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Optimization of extraction conditions for colchicine from *Gloriosa superba* tubers using response surface methodology

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Gloriosa superba is a medicinal plant used in traditional medicine for the treatment of various diseases. The tuber of *Gloriosa superba* is a rich source of colchicine which is used for the treatment of gout, cirrhosis and also used in plant breeding studies to produce polyploidy. Optimization of various extraction parameters using response surface methodology (RSM) was performed to assess maximum yield of colchicine from *Gloriosa superba* tubers. Plackett-Burman design criterion was applied to identify the significant effect of various extraction parameters such as temperature, time, mean particle size, solvent-solid ratio, solvent composition, pH and number of extraction steps on extraction of colchicine. Among the seven variables tested extraction time, mean particle size, solvent-solid ratio and solvent composition were found to have significant effect on colchicine extraction. Optimum levels of the significant variables were determined by using Box-Behnken Design (BBD). The most suitable condition for extraction of colchicine was found to be single step extraction at temperature 35^0C, pH 7, extraction time 70 minutes, solvent-solid ratio 50:1, mean particle size 0.5 mm and solvent composition 70% ethanol in ethanol-water mixture. At these optimum levels of extraction parameters, the maximum yield of colchicine obtained experimentally (0.91% dry weight of tubers) was found to be very closed to its predicted value of 0.97% dry weight of tubers. The mathematical model developed was found to fit well with the experimental data of colchicine extraction.

Key words: *Gloriosa superba,* colchicine, solid-liquid extraction, Box-Behnken Design, response surface methodology.

Introduction

Gloriosa superba L. (Liliaceae) is an ornamental climbing herb native of tropical Asia and Africa often been cultivated for its beautiful flowers. The roots and tubers of this plant have been used in traditional Indian medicine for the treatment of gout, rheumatic arthritis, in diseases of skin and liver and

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several other purposes (Finnie and Staden, 1994). Since the detection of colchicine in *Gloriosa* (Clewer *et al*., 1915) a number of researchers have suggested that *Gloriosa* could serve as a commercial source of colchicine (Sarin *et al*., 1977; Srivastava and Chandra, 1977) as the colchicine content in the genera *Colchicum* has been reported to be lower than in *Gloriosa* (Bellet and Gaignault, 1985). Colchicine, the main alkaloid of *Gloriosa superba*, was useful agent in the treatment of acute attacks of gout (Box and Wilson, 1951) cirrhosis of the liver (Roberts *et al*., 1987) and familial Mediterranean fever (Kershenobich *et al*., 1988; Goldfinger, 1972). Colchicine and its analogues were used clinically for the treatment of certain forms of leukemia and solid tumers (Alexander *et al*., 1994). Due to its potent affinity for tubulin, colchicine is used in biological and breeding studies to produce polyploidy, multiplication of the chromosomes in cell nucleus and in tubulin binding assays as a positive control (Trease and Evans, 1977).

Extraction is the first important step in the recovery and purification of active ingredients of plant materials. Many techniques have been developed to extract colchicine from different members of family colchicaceae, among which the soxhlet (Husek *et al*., 1990) and solid –liquid extraction (Husek *et al*. 1989) are the most commonly used techniques. Extraction of colchicine from different member of family colchicaceae i.e *Sandersonia aurantiaca*, *Colchicum autumnale*, *Androcymbium melanthioides* by using methanol (Finnie and Staden, 1991), *Gloriosa superba* by aqueous methanol (Kanna *et al*., 2007) and ethanol (Ellington *et al*., 2003) have been reported. Many factors contribute to the efficacy of solvent extraction, such as the type of solvent, pH, temperature, number of steps, liquid-to-solid ratio and particle size of the plant material (Shi *et al*., 2005).

When many factors and their interactions affect desired response, response surface methodology (RSM) is an effective tool for optimizing the process, which was originally described by Box and Wilson (1951) RSM is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes (Atkinson and Donev, 1992). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). Response surface methodology has been successfully used to model and optimize biochemical processes (Boyacy, 2005; Kim *et al*., 2004) including extraction processes, such as effective substances from the stem of *Opuntia fiscus-indica* (Lee *et al*., 2005), anthocyanins from black currants (Cacace and Mazza, 2003), phenolic compounds from wheat (Pathirana, 2005), oleanolic acid from *Lantana camara*

root (Banik and Pandey, 2008) and protein from germinant pumpkin (Li and Fu, 2005). The optimization of extraction parameters of colchicine from *Gloriosa superba* using response surface methodology has not been reported yet.

The objective of the present paper was to choose a suitable solvent for extracting colchicine from dried tuber of *Gloriosa superba*. Moreover, Response surface methodology (RSM) was employed to optimize the effects of extraction time, particle size, solvent-solid ratio and solvent composition for the extraction of colchicine from dried tubers of *Gloriosa superba.*

Material and methods

Plant material

The field study was carried out in 2006 at the medicinal plant garden, B.H.U., Varanasi, India (25^{o} $18^{''}$ N, 83^{o} $50^{''}$ E). The experimental location experiences semi-arid tropical climate. The soil of the experimental field was sandy loam texture; organic Electrical conductivity 0.42 dSm^{-1}, available carbon 0.38 %, available nitrogen 180 kg ha^{-1}, available phoshorus 21 kg ha^{-1}, pH 7.3. Plants used in the study were propagated from its underground, V shaped tuber. The tubers were planted in first week of July 2006 at a depth of 6-8 cm, keeping a plant to plant distance of 30 to 40 cm. After six month, December 2006, plants were harvested from the field. The arial and tuber parts of the plants were separated and the tubers were washed with tap water, shade dried and kept in cellulose bags for further experiment.

Selection of solvent for extraction of colchicine from Gloriosa superba tubers

Before the development of the study by RSM, a first set of tests was performed to select the appropriate solvent for extraction of colchicine from *Gloriosa superba* tubers. The influence of solvents i.e water, ethanol, acetone, 50 % aqueous ethanol and 50 % aqueous acetone on the extraction was investigated, by considering 50:1 solvent solid ratio, 0.5 mm mean particle size, extraction time of 60 min, pH 7.0 at room temperature.

Extraction procedure

The dried tuber parts of *Gloriosa superba* was milled with the help of grinder. Solvent extraction of *Gloriosa superba* tubers was carried out in temperature controlled water bath by stirring at the constant speed of 200 rpm. The independent variables were temperature (25^{0}C - 50^{0}C), mean particle size

(0.3 mm- 1.2 mm), solvent composition (30%– 90% ethanol in ethanol-water mixture), solvent – solid ratio (10:1- 75:1), pH (5.0 -9.0) and extraction steps (1-3). The milled particles were sieved with a sieve shaker of different size. *Gloriosa superba* tubers powder of different mean particle size were taken into 150 ml Erlenmeyer flask, then different proportion of ethanol in ethanol-water mixture was added in different solvent - solid ratio and put in temperature controlled water bath at selected temperatures for different periods of time. 1 g of the tubers sample was used for each treatment.

Quantification of colchicine by HPTLC method

The amount of extracted colchicine from *Gloriosa superba* tuber was analysed by high performance thin layer chromatography (HPTLC) as described by Bodoki *et al.*, 2004 with some modification. Standard colchicine and the samples were spotted on precoated silicagel F_{254} aluminium plate (E-Merck grade) as narrow bands 4 mm wide at a constant rate of 10 µl s^{-1} using Camag Linomat IV model applicator under nitrogen atmosphere. A mixture of toluene and methanol (85:15 v/v) was used as the mobile phase. For detection and quantification of colchicine (at R_f 0.2), scanning densitometry was performed using a Camag TLC scanner with CATS 4 software, in reflectance (at 360nm) and fluorescence modes (Hg lamp, 254 nm).

Selection of significant variables by. Plackett-Burman design

Plackett-Burman design criterion was applied to identify the significant variables responsible for extraction of colchicine from *Gloriosa superba* tuber. This design criterion assumes that there are no interactions between the different extraction parameters and is based on the first order model:

$$Y_i = \beta_0 + \sum_i \beta_i X_i \qquad (1)$$

Where Y_i is the estimated target function and β_i are the regression coefficients. β_0 is scaling constant. The effect of seven variables (temperature, extraction time, solvent composition, particle size, solvent: solid ratio, pH and number of extraction steps) on the extraction of colchicine was tested at two experimental levels high level denoted by (+) and a low level denoted by (-) as listed in Table 2. Seven variables were screened by conducting twelve experiments and the experimental design is given in Table 3. All experiments were conducted in

duplicate and the average value of extracted colchicine was used for statistical analysis.

The variables which were significant at 5% level ($P< 0.05$) from the regression analysis as given in Table 4 were considered to have greater impact on extraction of colchicine and were further optimized by Box-Behnken design.

Optimization of response surface methodology

A Box-Behnken design was applied to determine the optimum level of four significant extraction parameters screened from Plackett-Burman design criterion. As shown in Table 4 the effect of four parameters (extraction time, solvent composition, mean particle size and solvent: solid ratio) on the extraction of colchicine was studied at three experimental levels: –1, 0, +1. The experimental levels for these variables were selected from our preliminary work, which indicated that an optimum could be found within the level of parameters studied. The levels of factors used for experimental design are given in Table 5. A total of 27 experiments were conducted. The experimental design scheme is given in Table 6. The response values (Y) in each trial were the average of the duplicates.

Statistical analysis and modelling

The data obtained from RSM on colchicine extraction were subjected analysis of variance (ANOVA). The experimental results of RSM were fitted via the response surface regression procedure, using the following second order polynomial equation:

$$Y_i = \beta_0 + \sum_i \beta_i X_i + \sum_{ii} \beta_{ii} X_i^2 + \sum_{ij} \beta_{ij} X_i X_j \qquad (2)$$

In which Y_i is the predicted response, X_iX_j are independent variables, β_o is the offset term, β_i is the ith linear coefficient, β_{ii} is the ith quadratic coefficient, and β_{ij} is the ijth interaction coefficient. However, in this study, the independent variables were coded as X_2, X_3, X_4 and X_5. Thus, the second order polynomial equation can be presented as follows:

$$Y = \beta_0 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{44}X_4^2 + \beta_{55}X_5^2$$

$$+ \beta_{23}X_2X_3 + \beta_{24}X_2X_4 + \beta_{25}X_2X_5 + \beta_{34}X_3X_4 + \beta_{35}X_3X_5 + \beta_{45}X_4X_5 \qquad (3)$$

The statistical software package, Design-Expert 7.0 (Stat- Ease, Inc., Minneapolis, MN, USA) was used for the regression analysis of the

experimental data, and also to plot the response surface graphs. The statistical significance of the model equation and the model terms was evaluated via the Fisher's test. The quality of fit of the second-order polynomial model equation was expressed via the coefficient of determination (R^2) and the adjusted R^2. The fitted polynomial equation was then expressed in the form of three-dimensional surface plots, in order to illustrate the relationship between the responses and the experimental levels of each of the variables utilized in this study. The point optimization method was employed in order to optimize the level of each variable for maximum response. The combination of different optimized variables, which yielded the maximum response, was determined in an attempt to verify the validity of the mode.

Results and discussions

Selection of solvent

Several solvents were used to extract colchicine from *Gloriosa superba tubers*, i.e. water, ethanol, acetone, 50 % aqueous ethanol and 50 % aqueous acetone. It was seen in Table 1 that 50 % aqueous ethanol was the best solvent.

Table 1. Selection of most efficient solvent for extraction of colchicine from *Gloriosa superba* tuber

S.N.	Solvent	% colchicine
1.	water	0.45
2.	ethanol	0.52
3.	acetone	0.31
4.	50 % aqueous ethanol	0.61
5.	50 % aqueous acetone	0.38

Screening of significant extraction parameters using Plackett-Burman design criterion

A total of seven variables were analyzed with regard to their effects on colchicine yield using a Plackett-Burman design (Table 2). The design matrix selected for screening of significant variables for colchicine extraction and the corresponding responses were shown in Table 3.

Table 2. Level of the extraction parameters for extraction of colchicine from *Gloriosa superba* tuber by using Plackett-Burman design criterion

Extraction Code	Extraction condition	High level	Low level (-)
X_1	temperature	(+)	25 ^{0}C
X_2	time	50 ^{0}C	30 min
X_3	mean particle size	60 min	0.6 mm
X_4	solvent: solid ratio	1.2 mm	10:1 ml/g
X_5	Solvent composition (% ethanol in ethanol water mixture v/v)	50:1 ml/g 70 v/v	35 v/v
X_6	pH 9	5	
X_7	extraction steps	3	1

Table 3. Yield of colchicine from *Gloriosa superba* tuber using the different levels of extraction variables of Plackett-Burman design criterion

run	X_1	X_2	X_3	X_4	X_5	X_6	X_7	Colchicine (%) dry weight of tuber
1	+	-	+	+	-	+	-	0.32
2	+	+	-	+	+	-	+	0.78
3	-	+	+	+	-	+	+	0.45
4	+	-	+	-	-	-	+	0.11
5	-	-	+	+	+	-	+	0.54
6	-	+	-	-	-	+	+	0.41
7	+	+	-	+	-	-	-	0.63
8	+	-	-	-	+	+	+	0.52
9	+	+	+	-	+	+	-	0.49
10	-	-	-	-	-	-	-	0.22
11	-	-	-	+	+	+	-	0.66
12	-	+	+	-	+	-	-	0.38

The adequacy of the model was calculated, and the variables evidencing statistically significant effects were screened via Student's t-test for ANOVA (Table 4). Factors evidencing *P*-values of less than 0.05 were considered to have significant effects on the response, and were therefore selected for further optimization studies. Among seven extraction parameters (temperature, extraction time, pH, solvent composition, mean particle size, solvent: solid ratio and number of extraction steps) studied, four parameters (extraction time, solvent composition, mean particle size, solvent: solid ratio) were found to have significant influence on colchicine extraction as evidenced by their *P* values (< 0.05, significant at 5% level) obtained from regression analysis. The coefficient of determination (R^2) of the model was 0.975 which indicates the model can explain up to 97.5% variation of the data. When the sign of the effect of the

tested variables is positive, the influence of the variable on colchicine yield is greater at a high level. And when negative, the effect of the variable is greater at a low level. One of the four significant variables screened, mean particle size, exerted a negative effect, whereas the other variables, extraction time, solvent-solid ratio and solvent composition, exerted positive effects on colchicine extraction. All other insignificant variables (extraction temperature, pH and number of extraction steps) were neglected, and the optimum levels of the four variables, (extraction time, mean particle size, solvent: solid ratio and solvent composition) were further determined by an RSM.

Table 4. Regression analysis of Plackett-Burman design criterion data for the prediction of significant extraction parameters

Term	Effect	Coef	SE Coef	T	P
Constant		0.45917	0.01127	40.73	0.000
temperature	0.03167	0.01583	0.01127	1.40	0.233
extraction time	0.12833	0.06417	0.01127	5.69	0.005
particle size ratio	-0.15500	-0.07750	0.01127	-6.87	0.002
solvent solid ratio	0.20833	0.10417	0.01127	9.24	0.001
solvent comosition	0.20500	0.10250	0.01127	9.09	0.001
pH	0.03167	0.01583	0.01127	1.40	0.233
extarction steps	0.01833	0.00917	0.01127	0.81	0.462

Optimization of significant variables using response surface methodology

Response surface methodology using *Box-Behnken design* was applied to optimize the levels of significant extraction parameters resulting from Plackett-Burman design experiments. The experiments conducted in the present study were targeted toward the construction of a quadratic model consisting of twenty seven trials. The design matrix and the corresponding results of RSM experiments to determine the effects of four independent variables (extraction time (X_2), mean particle size (X_3), solid-liquid ratio (X_4) and solvent composition(X_5)) were shown in Table 6, along with the predicted values. The ANOVA analysis of the optimization study indicated that the model terms X_2, X_3, X_4, X_5, X_2^2, X_3^2, X_4^2, X_5^2, $X_2 X_3$, $X_2 X_5$, X_3X_4 and $X_4 X_5$ were significant ($P < 0.05$). The model F-value was 255.48, and the F-value for lack of fit was 3.82 (Table 7). The high F-value and non-significant lack of fit indicate that the model was a good fit. The *P*-values for the model (<0.0001) and for lack of fit (0.1041) also suggested that the obtained experimental data was a good fit with the model.

Table 5. Treatment variables and their coded and actual values used for optimization of colchicin extraction from *Gloriosa superba* tuber by using Box-Behken design

Treatment variables	Coded levels			
	Symbol	-1	0	+1
time (minutes)	X_2	40	60	80
Particle size	X_3	0.3	0.6	0.9
Solvent-solid ratio(ml/g)	X_4	20	40	60
Solvent composition (% Ethanol in water)	X_5	30	60	90

Table 6. Box- behnken design criterion of extraction parameters with their corresponding experimental and predicted value

Run	X_2	X_3	X_4	X_5	% colchicine	
					Experimental	Predicted
1	80	0.6	40	30	0.523	0.519
2	60	0.6	60	90	0.812	0.841
3	60	0.6	20	30	0.352	0.328
4	60	0.6	20	90	0.476	0.472
5	60	0.9	20	60	0.364	0.365
6	60	0.9	40	30	0.312	0.315
7	60	0.6	60	30	0.463	0.472
8	40	0.6	20	60	0.421	0.425
9	40	0.6	60	60	0.732	0.711
10	60	0.3	60	60	0.812	0.806
11	40	0.6	40	30	0.465	0.464
12	60	0.9	40	90	0.576	0.559
13	40	0.3	40	60	0.640	0.642
14	60	0.3	40	30	0.470	0.487
15	60	0.6	40	60	0.876	0.886
16	60	0.3	20	60	0.460	0.462
17	40	0.9	40	60	0.480	0.497
18	80	0.6	60	60	0.810	0.807
19	60	0.6	40	60	0.884	0.886
20	80	0.9	40	60	0.580	0.583
21	80	0.3	40	60	0.820	0.808
22	60	0.9	60	60	0.540	0.533
23	60	0.6	40	60	0.897	0.886
24	60	0.3	40	90	0.760	0.757
25	80	0.6	40	90	0.850	0.846
26	40	0.6	40	90	0.650	0.649
27	80	0.6	20	60	0.560	0.581

Table 7. Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	estimate	Squares	df	Mean Square	F –value	p-value
Intercept	0.88		1			
X_2	0.063	0.048	1	0.048	168.17	<0.0001
X_3	-0.093	0.10	1	0.10	363.49	<0.0001
X_4	0.13	0.20	1	0.20	696.04	<0.0001
X_5	0.13	0.20	1	0.20	698.76	<0.0001
$X_2 X_3$	-0.02	1.600E-003	1	1.600E-003	5.66	0.0321
$X_2 X_4$	-0.015	9.302E-004	1	9.302E-004	3.29	0.0910
$X_2 X_5$	0.035	5.041E-003	1	5.041E-003	17.85	0.0008
$X_3 X_4$	-0.044	7.744E-003	1	7.744E-003	27.42	0.0001
$X_3 X_5$	-6.5E-003	1.690E-004	1	1.690E-004	0.60	0.4521
$X_4 X_5$	0.056	0.013	1	0.013	44.81	<0.0001
X_2^2	-0.081	0.043	1	0.043	152.00	<0.0001
X_3^2	-0.17	0.19	1	0.19	671.35	<0.0001
X_4^2	-0.17	0.19	1	0.19	683.18	<0.0001
X_5^2	-0.18	0.22	1	0.22	778.37	<0.0001
		1.01	14	0.072	255.48	< 0.0001
Model		3.955E-003	14	2.825E-004		
Residual		3.580E-003	10	3.580E-004		
Lack of Fit		3.748E-00	4	9.370E-005	3.82	0.1041
Pure Error		1.01	28			
Cor Total						

The regression equation coefficients were calculated and the data was fitted to a second-order polynomial equation. The response, colchicine extraction (Y) from *Gloriosa superba* dried tubers, can be expressed in terms of the following regression equation:

$$Y = 0.88 + 0.063X_2 - 0.093X_3 + 0.13X_4 + 0.13X_5 - 0.081X_2^2 - 0.17X_3^2 - 0.17X_4^2 - 0.18X_5^2 - 0.02\ X_2X_3 - 0.035X_2X_4 - 0.044\ X_3X_4 + 0.056X_4X_5 \quad (4)$$

The regression equation obtained from the ANOVA showed that the R^2 (multiple correlation coefficient) was 0.9961 (a value >0.75 indicates fitness of the model). This was an estimate of the fraction of overall variation in the data accounted by the model, and thus the model was capable of explaining 99.61% of the variation in response. The 'adjusted R^2' is 0.9922 and the 'predicted R^2' was 0.9791, which indicates that the model was good (for a good statistical model, the R^2 value should be in the range of 0–1.0, and the nearer to 1.0 the value was, the more fit the model was deemed to be). The 'adequate precision value' of the present model was 47.102, and this also suggests that the model can be used to navigate the design space. The 'adequate precision value' was an index of the signal-to-noise ratio, and values of higher than 4 are essential

prerequisites for a model to be a good fit. At the same time, a relatively lower value of the coefficient of variation (CV = 2.66 %) indicated a better precision and reliability of the experiments carried out.

Figures 1 to 6 showed the 3-dimensional response surface plot of colchicine extracted for each pair of extraction parameters by keeping the other two parameters constant at its middle level. The effect of extraction time and solvent composition on the extraction of colchicine was shown in Fig 1. Maximum colchicine is obtained at extraction time 70 min. and solvent composition 70 %. Further increase in solvent composition leads to deceleration of the colchicine yield. The result presented here on the effect of solvent composition (% ethanol in ethanol water mixture) were in good agreement with those of Cacace and Mazza (2003) for total phenolic extraction from black currant, were total phenolic compound content increased with ethanol concentration upto a maximum of about 60% and then decreased with further increase in solvent concentration. In Fig 2, effect of particle size and extraction time on the extraction of colchicine showed that maximum colchicine was extracted when particle size was 0.5 mm, further increase in the particle size leads to decrease in extraction of colchicine. Banik and Pandey (2008) reported the effect of particle size on the extraction of oleanolic acid in *Lantana camara* roots. This was indicating that diffusion of the solvent into the particle, and solvent-solute diffusion out of the particle may be limiting the extraction process. The increased particle size leads to decrease in exchange surface and increase in path length of the solute to reach the surface, which increases the extraction time. On the contrary, very small particles may lead to technical difficulties related to the permeability of the solid bed, during the mixing of the plant material and with solvent, as well as during the filtration. In Fig 3 response surface plot indicate that maximum colchicine extraction occurred at about solvent: solid ratio of 50:1 and extraction time of 70 min.. The extraction of colchicine increases with increase in solvent: solid ratio upto (50:1) further increase in the solvent:solid ratio decelerates the extraction of colchicines. Ficks second law of diffusion predicts a final equilibrium between the solute concentrations in the solid matrix and in the bulk solution after a certain time (Boyacy *et al.*, 2005). In Fig. 4 response surface plot indicate that maximum colchicine extracted at solvent-solid ratio (50:1) and solvent composition around 70 % ethanol in ethanol-water mixture. In Fig.5 maximum colchicine extracted when particle size was (0.5mm) and solvent composition (70% ethanol in ethanol-water mixture). Further increase in both the parameters leads to deceletration of colchicine extraction. In Fig. 6, response surface plot showed that maximum colchicine extracted at solvent-solid ratio (50:1) and mean particle size (0.5 mm).

Validation of the model

The experimental data were fitted in to equation (4) and the optimum values were found to be: extraction time (70 min), solvent-solid ratio (50:1), particle size (0.5 mm) and solvent composition (70 % ethanol in ethanol-water mixture). At these optimum levels of extraction parameters colchicine extracted from *Gloriosa superba* tubers was 0.91 %, which was very close to the predicted value of 0.97 % dry weight of *Gloriosa superba* tuber.

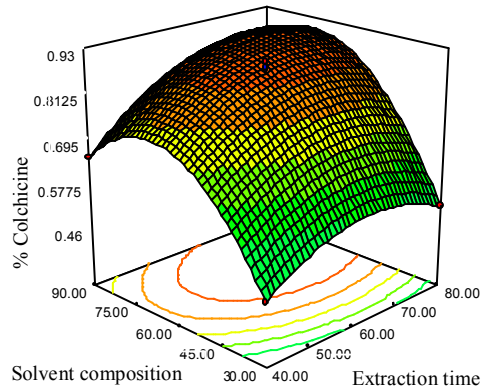

Fig. 1. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the time and solvent composition. Hold values: solvent:solid ratio 40; particle size:0.6mm.

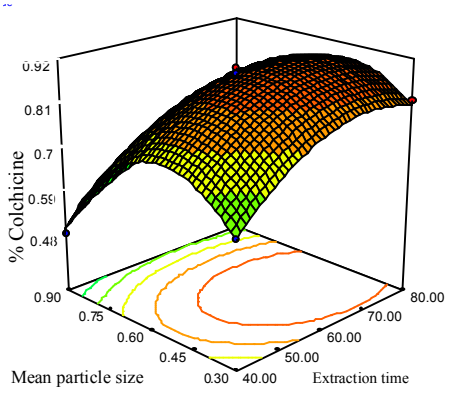

Fig. 2. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the particle size and time. Hold values: solvent:solid ratio: 40; solvent composition 60 v/v.

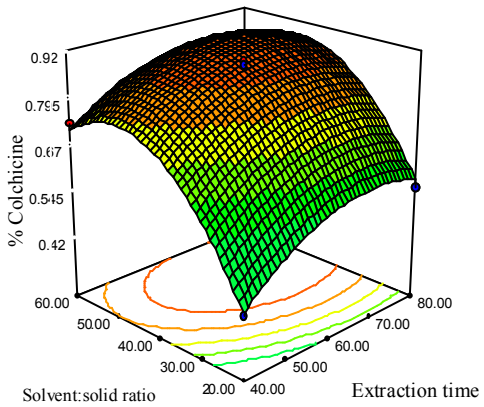

Fig. 3. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the solvent. solid ratio and time. Hold values: particle size: 0.6 mm; solvent composition: 60 v/v

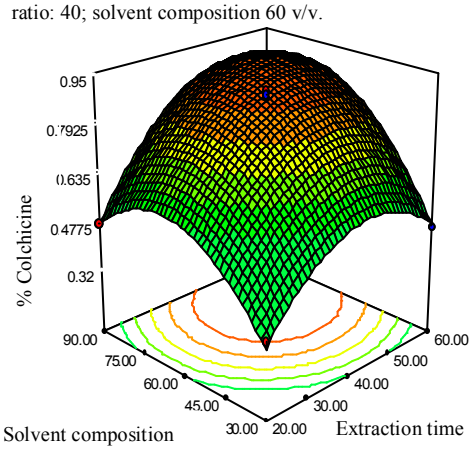

Fig. 4. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the solvent: solid ratio and solvent composition. Hold values: solvent:solid ratio : 40; solvent composition 60 v/v.

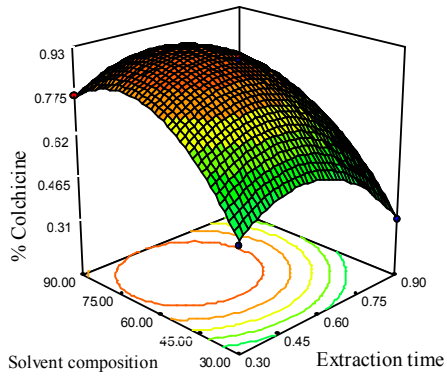

Fig. 5. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the particle size and solvent composition. Hold values: solvent:solid ratio :40; time: 60 min.

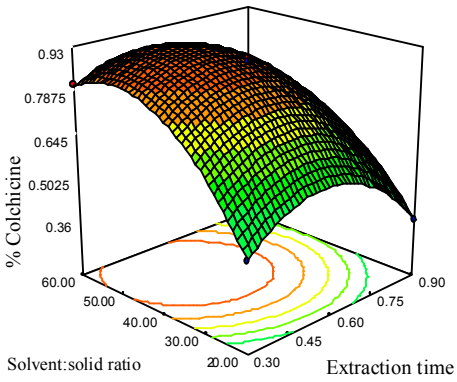

Fig. 6. Three-dimensional response surface plot for colchicine extraction showing the interactive effects of the solvent: solid ratio and particle size. Hold values: time: 60 min; solvent composition 60 v/v

Conclusion

Response surface methodology was successfully used to investigate the optimum extraction parameters for extraction of colchicine from *Gloriosa superba* tuber. To optimize various parameters for extraction of colchicine from *Gloriosa superba* tuber seven parameters viz temperature, time, solvent-solid ratio, solvent composition, mean particle size, pH and number of extraction steps were tested by using Plackett-Burman design criteria and four parameters time, solvent-solid ratio, mean particle size and solvent composition showed significant effect on extraction of colchicine. The extraction parameters were optimized by applying Box-Behnken design and the parameters for best extraction of colchicine from *Gloriosa superba* tuber was found to be extraction time (70 minutes), solvent-solid ratio (50:1), mean particle size (0.5 mm) and solvent composition (70% ethanol in ethanol-water mixture). The second order polynomial model was found to be satisfactory for describing the experimental data. The maximum colchicine from *Gloriosa superba* tuber was 0.91 % dry weight which was very close to the predicted value 0.97 %. This is the first report about the optimization of extraction of colchicine from *Gloriosa superba* tuber using response surface methodology.

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