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Extraction-Optimization of Hydrogel from Lallemantia Royleana Seeds Through Response Surface Methodology

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ABSTRACT: The aim of this study was to evaluate the parameters for the maximum extraction of hydrogel (LRH) from the Lallemantia royleana seeds by Box Behnken Design Response Surface Methodology (BBD-RSM). The influence of different parameters such as pH , temperature, water-to-seed ratio (S/W ratio) and water-to-seed contact-time (S/W time) on LRH yield was evaluated. ANOVA pvalues showed that these factors were the most significant constraints of the LRH yield. The Scenarios where the Temperature, Concentration and Time are involved in optimizing the aforementioned parameters were generated in the Construct 2 DesignExpert software, which is also capable of producing 2D and 3D contour maps. The maximum LRH yield of 13.73%, was observed when the seed-to-water ratio, contact period and pH were varied in a controlled manner using the RSM-BBD method. The optimal extraction conditions for the production of LRH were a temp. of extraction of 80 degree celsius, pH of 7 , contact time for S/W ratio of five hours and S-W ratio of 1 : 20. The second order polynomial equations were also discovered to fit better the response under consideration.

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I. INTRODUCTION

Hydrogels derived from Lallemantia rovleana seeds have gained significant attention for their applications in agriculture, medicine, and biotechnology, owing to their water-retention properties and biocompatibility[1–3]. The extraction of these hydrogels has become increasingly important in response to the growing demand for natural and environmentally friendly products. Traditional extraction methods for hydrogels are often energy-intensive and require the use of harmful chemicals, which raises concerns about their environmental impact[4]. Therefore, there is a pressing need for more efficient and sustainable extraction techniques that minimize environmental harm.

This work systematically assesses many independent variables such as temperature, pH , seed-to-water ratio (S/W ratio), and contact time and their interactions to improve hydrogel production from Lallemantia rovleana seeds. The Response Surface Methodology (RSM), specifically the Box-Behnken Design (BBD), was chosen for its ability to model complex relationships between variables and optimize processes efficiently[5]. The preference for BBD over other statistical methods, such as Central Composite Design (CCD), was predicated on its benefits in reducing experimental runs while delivering solid and dependable results. The findings of this study provide a scalable approach to hydrogel extraction, which could be beneficial for commercial hydrogel production[6].

Additionally, the study incorporates statistical validation through Analysis of Variance (ANOVA), confirming the significance of the parameters under investigation. The comparative study with prior experiments on hydrogel reinforces the effectiveness of the enhanced extraction conditions[7]. In comparison to previous reports, the yield of hydrogel in this study was remarkably higher, with optimized conditions resulting in a yield increase to 13.73%. This improvement in yield underscores the efficiency of the proposed extraction method over traditional techniques[8]. Future investigations may delve further into the functional features of the hydrogel, including its swelling behavior and biodegradability. Moreover, assessing its potential uses in medicine delivery and wound healing would expand the research's

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breadth and amplify its overall significance[3].

II. METHODOLOGY

A. Experimental Design

The study adopted Box Behnken Design to optimize the extraction conditions. Four independent factors were involved, and these included: - pH : The pH level of the extraction solution ranged from 5 to 9.
- Temperature: The temperature was tested ranging from 60°C to 90°C.
- S/W ratio: The seed-to-water ratio ranged from 1:10 to $1:30\text{w/v}$.
- Seed-to-Water Contact Time (S/W time): The contact time was varied from 3 to 6 hours.

B. Extraction Procedure

Hydrogel extraction was done through the soaking of *Lallemantia royleana* seeds in distilled water with very minor adjustments, LRH extraction was performed as described in the literature. To put it briefly, the seeds of *L.* royleana were soaked in deionized water (S/W, $1:20\text{w/v}$, interaction period of 5 hours, and temperature of 80°C). Using a knife for scraping and a piece of cotton fabric for isolation, hydrogel was removed from seeds. Extracted hydrogel was sieved 3 times using hexane and deionized water to remove polar and non-polar contaminants. After extraction, the residue paste was separated from the mucilage by the centrifugation at speed of 4000 rpm for one hour, producing *Lallemantia rovleana* hydrogel (LRH), which was then disassembled on petri plate and desiccated in a vacuum oven at 60°C. Finally, LRH was desiccated in a vacuum and then processed through mesh no. 60 to create a fine, homogenized powder that was stored for later use in a vacuum oven.

Using a formula, the yield of LRH extracted was determined.

$$\text{Extraction yield of LRH: } \frac{\text{weight of extracted LRH after drying}}{\text{weight of seeds taken for extraction}} \times 100$$

C. Data Analysis

It aims to find out how many parameters affect the yield of LRH extraction. Some preliminary investigations were made, such as the effects of temperature ($30-110°\text{C}$), seedwater contact period ($1-13$ hours), pH($4-10$), and seed/water ratio ($5-35\text{w/v}$). This study selected three levels of every factor: low (-1), moderate (0), and high (+1). The four parameters examined in this study were the seed/water ratio, isolation temperature, pH , and contact duration. The tests were structured in a Box-Behnken design. Statistical software, Design-Expert, was used to find the regression models, ANOVA, and graphical graphs 2D/3D. Linear and quadratic interactions were discovered, and using the data from 36 trials, a polynomial equation for predicting the LRH yield has been developed. This model depicts the effectiveness of RSM-BBD technique. The need of the RSM-BBD is shown by this polynomial equation. The equation's linear version is shown as follows:

$$\mathrm{Y} = \mathrm{Z}_0 + \mathrm{Z}_1\ \mathrm{A} + \mathrm{Z}_2\ \mathrm{B} + \mathrm{Z}_3\mathrm{C} + \mathrm{Z}_4\mathrm{D} + \mathrm{Z}_{11}\ \mathrm{A}^2 + \mathrm{Z}_{22}\ \mathrm{B}^2 + \mathrm{Z}_{33}\mathrm{C}^2 + \mathrm{Z}_{44}\mathrm{D}^2 + \mathrm{Z}_1\mathrm{Z}_2\mathrm{AB} + \mathrm{Z}_1\mathrm{Z}_3\mathrm{AC} + \mathrm{Z}_1\mathrm{Z}_4\mathrm{AD} + \mathrm{Z}_2\mathrm{Z}_3\mathrm{BC} + \mathrm{Z}_2\mathrm{Z}_4\mathrm{BD} + \mathrm{Z}_3\mathrm{Z}_4\mathrm{CD} + \mathrm{E}_\mathrm{i}$$

In above mentioned equation Y stands for the mucilage withdrawal yield, the dependent variable. The variables, which include S/W, temperature, medium pH & seed-water contact duration, are denoted by the letters A, B, C, and D. Regression intercept coefficient (Z0), linearity coefficient (Z1, Z2, Z3, Z4), squared co-

TABLE I: BBD & RSM comparison among actual & predicted yield of LRH in (%).

Experiments	Factors tested				Response/Yields (%)	
	pH	Temperature (°C)	Seed-water ratio (w/v)	Seedwater contact time (h)	Actual yield	Predicted yield
1	7	80	10	3	9.94	10.09
2	8	100	20	5	12.55	12.79
3	6	80	20	7	11.03	10.81
4	7	100	10	5	12.31	11.90
5	7	100	30	5	12.83	12.94
6	7	80	10	7	12.17	12.15
7	7	60	30	5	11.45	11.56
8	7	60	20	7	11.62	11.79
9	7	100	20	3	11.71	11.71
10	8	80	10	5	12.05	12.14
11	6	80	10	5	8.88	9.48
12	7	80	30	3	11.59	11.73
13	8	80	20	7	12.65	12.67
14	7	80	20	5	13.78	13.73
15	8	60	20	5	11.57	11.72
16	8	80	20	3	11.46	11.38
17	6	100	20	5	11.11	11.07
18	7	60	20	3	10.12	10.21
19	6	80	30	5	11.06	11.15
20	7	80	20	5	13.67	13.73
21	8	80	30	5	12.69	12.27
22	6	60	20	5	9.78	9.66
23	7	60	10	5	11.21	10.80
24	7	80	30	7	12.35	12.31
25	7	100	20	7	12.69	12.77
26	6	80	20	3	9.77	9.46

efficient (Z11, Z22, Z33, Z44), interaction coefficient (Z1Z2, Z1Z3, Z2Z3, Z2Z4, Z3Z4), and function model error (Ei) are all included. The relevance of ANOVA was further shown by equating the experiment's result data and estimated yield values and computing the prominence of polynomial model to fit for the calculation of coefficient of regression (R2), R2-adj (adjusted-R2) & R2-pred (predicted R2). CV (Coefficient of variation), PRESS (anticipated error sum of squares), ADP adequate precision, SE (standard error), a scattered plot of comparison between actual and predicted yield and lack of fit were used to assess the acceptability level of RSM-BBD. Using p-values and F-values, the mathematical significance of the BBD vs. ANOVA model was determined. Additionally, to illustrate the interaction among all the responses and to determine location of the ideality of the parameters of experiment and sustainability of model, the 3D (threedimensional) response surface and 2D (two-dimensional) contour plots were shown.

III. RESULTS

The results obtained showed that the most significant factors that influence the yield of the hydrogel production were temperature, seed-to-water ratio, and seed-to-water contact time. ANOVA showed significant effects of these parameters besides pH as the interaction of these factors had a significant role in maximizing hydrogel production yield.

A. Optimization of Parameters

The Design-Expert Software provided the optimal extraction conditions, which are represented as:

The graphs in Fig. 1 depicts the optimized conditions for extraction of hydrogel from *L.* royleana seeds. The optimized conditions are given as;
- Extraction temperature: 80°C
- **pH** : 7
- S/W ratio: 1:20 (w/v)
- S/W contact time: 5 hours

TABLE II: ANOVA representing conclusions of BBD-RSM for the optimized conditions of extraction for yields of LRH.

Source	SSE	DF	Mean	F-values	p-values
Model	35.33	14	2.52	22.14	< 0.0001
Linear relations					
A-Effect of pH	10.72	1	10.72	94.02	< 0.0001
B-Effect of Temperature	4.63	1	4.63	40.58	< 0.0001
C-Effect of S/W ratio	2.44	1	2.44	21.40	0.0007
D-Effect of S/W contact time	5.23	1	5.23	45.86	< 0.0001
Quadratic relations					
$\mathbf{A}^2$	9.54	1	9.54	83.72	< 0.0001
B^2	3.81	1	3.81	33.47	0.0001
C^2	4.26	1	4.26	37.33	< 0.0001
D^2	5.94	1	5.94	52.07	< 0.0001
Interaction relations					
AB	0.0306	1	0.0306	0.2687	0.6145
AC	0.5929	1	0.5929	5.20	0.0435
AD	0.0012	1	0.0012	0.0107	0.9193
BC	0.0196	1	0.0196	0.1720	0.6863
BD	0.0676	1	0.0676	0.5931	0.4574
CD	0.5402	1	0.5402	4.74	0.0521
Remaining	1.25	11	0.1140		
Lack of Fit	1.25	10	0.1248	20.62	0.1699
Pure Error	0.0060	1	0.0060		
Cor Total	36.59	25			

R^2 is 0.9657, R^2-adjusted is 0.9221, R^2-predicted is 0.8029, ±SD is 0.3376, %CV is 2.92%, Mean is **11.22**, ADP is **16.6419** and PRESS is 7.21

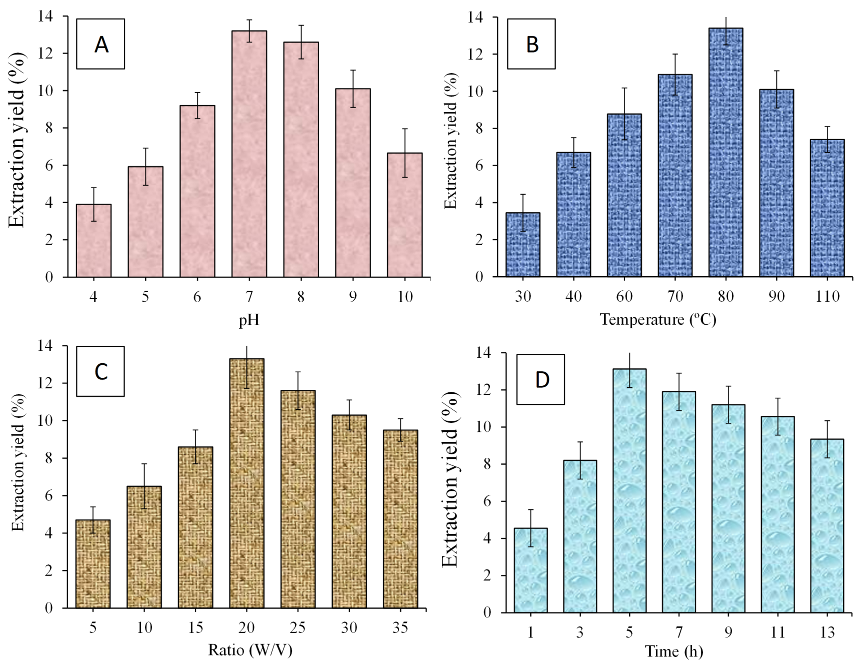

FIG. 1: (A) pH effect, (B) Temperature effect, (C) S/W ratio effect, (D) Contact time effect on LRH yield.

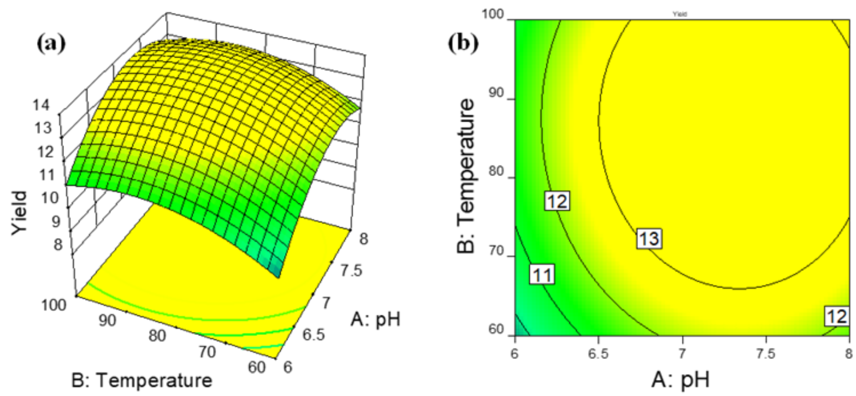

FIG. 2: 3-dimensional (a) & 2-dimensional (b) graphs indicate the mutual influence of pH & Temp on yield of the LRH

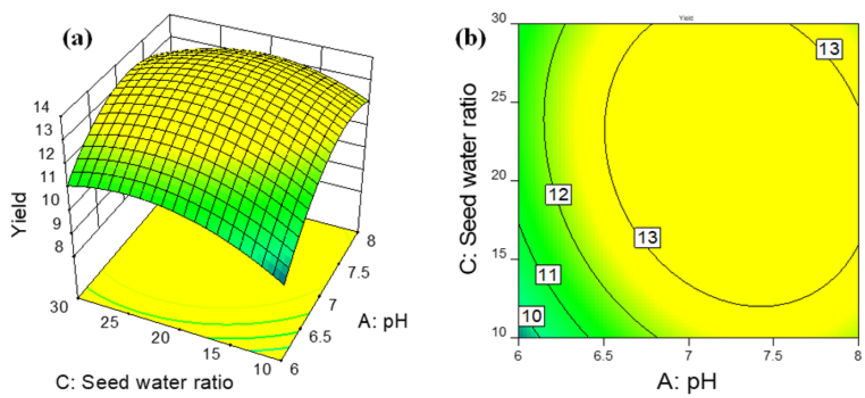

FIG. 3: 3-dimensional (a) and 2-dimensional (b) graphs indicate how pH and S/W ratio affect LRH yield.

These conditions resulted in a hydrogel yield of **13.73**%, which was the highest observed during the study.

B. ANOVA RESULTS

Consequently, three distinct values were chosen for each independent component based on the previously published optimised parameters indicated above. The stages are given as: least (pH6; temperature: 60°C; S/W : 1/10w/v, time of contact: three hours), moderate (pH 7 ; temperature 80°C;S/W1/20w/v, and contact time: 5 hours), and highest (pH : 8, temperature: 100°C; ratio of seed to water: 1/30w/v& time of contact 7 hours). Yields were 7.10%, 13.20%, and 9.10%, in that order. A BBD & RSM was created utilizing Design Expert in accordance with these conditions.

The ANOVA for the extraction yield of *Lallemantia ropleana* hydrogel (LRH) was interpreted to mean that the second-order quadratic equation gave the best fit to the experimental data, and the yields ranged from 9.46% to 13.73% (Table I). The p-value from the ANOVA was less than 0.001 , indicating that the model was satisfactory in representing the yield data. Substantial factors affecting LRH yield involved temperature, pH , seed-towater ratio, and seed-to-water contact time, with temperature having the most considerable impact. The quadratic terms $\left(A^2, B^2, C^2, D^2\right)$ and several interaction terms were found to be highly significant. The model's appropriateness was reinforced by high R^2 values (0.9657 and 0.9221 for attuned R^2), a low coefficient of variation (2.92%), and a high average deviance percentage (16.6419), representing its reliability in recognizing optimum conditions. Moreover, a non-significant lack of fit and a pure error of 0.0060% confirmed that the BBDRSM model was appropriate for optimizing LRH yield (Table I).

By calculating the signal-to-noise ratio and taking average deviation percentage (ADP) into account, the de-

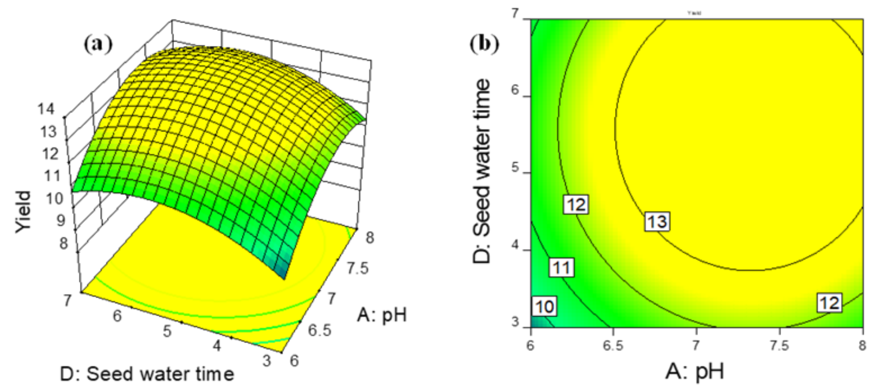

FIG. 4: 3-dimensional (a) & 2-dimensional (b) graphs show mutual influence of the pH and seed/water interaction time on LRH production.

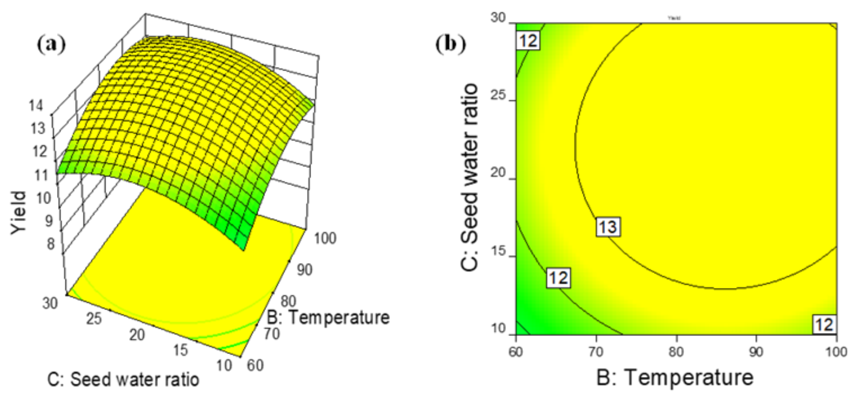

FIG. 5: 3-dimensional (a) and 2-dimensional (b) plots depicting mutual impact of temperature & seed to water ration on production of LRH.

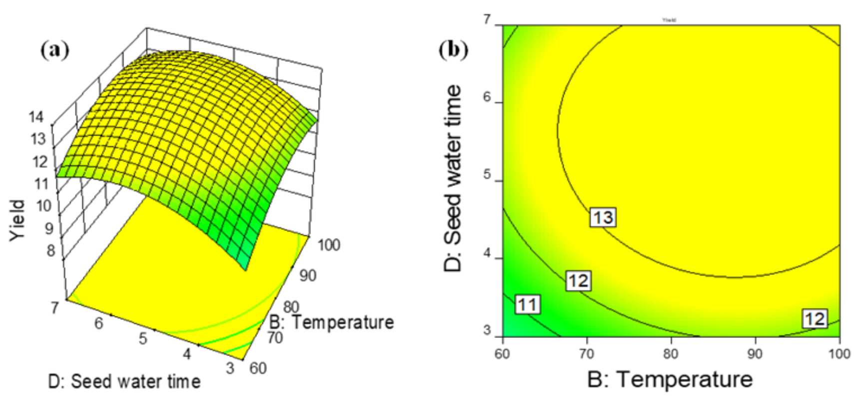

FIG. 6: 3-dimensional (a) and 2-dimensional (b) plots depicting the mutual impact of temperature & seed water time on production of LRH.

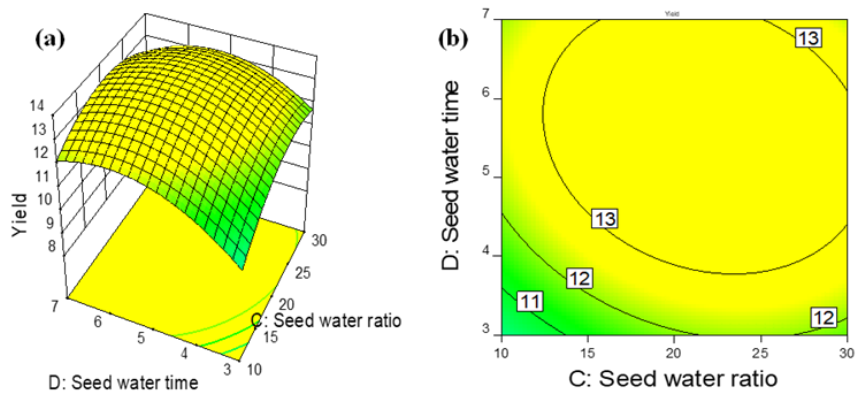

FIG. 7: 3-dimensional (a) and 2-dimensional (b) plots depicting the mutual impact of S-W ration & S-W contact time on LRH yield.

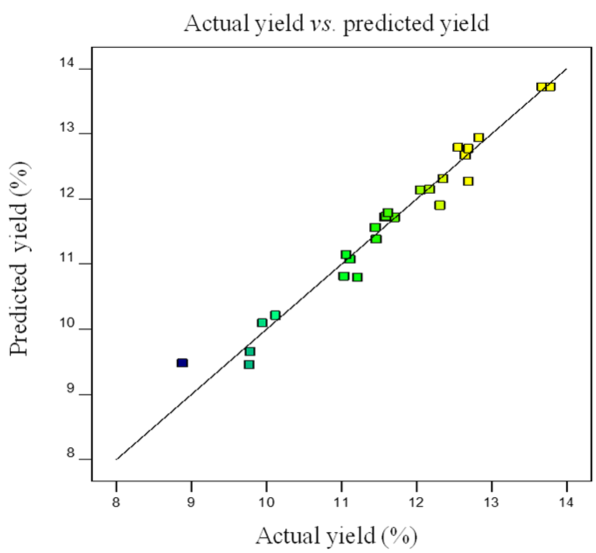

FIG. 8: Dispersed graph presenting the contrast amid Actual & Predicted yields of LRH.

sign's agreeability may be evaluated. ADP values often have a direct correlation with model accessibility and a negative correlation with the signal-to-noise ratio. This suggests that a low signal-to-noise ratio and better model attractiveness are the outcomes of higher ADP levels. The average value of the ADP is 4.0 . The system is deemed attractive if the ADP value is higher than 4.0 , or vice versa. ADP was found to be 16.6419 in this most recent study, which is higher than the average value (Table II). This demonstrates that the BBD-RSM can identify the ideal conditions for LRH extraction in order to maximize yield. When evaluating a model's ability to correlate effectively with experimentally obtained data, absolute errors and lack of fit are important considerations. The model architecture is unsuitable for response-predictor analysis if there is a significant "lack of fit," and experimental data should be ignored. However, in the event of a non-significant "lack of fit," the model may be effectively applied to the interpretation of experimental data, and the response generator should not be disregarded. With a 95% confidence interval, the pure error of the ANOVA for LRH yield optimization was found to be 0.0060% with a non-significant lack of fit (Table II). Consequently, it can be said that the BBD-RSM is an appropriate model for LRH yield optimization.

C. Explanation of Response Surface-Plots

BBD-RSM was used to generate 3D response surface & 2D-contor plots by maintaining the two variables constant at their optimal values while varying the other two variables in order to analyze the influence of the variables under inquiry in a binary format.

1. *MUTUAL IMPACT OF PH & TEMPERATURE YIELD OF LRH*

The contour plot and the 3D response surface of pH -temperature relationship are demonstrated in Figs. 2a and b , respectively. The data were demonstrated to increase gradually with the increase in both temperature and pH . The maximum yield in LRH production was reported at a pH of 7 and a temperature of 80°C, which was found to be 13.4%. However, at pH 10 and 110°C, the yield decreased to 6.65%.

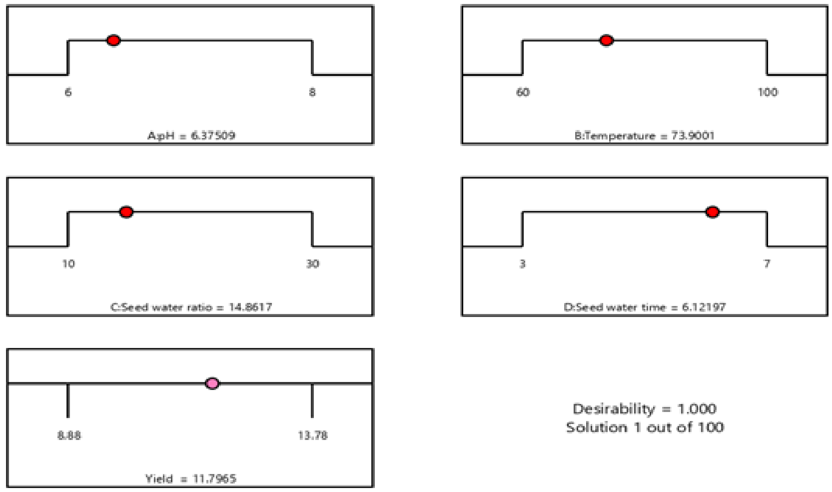

FIG. 9: Optimization sequences aimed at the extraction-optimization of LR hydrogel.

2. MUTUAL IMPACT OF THE PH & S/W RATIO ON THE YIELD OF LRH

Figs. 3a and b represent combined 3 D response surface and 2D contour plots of pH& S/W ration on the response variable LRH. Data reflects a positive direct linear upsurge in LRH production. Supreme LRH production was 13.4% obtained at seed/water ration of $1:20$ weight/volume and $\mathrm{pH}=7$, but with an onset of LRH decline when those ideal settings were surpassed.

3. MUTUAL IMPACT OF PH & SEED WATER CONTACT TIME ON YIELD OF LRH

The effects of the pH and the seed/water contact time on production of LRH were studied at an unaltered T of 80 degree Celsius and seed/water ration of 1/20 weight/volume. Fig. 4a and b depict the observations. These graphs indicate that LRH generation is insignificant at low pH& short seed-to-water contact periods. At increased pH& time of seed/water contact, the production of LRH also increased. The maximum LRH production was obtained at a pH value of 7.10& a five-hour seed/water contact duration, which was 13.4%. Beyond these optimal conditions, the LRH yield fell drastically, obtaining a minimum of 6.65% at pH 10 and 13 hours of contact time (Fig. 4a and b).

4. MUTUAL IMPACT OF T & S/W RATION ON THE YIELD OF LRH

Figures 5a (3D response surface plot) and b (2D contour plot) depict the interactive effect of temperature and seed-to-water ($\mathrm{S}-\mathrm{W}$) ratio, measured at constant $\mathrm{pH}=7.0$ & with a S/W contact time $=5$ hours. Both Temp & S-W ratio significantly affected the LRH production. The results indicated that, through an upsurge in temp from 30°C to 80°C and S-W ratio from 1/5 to 1/20 weight/volume, the LRH yield increased. At 80°C and 1/20 weight/volume $\mathrm{S}-\mathrm{W}$ ratio, the supreme LRH production of 13.4% was obtained. However, at 110°C and a 1/35 weight/volume S-W ratio, the LRH yield decreased to 7.4%.

5. MUTUAL IMPACT OF T & SEED TO WATER INTERACTION TIME ON PRODUCTION OF LRH

Quadratic impacts of temperature and S/W duration on LRH yield were evaluated at pH 7 and at a seed-to-water ratio of 1/20 weight/volume. Figures 6 a, the 3 D response surface plot, and Figure 6 b , the 2D contour plot, both of which exhibit the same trends of an increase or a reduction of LRH production as per the mutual effects of temperature & S-W duration, are shown in Fig. 6. Maximum LRH production of 13.4% was observed at a temp of 80 degree Celsius with a contact time 5 hr of $\mathrm{S}-\mathrm{W}$.

6. MUTUAL IMPACT OF S-W RATION & S-W INTERACTION TIME ON PRODUCTION OF LRH

At a pre-optimized temperature of 80°C and pH 7 , the interaction between LRH yield and the changing seed-to-water ($S-W$) ratios and S/W durations was investigated. The data was represented graphically using a 2D contour plot (Fig. 7b) and a 3D response surface graph (Fig. 7a). The graphs indicated that LRH production was lower, at value 8.6 percent, once the S-W ration was $1:15$ weight/volume and the S-W contact duration was three hours. Though, elsewhere this value, the yield of LRH was linearly increased. In this case, the maximum yield that was obtained was 13.4% at 1/20w/vS/W ratio and 5 -hour S/W duration.

In 2D-Contor graphs, the yellow regions were marked with obvious circular black lines that showed the areas of highest LRH production; however, in the 3D-Response surface graphs, an area of approximately 15% was stretched out to depict the ideal area of maximum LRH output. 3.4 Evaluating Model Appropriateness and Desirability

The sustainability of the BBD-RSM for LRH production was evaluated using the scatter plot with actual LRH yield (Ya) and the designed yield by Design-Expert, shown in Fig. 8. In the scatter plot, a straight line represented Yp while dots represented a random scatter of Ya. As there could be confusion for Ya and Yp along the straight line, therefore dots of Ya were suppressed. The plots have no significant difference between Ya and Yp (Figure 8 and Table I).

Besides, the 2D desirability graphs of Fig. 9 indicate that the total effect of studied factors for each scenario is 1 . This therefore confirms that, the quadratic-model proposed and BBD, RSM used were suitable & effective in optimization of the situations to obtain maximum yield of LRH.

D. OPTIMAL CONDITIONS

LR hydrogel yield was generated using the yield data provided by Design-Expert on the *L.* royleana seeds as being able to produce a yield of 13.4% under a pH of 7.12, 80.04°C, seed to water of 1/20w/v and 5hrS/W. Under the same conditions (pH7, 80°C, 1/20w/vS/W ratio, and 5 -hour S/W duration), the experimental yield was determined to be 13.67%, which is close to the predicted yield; hence, the optimal conditions obtained are confirmed correct (Table I, run 20).

The LRH yield data from the scatter plots comparing actual (Ya) and predicted (Yp) yields also almost matched the values mentioned earlier. Hence, it was concluded that the extraction circumstances given in the Table I for run 20 were best suited to obtain the maximum amount of LRH, yield by seeds of *L.* ropleana.

IV. DISCUSSION

The optimization of hydrogel extraction from *Lallemantia royleana* seeds using RSM successfully identified the critical factors that influence hydrogel yield. Temperature and S/W ratio were the most critical factors in maximizing yield; however, contact time and pH also played significant roles. The ultimate extraction conditions, which included an extraction temp. of 80 degree Celsius, pH of 7 , S-W ration of $1:20$, and a contact time of five hours, provided a hydrogel yield of 13.73%, which is a high yield for this process. The successful application of RSM-BBD optimized the extraction parameters and showed the possibility of eco-friendly hydrogel production from *Lallemantia rovleana* seeds. In the present study, the generated second-order polynomial model resulted in an acceptable representation of the extraction process. The contour plots showed very well defined optimal regions for achieving maximum yield hydrogel. These results are indicative of the fact that RSM could be used as a good tool for optimizing extraction processes, with an efficient and sustainable method for large-scale hydrogel production.

Implications for Industry

This optimized extraction method can be very helpful for industries like agriculture, pharmaceuticals, and biotechnology. The demand for eco-friendly and cost-effective hydrogel extraction methods is increasing in such industries. The results of this study deliver a basis for forthcoming research on large-scale hydrogel production and its applications.

Limitations and Future Work

Although the study achieved successful optimization of the extraction process, further research is needed to explore the scalability of these results. Investigating other variables, such as seed variety or geographical differences, may provide more insights into improving the extraction process. Additionally, exploring the functional properties of the hydrogel produced under these optimized conditions will be essential for assessing its practical applications.

V. CONCLUSION

This study demonstrated successful application of Response Surface Methodology with Box Behnken Design in optimizing hydrogel extraction from Lallemantia royleana seeds. The maximum hydrogel yield of 13.73% was obtained under optimal conditions, such as an extraction temperature of 80° Celsius, pH of 7, S/W ratio of $1:20$, and a contact time of five hours. This product offers a sustainable and efficient source of hydrogel extraction, widely applicable in various industrial industries. This work, therefore, used the second-order polynomial equation effectively as a modeling approach for predicting and optimizing the yield of hydrogel.

DECLARATION OF COMPETING INTEREST

The authors have no conflicts to disclose.

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