IJPSR (2019), Volume 10, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 May 2018; received in revised form, 06 July 2018; accepted, 22 November 2018; published 01 January 2019

MATHEMATICALLY DESIGNED BIOPROCESS FOR RELEASE OF VALUE ADDED PRODUCTS WITH PHARMACEUTICAL APPLICATIONS FROM WASTES GENERATED FROM SPICES INDUSTRIES

Meemansha Singh and Siddharth Vats *

Institute of Biosciences and Technology, Shri Ram Swaroop Memorial University, Lucknow - 225003, Uttar Pradesh, India.

Keywords:

The steam explosion, RSM, Cellulase, Xylanase, *Piper nigrum* and *Syzygium aromaticum*

Correspondence to Author:
Dr. (Er.) Siddharth Vats

Assistant Professor,
Institute of Biosciences and Technology, SRMU, Lucknow - 225003, Uttar Pradesh, India.

E-mail: vatssidd@gmail.com

ABSTRACT: A mathematical model optimizing bio-physico-chemical pre-treatment of steam exploded *Piper nigrum,* and *Syzygium aromaticum* lignocellulosic biomass waste was developed. The model was developed using, RSM, involving central composite face-centered design (CCD) with six parameters, three levels and 40 runs, at 95% confidence level. Six parameters optimized were quantity of each enzyme (a) Cellulase (ml); (b) Xylanase (ml) for enzymatic actions at 50 ºC; (c) Incubation time of 2-15 days for which substrate were dipped in various solvent systems; (d) Volume of solvent systems used; (e) Time of incubation at given steam pressure for steam explosion; and (f) Steam pressure 10-15 Psi. Cellulase and xylanase enzymes used were with activity (3.563 IU/mL), (33.32 IU/mL) respectively. ANOVA was applied for validation of the predicted model at 95% of confidence level. The Base 10 log transformation was selected from the comparative study, and the model predicted 13.79 µmoles/mL, the release of polyphenols at 2 days of incubation of substrate with 10 ml of the solvent system, under 15.0 psi steam explosion pressure for 15 min, with the treatment of 1.00 ml of cellulose. The released polyphenols were tested against human pathogenic microbes (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Micrococcus*) and found to possess strong antimicrobial properties.

INTRODUCTION: History has witnessed a maximum number of loss to human life either by war or infectious diseases. With the domestication of animals, humans have made microbes to modify them genetically and morphologically and evolving to human hosts.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.10(1).130-38

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.10(1).130-38

Microbes are evolving, and antibiotic resistance among microbes is one of the biggest challenges which have given a blow to all possible human medicine and drug system [1].

So, it's become an urgent and highest priority to discover new medicines and new drugs with better efficacy and target specificity. Effective and cheap medicines can be made available to everyone if the overall cost of the process decreases while productivity increases. More than 25 percent of total drugs available in the market are made up of phytochemicals or plant's derived substances [2]. Phytochemicals like polyphenols have protective

role against heart diseases, cancers and degenerative diseases [3, 4, 5]. Poly-phenols include flavonols, flavanones, flavones, flavon-3-ols, iso-flavones, *etc*. Herbs and spices are an integral part of South Asian diet and are very rich in phytochemicals. Ayurveda the oldest known medicinal practice exploits the goodness of phytochemicals present in plants, herbs and spices [6, 7]. In Indian traditional medicine system, Ayurveda, use of herbs and spices for health benefits includes the use of turmeric, ginger, cinnamon, basil, and mace, *etc*. [8] Phyto-chemicals obtained from spices can be a source to various drugs and medicines. *Piper nigrum* and *Syzygium aromaticum* lignocellulosic biomass wastes generated from industries associated with oil, and its derivative products can be used for the extraction of phytochemicals by employing bioprocess based techniques.

According to the report released by Spices Board of India, 37000 tonnes of pepper and 2060 tonnes of clove were produced in the year [14, 15] respectively. Even 1% waste of this huge amount would be a large quantity. This biomass can be exploited for the extraction of valuable phytochemicals. Like in other lignocellulosic biomass lignin provide a structural framework, which holds cellulose and hemicelluloses combined and embedded within it [9]. To disturb this structural framework, pre-treatment of lignocellulosic biomass is must to ease the extraction of phytochemicals [10]. Biological pre-treatment methods for *Piper nigrum* and *Syzygium aromaticum* lignocellulosic biomass wastes generated from industries is better than chemical and physical treatment methods, being eco-friendly, specific and producing fewer side products. Steam explosion based enzymatic pre-treatment process is significantly influenced by factors like steam pressure, the ratio at which each enzyme used and incubation time with solvent system, incubation time during the steam explosion, steam explosion pressure and solvent system volume.

Practically, optimizing "One variable at a time" approach disregards the complex interactions among parameters. Out of various new statistical optimization methods, RSM combines mathematical and statistical techniques for analyzing the problem with several independent variables having control on a dependent variable [11, 12]. In the present study, optimization was done through stepwise experimental strategy. First, few wet lab experiments were conducted and based on them, screening of most significant factors was done and then optimization of significant components. A mathematical model has been generated with all possible relations among optimized factors to maximize polyphenols release.

MATERIALS AND METHODS:

Chemicals and Materials: All chemicals used in this study were of analytical grade and purchased from Himedia and S. D. Fine chemicals.

Enzyme Unit: Enzyme activity was calculated in units/mL which is defined as the amount of enzyme catalyzing the production of one-mole micromole of colored product per ml/min.

***Piper nigrum* and *Syzygium aromaticum* Biomass Waste and Steam Explosion:** *Piper nigrum* and *Syzygium aromaticum* lignocellulosic biomass used were obtained from various small scale industries in and around Lucknow, producing pepper and clove oils. For pre-treatment of *Piper nigrum* and *Syzygium aromaticum* biomass waste was soaked with the different solvent system as mentioned in **Table 1**.

TABLE 1: USE OF DIFFERENT SOLVENT SYSTEMS FOR EXTRACT PREPARATION

S. no.	Conical flask (100 ml)	Solute: Solvent (w/v; 5g:20 ml)	Solvent (v/v)	Solution system (w/v)
1.	A	*Syzygium aromaticum*	Ethanol (100%)	5g/20ml
2.	B	*Syzygium aromaticum*	DW (100%)	5g/20ml
3.	C	*Syzygium aromaticum*	Ethanol + DW(1:1)	5g/10ml:10ml
4	D	*Piper nigrum*	Ethanol (100%)	5g/20ml
5	E	*Piper nigrum*	DW (100%)	5g/20ml
6	F	*Piper nigrum*	Ethanol + DW (1:1)	5g/10ml:10ml

This system was kept undisturbed for 2-15 days. This chemical time-based pre-treatment of *Piper nigrum* and *Syzygium aromaticum* was carried out to disturb its lignocellulosic structure and provide easy access to enzymes for biological pre-treatment. After this steam explosion was carried at

121 ºC temperature, 15 Psi, maintained for 8-10 min. Pre-treated solid cellulosic residues were collected and treated with enzymes, 0.5 g dry substrate / 15 ml of 100 mM sodium phosphate buffer pH 6-13, was taken and crude enzymes were added according to the values for hydrolysis [14, 15, 16] as suggested by RSM in a 100 ml flasks, incubated at 120 rpm for 12 h at 50 ºC [17] and filtered [18].

Experiment Description: 5 g *Piper nigrum* and *Syzygium aromaticum* wastes were taken in 250 ml Erlenmeyer flasks. To form mathematical model, it was necessary to perform few wet lab experiments, to pre-treat steam exploded *Piper nigrum* and *Syzygium aromaticum* with enzymes in different ratios for hydrolysis. The values suggested by RSM were obtained by setting RSM, CCD with replicated of factorial points of 1, and replicates of axial star point value as 1 and center points value 6 with K value >5 (alpha =1.56508), and 40 runs. A design was then suggested by the software

Design Matrix 10 (Stat-Ease, Minneapolis, MN), according to which practical values were set and total polyphenols values were calculated. 6 Factors namely A, B, C, D, E, F were used. Design Matrix with evaluation for Response Surface Quadratic Model. No aliases found for Quadratic Model. Aliases were calculated based on our response selection, taking into account missing data points, if necessary. The design had degree of freedom (df) evaluations for the model (27), residuals (12), lack of fit value (7) and pure error degree of freedom (5) where minimum valued recommended for df for lack of fit is 3 and, 4 for pure error. This ensures a valid lack of fit test. Fewer df will lead to a test that may not detect lack of fit. Data collected was then, used in the software, Design expert 10 (Stat-Ease, Minneapolis, MN), the range was filled in the software as shown in **Table 2.1**, to help Software to generate a model, which was used to perform wet lab experiments as shown in **Table 2.2**, and experimental values of polyphenols (PP) release was fed. Once the practical was performed for all set as per the model suggestion the value obtained was entered in the design layout the view.

A response was chosen by clicking on the corresponding node under analysis. Six steps were performed. The first step was transformation where response node and transformation was chosen. As in our case, we have chosen base 10 Log, after comparing with other values of other statistical parameters. Fit summary data was obtained and used to evaluate models in step 2. Step three involves the choosing of the model order and desired terms from the list. After this, analysis of variance was performed based on which a mathematical model was developed based on results, showing coded and actual value equations.

Diagnostics of the model was the fifth step where model fit values and transformation choice was evaluated. In the lasts step, model graphs were obtained and analyzed for interpretation and evaluating the final model. To optimize the parameters, the software was then fed priority values for each factor and based on that; software predicted values for PP release at a different set of conditions.

TABLE 2.1: CODED AND ACTUAL VALUES OF PARAMETERS

Name / Units	Incubation (Days)	Volume (ml)	Steam explosion (Psi)	Psi time (min)	Cellulase (ml)	Xylanase (ml)
LOW	2	10	10	8	0	0
HIGH	15	20	15	15	1	1
(-)ALPHA	-1.67305	7.174577	8.587289	6.022204	-0.28254	-0.28254
(+)ALPHA	18.67305	22.82542	16.41271	16.9778	1.282542	1.282542

Developing Matrix and Empirical Relationship: For evaluation software used was Design expert 10. Using response surface methodology, the six parameters of Incubation time, the volume of the solvent system, steam Explosion pressure, Psi time of incubation, the volume used of cellulase and xylanase enzymes. Representation of independent factors in quantitative form can be given as:

$$Y = Ø\ (x_1, x_2, \ldots\ldots\ldots\ldots x_k) \pm e_r \quad (1)$$

Where, Y and x_1, x_2, x_3.....x_k with k quantitative factors, e_r is a measure of experimental error. Ø represents response functions.

Representing the PP (Poly-phenol) released and the responses as functions of A, B, C, D, and E; PP= *f* (A, B, C, D, E, F)

The second order polynomial (regression) equation used to represent the response surface PP is:

$$Y= b_0+\sum b_i x_i + \sum b_{ii} x^2_i + \sum b_{ij} x_i x_j + e_r \quad (2)$$

TABLE 2.2: EXPERIMENTAL SETUP AS SUGGESTED BY SOFTWARE BASED ON RSM

Std	Block	Run	Factor 1 A incubation (Days)	Factor 2 B Volume (ml)	Factor 3 C Steam Expl. (Psi)	Factor 4 D: Psi Time (min)	Factor 5 E. Cellulose	Factor 6 F Xylanase (ml)	Response 1 Total Poly - Phe mg/ml	O.D @405 nm
16	Block 1	1	15	10	10	8	1	1	3.6421	1.147
27	Block 1	2	8.5	15	8.587289	11.5	0.5	0.5	0.9375	0.295
1	Block 1	3	2	10	15	15	0	1	1.039	0.327
21	Block 1	4	15	20	15	8	1	0	1.3222	0.416
3	Block 1	5	2	10	10	8	1	0	1.2324	0.388
19	Block 1	6	2	10	10	15	0	0	0.9186	0.289
10	Block 1	7	15	10	15	15	1	1	3.8734	1.220
11	Block 1	8	15	10	10	15	1	0	1.3288	0.418
9	Block 1	9	15	10	15	8	0	0	1.1134	0.350
5	Block 1	10	2	20	10	15	0	1	1.2596	0.397
28	Block 1	11	8.5	15	16.41271	11.5	0.5	0.5	1.2596	0.397
37	Block 1	12	8.5	15	12.5	11.5	0.5	0.5	1.2261	0.386
8	Block 1	13	2	20	10	15	1	0	1.2594	0.397
39	Block 1	14	8.5	15	12.5	11.5	0.5	0.5	1.242	0.391
12	Block 1	15	15	20	10	15	1	1	4.0111	1.264
14	Block 1	16	15	20	15	8	0	0	0.8126	0.256
18	Block 1	17	2	10	10	8	0	1	1.2466	0.393
7	Block 1	18	15	10	10	15	0	1	1.3296	0.419
40	Block 1	19	8.5	15	12.5	11.5	0.5	0.5	1.25	0.394
24	Block 1	20	18.67305	15	12.5	11.5	0.5	0.5	1.0126	0.319
20	Block 1	21	2	20	15	15	1	1	3.9123	1.232
38	Block 1	22	8.5	15	12.5	11.5	0.5	0.5	1.256	0.396
29	Block 1	23	8.5	15	12.5	6.022204	0.5	0.5	1.0288	0.342
36	Block 1	24	8.5	15	12.5	11.5	0.5	0.5	1.299	0.409
35	Block 1	25	8.5	15	12.5	11.5	0.5	0.5	1.312	0.413
30	Block 1	26	8.5	15	12.5	16.9778	0.5	0.5	1.0122	0.319
25	Block 1	27	8.5	7.174577	12.5	11.5	0.5	0.5	0.9299	0.293
2	Block 1	28	15	20	10	8	0	0	0.8284	0.261
22	Block 1	29	2	10	15	8	1	1	3.8122	1.201
17	Block 1	30	2	20	15	8	0	0	0.8126	0.256
33	Block 1	31	8.5	15	12.5	11.5	0.5	-0.28254	0.5369	0.169
23	Block 1	32	-1.67305	15	12.5	11.5	0.5	0.5	1.0298	0.324
15	Block 1	33	2	10	15	15	1	0	1.2288	0.387
13	Block 1	34	2	20	10	8	1	1	3.7124	1.169
6	Block 1	35	15	20	15	8	0	1	1.3129	0.413
34	Block 1	36	8.5	15	12.5	11.5	0.5	1.282542	1.4111	0.444
4	Block 1	37	15	20	15	15	0	0	0.8389	0.262
32	Block 1	38	8.5	15	12.5	11.5	1.282542	0.5	1.4126	0.445
26	Block 1	39	8.5	22.82542	12.5	11.5	0.5	0.5	1.0349	0.326
31	Block 1	40	8.5	15	12.5	11.5	-0.28254	0.5	0.5169	0.163
41	Block 2	41	5.25	17.5	12.5	9.75	0.25	0.25	0.12	0.038
42	Block 2	42	8.5	12.5	11.25	13.25	0.75	0.75	0.13	0.041

PP released based on six factors, the selected polynomial could be expressed as:

$$b_0 + b_1(A) + b_2(B) + b_3(C) + b_4(D) + b_5(E) + b_6(F) + b_{11}(A^2) + b_{22}(B^2) + b_{33}(C^2) + b_{44}(D^2) + b_{55}(E^2) + b_{66}(F^2) + b_{12}(AB) + b_{13}(AC) + b_{14}(AD) + b_{15}(AE) + b_{16}(AF) + b_{23}(BC) + b_{24}(BD) + b_{25}(BE) + b_{26}(BF) + b_{34}(CD) + b_{35}(CE) + b_{45}(DE) \quad (3)$$

Where, Intercept is A- Incubation, B- Volume, C- Steam Explosion, D- PsiTime, E- Cellulase, F- Xylanase.

Preparation of Microbial Culture and Plates for Test: Microbial culture of 10^6 colonies forming unit (CFU) for analysis was freshly prepared from stock solution. Muller Hinton broth (MHB) was sterilized and autoclaved. 15 g / 1000 ml of (MHB: DW) distilled water of MHB was used as a media for inoculation of pathogenic microbes. Media prepared had a pH 7.0 and post inoculation, incubated for 24 h at 28 ± 2 ºC @ 120 rpm in an incubator shaker. Plates were prepared of Muller Hinton agar (MHA) to check the antimicrobial activity of extracts.

Assay for Antimicrobial Activity of Against Gram Positive and Gram Negative Microbes: Modified Bauer-Kirby well diffusion method was employed [18, 19]. Muller Hinton agar (MHA) plates made up of autoclaved MHA media and had bacteria swabbed (100 µl), were subjected to well preparation of 8 mm diameter size. These wells were then impregnated with 20, 30 50 µl of phytochemical extract. These plates were then incubated overnight at 28 ± 2 ºC, and the zone of inhibition around the well was measured. Large zones of inhibition around the disc indicated susceptibility of microbe toward the polyphenol extract. While, small zones of inhibition or no zones of inhibition were an indicator of resistive microbes. The method followed was according to the Clinical Laboratory standards Institute (CLSI) recommended.

RESULTS AND DISCUSSION:

Standardization of Moisture Content: *Syzygium aromaticum* and *Piper nigrum* waste in powdered form was taken, dried at room temperature 25 ± 5ºC, to prevent loss of valuable phytochemicals. The substrate was weighed at an interval of one hour as gap time where they were kept in plastic bags. The procedure was repeated until the weight was standardized as a measure of loss and gain of moisture content was uniform for each hour. The work was carried out with two sets (one was oven dried, and one was microwave dried) to find the average weight **Table 3**.

TABLE 3: STANDARDIZATION OF MOISTURE CONTENT IN THE BIOMASS

Sets**	Glass plates (g)	Powder and watch glass (g)	Dried with	Weight loss	Permissible limit
1	67.5464	86.4852 g	Oven	1.8 g	6.1% / 10%
2	65.0954	86.5815 g	Microwave	2.1 g	5% / 10%

Central Composite Design: Co-efficient were obtained by applying central composite design, using the Design Expert Statistical software package (Stat-Ease, Minneapolis, MN), at 95% confidence level, significant coefficients were determined, used for finding final empirical relationships to estimate polyphenols (PP), and with equations showing empirical relationships for polyphenol (PP). In case of the mixture of design coded equations are determined first and then from this the actual equations, by replacing each term in coded equations with its coding formula:

$$X_{coded} = X_{actual} - \bar{X} / [(X_{high} - X_{low})/2] \quad (4)$$

While substituting in the quadratic term will generate the result in new quadratic coefficients and correction in the intercept.

TABLE 4: STATISTICAL DATA RELATED TO DIFFERENT TRANSFORMATION TO CHOOSE THE MOST SUITABLE TRANSFORMATION

S. no.	Transform	Lack of fit P-value		Adjusted "R" square		Predicted "R" squared		F-value	Prob>F	Model	Lack of fit significant or not
		Linear (< 0.0001)	Quadratic (< 0.0001)	Linear (<0.0001)	Quadratic (<0.0001)	Linear (<0.0001)	Quadratic (<0.0001)				
1	None	< 0.0001	< 0.0001	0.5639	0.7319	0.3581	-3.7624	9.62	<0.0001	Quadratic	Sign.
2	Square root	<0.0001	<0.0001	0.6227	0.7299	0.4382	-2.9608	12.0	<0.0001	Quadratic	Signit
3	Natural log	<0.0001	< 0.0001	0.6725	0.6943	0.5193	-2.4233	14.69	<0.0001	Quadratic	Sign.
4	Base 10 log	<0.0001	<0.0001	0.6725	0.6943	0.5193	-2.4233	14.69	<0.0001	Quadratic	Sign.
5	Inverse square root	<0.0001	<0.0001	0.6878	0.6121	0.5836	-2.6828	15.68	<0.0001	Quadratic	Sign.
6	Inverse	<0.0001	<0.0001	0.6458	0.4940	0.5700	-4.1575	13.16	<0.0001	Quadratic	Sign.
7	Power	<0.0001	<0.0001	0.5639	0.7319	0.3581	-3.7624	9.62	<0.0001	Quadratic	Sign.
8	Logit	--	--	--	--	--	--	--	--	--	--
9	Arc sine square root	--	--	--	--	--	--	--	--	--	--

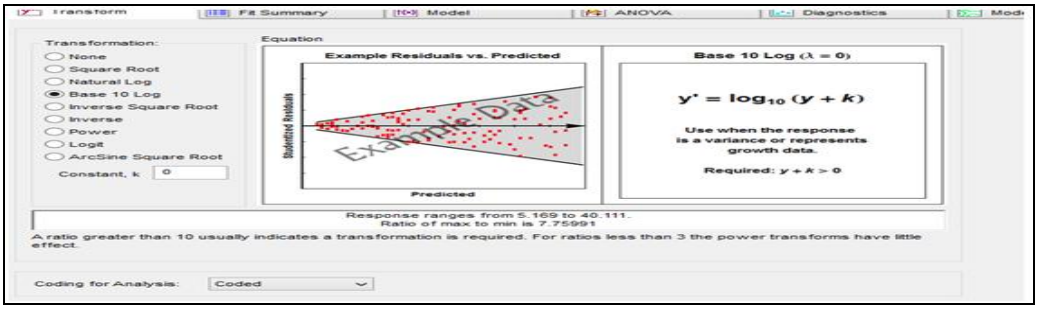

FIG. 1: Base Log 10 TRANSFORMATION

Choice of a Transformation: After comparing different statistical parameters as shown in **Table 4** the most suitable transformation was chosen. Base 10 Log was chosen after analyzing the obtained data **Fig. 1**.

Final Equation in Terms of Coded Factors:

Log 10 (Total Poly Phenol) = + 1.00 + 0.012*A - 4.507E - 003*B + 0.011*C - 4.512E - 003*D + 0.16*E + 0.15*F - 9.727E - 003*AB + 5.688E - 003*AC + 5.104E - 003*AD - 2.951E -03*AE + 2.513E - 003*AF - 6.343E -004*BC + 0.010*BD + 0.010*BE + 0.016*BF - 0.012*CD + 0.012*CE - 7.929E - 004*CF + 3.646E - 003*DE - 2.997E - 003*DF + 0.083*EF + 0.037*A^2 + 0.030*B^2 + 0.048*C^2 + 0.036*D^2 + 5.029E003*E^2 + 8.300E - 003*F^2 .....(6)

Preliminary one factorial wet lab experiments were perfomed to get the ranges, and it had been identified that parameters described in the **Table 3** were chosen out of various parameters and were focussed to be optimised. Statistically model was evalueated by the F-test for analysis of variance (ANOVA) as shown in **Table 5** and **6**. The model F-value of 4.80 implies the model is significant. There is only a 0.33% chance that an F-value this large could occur due to noise. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case E, F, EF is significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

If there are many insignificant model terms (not counting those required to support hierarchy), the model reduction may improve your model. The "Lack of Fit F-value" of 174.02 implies the lack of fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is bad- we want the model to fit.

TABLE 5: ANOVA FOR RESPONSE SURFACE QUADRATIC MODEL (ANALYSIS OF VARIANCE TABLE [PARTIAL SUM OF SQUARES - TYPE III])

Source	Sum of Squares	df	Mean Square	F Value	P-value Prob>F	
Model	1.76	27	0.065	4.8	0.0033	Significant
A-Incubation	2.66E-03	1	2.66E-03	0.2	0.6659	
B-Volume	3.78E-04	1	3.78E-04	0.028	0.8704	
C-Steam Explosion	2.45E-03	1	2.45E-03	0.18	0.6785	
D-PsiTime	3.78E-04	1	3.78E-04	0.028	0.8703	
E-Cellulase	0.45	1	0.45	33.46	< 0.0001	
F-Xylanase	0.42	1	0.42	30.95	0.0001	
AB	1.58E-03	1	1.58E-03	0.12	0.7389	
AC	5.41E-04	1	5.41E-04	0.04	0.8452	
AD	4.36E-04	1	4.36E-04	0.032	0.8609	
AE	1.46E-04	1	1.46E-04	0.011	0.9193	
AF	1.06E-04	1	1.06E-04	7.77E-03	0.9312	
BC	6.73E-06	1	6.73E-06	4.95E-04	0.9826	
BD	1.82E-03	1	1.82E-03	0.13	0.7207	
BE	1.78E-03	1	1.78E-03	0.13	0.7235	
BF	4.14E-03	1	4.14E-03	0.3	0.5909	
CD	2.27E-03	1	2.27E-03	0.17	0.69	
CE	2.42E-03	1	2.42E-03	0.18	0.6807	
CF	1.05E-05	1	1.05E-05	7.73E-04	0.9783	
DE	2.22E-04	1	2.22E-04	0.016	0.9004	
DF	1.50E-04	1	1.50E-04	0.011	0.918	
EF	0.11	1	0.11	8.38	0.0134	
A^2	0.018	1	0.018	1.32	0.2737	
B^2	0.012	1	0.012	0.85	0.3736	
C^2	0.03	1	0.03	2.23	0.1614	
D^2	0.018	1	0.018	1.31	0.2752	
E^2	3.37E-04	1	3.37E-04	0.025	0.8774	
F^2	9.19E-04	1	9.19E-04	0.068	0.7993	
Residual	0.16	12	0.014			
Lack of Fit	0.16	7	0.023	174.02	< 0.0001	Significant
Pure Error	6.67E-04	5	1.33E-04			
Cor Total	1.92	39				

TABLE 6: STATISTICAL DATA

Std. Dev.	0.12	R-Squared	0.9152
Mean	1.11	Adj R-Squared	0.7245
C.V. %	10.46	Pred R-Squared	-1.8072
PRESS	5.4	Adeq Precision	8.321
-2 Log Likelihood	-106.58	BIC	-3.29
		AICc	97.06

A negative "Pred R-Squared" implies that the overall mean a better predictor of your response than the current model. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. This ratio of 8.321 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Actual Factors:

Log 10 (Total Poly Phenol) = + 2.71928 - 0.015292 ***** Incubation - 0.045202 * Volume - 0.17738 * Steam Explosion - 0.064210 * Psi Time - 0.070795 * Cellulase + 0.029204 * Xylanase - 2.99295E - 004 * Incubation * Volume + 3.50050E -004 * Incubation * Steam Explosion + 2.24374E - 004 * Incubation * Psi Time - 9.08104E – 004 * Incubation * Cellulase + 7.73276E - 004* Incubation * Xylanase - 5.07464E – 005 * Volume * Steam Explosion + 5.96364E - 004 * Volume * Psi Time + 4.13134E - 003 * Volume * Cellulase + 6.29789E - 003 * Volume * Xylanase - 1.33166E - 003 * Steam Explosion * Psi Time + 9.61954E - 003 * Steam Explosion * Cellulase - 6.34284E - 004 * Steam Explosion * Xylanase + 2.08330E - 003 * Psi Time * Cellulase - 1.71270E - 003 * Psi Time * Xylanase + 0.33024 * Cellulase * Xylanase + 8.66754E-004 * Incubation 2 + 1.18022E-003* $Volume^2$ + 7.62332E - 003* Steam Explosion 2 + 2.97953E - 003 *$Psi\ Time^2$ + 0.020116 * $Cellulase^2$ +0.033201* $Xylanase^2$ ....(7)

Effect of Enzyme on the Structure of *Piper nigrum* and *Syzygium aromaticum* lignocellulosic Biomass Waste: After optimizing the parameter for enzymatic pretreatment, strucutral change in the *Piper nigrum* and *Syzygium aromaticum* lignocellulosic biomass waste were studied and change in size was noted, and this change in the structure of the biomass might be one reason for enhanced release of polyphenols after pre-treatment. Disturbance in the crystallinity of the lignocellulosic biomass makes them more prone to enzymatic hydrolysis. Crystallinity of biomass influences the hydrolysis [20].

Steam explosion and then further enzymatic treatment of *Piper nigrum* and *Syzygium aromaticum* lignocellulosic biomass by disrupting inters and intra hydrogen bonding of cellulose fibrils [21] can be the reason for enhanced polyphenol release. Other reason could be amorphous sites on lignin which because of steam explosion got disturbed and enzyme were prevented from being getting bonded to lignin, instead of attacking the cellulose and increasing their efficiency [22].

Synergistic Antimicrobial Activity of Extract Prepared: All the extracts prepared were tested for antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Micrococcus.* The results obtained are listed in **Table 7**.

Need of Polyphenol in Combating the Diseases: Pathogens and diseases have affected human and its live stocks since ages. The ultimate source of drugs is medicinal plants and herbs which are abundant. Antibiotics are losing their edge in the fight against diseases and pathogens. Many antibiotic resistance microbes like vancomycin-resistant *Enterococcus* (VRE), PRSA, MRSA, quilone, and ciprofloxacin resistance *P. Aeruginosa* (QCPRA) pose a challenge to our well being. Many foodborne pathogens such as *E. coli*, *Salmonella*, and *Campylobacter* responsible for diarrhea and gastroenteritis have resistance towards antibiotics. The stem of *Fadogia agrestis* showed the presence of saponins, steroids, terpenoids, flavonoids, tannins, anthraquinone, glycosides and alkaloids. Extract demonstrated antibacterial activity against *S. aureus, S. spp. B. subtilis* and *E. coli* [2]. A diet rich in phytochemicals may decrease the chances of deadly diseases like heart diseases & cancers [5].

Vegetables, berries and fruits, and beverages are good sources of flavonoids and are associated with reducing the risks of no. of diseases, flavonoids have shown their effects on immune system both *in-vitro* as well as *in-vivo* [23]. Polyphenols of low molecular weight and are having three-ring structures and are of various types based on the different substitutions [23]. Flavonoids play a number of important roles in plants as antimicrobials, antioxidant, attractors, light receptors, and many other biological activities [24]. And the main possible mechanism is their antioxidant activity.

TABLE 7: ANTIMICROBIAL ACTIVITY OF EXTRACT PREPARED UNDER DIFFERENT SET OF CONDITIONS

Combination	Sample	A		B		C		D	
		Min(mm)	Max(mm)	Min(mm)	Max(mm)	Min(mm)	Max(mm)	Min(mm)	Max(mm)
8.P.N.	D.W.E	4	8	5	9	5	12	5	12
15.P.N.	D.W.E	4	8	5	9	5	10	4	13
8.S.A	D.W.E	4	7	5	10	4	8	3	9
15.S.A.	D.W.E	5	9	5	10	5	9	4	9
8.P.N.	E	5	10	5	9	5	10	4	11
15.P.N.	E	5	10	5	9	4	8	4	10
8.S.A	E	5	11	5	9	5	9	5	9
15.S.A.	E	5	10	4	8	4	8	5	10
8.P.N.	D.W.	5	9	5	9	5	10	5	11
15.P.N.	D.W.	5	10	5	9	5	10	5	8
8.S.A	D.W.	4	8	4	8	5	10	4	8
15.S.A.	D.W.	5	9	4	8	5	10	4	8

Where A, B, C, and D are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micro coccus*. 8P.N.= 8 min steam explosion treated *Piper nigrum*, 15P.N. = 15 min steam explosion treated *Piper nigrum*, where as S.A. stands for *Sygium arromaticum*, D.W. distilled water E = ethanol

Antioxidants are evolved as an important part of natural defense mechanism among living organisms [25]. These are the molecules which scavenge the free radicals species and inhibit the chain reactions which can damage vital molecules of living organisms. Intake of flavonols and flavones can reduce the chances of heart disease like myocardial infarction and strokes. Methods to increase the production of phytochemicals can be done by understanding the phytochemical pathway genes, which lead to the synthesis of these compounds in fruits and other vegetables. Biochemical and molecular techniques can be used to enhance the productions. Isoflavones are the subclass of flavonoids and are scarcely distributed in nature.

The objective of using RSM CCD design was to optimize the hydrolysis conditions for enhancing the release of phytochemicals like polyphenol. One parameter at one time, based optimization cannot study the combined effect of all variables. There have been various studies for optimization of reducing sugar released and bioethanol production from lignocellulosic wastes. And pre-treatment of lignocellulosic materials is must to remove lignin and disturb the crystallinity of hemicellulose [22]. Numbers of pre-treatment methods have been introduced including physical, chemical and biological, which are used either singularly or in different combinations. Sindhu *et al*., 2012 studied surfactant assisted acid pre-treatment of sugarcane tops for bioethanol production with the help of Box-Benkhen Design and 0.798 g/g sugarcane tops, reducing sugar were obtained [26].

Similarly RSM based statistical tool was used for optimizing enzymatic hydrolysis of alkaline pretreated peroxide wheat straw, by Qi *et al*., (2009) [27], where they noted conditions like cellulase loading (40.00 FPU/g), substrate concentration 22.00 g/L, surfactant concentration 6.676 g/L with hydrolysis time of 72 h. Use of acid, chemicals, and surfactant affects environment & lead to the production of more inhibitors, while the use of a large amount of water at pre, during and post-treatment can add to cost and make it more energy consuming [22]. Presence of various phytochemicals in *Piper nigrum* and *Syzygium aromaticum* if purified can be used in pharma industries also which can turn waste in to gold. This model predicted satisfactory the minimum use of chemicals and wastage of energy with the production of value-added producers from agricultural wastes also give it an edge over other process.

CONCLUSION: From this investigation, the following important conclusions were derived:

- An empirical relation among different parameters (Where, Intercept is A-Incubation, B-Volume, C-Steam Explosion, D-PsiTime, E-Cellulase, F-Xylanase) was developed to predict the polyphenols release, at 95% confidence level, incorporating central composite design (CCD), design mode was quadratic, based on response surface study.
- The Base 10 Log transformation was selected from the comparative study, and the model predicted 13.79 µmoles/mL, the release of polyphenols at 2 days of incubation of substrate with 10 ml of the solvent system, under 15.0 Psi steam explosion pressure for 15 min, with the treatment of 1.00 ml of cellulose.

- The released polyphenols were tested against human pathogenic microbes (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Micrococcus*) and had shown antimicrobial activity against them.

ACKNOWLEDGEMENT: The authors gratefully acknowledge the SRMU, Lucknow for providing all the necessary help and support.

CONFLICT OF INTEREST: The authors declared no competing interests.

REFERENCES:

1. Ojha and Animesh K: Synthesis of well-dispersed silver nanorods of different aspect ratios and their antimicrobial properties against gram-positive and negative bacterial strains. Journal of Nanobiotechnology 2013; 11(1): 42.
2. Ameen OM, Olatunji GA, Atata RF and Usman LA: Antimicrobial activity, cytotoxic test and phytochemical screening of the extracts of the stem of *Fadogia agrestis*. Nigerian Society for Experimental Biology J 2011; 11(1): 79-84.
3. Wang H, Cao G and Prior RL: Oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural Food Biochemistry 1997.
4. Manach C, Scalbert A, Morand C, Rémésy C and Jiménez, L: Polyphenols: food sources and bioavailability. The American J of Clinical Nutrition 2004; 79(5): 727-747.
5. Boyer J and Liu RH: Apple phytochemicals and their health benefits. Nutritional J 2004; 12: 3-5.
6. Moon K, Khadabadi SS, Deokate UA and Deore SL: *Caesalpinia bonducella* F - An overview. Report and Opinion, Scientific publication J 2010; 2(3): 83-90.
7. Gupta M, Shaw BP and Mukherjee A: A new glycosidic flavonoid from *Jwarhar mahakashay* (antipyretic) Ayurvedic preparation. International J of Ayurveda Research 2010; 1: 106-111.
8. Tapsell, Linda C, Hemphill I, Cobiac L, Sullivan DR, Fenech M, Patch CS and Roodenrys S: Health benefits of herbs and spices: the past, the present, the future 2006.
9. Faulon J, Carlson GA and Hatcher PG: A three dimensional model for lignocellulosic from Gymno-spermous wood. Orga Geochem 1994; 21(12): 1169-1179.
10. Sonderegger M, Jeppsson M, Larsson C, Gorwa-Grauslund ME, Boles E, Olsson L, Spencer-Martins I, Hahn-Haegerdal B and Sauer U: Fermentation performance of engineered and evolved xylose-fermenting *S. cerevisiae* strains. Biotechnol Bioeng 2004; 87: 90-98.
11. Cochran WG and Cox GM: Experimental design. Jhon wiley, London, 1957.
12. Rajakumar S, Muralidharan C and Balasubramanian V: Optimization of the friction-stir-welding process and tool parameters to attain a maximum tensile strength of AA7075-T6 aluminum alloy. Proceedings of the Institution of Mechanical Engineers, Part B: J of Engineering Manufacture 2010; 224(8): 1175-1191.
13. Waeonukul, R, Kyu KL, Sakka K and Ratanakhanokchai K: Isolation and characterization of a multienzyme complex (cellulosome) of the *P. curdlanolyticus* B-6 grown on Avicel under aerobic conditions. J Biosci Bioeng 2009; 107(6): 610-614.
14. Ghose TK and Bisaria VS: Measurement of hemicellulase activities part 1: xylanases. Pure Appl Chem 1987; 59(12): 1739-1752.
15. Ghose TK: Measurement of cellulase activities. Pure Appl Chem 1987; 59(2): 257-268.
16. Jadhav A, Vamsi KK, Khairnar Y, Boraste A, Trivedi S, Patil P, Gupta G, Gupta M, Mujapara AK, Joshi B and Mishra D: Optimization of production and partial purification of laccase by *P. chrysosporium* using submerged fermentation. Int J Mic Res 2009; 1(2): 09-12.
17. Janu KU, Sindh R, Binod P, Kuttiraja M, Sukumaran, RK and Pandey A: Studies on physicochemical changes during alkali pre-treatment and optimization of hydrolysis conditions to improve sugar yield from bagasse. J Sci Ind Res 2011; 70: 952-958.
18. Vats, Siddharth and Miglani K: Synergistic antimicrobial effect of cow urine and *Azadirachta indica* on infectious microbes. International Journal of Pharmaceutical Sciences and Research 2011; 2(7): 1781.
19. Vats, Siddharth, Kumar R and Negi S: Natural food that meets antibiotics resistance challenge: *In-vitro* synergistic antimicrobial activity of *Azadirachta indica*, *Terminalia chebula*, *Piper nigrum*, and photo activated cow urine. Asian Journal of Pharmaceutical & Biological Research (AJPBR) 2012; 2(2).
20. Kim, Hyun T and Lee YY: Pretreatment and fractionation of corn stover by ammonia recycle percolation process. Bioresource Technology 2005; 96(18): 2007-2013.
21. Jeoh T, Ishizawa CI, Davis MF, Himmel ME, Adney WS and Johnson DK: Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. Biotechnol Bioeng 2007; 98(1): 112-22.
22. Kumar P, Barrett DM, Delwiche MJ and Stroeve P: Methods for pre-treatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 2009; 48(8): 3713-29.
23. Middleton, Elliott, Kandaswami C and Theoharides TC: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 2000; 52(4): 673-751.
24. Pietta and Pier-Giorgio: Flavonoids as antioxidants. Journal of Natural Products 2000; 63(7): 1035-1042.
25. Jovanovic and Slobodan V: Flavonoids as antioxidants. J of the American Chemical Soc 1994; 116(11): 4846-4851.
26. Sindhu and Raveendran: A novel surfactant-assisted ultrasound pretreatment of sugarcane tops for the improved enzymatic release of sugars. Bioresource Technology 2013; 135: 67-72.
27. Qi and Benkun: Optimization of enzymatic hydrolysis of wheat straw pretreated by alkaline peroxide using response surface methodology."Industrial & Engineering Chemistry Research 2009; 48(15): 7346-7353.

How to cite this article:
Singh M and Vats S: Mathematically designed bioprocess for release of value added products with pharmaceutical applications from wastes generated from spices industries. Int J Pharm Sci & Res 2019; 10(1): 130-38. doi: 10.13040/IJPSR.0975-8232.10(1).130-38.