Extraction of *Lepidium Sativum* seed mucilage: optimization of extraction process with maximum yield by using full factorial design

ABSTRACT

Objective

The present investigation helps to isolate the seed mucilage of *Lepidium Sativum* by using an economic extraction process with utmost yield. Diligence effort was given to get the maximum yield of the mucilage with good flow properties without affecting the intrinsic properties of the extracted mucilage. The yield of the seed mucilage of *Lepidium Sativum* was optimized by using a quality by design approach. The full factorial design was used using three variables (pH of extract, water to seed ratio, and drying temperature) at two levels (high and low). Overall, ten formulation trials were generated through statistical software Minitab placing 2 center points, 1 replicate, and 1 block. All trials were executed to evaluate the percentage yield.

Results

The extraction process was evaluated with different solvents (such as ethanol and acetone) and different techniques to monitor the yield of the process. The maximum yield was achieved by soaking *Lepidium Sativum* seeds in water (1:30 ratio), maintaining an alkaline pH, blending by hand blender, and using acetone as a solvent. A factorial design was used to observe the responses like the yield of the isolated mucilage for different levels of all the independent variables. A polynomial equation was developed and model plots (contour plot and Pareto chart) were generated to study the impact of the critical variables on the response yield. ANOVA analysis of the model suggested that the independent variables had significantly affected ($p < 0.05$) in predicting the response (% yield) and the coefficient terms with a p-value less than 0.05 had a significant effect on the prediction efficacy of the model. Finally, the optimized parameter was established based on statistical evaluation obtained from the polynomial equation as well as the recommendation obtained from the response optimizer. The study indicates that the obtained linear regression equation has a linear relation with a regression coefficient of 0.9869 % for response % yield.

Conclusion

A cost-effective extraction and isolation process was established to extract the seed mucilage of *Lepidium Sativum* with maximum yield. The isolated mucilage was evaluated for flow

properties and demonstrated that it is having good flow properties and can be used as an excipient or adjuvant in different pharmaceuticals and food industries. The applicability of factorial design was well demonstrated to optimize the yield by optimizing the critical factors.

Keywords: Seed mucilage, Isolation, Extraction, Garden cress seeds, *Lepidium Sativum,* maximum yield.

INTRODUCTION

The plant-derived products have drawn a lot of attention due to their abundance, and biocompatibility with a wide range of active medicinal ingredients, paving the way to innovative and sustainable applications. India is accomplished with ancient traditional plant-derived products for the treatment of various diseases [1]. Plant-derived polymers have also attained high demand in food and other industries. Plant-derived mucilage, due to its distinctive health and food properties, is widely used as an active ingredient for the formulation of pharmaceutics, functional, and nutraceutical products [2]. Recent investigations on different plant-derived products demonstrated that these naturally obtained products can be used as an alternative to different excipients used in pharmaceuticals and food industries [3].

Mucilages are polysaccharide-based compounds and significantly vary in composition [4, 5]. The mucilage is a water-soluble edible adhesive material that constitutes carbohydrates and uranic acid units present in different parts of plants including the mucous epidermis of the outer layer of seeds, bark, and leaves [6]. *Lepidium Sativum* is a medicinal herb readily available in different parts of India. It is popularly known as halon and is categorized as an edible plant. In India, the *Lepidium Sativum* plant is mainly utilized for seeds, roots, and leaves. However, the plant is recognized for seeds [7]. The seeds of the halon plant are supplemented with different alkaloids which enable its use as an excipient and adjuvant in different pharmaceutical and food preparations [7].

The recent investigation on the *Lepidium Sativum* plant mainly focused on the use of seed mucilage in various medicinal products [7-9]. The use of seed mucilage of *Lepidium Sativum* is gaining popularity and researchers already explored the use in pharmaceutical formulations with various distinctive roles in tablet dosage forms and liquid dosage forms [10-14]. However, designing innovative medicinal products containing mucilage is becoming extremely prevalent. Moreover, the mucilage of *Lepidium Sativum* is obtained from the seed as a natural source. However, diminutive information is available on how to get the maximum percentage of mucilage from the seeds.

In recent years, many reports have explored mucilage from various plant seeds of Salvia hispanica, taro mucilage, and psyllium mucilage [15-17]. A major emphasis in all these studies has been channeled toward investigating mucilage extraction from novel sources, and the effect of various parameters, such as temperature, time, water/seed ratio, pH, and stirring modes for the release of hydrosoluble compounds. Various such reports indicated varying levels of yields usually dependent on extraction methods and parameters employed [18]. To analyze the effect of extraction conditions on the extraction yield obtained, the quality by design by using statistical software is a widely accepted method [18, 2].

Based on this assurance, the present study focused on the development of a cost-effective isolation and extraction process of mucilage from *Lepidium Sativum* seeds. The current research emphasized the critical attributes that are affecting the yield and utilizes the quality by design (QBD) approach to explore the study. The present work was carried out to systematically investigate the extraction optimization of mucilage using full factorial design, from *Lepidium Sativum* seeds. Special attention and focus have been given to optimizing the critical factors and based on the study, recommendations were proposed. Extraordinary effort and consideration were given to discussing the flow properties of the obtained powdered mucilage to enable it to use as an excipient in different pharmaceutical, ayurvedic and food preparations, and formulations.

MATERIALS AND METHODS

Materials

Seeds of *Lepidium Sativum* were procured from the nearest retail store in Mumbai, India. All the chemicals and other reagents such as ethanol, acetone, HCl, and NaOH utilized were of analytical quality and were procured from S.D. Fine chemicals, Mumbai, India. Distilled water was produced *in-house* in the laboratory and this water was employed in all the experiments.

Methods

Extraction and Isolation of mucilage from seeds of *Lepidium Sativum*

The mucilage extracted from the seeds of *Lepidium Sativum* is popularly used as an excipient in different pharmaceutical and food preparations. The mucilages are mainly present around the out layer of seeds of *Lepidium Sativum*. From the literature, it was identified that it is very difficult to extract the mucilage from the seeds as it swells around the seeds but does not separate easily. In literature, different methods were adopted to find out the suitable extraction process utilizing various organic solvents. In our study, we evaluated the available literature database by using prior art information. At the initial stage, it was decided to adopt the methods that are feasible on the laboratory scale, easy to use, and produce a better yield. Thus, selected trials were planned (named screening trials) and are presented in table 1. Screening trials were performed by taking small quantities (50 g for each method) of seeds. The main purpose behind this is to understand the feasibility of the extraction process and ease of operation. The different techniques adopted for the extraction of seed mucilage from *Lepidium Sativum* were summarized below.

Table 1: Details of screening trials for extraction and isolation of mucilage from seeds of *Lepidium Sativum*

Sr. No.	Extraction Process	References
Method-1	Precipitation of mucilage in acetone after boiling in water and blending by using a hand blender	[4,14,19]
Method-2	Precipitation of mucilage in ethanol by using soaked seed	[4,14,19,20,21]
Method-3	Extraction and isolation of mucilage by boiling seeds in water and using ethanol as a solvent	[4,19, 20]
Method-4	Extraction and isolation of mucilage by soaking crushed seeds in water and using ethanol as a solvent	[22,23]

From the screening methods, three methods were finally selected for the pilot-scale study. The details of observation and yield of the pilot-scale study were enumerated in the results section (table 5). Based on the results of the pilot study the quality by design approach was performed to identify the critical factors and based on the study design finally a modified method was proposed.

Experimental design for the extraction process to get maximum yield by using full factorial design

A full factorial design was used for the development of a systematic extraction and isolation of seed mucilage of *Lepidium Sativum* using software Minitab 173.0. Three factors having two levels (+1and -1) of the critical extraction process variables were used, designated as X1: pH of extract (5-10) and X2: Water to seed ratio (10:1-30:1), and X3: Drying temperature (40-60˚C) keeping all other extraction processes same. A full factorial design was used to evaluate the influence of critical factors on the response yield. The model was further examined through the obtained polynomial equation, ANOVA study, and percent of the coefficient variance. A total of 10 experimental runs were generated having 2 center points, 8 base designs, 1 replicate, and 1 block. The different level of independent variables (factors) is given in table 2. The full factorial study design considering the three factors using statistical software Minitab 17.3.0 are presented in table 3. For each experimental trial, the extraction process adopted was the same and only the critical factors are changed as per the full factorial study design. The obtained extracts of each trial were subjected to drying and sizing. All operations were carried out as per the standard extraction process.

Table2: Levels of independent variables for optimization

Code	Variables	Level of variables		
		-1	0	+1
X1	pH of extract	5	7.5	10
X2	Water to seed ratio	10:1	20:1	30:1
X3	Drying temperature	40	50	60

Table3: Full factorial design obtained by statistical software Minitab 17.3.0.

Run order	**pH of extract**	**Water to seed ratio**	**Drying temperature**
1	5.00	10:1	60
2	5.00	30:1	60
3	10.00	30:1	60
4	10.00	10:1	60
5	5.00	10:1	40
6	10.00	10:1	40
7	7.50	20:1	50
8	5.00	30:1	40
9	10.00	30:1	40
10	7.50	20:1	50

A modified method for extraction and isolation of mucilage from seeds of Lepidium Sativum

During the pilot-scale evaluation, three methods were studied to evaluate the scalability effect as well as % the yield of dried mucilage. Finally, extraction and isolation of mucilage by boiling seeds in water using acetone as a solvent was selected as a final method. This method was selected due to its ease of operation and a higher % of yield (table 5). The recommended critical steps for the modified method as compared to the existing process are

- Adjustment of pH to 10 by using 0.1 M HCl or NaOH
- Maintaining the water to seed ratio of 30:1
- Inclusion of soaking of seeds before boiling step

- Keep the filtrate in the refrigerator for five hours before the removal of mucilage from the filtrate.
- Optimization of drying temperature

The details of the modified method was enumerated below

Extraction and isolation process (Modified method)

In this method, about 100 g of *Lepidium Sativum* seeds were soaked for overnight at least 12 hours in 3000 ml of distilled water. The pH was adjusted to 10. The soaked seeds were boiled in distilled water for 15 minutes at a temperature of 60°C and kept aside for half an hour to settle down the foam generated during heating. The seeds were then blended by using a hand blender which enables easy removal of the swollen mucilage from the sides of the seeds. Hand blending of seeds was performed for 15 minutes by using a 175-watt Philips HR 3700 hand blender. The mucilage from the seeds was separated by filtering through a muslin cloth. The retained blended seeds were again blended with additional 200 ml of hot water for 15 minutes by using a 175 watt Philips HR 3700 hand blender. To get the best yield, the blended seeds were re-filtered using a muslin cloth. The filtrate from both the steps was collected in a beaker and treated with an equivalent amount of acetone (1:1 ratio of liquid containing mucilage and acetone). After the addition of an equivalent amount of acetone, the beaker was kept in a deep refrigerator for 5 hours. This helps to get the maximum amount of mucilage from the treated liquid mixture. After 5 hours the liquid was removed and the white supernatant coagulant mass was separated above the beaker. The precipitated mucilage was collected in a petridish and drying was done at 45°C for 6 h in a hot air oven till it was completely dried. A mortar and pestle were used to grind the dried material. The triturated material was pulverized using an 80 # mesh and weighed to determine the yield. The finalized powder is stored in a desiccator until it is further used. The final seed mucilage powder discussed in the modified method can be easily illustrated from the pictorial diagram as shown in figure 6 and figure 7. Further, the following precautions were proposed to maximize the yield-

1. Time for soaking of seeds fixed for NLT 8 hours.
2. Adjustment of pH to 10 and boiling of seeds for at least 30 minutes at 60°C.
3. After boiling the seeds, it was allowed for half an hour to settle down the foam.
4. During hand blending crushing of seed was avoided.
5. Storage of the filtrate in a deep freezer before precipitation or separation of mucilage

Evaluation of flow properties of extracted mucilage

The extracted mucilage was evaluated for flow characteristics and compressibility index. To evaluate these parameters, the sample was characterized for bulk density (BD), Tapped density (TD), Compressibility index (CI), Hausner's ratio (HR), and angle of repose [24,25]. Approximately 1 g of sample was placed in the sample pan of a sartorius moisture analyzer and the sample was heated at 105^{0}C temperature till constant weight was achieved. Then the final value was noted and considered as the percentage of moisture content present in the blend.

RESULTS

In the present study, the extraction and isolation were carried out by using seeds of *Lepidium Sativum* as the base material with the help of suitable solvents (ethanol and acetone), using an appropriate extraction process with suitable reagents and analytical instruments.

Extraction and isolation of mucilage from seeds of *Lepidium Sativum*

Different methods and techniques; have been reported for the isolation and extraction of seed mucilage from *Lepidium Sativum*. Thus, it is highly essential to screen the available methods to optimize the robust process. The observations of different methods used for screening the extraction of seed mucilage from *Lepidium Sativum* were summarized below in table 4.

Table 4: Observation and percentage yield of screening trials for extraction and isolation of mucilage from seeds of *Lepidium Sativum*

Sr No.	Extraction process	Observation	Observed Yield (g)	% yield
1.	**Extraction and isolation of mucilage by boiling seeds in water and using acetone as a solvent**			
	Method 1	Some of the seeds broken during hand blending	0.846	8.46
2.	**Extraction and isolation of mucilage by soaking seeds in water and using ethanol as a solvent**			

Sr No.	Extraction process	Observation	Observed Yield (g)	% yield
	Method 2	A very less amount of mucilage was precipitated.	0.544	5.44
3.	**Extraction and isolation of mucilage by boiling seeds in water and using ethanol as a solvent**			
	Method 3	Heating of seeds generated foam which was settled down after cooling.	0.711	7.11
4.	**Extraction and isolation of mucilage by soaking crushed seeds in water and using ethanol as a solvent**			
	Method 4	In the extracted mucilage some colored particles were observed which seem to be crushed parts of the seed.	0.628	6.28

Pilot-scale manufacturing of selected methods for extraction and isolation of mucilage from seeds of *Lepidium Sativum*

From the screening trials, three processes were selected for the further pilot-scale study. The observations of each process with their % yield were summarized below in table 5.

Table 5: Observation and percentage yield of screening trials of extraction and isolation of mucilage from seeds of *Lepidium Sativum*

Sr No.	Extraction process	Observation	Observed Yield (g)	% yield
1.	Precipitation of mucilage in acetone after boiling in water and blending by using a hand blender	The hand blender very effectively helped to remove the swelled mucilage around the seeds.	1.318	13.18
2.	Precipitation of mucilage in ethanol by using soaked seed	With the increase in batch size, the ethanol consumption was very high.	0.924	9.24
3.	Extraction and isolation of mucilage by boiling seeds in water and using ethanol as a solvent	Heating of seeds generated foam which was settled down after cooling.	1.048	10.48

Based on the results of the pilot study method (Table 5, Sr No. 1) was selected for optimization study by using an experimental design approach.

Experimental design

In the present study, the critical process attributes were optimized by using a full factorial design. The details of the factors with their studied level are presented in Tables 2 and 3. As per the factorial study design, 10 trials were planned and the % yield obtained was evaluated. Quadratic models were applied to study the relationships of factors pH of extract, water to seed ratio, and drying temperature on response % yield. A statistical model summary of the response variable (% yield) is presented in table 7.

Table 6: Summary of the response variable (% yield) obtained with different levels of studied factors i.e., pH of extract, water to seed ratio, and drying temperature

Run order	Factors (Independent variable)			Response (Dependent variable)
	pH of extract	Water to seed ratio	Drying temperature	% Yield
1	5.00	10:1	60	6.21
2	5.00	30:1	60	8.81
3	10.00	30:1	60	20.12
4	10.00	10:1	60	8.46
5	5.00	10:1	40	5.68
6	10.00	10:1	40	13.32
7	7.50	20:1	50	14.81
8	5.00	30:1	40	8.11
9	10.00	30:1	40	20.16
10	7.50	20:1	50	13.66

Table 7: Statistical model summary of pH of extract, water to seed ratio, and drying temperature on yield

Source of Variation	DF	Adj Sum of square	Adj Mean square	F value	P-value summary	
					Value	Significant
Model	7	252.813	36.116	21.47	0.045	Model significant
Linear	3	209.087	69.696	41.44	0.024	
pH of extract	1	130.195	138.195	82.16	0.012	
Water to seed ratio	1	69.208	69.208	41.15	0.023	
Drying temperature(˚C)	1	1.684	1.684	1.00	0.422	
2-Way Interaction	3	30.490	10.163	6.04	0.145	
pH of extract* Water to seed ratio	1	22.680	22.680	13.48	0.067	
pH of extract* Drying temperature(˚C)	1	4.697	4.697	2.79	0.237	
Water to seed ratio *Drying temperature(˚C)	1	3.113	3.113	1.85	0.307	
Curvature	1	13.237	13.237	7.87	0.107	
Lack-of-Fit	1	2.703	2.703	4.09	0.292	Lack-of-Fit not significant

Model Summary		
S	R-sq	R-sq(adj)
1.29693	98.69%	94.09%

Effect pH of extract, water to seed ratio, and drying temperature on yield

The result of the response variable yield is presented in table 6 and the statistical model summary is presented in table 7. The full quadratic polynomial equation for the measured response (yield) is given below:

Hardness (N) = 0.15 + 1.85 pH of extract + 0.523 Water to seed ratio

+ 0.059 Drying temperature + 0.0673 pH of extract*Water to seed ratio

- 0.0307 pH of extract*Drying temperature

+ 0.00624 Water to seed ratio*Drying temperature + 2.88 Ct Pt

The above polynomial equation represents the quantitative effect of the critical attributes (factors) on the studied response yield. The above-studied equation showed a good fit with the studied factors and response due to the close concurrence of R^2 between R^2 and adjusted R^2. The result of multiple linear regression analysis for yield demonstrated a positive sign for all three studied variables. This suggested that with an increase in the level of all three studied variables, the response yield increases. The ANOVA analysis of the model indicated that the applied model is significant and all the studied factors had significantly affected ($p < 0.05$) in envisaging the response (yield). The relationship between the factors and the studied response can be easily exemplified from the Pareto chart and contour plot as shown in fig 1, fig 2, fig 3, and fig 4.

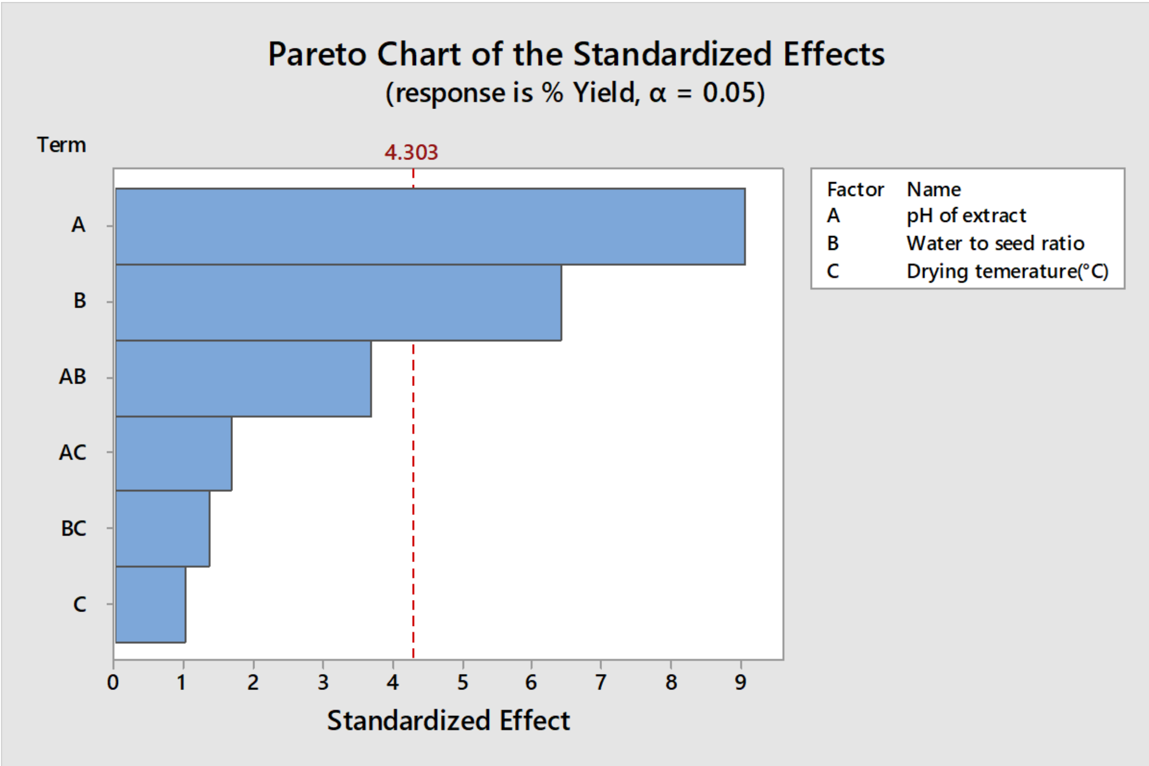

Figure 1: Contour plot of yield on the variables pH of extract, water to seed ratio, and drying temperature

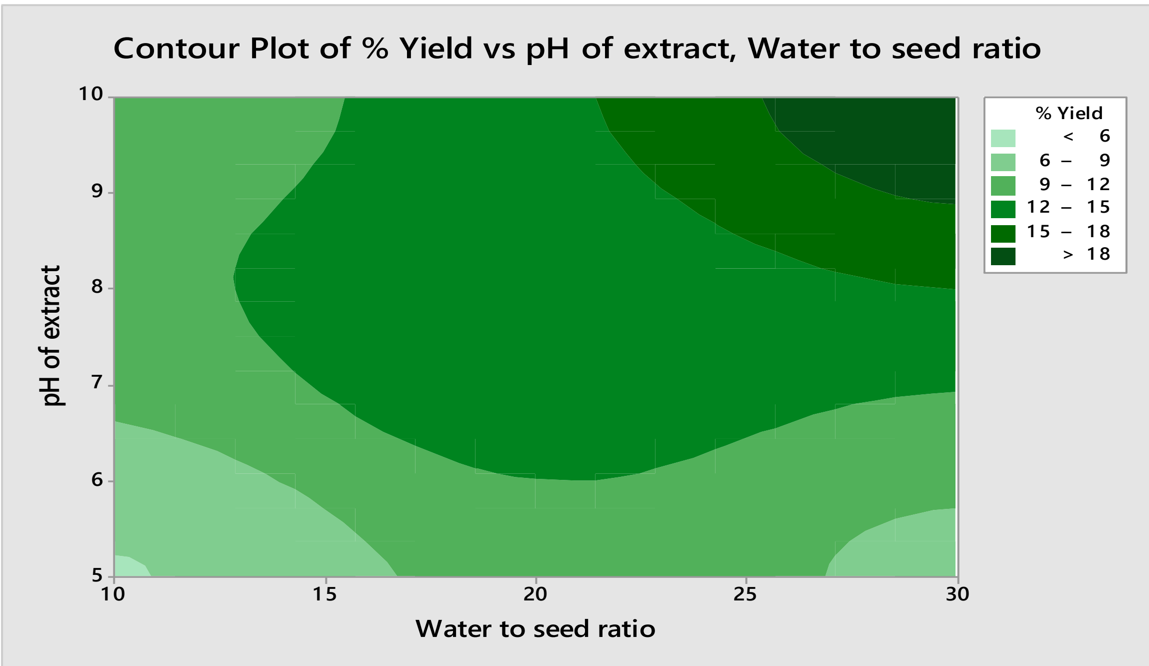

Figure 2: Contour plot of % yield on the variables pH of extract and water to seed ratio

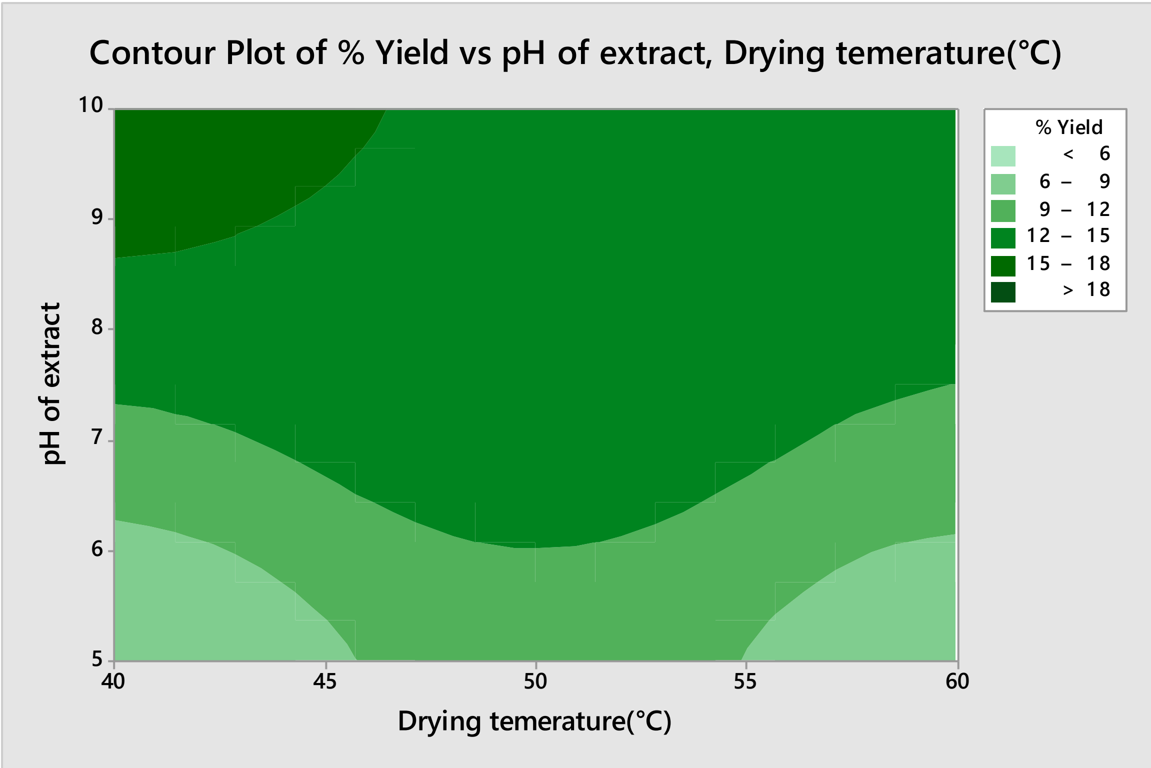

Figure 3: Contour plot of % yield on the variables pH of extract and drying temperature

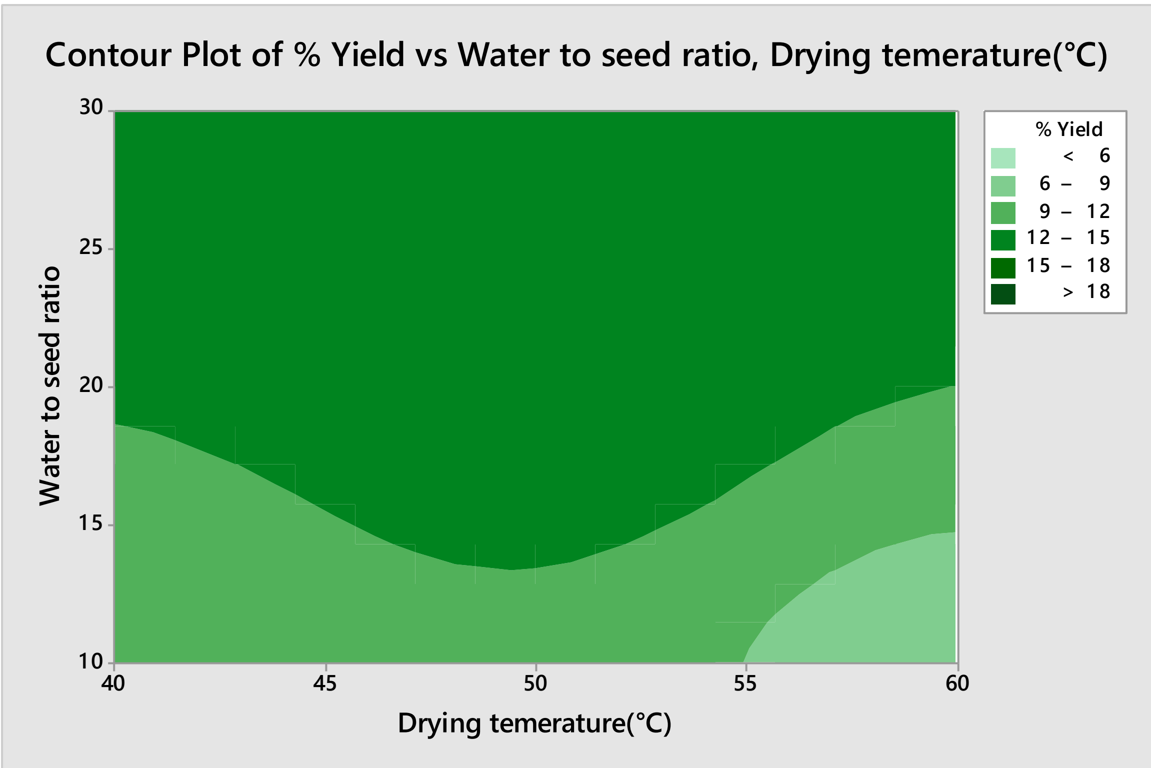

Figure 4: Contour plot of % yield on the variables water to seed ratio and drying temperature

Factorial plot for % yield

An interaction effect is used to study the synchronous effect when two or more independent variables have a combined influence on at least one dependent variable. In the present study, the factorial plot was used to study the main effects and interaction effects between the studied variables and the responses % yield (figure 5). The main effect plot indicated that the factor pH of the extract and water to seed ratio had a significant effect on the response yield as compared to the factor drying temperature.

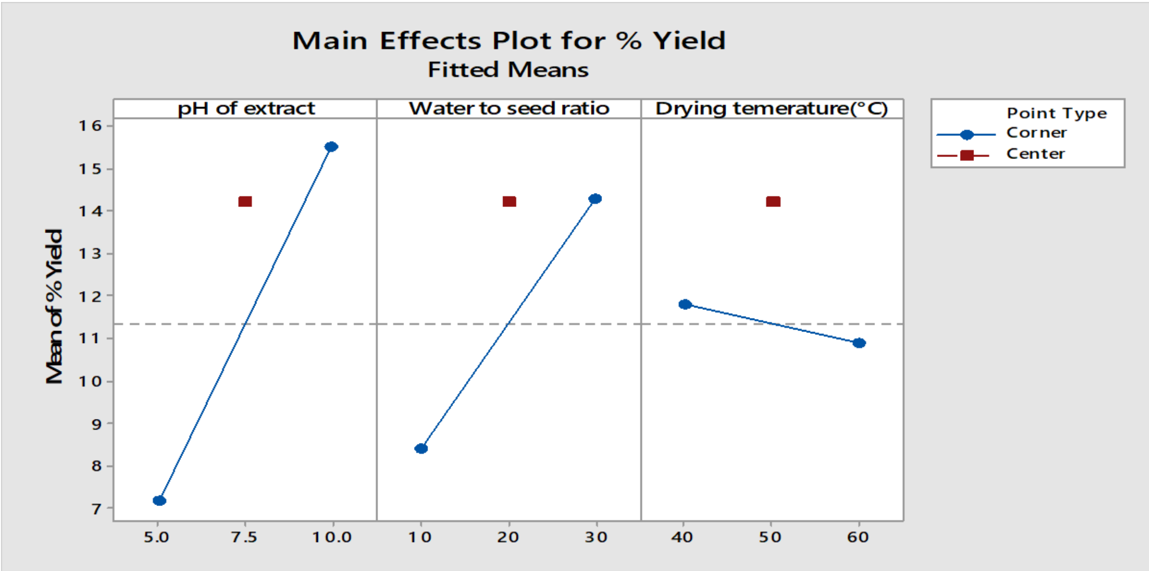

Figure 5: Main effects plot for % yield against studied variables pH of extract, water to seed ratio, and drying temperature on yield

Extraction and isolation of *Lepidium Sativum* seed mucilage by using a modified method

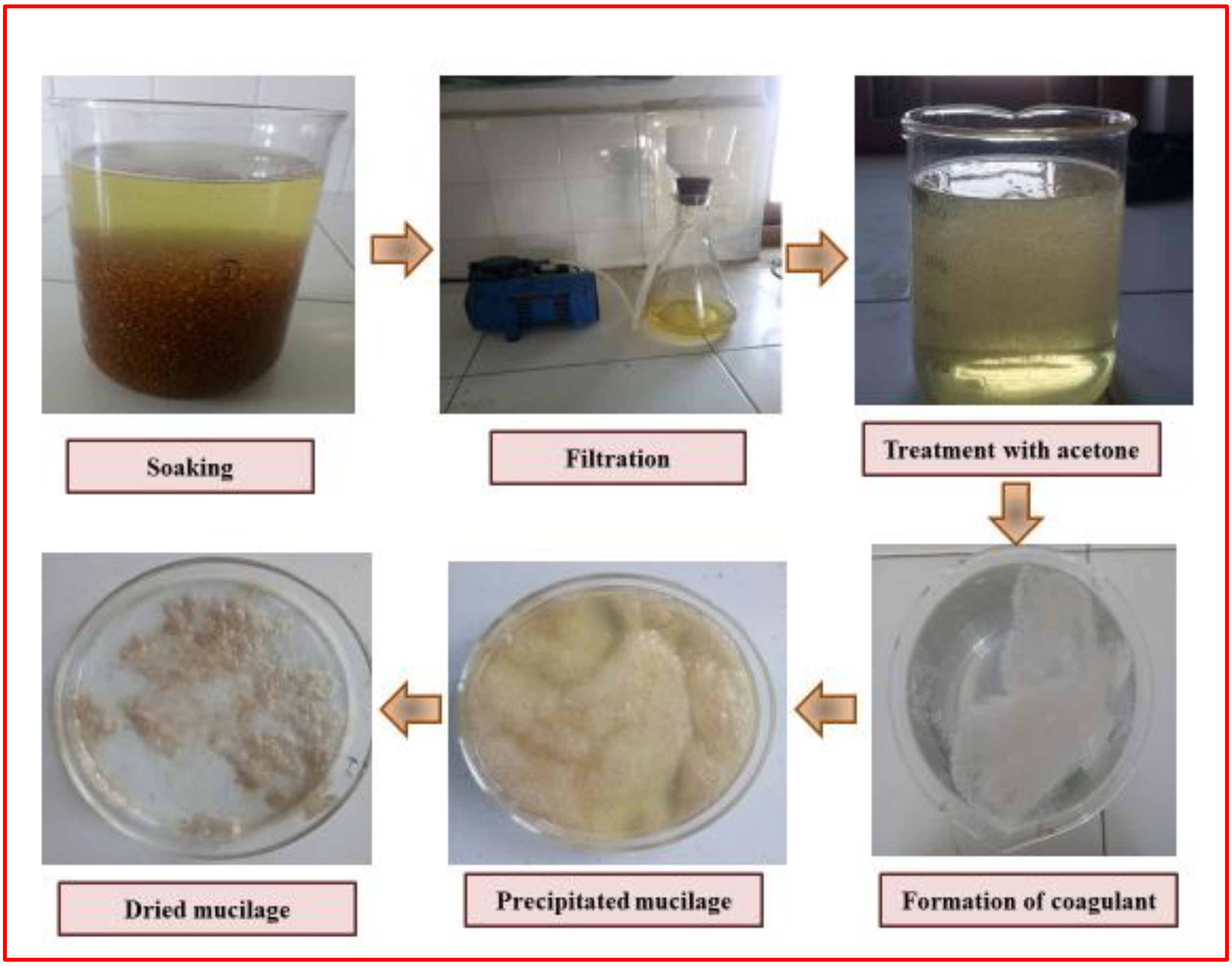

Figure 6: Pictorial diagram of final extraction process showing the dried mucilage

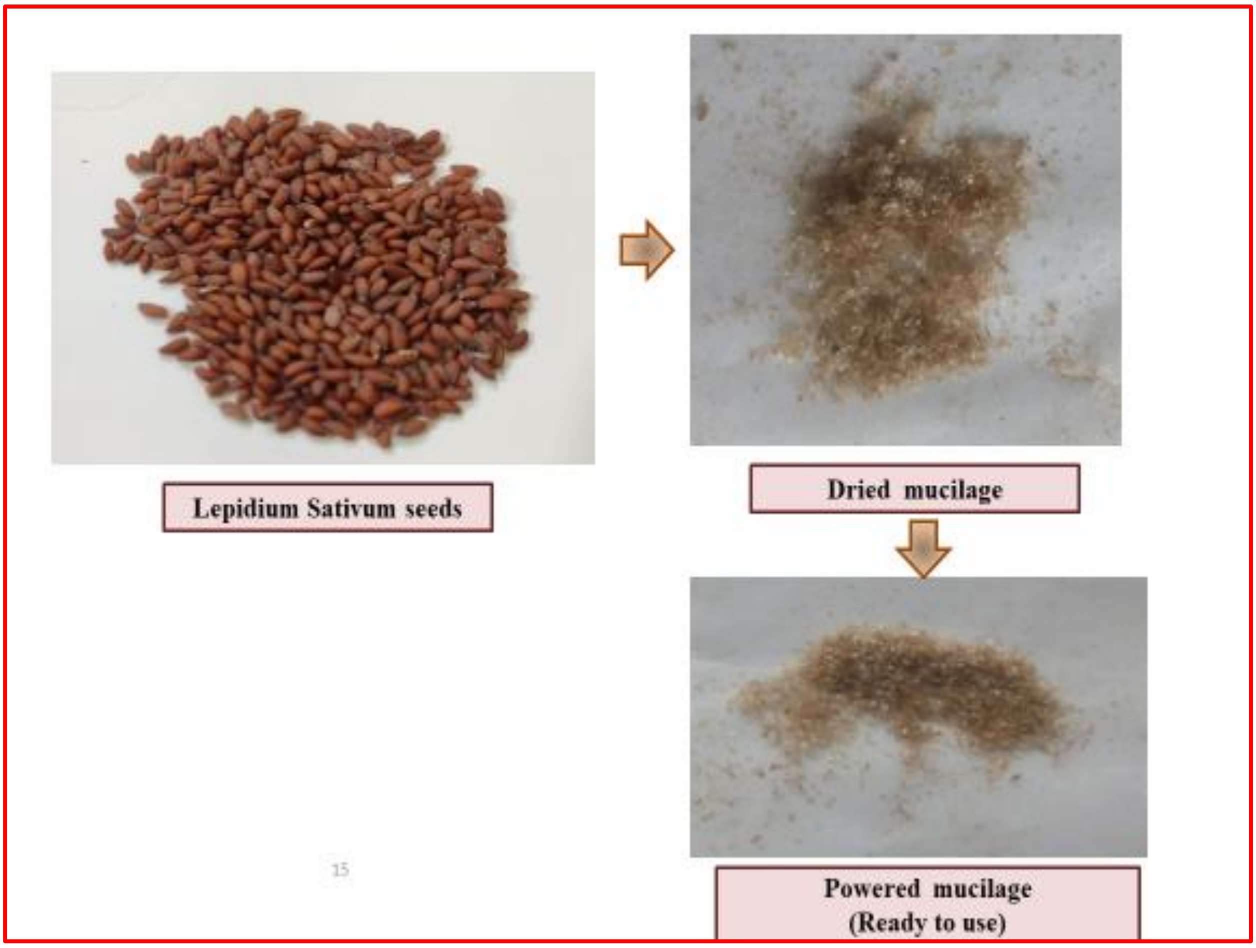

Figure 7: Pictorial presentation of raw seeds of *Lepidium Sativum* to powdered mucilage

Evaluation of flow properties of extracted mucilage

The micrometric parameters for the extracted seed mucilage were accomplished and the results are presented in table 8. The bulk density and tapped density (0.36 and 0.45 g/cc), angle of repose (38.21°), Hausner's ratio (1.25), and Carr's index (20.00 %) were found to be satisfactory.

Table 8: Evaluation of flow property of extracted seed mucilage of *Lepidium Sativum*

Evaluation of flow property of extracted seed mucilage of *Lepidium Sativum*	
Bulk Density	0.36±0.06 g/cc
Tapped Density	0.45±0.08 g/cc
Carr's Compressibility Index	20.00±1.1 %
Hausner ratio	1.25±1.4
Angle of repose	38.21±1.1

DISCUSSION

Preparation and optimization of extraction process of seed mucilage of *Lepidium Sativum*

In the current research, an attempt has been made to extract seed mucilage of *Lepidium Sativum*. A screening study was performed and the selected methods were evaluated. Four methods were evaluated in the preliminary stage (screening study) and out of these four methods three methods were finalized based on steps involved, ease of processing, cost, and finally yield of the mucilage obtained. Out of three methods, the first method was selected for the extraction process. The method selected was based on the feasibility of the extraction process as well as observed yield. The observed yield was 11.18 % by using acetone as a solvent and using a hand blender as compared to other methods. Based on this fact method 1, involved boiling of seeds in water and precipitation of mucilage using acetone as the final method.

It is always censorious and laborious to develop and optimize complex extraction processes for the isolation and extraction of any natural plant origin product. Thus, to arrive at a cost-effective process full factorial design was used for statistical process control. Based on the selected design, a total of 10 formulation combinations were made and evaluated for the response percentage yield. The above-discussed extraction method was used to perform the trials as per the suggested design.

The effect of each factor was studied with respect to the dependent variable percentage yield. From the study, it has been observed that the response varies significantly with the change in the independent variables. There was a clear indication from the results that the factor pH of extract and water to seed ratio imposes a great impact on the response variable yield. A trend of increase in the yield was observed with an increase in the pH of the extract from 5 to 10. It was observed that an alkaline environment helps to increase the yield. The alkali condition promoted hydrolyze of the insoluble component that was originally associated with mucilage and thus increased the extractability of the mucilage which enables to increase in the extraction yield. The same type of pH-based extraction study concerning yield was proposed by Karazhiyan et al.[26,27]. They extracted mucilage from cress seed (*Lepidium Sativum*) by optimizing pH, temperature, and water: seed ratio by using response surface methodology.

Differently, the extraction yield of mucilage increased at higher pH and this effect was less affected by other variables (Figures 2, 3, 4, and 5). Likewise, Ibrahim et al. found a similar type of observation while extracting mucilage from Talinum paniculatum. They reported that the highest extraction yield of mucilage from Talinum paniculatum was obtained in the alkaline solution with an insignificant influence on temperature [28].

A similar trend was observed for the water to seed ratio where with increasing the water to seed ratio from 10:1 to 30:1, the yield drastically increases. An increase in water/seed ratio up to 30:1 increased the yield to a certain maximum value of 20.16g/100 g. The increase in solvent quantity leads to increased exposure of seeds to an aqueous medium [29]. Fig. 2 and fig. 4 show an increase in the yield with an increase in water quantity. The contour plot shows the effect of time is more pronounced at higher water/seed ratios. Extraction time influences the extraction efficiency and selectivity of the fluid. The seed mucilage *of Lepidium Sativum* is a rich source of polysaccharides. A significantly higher fluid content increases the extraction time and has a positive effect on the yield of polysaccharides [30]. Similar results were also observed with a larger solvent quantity favored the polysaccharide production from Ocimum basilicum seeds [31]. The increase in the yield with an increase in the ratio of seed to water is attributed due to the hydrolysis capability of the solvent. The capacity to hydrolyze the polysaccharides increases as the amount of solvent increases. The same type of solvent effect was studied by Karazhiyan et al.[26]. The higher yield may be due to the availability of more liquid that acts as a driving force to exude mucilage out of the seeds as the volume of water/seed ratio was increased [32]. A greater mucilage yield was also reported from Alyssum homolocarpum, wild sage seed gum, and Opuntia spp. seeds as a function of water ratio [33]. The results obtained were in agreement with the early work with mucilage extraction from Ximenia americana seed using response surface methodology performed by bazezew et al.[34]

The drying of mucilage is an important step in the extraction process. However, there was a clear indication from the results that there was no significant effect of the factor temperature on the response yield (fig 5). It was anticipated that a higher temperature may cause fiber degradation during the drying process at high temperatures and may show a charring effect. However, the efficiency of drying also depends on the type of solvent used. In our process, we are using acetone as a solvent for the extraction process. As expected, samples showed no significant difference in yield regarding drying temperature as yield is related to the method of extraction and separation of mucilage from seeds or husk. Acetone can be evaporated at a low temperature. Thus, a drying temperature of 45^0C was selected and a drying time of 6 hours was finalized. Maintaining the temperature during the extraction process is highly essential as high temperatures may lose the functional characteristics of the extracted mucilage, and may hydrolyze the polysaccharides which ultimately affect the yield. Yield is directly influenced by the content of polysaccharides present in husks or seeds. We suggested that the drying process influences the content in the chemical structure of polysaccharides, and this might be responsible for the significant changes. The results obtained were in agreement with the early work performed to study the effects of drying methods on physicochemical properties and antioxidant activities of polysaccharides from Astragalus cicer L.[35]. The neutral effect of temperature can also be confirmed from the main interaction plot. The main effect was evaluated for % yield with the factor temperature. In our study, it has been observed that the line is vertical which signifies that the main effect is not present for the studied factor. Many other studies have reported a decrease in mucilage yield at the increasing temperature of the extraction medium which may be due to thermal degradation of the polysaccharides in the mucilage at high temperature [36]. Similar findings have been demonstrated for extraction optimization of chia seed mucilage [32] and Alyssum homolocarpum seed mucilage [37].

A similar type of trend was observed in the main effect plot (fig. 5). The main effect was evaluated for % yield with three studied factors pH of the extract, water to seed ratio, and drying temperature. In our study, it has been observed that the line is horizontal for the pH of the extract, and the water to seed ratio which signifies that the main effect is present for these two factors only. In the main effect plot, the pH of the extract 10 appears to be associated with the highest % yield, and a decrease in the value from 10 to 5 decreases the % yield. The same trend was observed for the water to seed ratio. It followed a linear trend where with an increase in water to seed ratio the percentage yield increases.

Response optimization was used to find the best combination of variables studied for the response yield. This is useful to assess the best possible combinations of the variable to get the highest percentage yield. In our study, we used response optimization recommendations to arrive at a suitable extraction process parameter. The standard error of the regression equation (S) which represents the relation between actual and predicted response was found to be 1.29693 for the response percentage yield. It represents the average distance of the data points from the fitted line and was found to be 1.29 % for response yield. The adjusted regression value (R^2) was 94.09 % for the studied response yield. The lower S value and higher adjusted regression values (R^2) reflected the appropriateness or goodness of the model. Based on the factorial design study and the recommendation obtained by the response optimization run 3 and 9 are selected as an optimization process. However, the process was modified to ease the process of operation and improve the yield. The modified process implemented some modifications as compared to other reported methods such as maintaining pH to 10, optimizing the extraction temperature, soaking time of seeds to a minimum of 8 hours, time for settling down the foam, precaution to avoid crushing of seeds during hand blending, and storage of filtrate in the refrigeration before precipitation of mucilage. The extraction temperature allows better penetration of water into the solid matrix to solubilize the compounds. As a result, the mucilage was easily released and the extraction yield increased [33].

The modified method based on the discussed recommendations produced a yield of 21.00 %. Many researchers worked on the extraction process of seed mucilage of *Lepidium Sativum*. However, such type of higher yield was never reported. Thus, our recommended process can be used for extraction and isolation of the *Lepidium Sativum* mucilages present around the seeds.

From the micromeritic study presented in table 8, it can be concluded that the good angle of repose (Fair- aid not needed) enables the dried mucilage to have good flow properties, and also the fair CI and HR showed that it is having good compressibility. The Bulk density and tapped density were observed as 0.36 and 0.45 g/cc respectively. Furthermore, Archana et al.[20] reported bulk and tapped densities of 0.2857 and 0.3389 (g/cc) respectively. Meanwhile, Bhatia et al.[5] reported 0.673 and 0.7 g/ml and Hassan et al.[38] reported 0.1396 and 0.2178g/ ml for bulk and tapped densities respectively. It is observed that the reported bulk densities and tapped densities vary in a wide range. The bulk and tapped densities predict the capacity of a powder to be compressed and reflect the importance of inter particulate interactions [39].

The CI and HR are used to estimate powder flow because they are correlated with powder density and internal friction. Specifically, they describe the extent of interparticle friction against the gravitational force (for bulk density), as well as the inertial force triggered by tapping (for tapped density) [40]. The bulk density and tapped density of material depend on the porosity and particle size of the studied material [41]. It is well known that extraction processes may change the porosity and particle size of the final material. Thus, it is believed that the different bulk densities and tapped densities observed might be due to the extraction process adopted. All these studied flow properties with good flow properties and compressibility index indicated that it can be recommended as an excipient for a direct compression formulation in the pharmaceutical and food industry manufacturing process.

CONCLUSION

The seed mucilages of *Lepidium Sativum* were extracted by using cost-effective and easily accessible solvents. The method selected was based on the feasibility of the extraction process as well as observed yield. The observed yield was 20.16 % which was relatively higher compared to other reported yields. A full factorial design was used to investigate the impact of formulation factors on response variables (% yield) using a quadratic model. Statistical analysis revealed that the model was capable to measure the response with a small change in the independent variable. Statistical analysis of the proposed model showed a good coefficient of regression for response percentage yield (0.9869). The F-ratio of the regression for all test variables against response variables was significant except for drying temperature. The statistical model represented a good relationship between the studied factors with response % yield as the actual and predicted results are in agreement with a 95% confidence interval. The optimal condition predicted an extraction yield of 20.16 g/100 g at 40-60^{0}C, at a pH of 10, and a water/seed ratio of 30:1. Optimal conditions were determined to obtain the highest extraction yield. Results indicated that the water/seed ratio and the pH of the extract was the most significant parameter, followed by temperature. The study demonstrated that seed mucilage of *Lepidium Sativum is* extracted by using an economic extraction process.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare

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ETHICAL APPROVAL

Ethical approval is not required for this study

AUTHOR'S CONTRIBUTIONS

We declare that this work was done by the authors named in this article: APKM conceived, designed the study, and carried out the laboratory work. SKP and BP collected and analyzed the data. SNM drafted the manuscript. PNM supervised the work and assisted in the data analysis. PNM contributed to the final revision of the manuscript. All authors have read and approved the final manuscript. All authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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