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Open Access Research Article

Optimization of Ultrasound-Assisted extraction for *Tephrosia purpurea* by Response Surface Methodology and evaluation of its antioxidant activity

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ABSTRACT

Objective: The aim of the present research work is to optimize the Ultrasound Assisted Extraction (UAE) of whole plant of *Tephrosia purpurea* by Response Surface Methodology (RSM) and to evaluate its antioxidant activity by DPPH radical scavenging assay and Ferric (Fe^{3+}) Reducing Power Assay.

Methods: *T. Purpurea* was subjected to UAE by Box-Behnken experimental design using independent factors/variables. The design matrix developed by software consists of 15 runs. All batches were subjected to DPPH radical power assay to determined antioxidant activity.

Results: The optimal conditions for batch which showed highest extraction yield i.e. 9.82 % were 30 min irradiation time, 15 min soaking time, and 6.665% solid: solvent ratio. Statistical analysis of experiments indicated that the irradiation time and solid: solvent ratio has significantly affected the extraction ($p < 0.01$). The Box-Behnken experimental design shows that the polynomial regression models are in good agreement with the experimental results and with the coefficients of multiple determination of 0.9499 for extraction yield. The batch showing highest extraction yield were also showed highest DPPH scavenging activity i. e.87.96% and batch having optimal conditions viz 10 min irradiation time, 15 min soaking time, and 6.665% solid: solvent ratio showed 98.08 % ferric reducing power assay.

Conclusion: This research work showed the UAE can be a method of extraction of *T. purpurea*. The antioxidant activity done by DPPH radical scavenging assay and Ferric (Fe^{3+}) Reducing Power Assay may be useful for further research work of *T. purpurea*.

Keywords: *Tephrosia purpurea*, Ultrasound Assisted Extraction, Response surface methodology, Antioxidant activity

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INTRODUCTION

Plants have been used for the medicinal purpose from ancient period. Medicinal plants play a basic role in human healthcare. Ancient *Unani* manuscripts, Egyptians papyrus, and Chinese writings described the use of herbs. Evidence exists that *Unani Hakims*, Indian *Vedas*, and European and Mediterranean cultures were using herbs for over 4000 years as medicine. One of the oldest systems of medicine known as *Ayurveda* originated in India. India is a rich source of herbal medicines as it has great biodiversity. Herbal medicines are termed as safe because it has natural ingredients which are with less or no side effects. Recently there is a trend in the world that 'return to nature' this increases the demand for herbal drugs. Herbal drugs contain various chemical constituents termed as phytoconstituents/phytochemicals. The plant contains phytochemicals such as alkaloids, glycosides, phytosterols, flavonoids, saponins, polyphenols, etc. Phytochemicals are used as the development of novel drugs. Phytochemicals can be obtained from a natural source using various extractions techniques. Extraction, because the term is employed pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products, therefore, obtained from plants are comparatively impure liquids, semisolids or powders intended only for oral or external use. Extraction is carried out in various ways, it can be conventional (traditional) or non-conventional (modern) way. Methods such as maceration, percolation, soxhlet extraction, etc are termed in the conventional way of extraction while ultrasonic-assisted extraction (UAE), Accelerated solvent extraction (ASE), microwave-assisted extraction (MAE),

supercritical fluid extraction (SFE), etc are termed as non-conventional methods of extraction [1, 2, 3].

Conventional methods of extraction are, however, somewhat time-consuming for extraction, gives less extractive yield as well as phytochemicals yield. The phytochemicals which are temperature sensitive may also get affected due to high temperature. Furthermore, these processes cannot be accelerated by agitation and need a large amount of solvent. Thus, a large amount of energy for the evaporation/concentration step is required[4,14]. So there is increasing demand for new extraction techniques to reduce the extraction time, minimize the solvent consumption and increase the extraction yield. In another way, UAE is simple, rapid, environment-friendly, highly efficient and less time and energy consuming. it is operated on frequency range 20 KHz to 2000 KHz, in which the mechanical effect of ultrasound penetrates solvent into the cell wall of plant material and brings the cavitations in the cell membrane and which carry out the extraction. UAE gives high reproducibility, short extraction time, low temperature and energy requirement with low solvent consumption. Therefore UAE is the most suitable technique of extraction[5,6,7]. Response surface methodology (RSM) is an effective statistical methodology for the optimization of complex processes. RSM is used to simultaneous optimization or evaluation of the interaction of the several variables in various experimental processes to reduce the number of experimental runs, cost and time as compared to other methods[11, 14, 15].

Tephrosia purpurea (L.) Pers. (*T. purpurea*) is a wild perennial plant belonging to family Fabaceae. It is known as Sarphenka in Hindi, Fish poison in English, Unhali in Marathi, Kolanji in Tamil. It is widely distributed among India, Australia, China, and Sri Lanka up to 400 m to 1300 m altitude. It occurs naturally in the waste places along the roadsides and it prefers to grow in dry, gravelly or rocky and sandy soil[9]. *T. purpurea* has used folk medicine for the treatment of a number of diseases. In *Ayurveda* it is described as '*Sarva warnavishapak*' means it has a property to heal all types of wounds[8]. The plant has been claimed to cure diseases of the kidney, liver spleen, heart, and blood[10].

Till today, there is no practical relevant data available about the extraction of *T. purpurea* using UAE. Therefore this research work emphasizes on optimization of UAE using Box-Behnken experimental design by Response Surface Methodology (RSM).

MATERIALS AND METHODS:

Collection and Authentication of plant:

The plant material was collected in the month of September-October of 2018, from the region of Aurangabad District, The authentication and identification were done at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. The accession number 0704 and the voucher specimen were deposited for future reference. The plant material washed and dried in tray dryer (Microtray, S. B. Panchal & Company, Dadar (West), Mumbai.), it was ground to powder using a mixer grinder (Havells India Ltd. Delhi, India). Sifting was done through sieve shaker (CIP Machineries, Ahmedabad, GJ, India) 40# sieve (Swastika Electric and Scientific Works, Ambala, HR, India) and finally stored at room temperature for further use.

Chemicals and Reagents

Methanol was purchased from S. D. fine-chem. Ltd. India of HPLC grade, 2, 2- Diphenyl-1-picrylhydrazyl (DPPH) purchased from Himedia of LR (Laboratory Reagent) grade, potassium ferricyanide. Trichloroacetic acid, ferric chloride, potassium di-hydrogen phosphate, sodium hydroxide (AR grade) were purchased from Merck life science.

Preliminary batches

For the Box-Behnken experimental design, the preliminary batches of *T. purpurea* by ultrasound-assisted extraction was carried out using independent factors/variables. The preliminary design is shown in Table 1.

Table No. 1: Preliminary batches of *T. purpurea* extract

Sr. No.	Independent factor	Batch No.1	Batch No. 2	Batch No. 3
	Irradiation time (min)	15	20	25
	Soaking time (min)	5	25	15
	Solid: Solvent ratio	2	5	7

Experimental design

RSM with Box-Behnken Design (BBD) was used to design the experiment and investigate the three independent parameters: irradiation time (A), soaking time (B) and solid: solvent ratio (C). The experimental design was done by using Design-Expert® Version 11.1 (State-Ease, Inc, Minneapolis) two level three factorial design was used. The independent variables are shown in Table 2. The design matrix developed by software consists of 15 runs are shown in Table 3. The design was developed to study the interaction of independent variables on the percent extraction yield of the plant *T. purpurea* using ultrasound-assisted extraction by taking 15 runs of different combinations of three independent variables indicated as A, B, and C.

Table No. 2: Independent factors for experimental design

Sr. No.	Independent Factors	Units	Levels	
			Low	High
1	Irradiation time (A)	Minutes	10	30
2	Soaking time (B)	Minutes	15	120
3	Solid: Solvent ratio (C)	% W/V	3.33	10

Table No. 3: Box-Behnken Design matrix and extractive yield of *T. purpurea*

	Factor 1	Factor 2	Factor 3	Response
Run	**A:Irradiation time (Minutes)**	**B:Soaking time (Minutes)**	**C:Solid:Solvent ratio (% W/V)**	**Extractive value (Percent)**
1	-1	1	0	8.86
2	0	-1	-1	9.51
3	0	-1	1	9.2
4	1	1	0	9.09
5	0	1	-1	9.12
6	-1	0	-1	8.25
7	1	-1	0	9.82
8	0	0	0	9.2
9	1	0	-1	8.49
10	-1	-1	0	8.84
11	0	1	1	8.8
12	0	0	0	8.87
13	0	0	0	9.11
14	1	0	1	8.32
15	-1	0	1	7.3

Ultrasound-Assisted Extraction (UAE)

The extraction of *T. purpurea* performed by using Ultrasonic bath cleaner (LABMAN Pvt. Ltd. Model LMUC-6, 40 KHz, 150 W). The experimental design which was previously developed on the Design-Expert was used for the ultrasonic extraction of the plant material. The sample material (2 gm) were weighed accurately and placed in Erlenmeyer flasks (150 ml) and kept for the soaking in solvent (methanol) for the designed period of time (15, 67,5 and 120 min). It was then irradiated in ultrasonic waves for various time periods (10, 20 and 30 min). For UAE of 15 batches constant ultrasonic temperature (35^{0}C) was maintained by circulating water. After UAE, it was filtered using Whatman filter paper 1. Then the solvent was evaporated using a Rotary vacuum evaporator (Rotavapor R-210 Buchi Labortechnik AG), the semi-solid greenish extract of *T. purpurea* was obtained. The extractive yield which was required response value of BBD was calculated. It was then kept in a freeze at temperature 4-5^{0}C. The percent extractive yield was calculated using the following formula:

Extractive yield (%) = obtained yield / total weight of plant material × 100.

DPPH radical scavenging assay

DPPH radical scavenging activity was done by methods elucidated by Hanato *et al*., 1988 [12]. 1 gm sample was taken in 10 ml of methanol. It was kept overnight for extraction. This eluted extract (0.2 ml) was taken and to it, 1 ml of DPPH solution (80 µg/ml of methanol) was added. Sample sets were centrifuged for 15 min. In above solution, 1 ml of methanol was added. Absorbance was taken at 517 nm (UV-visible Spectrophotometer, UV-2600, Bio-Age) separately for blank and sample with pure methanol. The fresh solution was used for an activity. Care was to be taken that the DPPH solution did not come direct contact with light. The percentage inhibition activity was determined by the following formulae:

$$\text{DPPH inhibition activity (\%)} = \frac{Ab-As}{Ab} \times 100$$

Where, Ab =Absorbance for blank As = Absorbance for sample

Ferric (Fe^{3+}) Reducing Power Assay

The reducing power of *T. purpurea* was determined according to method previously described by Oyaizu, 1986 but with little modification[13]. The diluted methanolic extract of *T. purpurea* was taken in the same concentration (1mg/ml) and was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium ferricyanide (1% w/v) incubated in incubator at 50˚C for 30 min (Incubator, Stream series-091L, Medica Inst. Mfg. Co.). After that, 2.5 ml of trichloroacetic acid (10% w/v) was added; mixer was centrifuged at 3000 rpm for 10 min. After centrifugation 2.5 ml of supernatant was collected and mixed with equal amount of distilled water and 0.5 ml $FeCl_3$ (0.1% w/v) solution, it is then allowed to stand for 30 min at 25 ˚C. Then absorbance was measured at 700 nm. A blank was prepared. The ferric reducing power was measured by the following formula:

$$\text{Ferric } (Fe^{3+}) \text{ Reducing Power (\%)} = \frac{As-Ab}{As} \times 100$$

Where, Ab =Absorbance for blank As = Absorbance for sample

RESULTS AND DISCUSSION

Analysis of preliminary batches of *T. purpurea* by UAE

Analysis of preliminary batches of *T. purpurea* extract was carried out by UAE and analyzed for extraction yield and results are shown in Table 4.

Table No. 4: Results of preliminary batches of *T. purpurea*

Batch	Extractive yield (%)
1	6.352
2	7.981
3	10.252

Optimization of the extraction condition of *T. purpurea*

Response surface methodology using Box-Behnken design was developed to obtain the exact formula for the extraction of *T. purpurea* by UAE. Designed experiments are carried out to check the effect of independent variables that are irradiation time, soaking time and solid: solvent ratio on the extraction yield. This design was run with two levels and three factors which include three centers in order to optimize the extraction conditions. Three center points are runs to get the batch stability and batch to batch variability. The extraction yield was obtained in the range of 7.30% to 9.82% (shown in Table 3). The maximum yield was obtained

in experimental condition A = 30 min, B = 15 min, and C = 6.665%. By applying multiple regression analysis on experimental data coded polynomial equation was obtained. The coded equation given by software which was the function of independent variables shows the extraction yield of *T. purpurea* in selected ranges. The coded quadratic equation of the model is as shown below

Extractive yield (Y) = +9.06+0.3088A– 0.1875B -02187C– 0.1875AB+0.1950AC–0.0025BC–0.4875A^2+ 0.5800B^2– 0.48925C^2

Where, Y is response i.e. extraction value. A, B, C are the independent variables. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. The sign of individual factor indicates whether the effect of the corresponding factor is positive or negative.

Validation of the model

To evaluate the quality of the fitted model analysis of variance (ANOVA) was performed (Table 5). The linear terms and quadratic terms (except BC) were significant ($p < 0.05$), while the response was not significant indicating that the relationship between the response variable (extractive yield) and the test variables was not simply a linear one. The lack of fit was used to verify the adequacy of the model. ANOVA for the lack of fit was not significant ($p > 0.05$) for the model, indicating that the model could adequately fit the experiment data. The Lack of Fit F-value of 0.36 implies the Lack of Fit is not significant relative to the pure error. There is a 79.43% chance that a Lack of Fit F-value this large could occur due to noise.

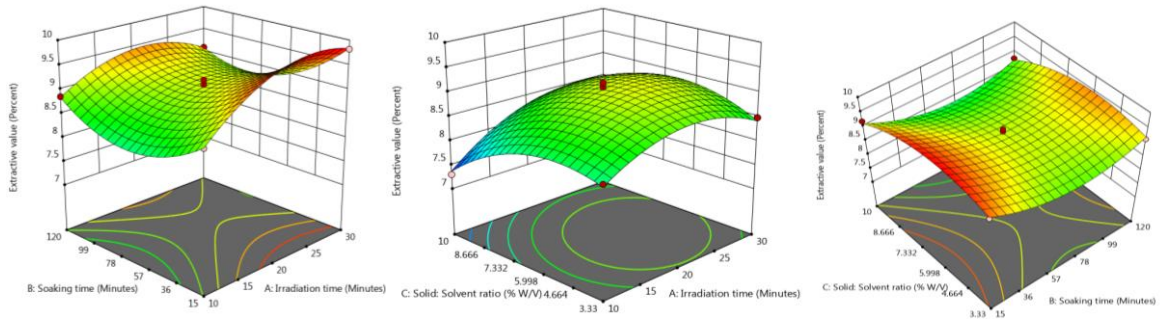
The Model F-value of 30.51 implies the model is significant. There is only a 0.08% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, AC, A^2, B^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In this study, the ratio was found to be 22.621 indicates an adequate signal. Therefore model was significant for the extraction process. This model can be used to navigate the design space. The value of Adjusted R^2 (0.9499) for the equation is reasonably close to 1, indicated a high degree of correlation between the observed and predicted values, therefore the model was suitable. A very low value of the coefficient of the variance (C.V.) (1.51) clearly indicated a very high degree of precision and reliability of the experimental values. The Predicted R^2 of 0.8741 is in reasonable agreement with the Adjusted R^2 of 0.9499; i.e. the difference is less than 0.2.

Table No. 5: ANOVA for the fitted model

Source	Sum of Squares	df	Mean Square	F-value	p-value	S
Model	4.91	9	0.5451	30.51	0.0008	**
A	0.7626	1	0.7626	42.69	0.0013	**
B	0.2813	1	0.2813	15.74	0.0107	*
C	0.3828	1	0.3828	21.43	0.0057	**
AB	0.1406	1	0.1406	7.87	0.0377	*
AC	0.1521	1	0.1521	8.51	0.0331	*
BC	0.0000	1	0.0000	0.0014	0.9716	NS
A^2	0.8775	1	0.8775	49.12	0.0009	**
B^2	1.24	1	1.24	69.53	0.0004	**
C^2	0.8596	1	0.8596	48.12	0.0010	**
Residual	0.0893	5	0.0179			
Lack of Fit	0.0311	3	0.0104	0.3565	0.7943	NS
Pure Error	0.0582	2	0.0291			
Cor Total	5.00	14				

df: degree of freedom, S: Significant; * $p < 0.05$, ** $p < 0.01$, NS; Not Significant

The combination of the analysis of variance (ANOVA) (Table 5) and response surfaces (Figure 1) indicated that the interaction effect between irradiation time and soaking time (AB), and irradiation time and solid: solvent ratio (AC) were statistically significant, but the interaction effect between soaking time and solid: solvent ratio (BC) was non-significant.



A B C

Figure No. 1: 3 D Response plots of extractive yield as a function of significant interaction between factors: (A) Irradiation time and soaking time: (B) Irradiation time and solid: solvent ratio; (C) Soaking time and solid: solvent ratio.

DPPH radical scavenging activity

The DPPH radical scavenging activity method was extensively used to determine the reduction capability of *T. purpurea*. The reduction capability of DPPH radical was determined by the decrease in absorbance at 517nm induced by antioxidants. The highest DPPH inhibition was obtained at 87.96%. DPPH radical scavenging activity of other batches is shown in Figure 2

.

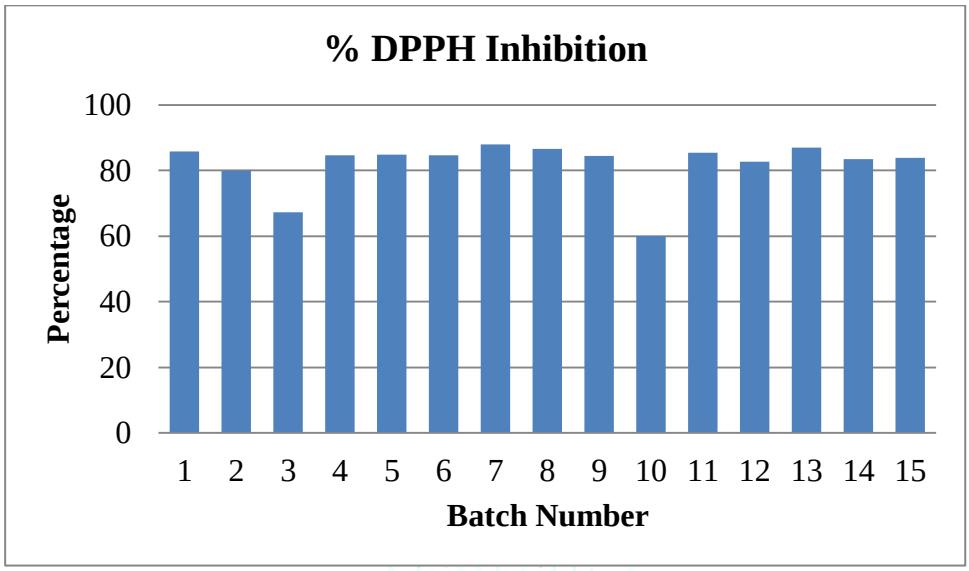


Figure No. 2: Results of DPPH scavenging assay

Ferric (Fe^{3+}) reducing power assay

The inhibition in reducing power assay denotes the yellow color of the test solution changes to various shades of green and blue depends upon reducing the power of each compound. The maximum reducing ability for the methanolic extract of *T. purpurea* was 98.80%. The ferric reducing power assay of other batches depicted in figure 3.

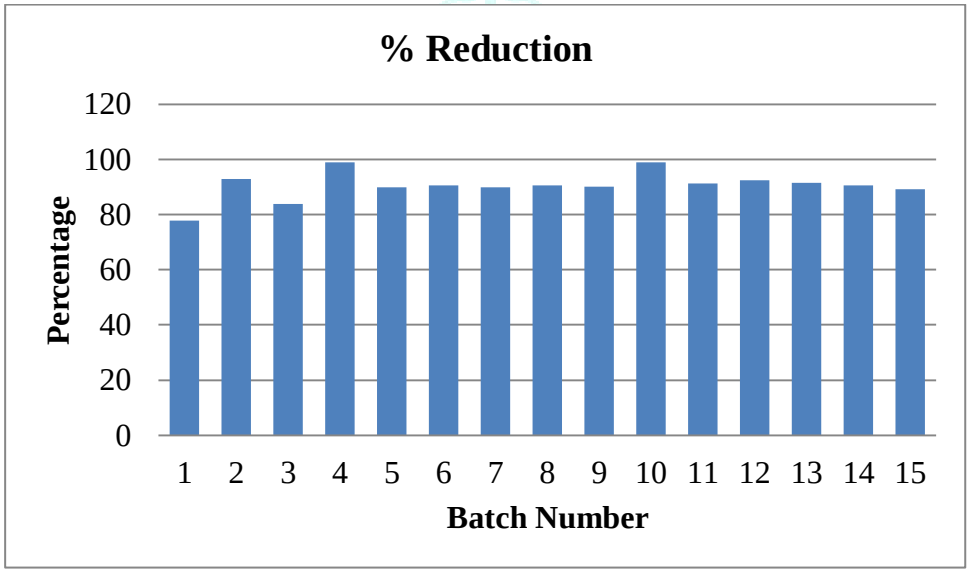


Figure No. 3: Results of ferric reducing assay

CONCLUSION

This experimental work firstly shows the ultrasound-assisted extraction of *T. purrpurea* by RSM. RSM is successfully employed for the extraction of *T. purpurea*. The best combination of response surface was obtained at the condition 30 min irradiation time, 15 min soaking time and 6.665% solid: solvent ratio. The extract obtained by this ratio shows the highest DPPH scavenging activity and moderate ferric reducing activity. This research work showed the UAE can be a method of extraction as well as the extract the antioxidants from *T. Purpurea*.

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