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EXTRACTION OF RESVERATROL: OPTIMIZATION OF PROCESS PARAMETERS USING RESPONSE SURFACE ANALYSIS

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ABSTRACT

Response surface methodology was employed to study the optimum conditions of Resveratrol extraction from ALR. The effect of solvent to solid ratio, temperature, particle size and reaction time was studied for extraction of resveratrol. From the analysis it was observed that the most relevant variable was solid to solvent ratio, extraction time, particle size and temperature. The co-efficient (R^2) determination was in goal agreement with the second order model.

Keywords: Extraction kinetics, *Artocarpus Lakoocha Roxb*., solvent to solid ratio, Diffusion, kinetic model.

INTRODUCTION

Resveratrol is an anticancer drug that is found in naturally occurring plants and is known as 3, 5, 4$^{/}$ trihydroxy stilbene (structure I). It has wide range of biological activities and can be used as antioxidant, cardioprotective, anti-inflamatory etc (Jang *et al*., 1997; Campagna and Rivas, 2010). From the natural sources resveratrol can be obtained by different extraction methods such as solvent extraction, supercritical fluid extraction, ultrasonication-assisted extraction etc (Robich *et al*., 2010).

Solvent extraction is a conventional technique for extraction of many naturally occurring molecules where water and organic solvents are used. For process optimization in solvent extraction, many factors have to be studied which require proper investigation by experimental and theoretical study. For process optimization of biomolrcule extraction from natural sources some factors such as solvent to solid ratio, particle size, extraction time, temperature and speed of agitation are to be studied.

Thus for optimizing the process parameter it is needed to study the effective factors. Response surface methodology is an effective tool for optimizing the process (Triveni *et al*., 2001) for a system. To use the minimum resources for quantitative data from experimental design and to solve a multivariable equation, statistical method like RSM can be used. With the help of Response surface experiment, it is easy to identify the responses. In response surface analysis, the central composite experimental design can be used to fit the empirical model. When central composite experimental design is completed with a full second order polynomial model it provides an adequate representation of Response surface as reported by Deming *et al*. (1990).

MATERIALS AND METHODS

Methods of extraction for resveratrol and the results were reported in our previous communication (Borah and Hazarika, 2015).

STATISTICAL ANALYSIS

In the statistical analysis, the average yield of the duplicate values were taken as the dependent variable or response. For our study, the proposed model is given as follows

$$Y_i = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{14} BC + \beta_{11} B^2 + \beta_{22} C^2 + \beta_{23} ABC + \beta_{33} B^2C + \beta_{44} BC^2 \quad (1)$$

Where Y_i is predicted response, B_o is offset terms, $\beta_1, \beta_2, \beta_3$ are called linear terms, $\beta_{12}, \beta_{22}, \beta_{33}$ are squared effects and $\beta_{12}, \beta_{13}, \beta_{23}, \beta_{123}$ are interaction effects.

The variation was explained by the polynomial models and is given by the multiple co-efficient of determination R^2.

The behavior of the surface was investigated for the response surface (Y_i) = g soluble resveratrol from extract using equation (1). For deducing workable optimum

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condition a graphical technique was used as reported by Flores and Chinnan (1988), Giovanni (1983) by fixing one variable at a predetermined optimum condition. Responses were monitored properly and results were compared with the predicted model. The fitted equation was expressed as surface plots in order to know the relationship between the response and experimented levels of each factor and for determining the optimum conditions.

Experimental design using Response Surface Methodology (RSM) for Optimization of Extraction Process

By using Response surface analysis we optimised the extraction parameters for extraction of Resveratrol. The Response Surface Methodology (RSM) used in this study was a central composite for central design involving different factors: Extraction time, extraction rate, solid to solvent ratio. Extraction of resveratrol from *Artocarpus Lakoocha Roxb* was assessed based on the face centered experimental plan as shown in Table 1. The results were analysed using Analysis of Variance (ANOVA) by Design Expert 9.0.1 software. Based on the effect of levels of three factors, three dimentional plots and their respective contour plots were obtained. From these three dimensional plots, simultaneous interaction effect of three factors on the response were studied and optimised region was identified based on the main parameters. The experiment was repeated five times randomly and the results were compared with the predicted values to determine the model accuracy.

RESULTS AND DISCUSSION

Model Fitting

A central composite design CCD was used to develop a correlation between the extraction time, extraction rate and solvent to solid ratio to improve the extraction rate of resveratrol. A total of 20 experiments with different combinations of the three variables were carried out. The results of experimental design are shown in Table 2 with multiple regression analysis, the quadric equation with the co-efficients of the full regression model equation and their statistical significance were determined and evaluated using Design expert 9.1.9 software. The final model equation in terms of coded values is given in equation (2). In this equation the positive sign indicates the synergetic effect and negative sign indicates an antagonistic effect.

$$Y = 14.01 + 0.23A + 0.059C + 0.65C + 1.32AB + 1.81AC + 3.06BC - 0.25B^2 + 0.15C^2 - 0.72ABC - 7.87B^2C - 4.44BC^2 \quad (2)$$

Where A, B, C are the coded variables, A = Time, B = Temperature, C = Solvent to solid ratio.

Table 1. Levels of the tested variable in the 2^4 Central Composite Design.

Independent variables	Code	Range and levels				
		-α	-1	0	+1	+α
Time	A	-1.0453	1	3.5	7	9.04538
Temperature	B	16.3641	30	40	50	83.6359
Solid to solvent ratio	C	8.29552	10	12	14	16.7045

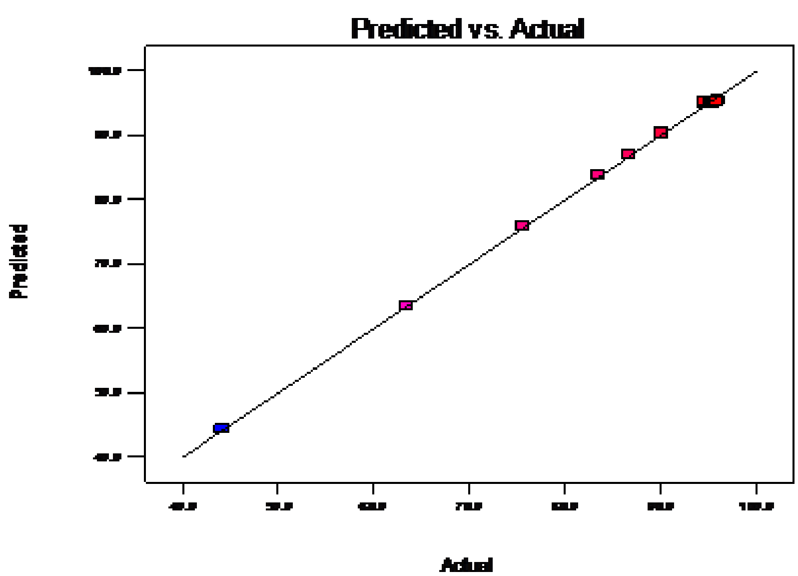

Fig. 1. Comparison between predicted and observed values of extraction.

Here each of the observed values are compared with the predicted values calculated from the model and it is seen from Figure 1 that predicted values accord with the calculated values. Table 3 represents the variations in the corresponding coded values of three parameters and the response based on experimental runs and predicted values proposed by CCD design. From Table 2, it was observed that the extraction rate was in the range of 13.23 $mmolL^{-1}h^{-1}$ to 44.12 $mmolL^{-1}h^{-1}$. The ANOVA for this model is significant ($p< 0.0001$) with a model F-value of 141.59.

From Table 3, it was seen that, Solvent to solid ratio (C) was highly significant than other two parameters i.e. temperature and extraction rate. However these two variables cannot be eliminated and it is seen that A^2 and C^2 are much more significant than B^2. The co-efficient of variance which is the ratio of the standard error of the extraction to the mean value of the observed response and in considered to be the reproducible while it is less than 10%. In our work, co-efficient of variance was found to be 4.09%. The value of "Ad eq precission" measures the signal to noise ratio which is found to be 4 in our case and

Table 2. The coded variables, yield (experimental and predictive) and residuals for each run.

Std. order	Run	Coded variables			Extraction rate		
		A	B	C	Experimental $mmolL^{-1}h^{-1}$	Predicted $mmolL^{-1}h^{-1}$	Residuals $mmolL^{-1}h^{-1}$
1	1	-1	-1	-1	20.86	20.86	0.000
2	2	1	-1	-1	25.17	25.17	0.000
3	3	-1	1	-1	14.32	13.98	0.34
4	4	1	1	-1	13.56	13.56	0.000
5	5	-1	-1	1	17.33	17.33	0.000
6	6	1	-1	1	13.49	13.49	0.000
7	7	-1	1	1	14.98	13.98	1.000
8	8	1	1	1	44.12	44.12	0.000
9	9	-1.682	0	0	31.66	31.66	0.000
10	10	1.682	0	0	13.24	13.98	-0.74
11	11	0	-1.682	0	15.00	15.00	0.000
12	12	0	1.682	0	13.39	13.98	-0.59
13	13	0	0	-1.682	14.55	14.45	0.10
14	14	0	0	1.682	13.23	13.23	0.000
15	15	0	0	0	14.33	14.33	0.000
16	16	0	0	0	14.34	14.45	-0.11
17	17	0	0	0	13.23	13.23	0.000
18	18	0	0	0	14.55	14.55	0.000
19	19	0	0	0	13.43	13.43	0.000
20	20	0	0	0	13.99	13.99	0.000

Table 3. Analysis of Variance (ANOVA): Data of Response Surface Model for Extraction of Resveratrol.

Source	Sum of square	df	Mean Square	F value	P-value Prob>F
Model	1007.63	14	71.97	141.59	0.0001
A- Time	0.29	1	0.29	0.57	0.4929
B-Temperature	0.020	1	0.020	0.039	0.8524
C- solvent to solid ratio	0.024	1	0.024	0.048	0.8380
AB	13.91	1	13.91	27.37	0.0064
AC	26.32	1	26.32	51.77	0.0020
BC	74.85	1	74.85	147.24	0.0003
A^2	0.70	1	0.70	1.37	0.3065
B^2	1.24	1	1.24	2.45	0.1929
C^2	2.5×10^{-5}	1	2.5×10^{-5}	4.9×10^{-5}	0.9947
ABC	4.13	1	4.13	8.13	0.0463
A^2B	65.27	1	65.27	128.41	0.0003
A^2C	205.20	1	205.20	403.67	<0.0001
AB^2	22.48	1	22.48	44.23	0.0027
A^2B^2	77.83	1	77.83	153.11	0.0002

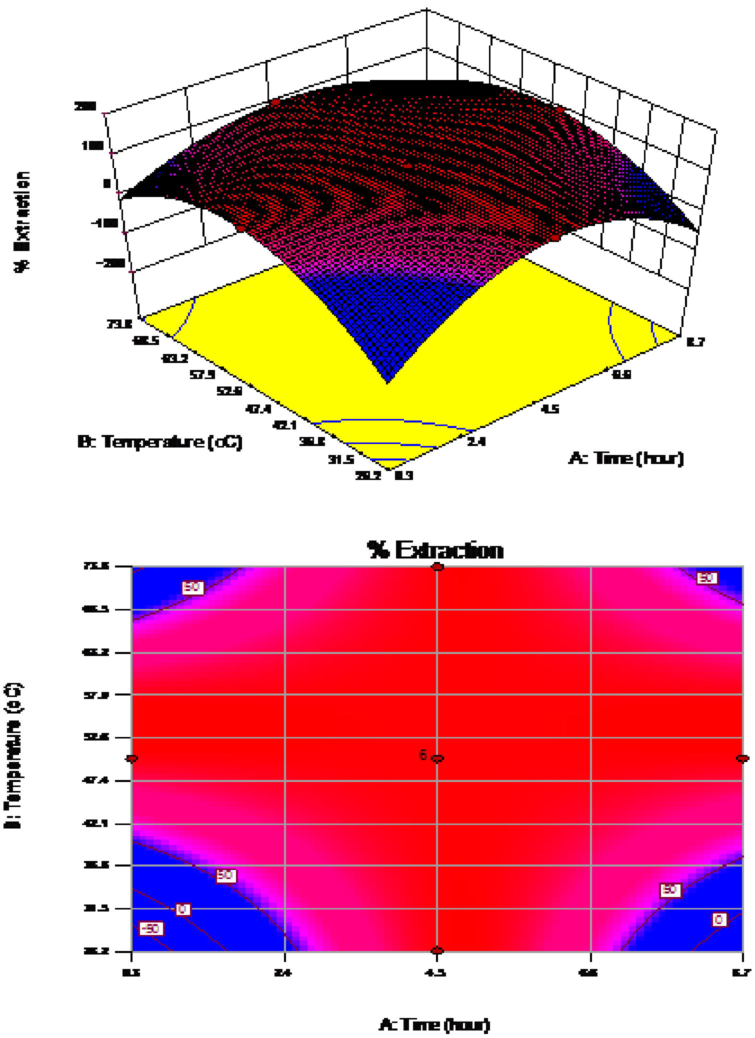

Fig. 2. Response surface and contour plots for the effect of temperature and time on.

it is desirable value. The model F value of 141.59 implies that the model is significant. In case of AB, AC, BC, ABC, A2B, A2C, AB2, A2B2, the values of "Prob>F" is less than 0.0500 and indicates that these model terms are significant and others are not significant.

From the CCD design, the optimum condition for extraction rate of resveratrol was chosen and the real model is presented as follows in terms of coded factors:

$$\text{Extraction rate} = + 14.21 + 0.23A + 0.059B + 0.065C + 1.32AB + 1.81AC + 3.06BC – 0.3A^2 – 0.39B^2 – 0.0017C^2 – 0.72ABC – 4.44A^2B – 7.87A^2C – 2.60AB^2 + 9.36A^2B^2 \quad (3)$$

From Table 2, it was observed that the predicted value of optimum conditions for highest extraction rate of resveratrol are 8.66 hours at 52.6°C and 1:15 solvent to solid ratio.

Optimization of the process

To describe the main and interactive variables on the dependent variables it is required to draw the 3D surface plots and hence we have drawn the 3D surface plots and contour plots by imposing a constant value.

The effect of %extraction, temperature and solvent to solid on response are shown in Figure 2, 3 and 4 wherein the contour plots for extraction rate of resveratrol are also shown. The plots are based on the interactions between the variables within the studied parameter. From the figure it was seen that rate of extraction increases up to 8.66 hours and beyond this point it remains constant or drops steadily. Solvent to solid ratio also indicates the same trend and gives the optimum value at 1:15 ratio. Similarly, when temperature became high, the extraction time decreased and optimum temperature is found to be 50.6°C for extraction of resveratrol.

Table 4. Validation of data and Model.

Run	Rate of extraction of Resveratrol		Residual	Error%
	Experimental	Predicted		
1	20.86	20.86	0	0
2	25.17	25.17	0	0
3	14.32	14.28	0.04	0.2
4	13.56	13.56	0	0
5	17.33	17.33	0	0
6	13.49	13.49	0	0
7	14.98	14.98	0	0
8	44.12	44.12	0	0
9	31.66	31.66	0	0
10	13.24	13.18	0.06	0.45
11	15.00	15.00	0	0
12	13.39	13.38	0.01	0.07
13	14.55	14.55	0	0
14	13.23	13.23	0	0
15	14.33	14.33	0	0
16	14.34	14.32	0.02	0.13
17	13.23	13.23	0	0
18	14.55	14.55	0	0
19	13.43	13.43	0	0
20	13.99	13.99	0	0

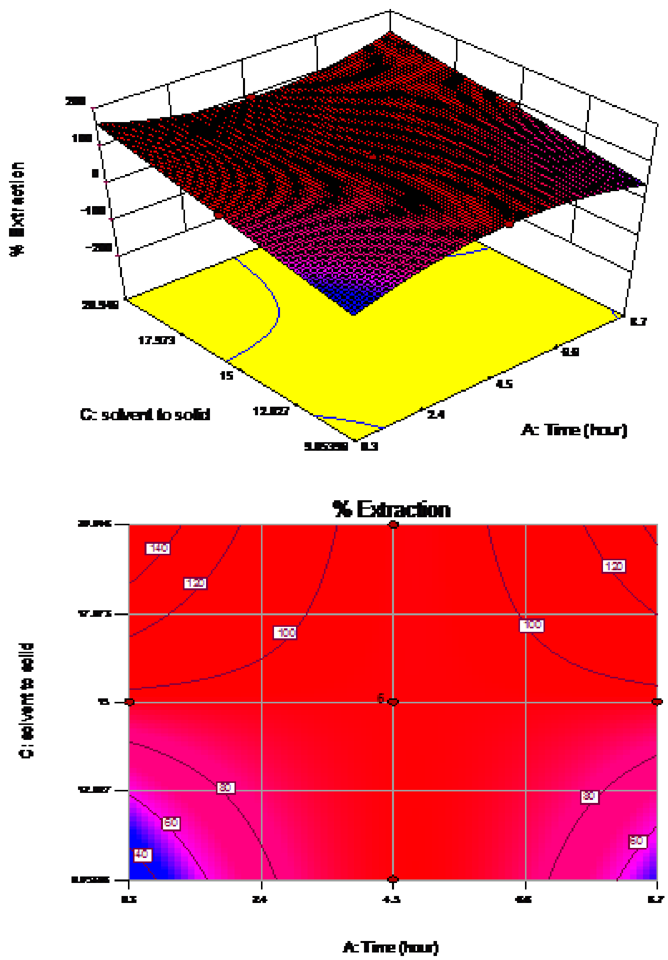

Fig. 3. Response surface and contour plots for the effect of solvent to solid ratio and time %Extraction of Resveratrol.

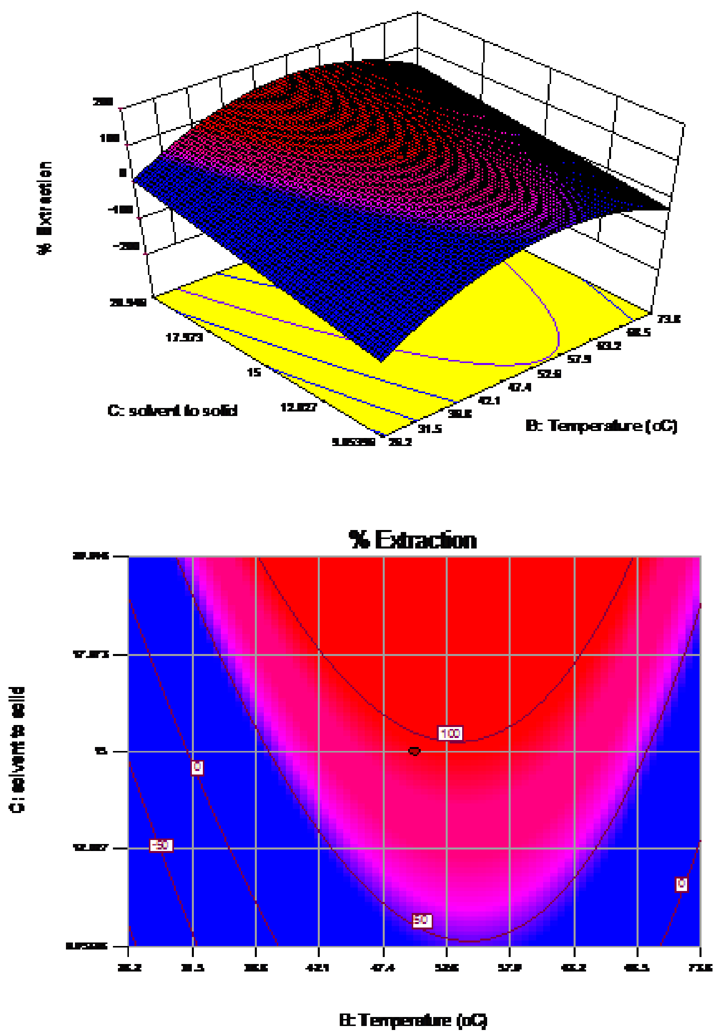

Fig. 4. Response surface and contour plots for the effect of solvent to solid ratio and temperature on %Extraction of Resveratrol.

Verification of experiment

To validate the accuracy of the model, we were carried out twenty experiments randomly for data at optimum conditions. The errors between experimental and predicted values were shown in Table 4 and it was observed that the values were in the range of 0-0.45% which are <1%. Thus it was confirmed that the proposed model was adequate for obtaining an optimal value in the range of the studied parameters.

CONCLUSION

Extraction of Resveratrol from the heartwood of *Artocarpus Lakoocha Roxb* was optimized using Design Expert version 9 software using response surface methodology to obtain the maximum yield of extraction. The independent variables involved in the optimization process are Solid to solvent ratio, time and temperature. From the RSM results, optimum parameters were found to be 8.66 hour extraction time, 50.6^{o}C temperature and 15:1 solvent to solid ratio to obtain the maximum yield of extraction of 50 mmolL^{-1}h^{-1}.

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