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OPTIMIZING THE PROCESS VARIABLES FOR EXTRACTING SEEDS OF *CASSIA TORA* BY PLACKETT-BURMAN DESIGN AND EVALUATION OF ANTIBACTERIAL ACTIVITY

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ABSTRACT

The purpose of the present study was to develop an optimized process for extracting seeds of *Cassia tora*, and evaluate its antibacterial activity. Plackett-Burman design was employed for extracting *Cassia tora* with the type of apparatus used (X_1), powder size, (X_2), duration of extraction (X_3), type of water for extraction (X_4), method of removal of solvent (X_5), and method of drying the extract (X_6) as independent variables. The percentage yield was considered as dependent variable. The main effect and interaction terms were quantitatively evaluated using a mathematical model. The results indicate that X_1 and X_2 significantly affected the percentage yield. But the use of different types of water was non-significant. Regression analysis and numerical optimization were performed to identify the best method. The predicted values agreed well with the experimental values, and the results demonstrate the practicability of the model in the development of extraction process. Antibacterial evaluation revealed that aqueous extract was more effective and *S. aureus* was the most susceptible organism. The present study supports the use of aqueous extracts of *Cassia tora* seeds for treating bacterial infection and skin diseases like psoriasis.

Key words: *Cassia tora*, Plackett-Burman, Process validation, Extraction

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INTRODUCTION

Cassia tora,(F : Cesalpinaceae), commonly known as 'Chakunda' is a small plant growing on dry soil in Bengal and throughout the tropical parts of India[1]. Leaves, seeds and root are used in internal as well as external preparations. Seeds constitute valuable remedy in bacterial infections and skin diseases[2]. It is reported to be highly useful in psoriasis, leprosy and sciatic pain in joints[3,4]. Apart from skin disease, the seeds are useful for various ailments[5].

With increasing demand for the herbal drugs and rising cost of solvents and the process of extraction, it is worthwhile to optimize the process variables and optimize the extraction process, so as to obtain high yield of the extract[6-7]. There are various factors which affect the extraction process either alone or by interaction with one another such as type of apparatus, type of solvent, powder size of the drug, duration of extraction, method of removal of solvent, method of drying to list a few depending upon the nature of the drug[7-10]. These factors eventually affect the percentage yield of the extract and alter the process. The process is said to be better, if it gives high percentage yield of the extract.

Classical optimization studies use one-factor-at-a-time approach, in which only one factor is variable at a time while all others are kept constant. This approach is time consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated and misleading conclusions may be drawn. Statistical design of experiments such as Placett-Burman design can overcome these limitations, since it allows accounting for possible interaction effects between variables[11]. If adequately used, this powerful tool can provide the optimal conditions to improve the process[12]. The optimization of the extraction process using Plackett-Burman design by establishing a mathematical model would not only serve as a visual aid to have a clearer picture about the effects of various factors on extraction but also help us to locate the region where the extraction is optimized.

In the present experiment, various factors affecting the extraction process such as type of apparatus, extraction time, method of removal of solvent and method of drying which might have significant influence on the yield of extract from raw materials were considered. In order to develop an optimal way for extraction process, the influence of various extraction parameters were investigated by using statistical design of experiments for seeds of *Cassia tora* were evaluated by the application of 2-level factorial Plackett-Burman design with type of apparatus, powder size of seeds, duration of extraction, type of water for extraction, method of removal of solvent and method of drying the extract as independent extraction variables. Percentage yield of the extract was considered as dependent variable. Finally, regression analysis was performed to identify the best formulation and to validate the model by comparing the experimental results with the theoretical values of the response.

Antibacterial activity of the extract obtained by optimized process was evaluated against both gram positive and gram negative organisms.

EXPERIMENTAL

Cassia tora seeds were obtained from Natural Remedies, Veer Sandra Industrial area and authenticated in Regional Research Institute, Bangalore, were used for the study. Reverse osmosis water, demineralized water (Strides Arco Labs, Bangalore) was used.

Apparatus and chemicals: Soxhlet apparatus, reflux condenser (Borosil), rotary flash evaporator Buchi type manufactured by positive Infotech , vacuum oven manufactured S.D (India corporation), copper sieve of mesh size of #10 and #44. Reverse osmosis water, demineralized water (Strides Arco Labs, Bangalore) All the chemicals and solvents used were of AR grade from Rankem Ltd., India.

Experimental design for extraction

The Plackett-Burman design belongs to the class of non-geometric orthogonal two-level arrays with 12 runs and may be used for an experiment containing up to 11 factors and the design allow the estimation of all main effects and interactions for any factors free of aliasing if other factors can be assumed inert. These designs are very useful for economically detecting large main effects. In accordance with the experimental design, 12 experiments were conducted and the data points obtained were then statistically analyzesd to determine the effect of studied variables. The 6 independent process variables selected for this study were

X_1: Type of the apparatus used; X_2: Powder size; X_3: Duration of extraction; X_4: Type of water for extraction; X_5: Method of removal of solvent;X_6: Method of drying the extract; The dependent variable was percentage yield (Y) of the *Cassia tora* seed extract.

Extraction of seeds of *Cassia tora*

The seeds of the *Cassia tora* were extracted following Placket-Burman design. Table 1 shows the level of variables according to experimental design. The process of extraction involved size reduction, size separation, extraction, concentration and drying of the *Cassia tora* seeds. The seeds were powdered and weighed accurately. Powder was loaded in the thimble made of filter paper, and placed in the Soxhlet apparatus, where as the accurately weighed powder was directly taken into the round bottom flask for refluxation. 12 sets of experiments were carried out in duplicate as per the experimental design and average percentage yield of extract obtained in each process was calculated separately. The design and the percentage yield obtained are as shown in Table 2.

Table 1. Level of investigated variables

Independent variables	Level (-1)	Level (+1)
X_1: Apparatus used	Soxhlet (S)	Reflux condenser (R)
X_2: Powder size	44	10
X_3: Duration of extraction	6 hrs	12 hrs.
X_4: Type of water for extraction	Reverse osmosis (RO)	Demineralized water (DM)
X_5: Method of removal of solvent	Water bath	Rotary evaporator
X_6: Method	Water bath	Vaccum oven

Table 2. Average percentage yield of extract obtained by extracting *Cassia tora* as per Plackett and Burman Design

Independent variables						Response / Dependent variable
Apparatus	**Mesh size of powder**	**Extraction time (hrs)**	**Type of water**	**Removal of solvent**	**Drying method**	**% Yield**
(+1)	(+1)	(+1)	(-1)	(-1)	(-1)	29.09 ± 2.59
(+1)	(-1)	(-1)	(+1)	(-1)	(-1)	35.79 ± 2.68
(-1)	(-1)	(+1)	(-1)	(+1)	(-1)	9.33 ± 1.95
(+1)	(+1)	(+1)	(+1)	(-1)	(+1)	29.53 ± 1.21
(+1)	(-1)	(-1)	(+1)	(+1)	(-1)	23.92 ± 1.64
(+1)	(-1)	(+1)	(-1)	(+1)	(+1)	38.26 ± 1.82

Cont…

Independent variables						Response / Dependent variable
Apparatus	Mesh size of powder	Extraction time (hrs)	Type of water	Removal of solvent	Drying method	% Yield
(-1)	(-1)	(+1)	(+1)	(-1)	(+1)	3.69 ± 1.91
(-1)	(+1)	(+1)	(+1)	(+1)	(-1)	4.69 ± 2.67
(-1)	(+1)	(-1)	(+1)	(+1)	(+1)	5.74 ± 2.53
(+1)	(+1)	(-1)	(-1)	(+1)	(+1)	19.43 ± 2.95
(-1)	(-1)	(-1)	(-1)	(-1)	(+1)	8.66 ± 2.57
(-1)	(+1)	(-1)	(-1)	(-1)	(-1)	6.11 ± 2.69

Statistical analysis

The effect of formulation variables on the response variable were statistically evaluated by applying one way ANOVA at 0.05 level using a commercially available software package Design-Expert® version 6.05 Stat-Ease Inc. A model consisting of main effects and interactions is assumed to give an adequate approximation of the response hence, the design was evaluated by a factorial linear interactive first order model.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3 \ldots + b_6 X_6 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3 \ldots + b_{56} X_5 X_6$$

where Y is the response variable, b_0 the constant and b_1, b_2, . . ., b_6 the regression coefficient. X_1 and X_2 stand for the main effect; X_1X_2 are the interaction terms, which show how response changes, when two factors are simultaneously changed.

Antimicrobial activity of the different extract

Staphylococcus aureus (ATCC 2267), *Bacillus subtillus* (ATCC 6633), *Pseudomonas aeruginiosa* (ATCC 25619) and *Escherichia coli* (ATCC 10536) collected from Institute of Microbial Technology, Chandigarh, India.

200 mgs of *Cassia tora* seeds aqueous extract was dissolved in 10 mL of DMSO to get the concentration of 20 mg/mL and used as sample. Streptomycin of 100 μg/mL concentration in sterilized double distilled water was used as standard.

Suspension of organism was prepared as per McFarland nephelometer standard. A

24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contained approximately 1.5 x 10^8 cells/mL. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05 mL of 1.175% of barium chloride and 9.95 mL of 1% sulphuric acid. Antibacterial activity of the extract was evaluated for the sample by cup-plate technique as per the standard procedures[13].

RESULTS AND DISCUSSION

Statistical design of experiments is a powerful tool to achieve breakthrough improvements in process efficiency. Hence, in the present research work we have attempted to improve the percentage yield of *Cassia tora* aqueous extract by simultaneous comparison between two levels of six factors by applying Plackett-Burman design. According to the design totally 12 experiments were conducted and as per the experimental design, each row represents an experiment and each column represents an independent variable. The signs + and – represent the two different levels (higher and lower) of the independent variable under investigation. The percentage yield of the extract was considered as response variable and the results obtained from the experiment are shown in Table 2. To understand the mathematical relationship between the studied independent variables and the depended variables, the design was evaluated by a factorial linear interactive first order model. Coefficients less than 0.05 were considered for the generation of equation and positive sign indicates that the response increases with the factor where as negative sign indicates the response and factors have reciprocal relation.

Effect of process variables on percentage yield of the extract

$$Y = +17.85 + 11.77 X_1 + 1.25 X_3 - 2.07 X_5 + 2.21 X_6 - 3.34 X_3X_4 + 4.21 X_3X_5$$

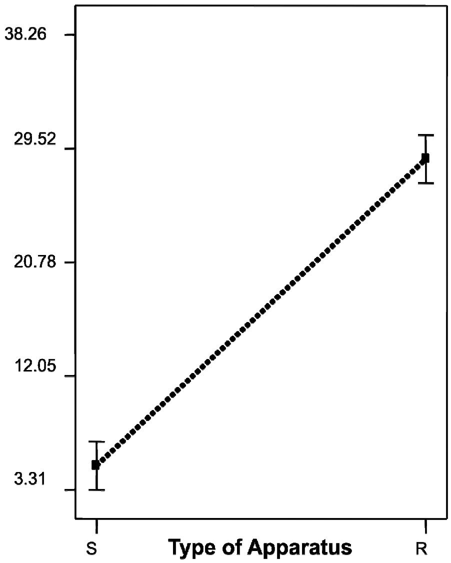
From the ANOVA analysis, it is observed that the model was found to be highly significant ($p < 0.0001$), with an F-value of 149.28 and a high R^2 of 0.9962 also indicate adequate fitting to the model (Table 3). The above equation presents the direction and magnitute of the studied significant factors on the response prcentage yield (Y). Factors X_1, X_3, X_6 and interaction factor between X_3X_5 exhibit positive sign, indicating a synergetic influence on the response and where as factor X_5 and interaction factor X_3X_4 show antagonistic effect on the studied response. The design expert software also provides the main effect with intraction plot, which helps to study the effect of independent variables as we change from -1 to +1 level on the response. Fig. 1 displays the main effect plot of the significant variables on the percentage yield and shows that among the studied variables, factor X_1 (type of apparatus) were found to be highly significant on the response percentage yield. i.e. when all the studied variables were kept constant (either low or higher level) and factor X_1 were increased form -1 to +1 level, the percentage yield increases significantly[14].

Table 3. Summary of ANOVA for dependent variable for the process of extraction of *Cassia tora* from Plackett and Burman design

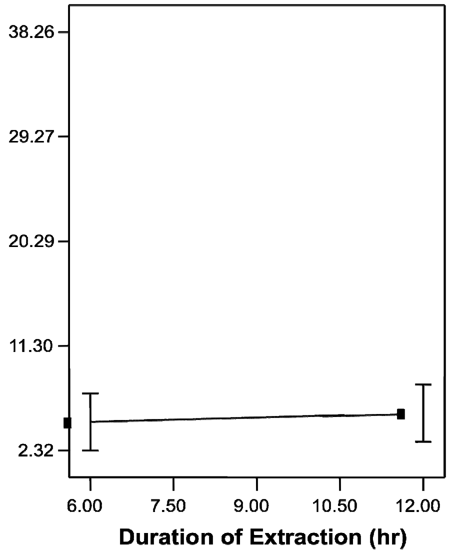
Source	df	Sum square	Mean square	F- Value	Prob. > F*
Model	7	1848.65	264.09	149.28	0.0001
X_1	1	1247.38	1247.38	705.08	0.0001
X_3	1	18.60	18.60	10.51	0.0316
X_4	1	6.19	6.19	3.50	0.1347
X_5	1	44.13	44.13	24.95	0.0075
X_6	1	44.11	44.11	24.93	0.0075
X_3X_4	1	89.20	89.20	50.42	0.0021
X_3X_5	1	141.62	141.62	80.05	0.0009

*Prob. > F less than 0.05 indicate model terms are significant

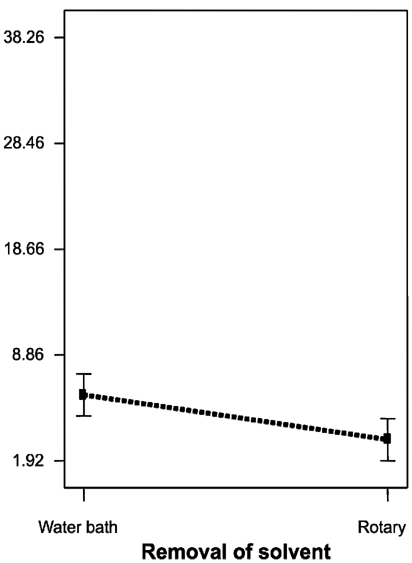
But such type of effect was not observed in case of changing the factor X_3 from lower to higher level, instead a minimal effect was observed, which may be due prescence of carbohydrates, saponins, glycosides, triterpenoids and phenols which readily dissolves in water[15], and extracting for more hours may not increase the yield. Similar but opposite effect was observed in case of factor X_5, In this case, the % yield decreases as the method of removal of solvent is changed from water bath to vacuum, and this result also compiles with the earlier results published elsewhere. In case of factor X_6, changing the type of drying method from IR to vacuum oven, the percentage yield increases, which may be due to low and efficient drying temperature in a closed chamber and also prevents the loss of product[16]. The effect of interaction factors can be further studied with the help of interaction graphs (Fig. 1) and from the graph, it may be concluded that a high percentage yield may be obtained, if the duration of extraction is decreased and using water bath as a method of solvent removal.


38.26
29.52
20.78
12.05
3.31
S
Type of Apparatus
R

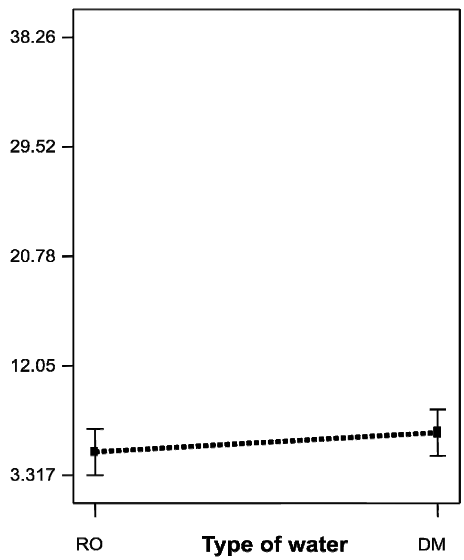
(a)


38.26
29.27
20.29
11.30
2.32
6.00
7.50
9.00
10.50
12.00
Duration of Extraction (hr)

(b)

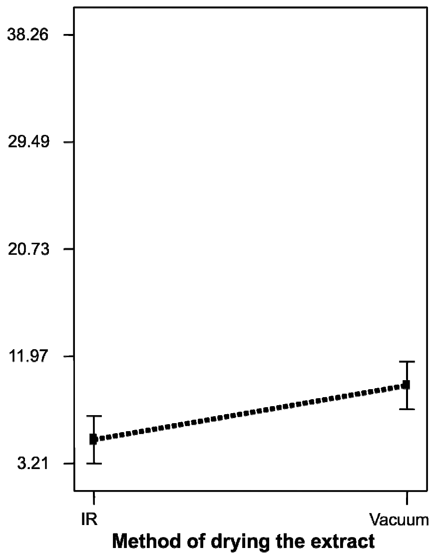

38.26
28.46
18.66
8.86
1.92
Water bath
Rotary
Removal of solvent

(c)

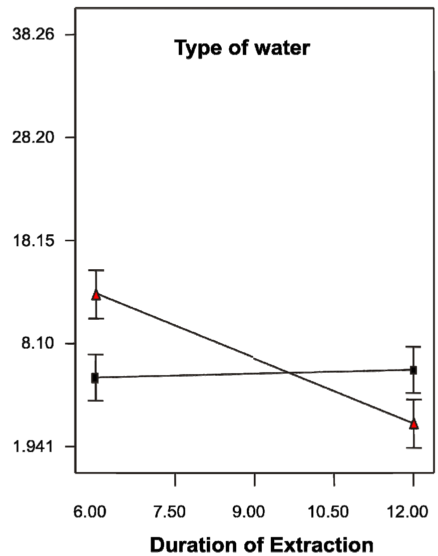

38.26
29.52
20.78
12.05
3.317
RO
Type of water
DM

(d)

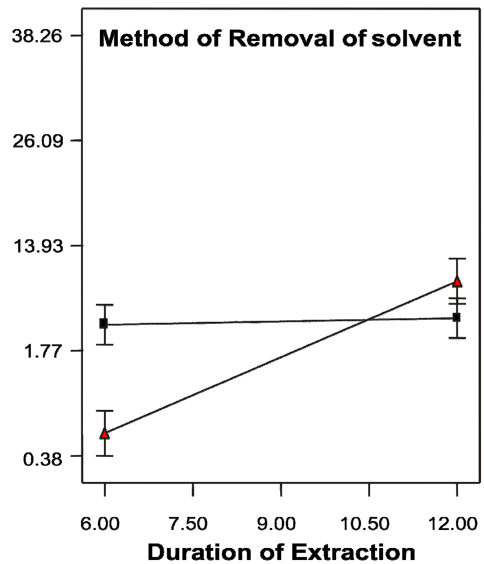
Cont…



(e)



(f)



(g)

Fig. 1: Main effect and interaction plots depicting the effect of different variables on the % yield in the extraction process of *Cassia tora* from the screening design

Optimization of conditions for the extraction process

A multi-criteria decision approach, like numerical optimization technique by the desirability function was used to generate the optimum settings for the extraction process. The variables were optimized for a high percentage yield and the optimized extraction process was arrived by ranging the studied independent variables. The optimized extraction process thus obtained is presented in Table 4. To verify the reproducibility and to validate, the optimized process was performed according to the predicted levels and evaluated. The results are given in Table 5, which showed a good relationship between the experimental and predicted values, which confirms the practicability of the model.

Table 4. Method of optimized process for extraction

Independent variables	Optimized process
Type of the apparatus used	Reflux condenser
Powder size	#44
Duration of extraction	7.30 hrs
Type of water for extraction	DM water
Method of removal of solvent	Water bath
Method of drying the extract	Vacuum oven

Table 5. Comparison between the experimental and predicted values for the most probable optimal process of extraction

Response (%)	Experimental (%)	Predicted (%)	Residual
Percentage yield	36.482 ± 2.63	38.2599	-0.6469

Antibacterial evaluation of *Cassia tora* extract

Standardized aqueous extract obtained from optimized process of extraction exhibited antibacterial activity against both Gram positive as well as Gram negative organisms tested. The activity was highest against *Staphylococcus aureus* as compared to other organisms tested. According to American Association of Dermatology, antibacterial therapy is included in treating psoriasis. Many of the antibacterial agents, including those obtained from the herbal source are used for treating different skin diseases like psoriasis[17]. *S. aureus* has been found to be the causative organism for various infections and is known to aggravate skin conditions like psoriasis, atopic dermatitis, erythroderma etc.[18,19] As the extract has shown excellent activity against *S. aureus* and has history of usage for treating skin diseases, this study could explain the use of aqueous extract of seeds of *Cassia tora* for treating various skin diseases like psoriasis and other bacterial infections. (Table 6).

Table 6. Antibacterial evaluation of aqueous extract of seeds of *Cassia tora*

Organism	Zone of inhibition (mm*)	
	***Cassia tora* aqueous extract**	**Streptomycin**
S .aureus	15 ± 1.5	23 ± 0.50
B. subtillus	-	30 ± 0.45
E. coli	13 ± 0.65	22 ± 0.55
P. aeuriginosae	12 ± 0.62	19 ± 0.50

*Value are in terms of Mean ± SEM (n = 3); = No activity

This article discussed a positive application of a computer optimization technique for the development of an extraction process from the seeds of *Cassia tora.* Instead of changing levels of one factor (variable) at a time in an unsystematic way to try and find the optimum conditions for the extraction process, statistical design of experiment was adopted by using Plackett-Burman design in which all the pertinent factors are varied systematically. The subsequent analysis of the resulting experimental data will identify the optimal conditions, the factors that influence the results and those that do not. Most importantly, the statistically designed experiments provide a strict mathematical framework for changing all factors simultaneously, in a small number of experimental runs and hence reduces time and cost of experiments. Based on the result, it was found that the type of water did not significantly affect the percentage yield. However, the type of apparatus used, powder size, duration of extraction and method of drying, significantly affect the studied dependent variable with predominant effect by changing the type of apparatus. To check the reproducibility of new derived formulae obtained by the application of optimization for extraction of *Cassia tora* was carried out and the results showed a good relationship between the experimental and the predicted values, which confirms the practicability of the mode l.

The extract exhibited good antibacterial activity against the tested bacteria except

B. subtillus. In conclusion, results from the present study supports the use of aqueous extract of *Cassia tora* seeds for treating bacterial infection and skin disease like psoriasis. This is the first report of the optimization of extraction process of seeds of *Cassia tora* using Plackett-Burman design.

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