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Testing the Influence of Temperature and Exposure on *Opuntia ficus-indica* and *Opuntia dillenii* Cladode Extraction on Percent Yield Using Design Expert Software

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A B S T R A C T

The main aim of the present study is to investigate the effects of temperature and exposure time on the extraction of *Opuntia ficus-indica* and *Opuntia dillenii* cladodes. Cladodes and other parts of plants were rarely extracted for their constituents from literature. Screening, however, was not conducted to determine the influence of dependent variables on independent responses. The impact of the dependent variable on the response was determined by the use of QbD software namely Design Expert. A plot of *Opuntia ficus-indica* and *Opuntia dillenii* are poised, authenticated, and drenched with water to determine the effect of dependent variables (temperature and exposure time) on the response (% yield). The equation coded from this study for the percent yield was generated as $+54.30+0.4061A-0.6432B+0.1500AB+0.8375A_2+0.3875B_2$. For extraction of the contents of cladodes, 65°C is the optimal temperature and exposure time is directly proportional.

***Key words*:** Extraction, *Opuntia*, Response, Screening, Variables

Natural plant substitutes have been comprehensively studied as an excipient in the pharmaceutical industry since decades [1]. Herbal products are primarily purchased by the pharmaceutical, nutraceutical, food, and cosmetic industries [2]. A lot of demand is there for botanical extracts as well as semi-finished herbal products. Plants of the desert stand out among the *Opuntia*. *Opuntia* has been used in remedy since past era [3-5]. All over the world, *Opuntia ficus-indica* breeds in dry, hot weathers. It has tall flowers/fruits, and this plant is also familiar as the Barbary fig. Therefore, it is well adapted for storing large amounts of rain during irregular rainstorms in dry areas [6]. *Opuntia dillenii* is another species of *Opuntia* that grows in the wild in south India. Additionally, it has pear bush, and bears fruits round the year. Designed to quickly absorb and store rainwater from uneven rains, it is ideally appropriate for dry areas. Pads, joints, and cladodes are all common terms for these plants' vegetative parts. The modified stems substitute the leaves in the photosynthetic process and have an ovoid or elongated shape of 18–25 cm in length. The chlorenchyma performs photosynthetic functions. Water is mostly stored in the inner part, which is made up of white medullar parenchyma. When leaves turn into spines, they become fine bristles called areoles. Plants with prickly areoles grow on these rocks. The large ones have sharp edges and are 2 cm long. There are 35 areoles in each internode [7]. The extracting, phytochemical screening, and biological screening of *O. ficus-indica* and *O. dillenii* cladodes have been studied extensively. Also, a comparison of the effects of temperature and time on the percent yield was conducted with a factorial design using Design of Experiment software, despite previous studies reporting that no investigation has been conducted.

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Factorial design (FD)

Due to its feasibility to manipulate statistically, traditional research approaches generally study the sway of one variable at a time. Nevertheless, only one factor can be studied at a time. Trying these two factors together leads to false results since they are interdependent. A part of multivariate analysis is the design of experiments (DOE). Nevertheless, DOE is understood to be a treaty with some factors but not all. An objective of DOE is to screen responses and optimize them [8]. The imitations explore possible amalgamations of the levels of the factors. FD's levels are designated as "high" (+1) and "low" (-1), and all input factors are called FD at two levels. The factors for a 2-level design may be 2, 3, 4, 5, 6, 7, 8 and so on. While

the number of runs will be 4, 8, 16, 32, 64, and 128. The number of runs will be extremely augmented above 5 factors, so fractional FD/ or Plackett-Burman design (PBD) is preferred. The screening goal for 2-4 factors is FD, and the response surface goal is Central composite (CCD) or Box-Behnken design (BBD). FD or PBD are appropriate screening goals for five factors and more, followed by response surface goals. DESIGN EXPERT / STATISTICA/ JMP / Unscrambler / Fusion / Minitab are the most widely used DOE software. Basically, the independent variable is entered to create the desired output, called the dependent variable [9].

MATERIALS AND METHODS

Opuntia ficus-indica cladodes and *Opuntia dillenii* cladodes were collected from plants growing in the dry hills surrounding Anantapur, Andhra Pradesh, India. A sample of cladodes measuring 7X20 cm was collected. It was identified by the department of botany of SK University, Anantapur, and authenticated. An Exemplar (SKBD/17/084 and SKBD/17/085) was deposited in the Herbarium.

Extraction of Mucilage

For mucilage extraction, cladodes from *O. ficus-indica* and *O. dillenii* were poised and cleaned. Manually separated and crushed medullar parenchyma was unglued from the medullar lining. 250 ml of water was added to 100 g of parenchyma. After stirring at 100 rpm at 50-80ºC for 60-90 min, the material was filtered through a domestic sieve (first filtrate). After precipitating with ethanol (95%), the mucilage was recuperated from the filtrate. This resulted in 300 ml of ethanol being added to 100 ml of the filtrate. The powder mucilage was attained by drying at 50°C for 3h. A triplicate of each extraction was carried out [10-11].

RESULTS AND DISCUSSION

Several industrial processes could be substituted with mucilage, which is a natural and inexpensive substance. The effectiveness of mucilage should therefore be investigated. Different methods of extraction were studied. Time and temperature were evaluated for yield impact.

Table 1 Factors and their levels used in the study

Factor	Levels		
	-1	0	+1
1 (A): Time (min)	60	75	90
2 (B): Temperature (ºC)	50	65	80

Mucilage extraction

The mucilage yields from the fresh cladodes using thermal extraction ranged from 50.3 to 52.5% and 54.1to 56.6%, respectively (Table 1). According to the investigational design data and mucilage % found for each condition, quadratic polynomials were calculated for *O. ficus-indica* and *O. dillenii* cladodes and validated by ANOVA analysis.

Initial risk assessment

According to ICH Q8 and Q9, the Quality Target Product Profile (QTPP) is vital for quality by design (QbD). Moreover, at the beginning of product development, it is important to separate unbiased views from biased ones. The QTPP entails the standards that must be seen for the item to be deemed quality. As a result of past investigations and appraisals of the literature, the QTPP and CQAs for extraction are robust.

Experimental design

In this study, a 9-run, 2 factor, 3-level full factorial design (FFD) was assumed for creation and judging quadratic response surfaces for screening the effect of temperature and exposure time (ET) on the extraction of contents from the cladodes of *O. ficus-indica* and *O. dillenii*. Stat-Ease Inc. used Design-Expert software (12.0) to assess the parameters. With the resultant quadratic model, the key, interface, and quadratic properties of independent variables on dependent variables were measured.

$$Y = B_0+B_1X_1+B_2X_2+B_{12}X_1X_2+B_1X_{12}+B_2X_2^2$$

Here Y is the dependent variable, B_0, B_1 and B_2 are the regression coefficients of independent variables and their mutual interactions and X_1 and X_2 are the independent variables (temperature and ET). A dependent variable/response was the percent yield of *O. ficus-indica* and *O. dillenii* cladodes. The variables and their levels used in the screening of the impact of independent variables on the response were exemplified in (Table 1) and central composite design experimental designs were expressed in (Table 2).

Table 2 The experimental design by Design-Expert software for studying the effect of temperature and exposure time for the extraction of contents from the cladodes of *O. ficus-indi*ca and *O. dillenii*

Trials	Factor 1 (A) Time (min)	Factor 2 (B) Temperature (°C)
1	60	50
2	90	50
3	60	80
4	90	80
5	53.7868	65
6	96.2132	65
7	75	43.7868
8	75	86.2132
9	75	65

The Response Surface Methodology (RSM) was utilized to test the influence of variables (time and temperature) on extraction. As the maximum values of the response surface of the temperature and time curves, the optimum settings for extracting mucilage were found. The untried designs were created and estimated with Stat-Ease-Design Expert V.12. Statistical validation was performed using one-way analysis of variance ANOVA with a confidence level of 95%. The models gained were based on a constrained central composite design constructed from a factorial design and star points. The response from the study was expressed as follows:

$$\text{Yield (\%)} = +50.70+0.3509A-0.5527\ B+0.0750AB+0.6063\ A^2+0.1062B^2$$

In terms of coded factors, the reckoning can be used to envisage the response to given levels of each influence. By default, the factors with high levels are coded as +1 and those with low levels as -1. When the coefficient of the factors is compared, the coded equation can show which factors have the greatest impact.

Fit summary

Fit summary for the response of percent yield of *O. ficus-indica* and *O. dillenii* cladodes were suggested Quadratic and Linear models (Table 3-4) .

Table 3 Fit summary for the response (Percent yield) of *O. ficus-indica* cladodes

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.0387	0.5492	0.2633	Suggested
2FI	0.8088	0.4659	0.1104	
Quadratic	0.0644	0.8570		Suggested
Cubic	0.3354	0.9517		

Table 4 Fit summary for the response (Percent yield) of *O. dillenii*

Source	Sequential p-value	Adjusted R^2	Predicted R^2	
Linear	0.0445	0.5276	0.3278	Suggested
2FI	0.6861	0.4532	0.2424	
Quadratic	0.0442	0.8861		Suggested
Cubic	0.1278	0.9944		

Table 5 ANOVA for Quadratic model for the response 1 (Percent yield) *O. ficus-indica*

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	4.90	5	0.9804	10.59	0.0401
A-Time	0.9850	1	0.9850	10.64	0.0471
B-Temperature	2.44	1	2.44	26.39	0.0143
AB	0.0225	1	0.0225	0.2430	0.6559
A^2	1.07	1	1.07	11.55	0.0425
B^2	0.0328	1	0.0328	0.3547	0.5934
Residual	0.2778	3	0.0926		
Cor Total	5.18	8			

Additionally, the ANOVA for Quadratic model for the response (percent yield) *O. ficus-indica* and *O. dillenii* cladodes were shown in (Table 5-6).

10.59 is a significant F-value for the model. The probability of an F-value this large occurring due to noise is as low as 4.01%. P-values below 0.0500 indicate that model terms are significant. Here, model terms A, B, and A_2 are significant. When the value exceeds 0.1000, model terms are not significant. If you have many insignificant terms (except those required for hierarchy support), reducing your model terms may make your models better. This indicates that the model is significant with a F-value of 13.45. An F-value of this size is only likely to occur due to noise by 2.88%. If the P-values for model terms are less than 0.05, then they are significant. A, B, and A^2 are significant model terms. If the value is greater than 0.1001, then the model terms must not be significant. It may be beneficial to reduce model terms (excluding those necessary to support the hierarchy) if you have many insignificant variables. The percent yields from *O. Ficus-indica* and *O. dillenii* cladodes were embodied in (Table 6).

Table 6 ANOVA for Quadratic model for the response (Percent yield) for *O. dillenii*

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	6.86	5	1.37	13.45	0.0288
A-Time	1.32	1	1.32	12.92	0.0369
B-Temperature	3.31	1	3.31	32.42	0.0107
AB	0.0900	1	0.0900	0.8816	0.4170
A^2	2.04	1	2.04	19.99	0.0209
B^2	0.4368	1	0.4368	4.28	0.1304
Residual	0.3063	3	0.1021		
Cor Total	7.17	8			

In fresh cladodes, the linear term for time has a positive coefficient, which indicates that mucilage extraction increases over time. According to the response surface, large variations in time in either direction result in increased extraction yields (positive quadratic terms). The linear term for temperature has no effect on mucilage yield, but the interaction between the two determines the level of mucilage extraction. As indicated by the quadratic terms, large temperature variations both in the positive and negative directions increase extraction yield. During the time range considered (50-80 min), mucilage extraction appears not to be significantly affected by time. The interaction between variables does not affect mucilage withdrawal when taking the confidence intervals into account. Temperature and ET interaction effects on the percent yield from the cladodes of *Opuntia ficus-indica* and *Opuntia dillenii* were expressed in the (Fig 1), whereas the contour plot and 3D response plots were represented in (Fig 2).

RSM was used to test the influence of variables (time and temperature) on extraction. We took the effects of temperature and time on yield as independent variables. A response surface whose maximum value represents the optimum conditions for extracting mucilage was attained. ANOVA with a 95% confidence level was used to generate and evaluate the experimental designs using Stat-Ease-Design ExpertV.12.

Based on a factorial design with few star points, we generated models with circumscribed central composite designs. The final equation in terms of coded factors as follows:

$$\text{Yield (\%)} = +54.30+0.4061A-0.6432B+0.1500AB+0.8375A_2+0.3875B_2$$

Using the equation in the form of coded factors, it is possible to envision the retort at dissimilar levels of each factor. Factors with high values are coded as +1 and those with low values as -1 by default. The coded equation can be used to determine how much each factor contributes to the outcome by comparing factor constants.

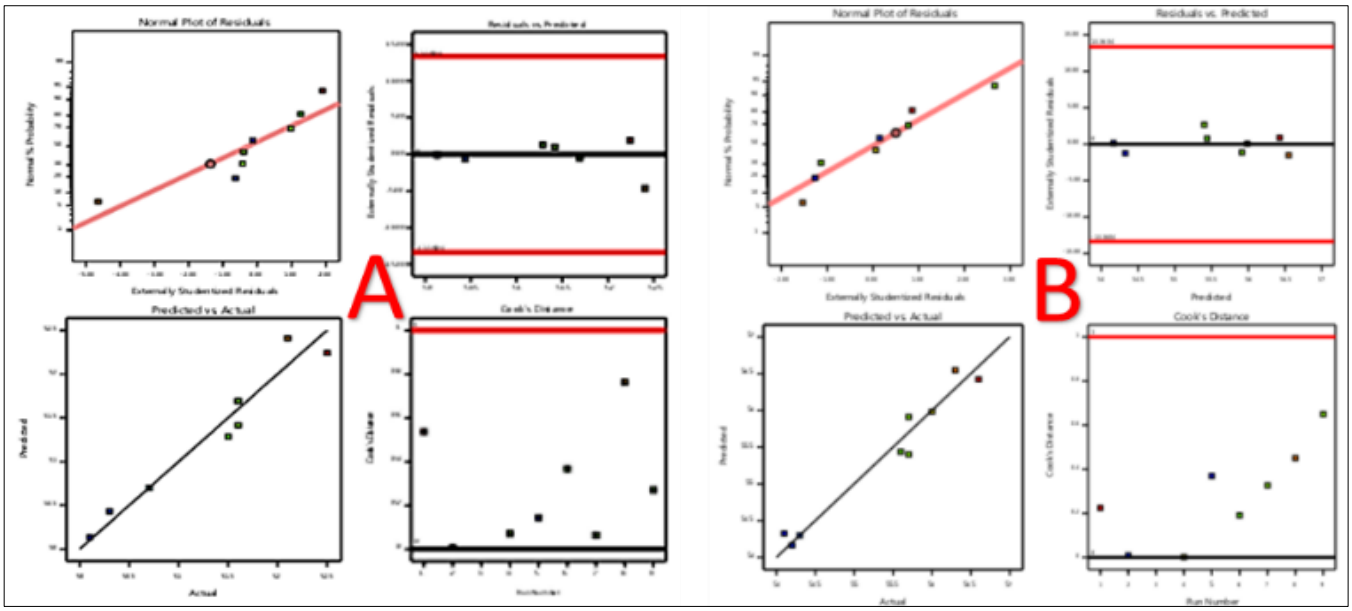

Fig 1 Plots showing the interaction effect of temperature and exposure time on the percent yield from the cladodes of *O. ficus-indica* and *O. dillenii*

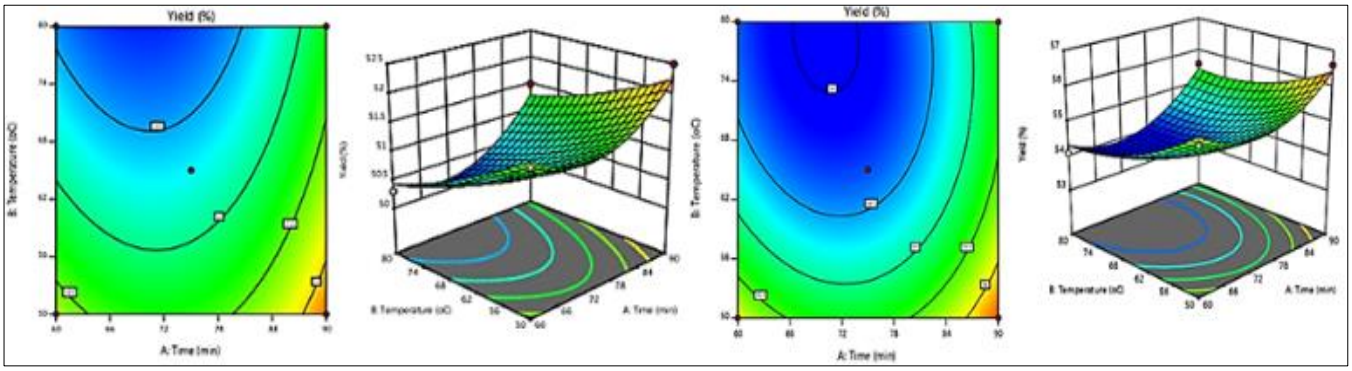

Fig 2 Contour plot and 3D response plot for the response (Percent yield) from *O. ficus-indica* (Left) and *O. dillenii* (right)

CONCLUSION

We designed an experiment using QbD software, namely Design Expert, in order to determine the impact of temperature and exposure time on the extraction of *Opuntia ficus-indica* and *Opuntia dillenii* cladodes. According to the study, 65°C is the optimal temperature for extracting the contents from cladodes and that the exposure time is directly proportional. According to the equation derived from the study (impact of temperature and exposure time on the percent yield) was found to be $+54.30+0.4061A-0.6432B+ 0.1500AB+ 0.8375A_2+0.3875B_2$.

Abbreviations

QbD: Quality by Design, FD: Factorial Design, DOE: Design of Experiments, PBD: Plackett-Burman design, CCD: Central composite Design, BBD: Box-Behnken design, ANOVA: Analysis of variance, QTPP: Quality Target Product Profile, CQAs: Critical Quality Attributes, ET: Exposure Time.

Declaration of competing interest

The author declared no conflict of interests.

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