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Original article

Development of response surface methodology for optimization of parameters and quantitative analysis of chebulinic acid from composition of medicinal herbs by HPLC

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ABSTRACT

The purpose of the research is to study the development of response surface methodology for optimization of chebulinic acid extraction from composition of medicinal herbs such as *Terminalia chebula*, *Phyllanthus emblica* and seeds of *Dimocarpus longan*. Optimization of extraction parameters such as weight dosages, pH and time were carried out by response surface methodology (RSM). The optimal conditions determined for extraction of chebulinic acid through response surface methodology were dosage (6.25 g), pH (5.7) and time (24.23 h). These results showed that the developed model is satisfactory and relevant for the extraction of chebulinic acid. The analysis of variance showed a high goodness of model fit and the performance of the RSM method for improving chebulinic acid extraction from the composition of medicinal herbs. Quantitative estimation of chebulinic acid in the composition of medicinal herbs by HPLC studies revealed that 0.712% w/w of chebulinic acid content was present in the composition of herbal powder.

1. Introduction

Polyphenols are natural, chemical synthetic and organic chemicals characterized by the presence of large units of phenol structures such as ellagic acid, tannic acid, phenyl propanoid, chebulinic acid and several other organic chemical components. Among them, chebulinic acid is a polyphenolic compound commonly found in the fruits of *Terminalia chebula* (Han et al., 2006), fruits and leaves of *Phyllanthus emblica* (Singh et al., 2011) and seeds of *Dimocarpus longan* (Rashed 2013) species, which has many potential uses in medicine. It is a yellowish crystalline powder and dissoluble in different organic solvents like methanol, ethanol and water. It exhibits various pharmacological activities such as anti-oxidant, anti-cancer, antihypertensive activities, etc.

RSM is a gathering of mathematical and statistical techniques, which provides important information concerning the optimum level of each process variable along with its interactions with other variables and their belongings on the response of the process such as product yield. With the use of RSM, the number of experiments can be minimizing without neglecting the interactions between the parameters of the process. This multivariate also improves statistical explanation possibilities and evaluates the comparative significance of several contributing factors even in the incidence of complex interactions. The current study was designed to optimize the extraction parameters of chebulinic acid from composition of medicinal herbs (*Terminalia chebula, Phyllanthus emblica* and *Dimocarpus longan*) through RSM method. Parameters optimized include different weight dosages, pH and extraction time period (hr).

2. Material and methods

2.1. Plant material

The dry fruits of *Terminalia chebula*, *Phyllanthus emblica* and seeds of *Dimocarpus longan* were collected from local market in Visakhapatnam, Andhra Pradesh state. These fruits and seeds were cleaned and cut into small pieces and powdered. The total powder

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was taken in to 120 mesh size. The different size powders were stored in the air tight small covers.

2.2. Extraction and determination of chebulinic acid

The above crushed powders of *Terminalia chebula* fruit (3g), *Phyllanthus emblica* fruit (1g) and *Dimocarpus longan* seeds (2g) are weighed and mixed in a flask (Pfundstein et al., 2010). Now add ethanol (80%) solvent at pH 6 and then, makeup the solution to 50 ml. Soak the solution for 24 h. After soaking, the solution is filtered by using a Whatman No. 1 filter paper and then the filtrate is heated at a temperature of 78 °C, so that the solvent which is taken in the glassware is evaporated. Then the solution is made up to 25 ml with distilled water. To this solution add 25 ml of hexane solvent (Choudhary, 2011) and mix the solution with care. With the help of a glass funnel, pour the entire mixture in the separating funnel. Incubate the solutions of ethanolic extract for 1hr (Manosroi et al., 2010). Then, 1 ml of ethanolic extract was reserved into 10 ml volumetric flask. 0.5 ml of Folin Denis reagent (Huang et al., 2014) and 1 ml of sodium carbonate solution were added and then the volume is made up to 10 ml with distilled water and the mixture was kept for 30 min at room temperature (Kesharwani et al., 2017). Then, measure the absorbance of the reaction mixture at 700 nm using spectrophotometer.

2.3. Optimization of the selected parameters using Central Composite design (CCD)

Once the variables having the greatest authority on the responses were recognized, a CCD (Box and Wilson, 1951) was used to optimize the levels of these variables. The CCD programme is based on three basic principles of an model experimental design, mainly it consists of (1) a complete 2^n factorial design, where n is the number of test variables, (2) n_0 center points ($n_0 \geq 1$) and (3) two axial points on the axis of each design programme variable at a distance of $2^{n/4}$ from the design center. So, the total number of design points is $N = 2^n + 2n + n_0$. In this programme, the statistical calculation of the variables X_i are coded as x_i according to Eq. (1):

$$x_i = \frac{X_i - \bar{x}_i}{\Delta x_i} (i = 1, 2, 3, \ldots\ldots\ldots\ldots, k) \quad (1)$$

where

x_i is dimensionless value of an independent variable
X_i is the real value of an independent variable,
$\bar{x}_i$ is the real value of the independent variable at the center point
Δx_i is the step change.

The second degree polynomials (Eq. (2)) are considered with the statistical package (Stat Ease Inc., Minneapolis, MN, USA) to estimate the response of the dependent variable:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (2)$$

where,

Y is predicted response
X_1, X_2, X_3, X_4 are independent variables
b_0 is offset term
b_1, b_2, b_3, b_4 are linear effects
b_{11}, b_{22}, b_{33}, b_{44} are squared effects
b_{12}, b_{13}, b_{14}, b_{23}, b_{24}, b_{34} are interaction terms

2.4. Experimental design and statistical analysis

The Central Composite Design (CCD) using statistical software package "Design Expert 7.1.5" was used to reach a quadratic model, consisting of factorial trials and star points to guess quadratic effects and central points to approximate the pure process changeability with extraction of chebulinic acid concentration as response (Prasad et al., 2011). The process of variables are screened through the Plackett-Burman (PB) designs, the parameters like weight dosages, pH, and time were selected as the most important (p values < 0.05) factors.

RSM was employed to optimize the process parameters namely

1. Different weight dosages (composition of medicinal herbs with 125 microns),
2. pH (various pH values of ethanolic extract)
3. Extraction time period

2.5. HPLC

The HPLC analysis was conducted at Venture labs, Hyderabad. It was performed with a auto-Sampler equipped with a Shimadzu LC (USA) reciprocating pumps connected to a DGU-20A 5 degasser and CBM-20Acolumn oven compartment. In this analysis process, UV–VIS detector, SPD-M20A diode array detector (Thomas et al., 2015) and Software LC solution 1.21 SP1 were used. It were carried out with a C18 ODS column (4.6 mm × 250 mm) packed with 5 µm diameter particles, volume injection of 20 µl and a flow rate of 1.0 ml/min. All analyses were performed at a ambient temperature of 38 °C, with a mobile phase of orthophosporicacid: acetonitrile (80:20 v/v), and was pumped into the column. The UV absorbance of the eluent was measured at 350 nm (Ghosal et al., 2013).

3. Results

The influence of various process parameters such as dosage of composition of herbs (X_1), pH (X_2) and time (X_3) for chebulinic acid extraction were studied by using full factorial CCD (Soni et al., 2015; Zhao et al., 2014). A central composite design with 16 experiments, which includes 8 cube point runs, 6 center point runs and 2 axial point runs, was used for the optimization of process variables for chebulinic acid extraction. For statistical calculations all independent variables were coded using Eq. (1). Based on the analysis of preliminary experimental results, the range of variables used in this design was selected and tabulated in Table 1.

Regression equation for the optimization of chebulinic acid is:

Chebulinic acid extraction is function of dosage of composition of herbs (X_1), pH (X_2) & time (X_3). The multiple regression analysis of the experimental data has yield the following Eq. (3).

$$Y = -52.3401 + 8.8477\,X_1 + 5.6504\,X_2 + 1.3427\,X_3 - 0.7244\,X_1^2 - 0.5122\,X_2^2 - 0.0304\,X_3^2 - 0.0088\,X_1X_2 + 0.0110\,X_1X_3 + 0.0110\,X_2X_3 \quad (3)$$

Table 2 represents the results obtained in Central Composite Design (CCD). The response is found in the form of investigation of variance (ANOVA) from regression Eq. (1) is put together in Table 3. Fischer's F-statistics value is defined as MS_{model}/MS_{error}, where MS indicates mean square. Fischer's 'F-statistics' value, having a low probability 'p' value, indicates the high significance.

Df-degree of freedom; SS-sum of squares; F-factor F; P-Probability.
R^2 = 0.97542; R^2 (adj): 0.93855

Table 1
Coded and actual levels of the independent variables for the design of experiment for extraction of chebulinic acid concentration from composition of medicinal herbs.

Independent variables	Range and level				
	−2	−1	0	+1	+2
Dosage (X_1), g	4	5	6	7	8
pH (X_2)	4	5	6	7	8
Time (X_3), min	12	18	24	30	36

Table 2
Central Composite Design (CCD) and the experimental responses of dependent variable of chebulinic acid concentrations.

S.No	Dosage (g)	pH	Time (hr)	Chebulinic acid (mg/ml)	
				Experimental	Predicted
1	5.000000	5.000000	18.00000	5.53000	5.313281
2	5.000000	7.000000	18.00000	4.80000	4.630737
3	5.000000	5.000000	30.00000	5.20000	5.221617
4	5.000000	7.000000	30.00000	4.70000	4.804073
5	7.000000	5.000000	18.00000	6.10000	5.934103
6	7.000000	7.000000	18.00000	5.30000	5.216559
7	7.000000	5.000000	30.00000	6.00000	6.107440
8	7.000000	7.000000	30.00000	5.50000	5.654895
9	4.300000	6.000000	24.00000	4.90000	5.024965
10	7.600000	6.000000	24.00000	6.30000	6.262466
11	6.000000	6.000000	13.90000	4.10000	4.447958
12	6.000000	6.000000	34.00000	5.00000	4.739474
13	6.000000	4.300000	24.00000	6.60000	6.720962
14	6.000000	6.000000	24.00000	7.50000	7.592500
15	6.000000	6.000000	24.00000	7.40000	7.592500
16	6.000000	6.000000	24.00000	7.60000	7.592500

Table 3
ANOVA of chebulinic acid extraction for entire quadratic model

Source of variations	SS	Df	Mean Square (MS)	F-value	p > F
Model	15.03006	9	1.50064	23.77063	0.0001
Error	0.37878	6	0.06313		
Total	15.40884	15			

Table 4
Comparison between optimum values from Experimentation and CCD.

S. No	Variables	Experimental	CCD
1.	Dosage (g)	6	6.25
2.	pH	6	5.7
3.	Time (hr)	24	24.23
4.	Chebulinic acid concentration (mg/ml)	7.7	7.7807

All the interaction terms ($P < 0.05$) are highly influential on adsorption capacity. Fig. 1 shows the pareto chart for the present data for chebulinic acid extraction.

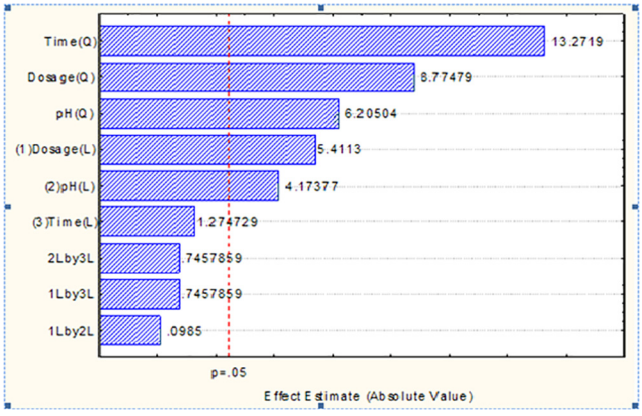

Fig. 1. Pareto chart for chebulinic acid extraction.

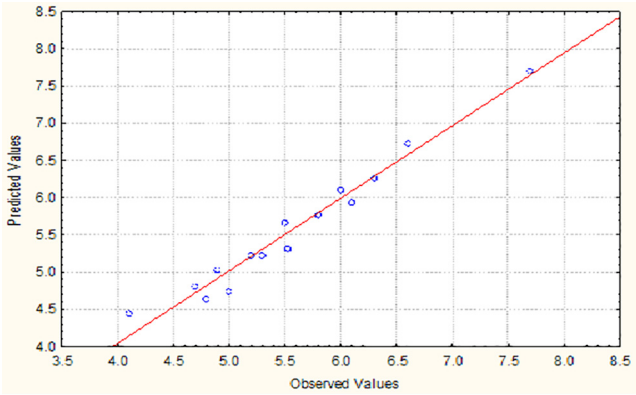

Fig. 2. Normal probability plot for chebulinic acid extraction.

3.1. Interpretation of residual graphs

Normal probability plot is a graphical technique used for analyzing whether or not a data set is usually disperced to greater extent. The difference between the observed and predicted values from the regression is termed as residual. Fig. 2 shows the normal probability plot for the present data for chebulinic acid extraction. It is evident that the experimental data are practically aligned implying normal distribution.

3.2. Interaction effects of chebulinic acid variables

Three-dimensional view of response surface contour plots (Figs. 3–5) exhibit chebulinic acid extraction for different combinations of dependent variables. All the plots are delineated as a function of two factors at a time, imposing other factors fixed at zero level. It is apparent from response surface contour plots that the chebulinic acid is minimal at low and high levels of the variables. The comparison between optimum values from Experimentation and CCD shown in Table 4. This behavior conforms that there is a

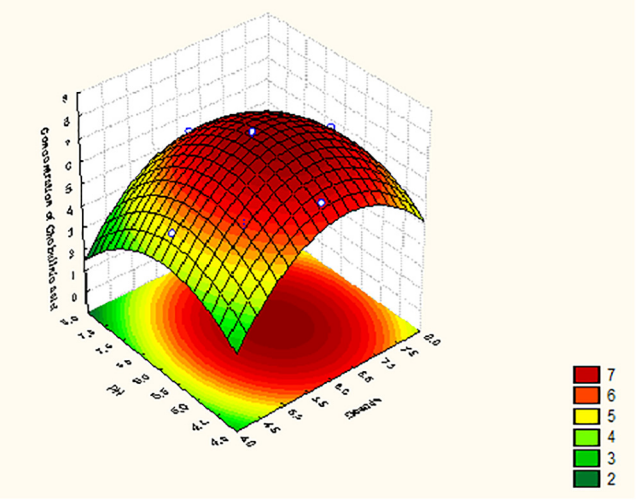

Fig. 3. Surface contour plot for the effect of Dosage (g) and pH on the extraction of chebulinic acid (mg/ml).

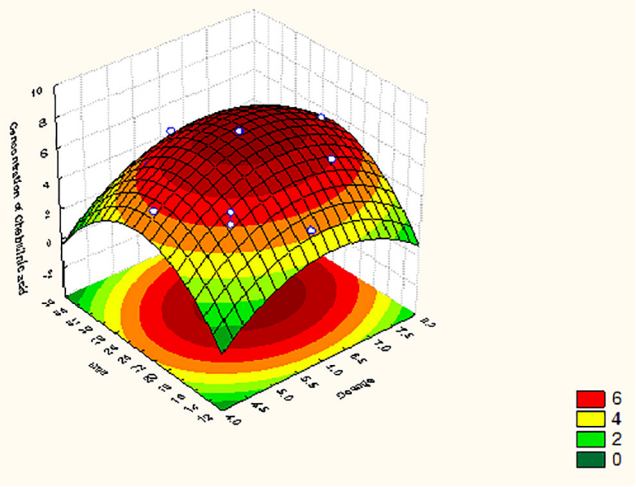

Fig. 4. Surface contour plot for the effect of Time (hr) and Dosage (g) on the extraction of chebulinic acid (mg/ml).

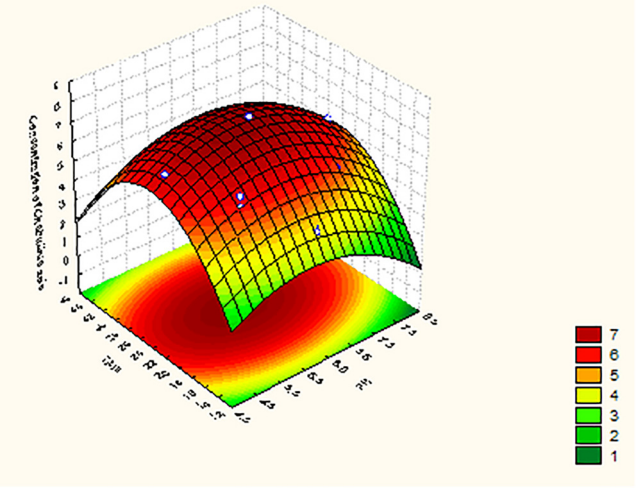

Fig. 5. Surface contour plot for the effect of Time (hr) and pH on the extraction of chebulinic acid (mg/ml).

presence of optimum for the input variables in order to maximize chebulinic acid extraction (Dwevedi et al., 2016). The predicted optimal set of conditions for maximum chebulinic acid extraction is:

Dosage = 6.25 g
pH = 5.7
Time = 24.23 h
Extraction of chebulinic acid concentration = 7.7807 mg/ml

3.3. HPLC analysis

Sample Weight taken: 10.0 mg
Area of sample: 310,101
Standard Weight taken: 1 mg
Area of Standard: 5,754,965
Assay of chebulinic acid std: 100%
Calculation of Assay of chebulinic acid in herbal composition sample

$$\begin{aligned}\text{Assay of chebulinic acid} &= (\text{Std Wt}/\text{Sample Wt})\text{x}(\text{Sample area}\ /\\ &\quad \text{Standard area})\text{xAssay of Standard}\\ &= (1.0/10.0)\text{x}(310101/5653856)\text{x}100\\ &= 0.712\%\text{w/w}. \qquad (4)\end{aligned}$$

Quantitative estimation of chebulinic acid in the composition of medicinal herbs - *Terminalia chebula* fruit, *Phyllanthus emblica* fruit and *Dimocarpus longan* seeds by HPLC analysis method. This method studies revealed that 0.712% w/w of chebulinic acid content in the composition of herbal powder, the assay of chebulinic acid was calculated according to Eq. (4) (Chhabra et al., 2017). The retention time observed for the authentic sample of chebulinic acid results was given in Figs. 6 and 7 and Tables 5 and 6.

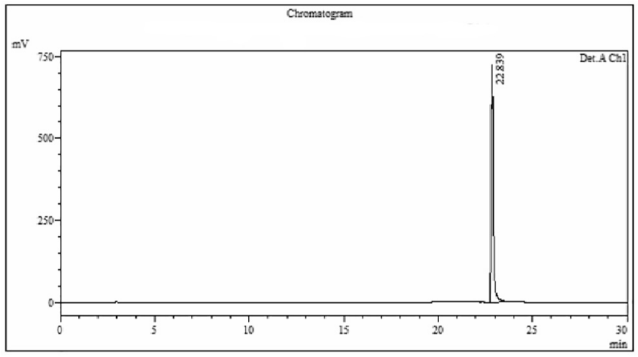

Fig. 6. Chromatogram analysis of standard chebulinic acid.

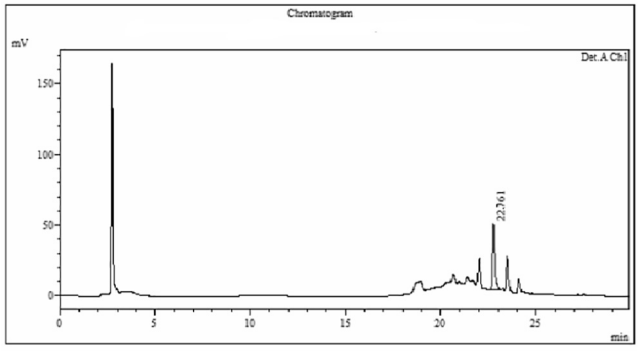

Fig. 7. Chromatogram analysis of chebulinic acid in herbal composition sample.

Table 5
Chromatogram analysis of standard chebulinic acid.

Peak Table D: \ hplc03-data-ddrive \ D \ chebulinic acid 145.1 cd detector A Ch1 350 nm						
Peak#	Name	Ret.time	Area	Theoretical plate #	Tailing Factor	Area %
1	Chebulinic acid	22.839	5653856	187305.87	1.50	100.00
Total			5653856			100.00

Table 6
Chromatogram analysis of chebulinic acid in herbal composition sample.

Peak Table D: \ hplc03-data-ddrive \ D \ chebulinic acid 146.1 cd detector A Ch1 350 nm						
Peak#	Name	Ret.time	Area	Theoretical plate #	Tailing Factor	Area%
1	Chebulinic acid	22.761	310101	170245.68	1.54	100.00
Total			310101			100.00

4. Discussion

The present study involves the use of statistical experimental design to optimize process conditions for maximum chebulinic acid extraction. These parameters were optimized using CCD involving RSM, Dosage = 6.25 g, pH of ethanolic extract = 5.7 and Time = 24.23 h. The significant interactions between the three parameters were observed from the contour plots. The maximum chebulinic acid extraction obtained with the above optimum values are 7.7807 mg/ml. A high similarity was observed between the predicted and experimental results, which reflected the preciseness and applicability of RSM to optimize for chebulinic acid extraction process. The reported HPLC method was found appropriate and accurate for chebulinic acid identification. In this method, orthophosporic acid: acetonitrile (80:20 v/v), was used as mobile phase with 1.0 ml/min elution flow rate, λ = 350 nm UV wavelength. Symmetry C18 ODS thermo (250 $\times$ 4.6 mm) column was used for quantification and recognition of chebulinic acid. The HPLC chromatograms for standard chebulinic acid and chebulinic acid content in herbal composition using the developed method are shown in Figs. 6 and 7. The retention time for standard chebulinic acid is 22.838 min and chebulinic acid content in herbal composition is 22.761 min. Anil D Mahajan and Nandini R Pai (Mahajan and Pai, 2011) reported that chebulinic acid retention time was 13.02 min and Ranjini et al. (2015) reported that chebulinic acid retention time was 23.8 min in *Terminalia chebula* species by HPLC method (Mahajan and Pai, 2011; Ranjini et al., 2015). Similarly working with *Phyllanthus embilica* reported that the chebulinic acid retention time was 21.16 min (Filipiak-Szok et al., 2012). Yean Soong and Philip John Barlow (2005) reported that chebulinic acid retention time was 15.7 min in *Dimocarpus longan* seeds (Soong and Barlow, 2005).

5. Conclusion

Using RSM, the maximum chebulinic acid was found to be 7.7807 mg/ml at optimum conditions of weight dosages, pH and time period are 6.257 gm, 5.723 and 24.233 h. These parameters were optimized using Central Composite Design involving response surface methodology. Determination of chebulinic acid in the composition of medicinal herbs like *Terminalia chebula* fruit, *Phyllanthus emblica* and *Dimocarpus longan* seed by HPLC method. HPLC studies revealed that 0.712% w/w of chebulinic acid content was present in the composition of herbal powder.

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Conflict of interest

The authors declare no conflict of interest.

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