual possible. The results of regression analysis were obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination (R^2), lack of fit, etc. These terms describe adequacy of predictive model and effect of independent parameters on the responses.

Results and Discussion

This research was undertaken with an objective to interpret the outcome of process factors on yield of colorant from walnut hull using Ultrasound assisted extraction. The experimental design used for the study was Box-Benhken design having four variables and each variable having three levels. The variables with levels were Ultrasound power (150, 200, 250 W), sonication time (10,20,30 mins), Solvent (Water) volume (20,30,40 ml) and particle size (150,300,450µm). Response surface methodology was utilized to enhance the process parameters. A complete second order mathematical model was fitted in the response. The competence of the model was confirmed using coefficient of determination (R2) and Fisher's test. The results obtained on numerous characteristics of the study are discussed in detail.

Effect of Independent Parameters on extract Yield

Bio colorant from walnut hull powder was extracted using Ultrasound assistance. To enhance the extraction process, parameters studied were Ultrasound power (150, 200, 250 W), sonication time (10, 20, 30 mins), Solvent (Water) volume (20, 30, 40 ml) and particle size (150,300,450µm). The quantity of extract yield for all the 29 experiments is showed in Table 4. The yield differed from 31.54 to 49.76% from sample amount of 15 g of sample over entire experimental conditions. Maximum extract yield of 49.76% for the hull sample was obtained at the experiment run 18 having experimental conditions of Ultrasound power at 200 W, Sonication time at 20 min, Solvent volume 1:20 and particles size was 150µm. On other hand, the minimum extraction yield of 31.54% was obtained at the experiment run no. 15 having independent variable conditions of Ultrasound power at 150 W, Sonication time at 10 min, Solid solvent ratio 1:30 and particles size was 300µm.Results of the present study in terms of extract yield, when compared with the past studies of (Agullo *et al.*, 2012) [10], was found 5.65% higher than their result of 44.11% extract yield. Increased extraction yield in the present study could be credited to the Ultrasound assisted extraction because it is effective for extraction as its mechanical effects on the process by increasing the penetration of solvent into the product due to disruption of the cell walls produced by acoustical cavitation (*Toma et al.* 2001) [9].

The experimental data was studied to note the remarkable consequences of numerous process variables on extract yield. The outcomes of variance for extract yield are given in Table 5.F-value was used to observe the significance of linear, quadratic and interactive terms. F_{cal} value for model was superior to F_{tab}, which infers that model was significant ($p < 0.01$). The outcome of independent variables on yield at linear level was observed to be highly significant at 1% level of significance ($F_{cal} > F_{tab}$), for quadratic and interactive terms it was also observed significant.

A second order polynomial equation was used to fit the coded variables (A, B, C, D) for extract yield using multiple regression analysis. Table 5 shows the coefficient of determination (R^2) for the independent variable, their interaction in coded form and their corresponding p value. The value of the R^2 for the extract yield was found as 0.9525 which implies that the model could account for 95.24% data. The lack of fit value for regression model was not significant, which indicates that the model equation was adequate to describe the extract yield. For better suitability of the model, the difference between the predicted and adjusted should be less than 0.2, the adequate precision should be greater than 4 and whereas C.V. should not exceed 10%. In this case, the "Pred R^2" of 0.7779 was in reasonable agreement with the "Adj R^2" of 0.9050, the adequate precision was found to be 16.981 and the C.V was 3.74% thereby verifying the accuracy and suitability of model. The coefficient of determination (R^2) and adjusted determination coefficient (Adj R^2) were reasonably close to 1, indicating a high degree of correlation between the observed and predicted values.

A second order polynomial equation (Eq. 2) was developed representing an empirical relationship between the response (Extract Yield) and the independent variables viz. Particle Size (A), Ultrasound Power(B), Sonication Time (C) and solvent volume (D). The equation for extract yield is given below:

$$\text{Extract Yield (\%)} = 45.79 - 2.21A + 2.90B + 1.12C - 1.85D + 0.10AB - 0.059AC + 0.47AD + 0.49BC - 0.059BD - 0.053CD - 1.27A^2 - 6.34B^2 - 5.71C^2 - 0.56D^2 \quad (2)$$

The equation included both significant and non-significant terms. Non-significant terms were removed from the model and then the equation (Eq. 2a) was regenerated, that describes only the effect of significant process variables on extract yield from walnut hull. The equation is as follows:

$$\text{Extract Yield (\%)} = 45.79 - 2.21A + 2.90B + 1.12C - 1.85D - 1.27A^2 - 6.34B^2 - 5.71C^2$$

Table 4: Experimental data on UAE of walnut hull extract

Run	Particle size (µm)	Ultrasound power (W)	Time (min)	Solvent volume (ml)	Yield (%)
1	150	150	20	30	36.90
2	300	200	20	30	43.96
3	450	150	20	30	32.05
4	150	200	10	30	39.13
5	300	200	30	20	41.83
6	450	200	10	30	35.05
7	300	250	20	40	39.08
8	300	200	10	40	35.86
9	300	150	20	20	37.28
10	450	250	20	30	38.20
11	300	200	20	30	47.09
12	300	250	30	30	39.68
13	150	200	20	40	45.37

14	300	200	20	30	46.22
15	300	150	10	30	31.54*
16	150	200	30	30	41.39
17	150	250	20	30	42.64
18	150	200	20	20	49.76**
19	300	200	10	20	39.53
20	300	200	30	40	37.94
21	450	200	20	20	44.37
22	300	150	30	30	32.95
23	300	250	10	30	36.33
24	300	200	20	30	47.04
25	300	200	20	30	44.62
26	450	200	20	40	41.88
27	450	200	30	30	37.08
28	300	150	20	40	33.52
29	300	250	20	20	43.08

* Minimum, ** Maximum

Table 4: Regression analysis for extract yield

Source	Sum of Squares	d.f.	Mean Square	F Value	p-value
Model	628.7153409	14	44.908239	20.043936	0.000001
A	58.73656512	1	58.736565	26.215946	0.000156
B	100.7147844	1	100.714784	44.952125	0.000010
C	15.01563896	1	15.015639	6.701944	0.021439
D	40.96942165	1	40.969422	18.285921	0.000768
AB	0.040401	1	0.040401	0.018032	0.895089
AC	0.013924	1	0.013924	0.006215	0.938281
AD	0.89718784	1	0.897188	0.400443	0.537060
BC	0.95004009	1	0.950040	0.424032	0.525477
BD	0.01411344	1	0.014113	0.006299	0.937863
CD	0.01127844	1	0.011278	0.005034	0.944441
A^2	10.4203299	1	10.420330	4.650916	0.048912
B^2	260.5012594	1	260.501259	116.269773	0.000000
C^2	211.1187983	1	211.118798	94.228852	0.000000
D^2	2.008561921	1	2.008562	0.896483	0.359783
Residual	31.36685957	14	2.240490		
Lack of Fit	23.25168856	10	2.325169	1.146085	0.486627
Pure Error	8.115171008	4	2.028793		
Cor Total	660.0822005	28			
R^2	0.9525				
Adj R^2	0.9050				
Pred R^2	0.7779				
Adeq Precision	16.981				
C.V. %	3.74				

Graphical Analysis

Graphical analysis was done for understanding the trend of various responses with respect to levels of significant process variables. To determine the operating range for the best result, graphs were drawn using software Design expert 10.0.1.

Effect of independent variables on Extract Yield

In Fig. 4.1a, at linear level the extract yield varies with particle size at optimum conditions of ultrasound power 201.815W, Time 18.002min, Solvent volume 20.504 for achieving maximum extract yield. The graph shows extract yield decreases with the increase in particle size in range from 150 µm to 450µm. Smaller the particle size greater the surface and hence the mass transfer efficiency increases. Grinding breaks the plant cell wall, thus facilating the active compound to release to the extraction solvent and enhance the yield. The readings are supported by (Yeop *et al*, 2017) [13]

In Fig. 4.1b, at linear level the extract yield varies with Ultrasound power at optimum conditions of particle size 257.125 µm, Time 18.002min, Solvent volume 20.504 for achieving maximum extract yield. The graph shows extract yield increases with the power and decrease after further increase in power and is in range from 150 W to 250 W. This is due ultrasound wave can facilitate the cell walls of target sample to disrupt, also can accelerate the diffusing and dissolving of target components in the liquid medium. However, higher ultrasonic power can weaken the cavitation effect because the cavitation bubbles in this case more likely grow too big to collapse Moreover, excessive cavitation bubbles production can hinder the mass transfer and lead the ultrasound waves to scatter, which weaken the effect of ultrasonic power. The readings are supported by (Carail *et al*, 2015) [2]

In Fig. 4.1c, at linear level the extract yield varies with Ultrasound power at optimum conditions of particle size 257.125 µm, Ultrasound Power 201.815, Solvent volume 20.504 for achieving maximum extract yield. The graph shows extract yield increases with the Sonication time and decrease after further increase in time and is in range from 10 min to 30 min. This is due broken cells are released at the early period of extraction, because ultrasound enhanced the release of those compounds into the exterior solvent and increased the yield in the first 20 min. However, longer extraction time with ultrasound treatment might induce the degradation of pigment. The readings are supported by (Tiwari *et al*, 2009) [8]

In Fig 4.1d at linear level the extract yield varies with solvent at optimum condition of Ultrasound power 201.815 W, Time 18.002 mins, Particle size 257.125 µm for achieving maximum extract yield. The graph shows extract yield decreases with the increase the solvent volume in range from 20 to 40 ml. This is due to the enhancement of the solubility of solute particle for a prolonged time interval which decreases the viscosity of extracting solvent and thus accelerating the release and dissolution of these compounds (Jawad and Langrish, 2012) [4]

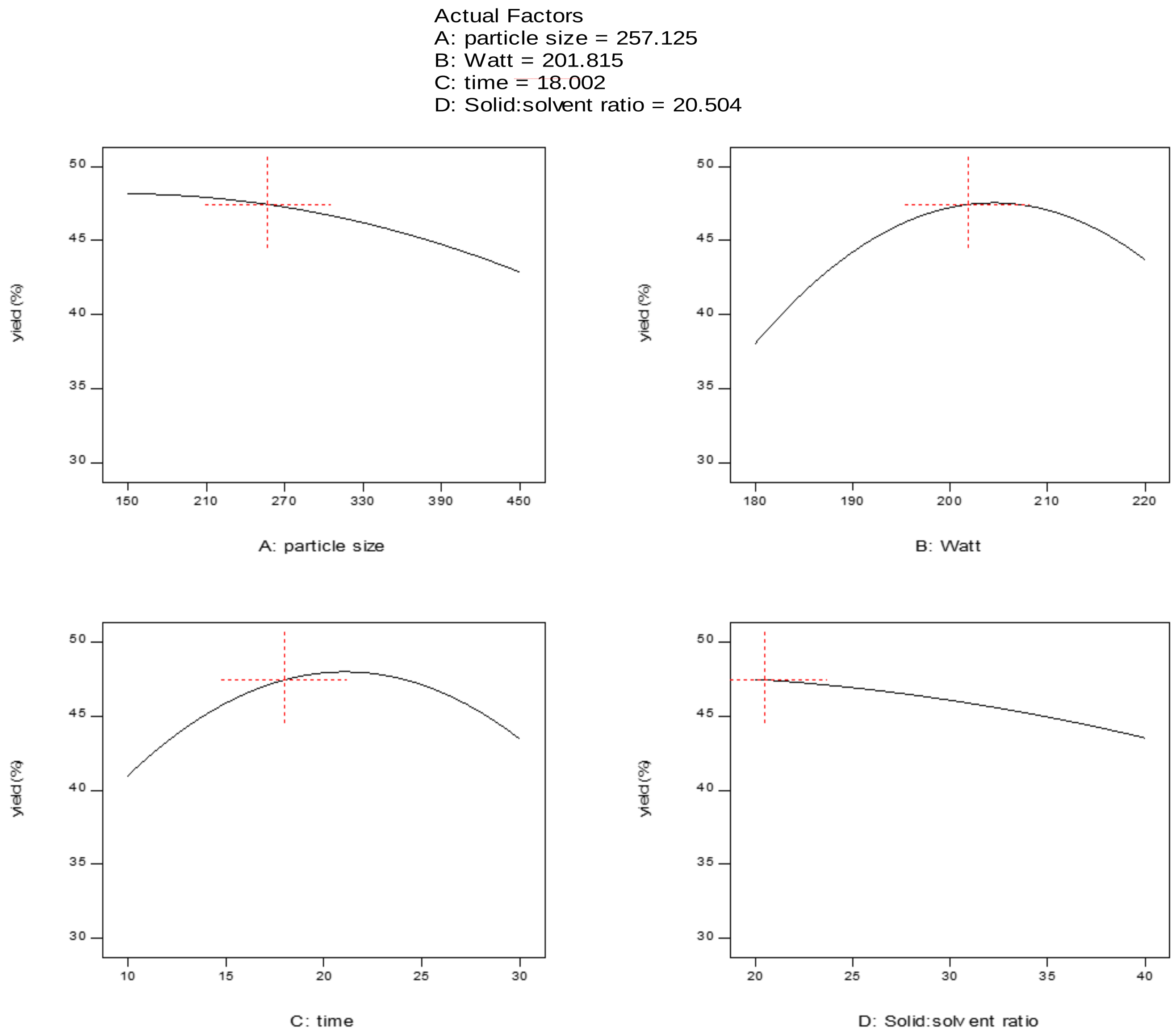

Fig 1: Effect of independent variables on Extract yield

Conclusion

Bio colorant is an important naturally occurring food additive that is widely utilized in the food industry. In this research, varied extraction conditions were studied for bio colorant extraction from walnut hull using Ultrasound assistance. The Box–Behnken design was utilized to correlate the effect of Ultrasound time, power, solvent volume and particle size on the yield. The outcome presents that the peak yield of biocolrant extracted at the ideal conditions of Ultrasound time of 20 min, Ultrasound power of 200 W, solvent volume 1:20 and particle size 150µm was 49.76%.

Acknowledgements

We are highly obliged to our fellow researchers and faculty members for their assistance and gratitude from the Department of Post-Harvest Process and Food engineering, GBPUAT, Pantnagar, India.

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