ORIGINAL ARTICLE

Development and Evaluation of Herbo-Mineral Supplement Fortified with Bhasma by Full Factorial Design

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ABSTRACT Herbs as nutritional supplements in drugs or food are in demand nowadays. Herbs are an excellent source of mineral elements, but no plant supplies all the desired elements. So, here we combined herb powder and ayurvedic formulas like bhasma because of the above matter. The Moringa tree is a miracle tree for its nutritional value. Research work aimed to formulate and evaluate powder using herbs incorporated with prepared classical formulations like 'Bhasma' to provide essential elements like Calcium, Iron, Zinc, and Copper. The formulation was prepared by trial and error method using herbs like Moringa leaf powder, Beetroot powder, Lauha bhasma, and Jasat bhasma in various combinations. Suitable formula from bathes was selected and evaluated further for consistency and stability as per standard guidelines.

Keywords: Nutrition, Herbal powder, Bhasma, Moringa, Minerals

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INTRODUCTION

Plant materials or their extracts have been utilized as drugs since ancient ages which are arguably poised for a comeback as sources of human health products following the endorsement by the World Health Organization (WHO) to use traditional plant-based products to fulfill needs unmet by modern systems (Winslow and Kroll, 1998). In 2013, WHO developed and launched 'WHO Traditional Medicine Strategy 2014-2023' and emphasized integrating traditional and complementary medicine to pr omit e universal healthcare and ensuring the quality, safety, and effectiveness of such medicine (WHO, 2013). Herbs or other botanicals.(1)

Moringa has a lot of minerals that are essential for growth and development, among which calcium is considered one of the critical minerals for human growth. While 8 ounces of milk can provide 300-400 mg, moringa leaves can provide 1000 mg, and moringa powder can provide more than 4000 mg. In addition, Moringa powder can substitute for iron tablets, hence treating anemia. Beef has only 2 mg of iron, while moringa leaf powder has 28 mg of iron. It has been reported that Moringa contains more iron than spinach.(2)

Moringa oleifera belonging to the family of Moringaceae is an effective remedy for malnutrition. Moringa is rich in nutrition due to various essential phytochemicals present in its leaves, pods, and seeds. For example, Moringa provides 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yogurt, 15 times more potassium than bananas, and 25 times more iron than spinach.(3) In the current era, modern lifestyles built around automation, reduced physical activity, and other socio-economic factors have contributed to the rising trend in non-communicable diseases.(4)

The intense red color of beetroots derives from high concentrations of betalains, a group of phenolic secondary plant metabolites.(5) Betalains are used as natural colorants

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by the food industry but have also received increasing attention due to possible health benefits in humans, especially their antioxidant and anti-inflammatory activities.(6)

It shows following pharmacological activity that it has not only in resorting hemoglobin level but in significantly increased body weight gain in *Bhasma*-treated animals and helpful in iron deficiency anemia. Furthermore, it is a powerful hematinic and tonic and is valuable in treating hemolytic jaundice and microcytic anemia 2, 7. The concept of using nanometal particles is prevailing since *Charakasamhita*.(9) For a metallic preparation of *Lauhadi Rasayana*, and iron is used to heat up until red hot and quenched in some liquid media immediately until flakes of iron become in fine powder form.(10)

Consumers now recognize the need to improve their diets with additional nutrition and healthy options. Supplements are increasingly being used to maintain an active lifestyle and address specific health concerns. However, advice by regulatory agencies to improve micronutrient intakes through a well-balanced diet may be inadequate.(11) Linking herbal supplements with traditional authentic medicines like bhasma may fulfill the need for all possible minerals in a single formulation. We want to try such combinations for making a simple but effective powder supplement fortified with ayurvedic bhasma.(12)

MATERIAL AND METHODS

Materials

The Moringa leaf powder and beetroot powder were purchased from Shashwat herbals Pvt. Ltd., but the care was taken that material should be fresh and hygienic. Lauha bhasma and Yashad bhasma (Baidyanath) were purchased from a local shop. Starch, sodium starch glycolate, magnesium stearate, and talc were purchased from chemdyes ltd. After collecting powder, they were passed through sieve No. 40.

Methods

Preparation of Powder Formulation

The powder composition of various herbal products shown in Table 1 was mixed and passed through sieve no. 80 to get the homogeneous mixture. Then, this mixture was utilized to form the granules by adding excipients.

Table 1: Composition of Powder Mixture (10 g)

S. No.	Content	Quantity
1	Moringa oleifera leaf powder	6.0 g
2	Beta Vulgaris root powder	3.7 g
3	Lauha bhasma	200 mg
4	Yashad bhasma	100 mg

Evaluation of Powder

Organoleptic Evaluation: A Five-Point Hedonic Rating Scale (Amerine *et al.*, 1965) was used for rating the attributes, viz., color, taste, flavor, and overall acceptability, and the evaluation was carried out by 10 panelists selected based on their sensitivity to different tastes.

Physicochemical and Phytochemical Evaluation: Various physicochemical parameters like pH and total sugars were performed.(13-15)

Determination of Moisture content: The AACC method measured the moisture content. A two-gram sample was placed in a preheated and weighed glass Petri-plate and then dried in a hot air oven at 130 °C for 2 hrs. Or till constant. Weight after drying glass Petri-plate was transferred to the desiccator to cool, and then petri-plate was reweighed. The loss in weight was calculated as a percentage of moisture content.

Determination of Ash Content: The ash content was measured, described by AACC (19) method. A two-gram sample was placed in a Pre-weighed crucible, and then the uncovered crucible was allowed to incinerate in a muffle furnace at 820 °C for 4 hours, and then the crucible was cooled in a desiccator and then weighed.

Determination of Extractive Value: Extractive Value was measured by cold maceration method, both aqueous and alcohol. The 4 g sample was mixed with 100 ml of water/ethanol and kept for 24 hours with occasional shaking. From total content, 25 ml was taken in Petri dish and evaporated to dryness.

Determination of Total Phenolics (16-18): Moringa leaf powder's total phenolic contents (TPC) were estimated using the Folin-Ciocalteu method described by Wojdylo et al. with minor modifications. The mechanism is based on the reduction of phosphotungstic acid to phosphotungstic blue, and as a result, absorbance increases due to rising in the number of aromatic phenolic groups. For this purpose, 50 L of each prepared extract was separately added to test tubes, each containing 250 L of Folin-Ciocalteu's reagent and 750 L of 20% sodium carbonate solution. The final volume was made up to 5ml with distilled water. After two hours, absorbance was measured at 765 nm using UV/visible light Spectrophotometer (Shimadzu) against control having all reaction reagents except sample extract. Total polyphenols were estimated, and values were expressed as gallic acid equivalent (GAE; mg gallic acid/100 g) using the following formula:

C = c × V/m

where C = Total phenolic contents (mg/g plant extract, in GAE)

c = Concentration of gallic acid (mg/mL)

V = Volume of extract (mL)

m = Weight of turmeric extract (g)

Method of Granules Preparation

Granules were prepared by the wet granulation method. First, powders and bhasma were taken in specified quantities mixed. This was followed by the addition of sodium starch glycolate (SSG). The granules were prepared by adding a sufficient starch paste to form a lumpy mass. The Granules were then prepared by passing through sieve No. 10 and dried in a hot air oven at 40 °C for 30 mins. After drying, the granules were retained on the sieve, and no 40 were collected. Talc and magnesium stearate was added at the end.

Optimization

Optimization was done by applying a design expert tool. For optimization, the concentration of sodium starch glycolate (A) and concentration of binder (B) was selected as independent factors. These two factors might affect the granule formulation. Three levels of each factor were selected and arranged according to 32 full factorial experimental designs. The disintegration time (Y1) and the friability of granules (Y2) were dependent factors. Based on the experimental design final formulation with the actual amount was displayed in Table 3. According to the design, 9 batches were prepared, and optimization was carried out using dependent factors.

Checkpoint Analysis

A checkpoint analysis was performed to confirm the role of derived polynomial equations and contour plots in predicting the responses in the preparation of granules. First, two checkpoint values of independent variables (A and B) were taken at any one point from each contour plot. Next, theoretical values of dependent variables were calculated by substituting the values to the respective polynomial Equation. Then, granules were prepared experimentally at 2 points and evaluated for the responses. Each batch was prepared three times, and mean values were determined.

Optimization of Formulation with Desirability Function

Optimization was performed to determine the independent variables (A and B) level that would yield Value of Disintegration time and % friability. The desirability function was used for the optimization of the formulation. During formulation optimization, the responses have been combined to produce a product of the desired characteristic. The application of the desirability function combines all the responses in one experiment and gives the possibility of predicting optimum levels for the independent variables. Combining the responses in one desirability function requires calculating the desirability function. By considering these facts, the optimization was performed through the software by setting the desired constraints for various variables to obtain the optimized batch, which has maximum desirability.(19-21)

EVALUATION OF GRANULES

Sensory Evaluation of Optimized Granules

A Five-Point Hedonic Rating Scale (Amerine *et al.*, 1965) was used to rate the attributes, viz., color taste, flavor, and overall acceptability.

Table 2: Independent Parameter with Levels

Independent Parameter	-1	0	1
Concentration of Disintegrants (%) (A)	4	6	8
The concentration of Binders (%) (B)	2	6	10

Table 3: Final Formulation Composition for Optimization

Ingredient	F1	F2	F3	F4	F5	F6	F7	F8	F9
Powder Mix (gm)	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
Sodium Starch glycolate (%)	6	6	4	8	4	4	6	8	8
Starch Paste (%)	10	6	10	6	6	2	2	2	10
Talc (mg)	5	5	5	5	5	5	5	5	5
Magnesium stearate (mg)	5	5	5	5	5	5	5	5	5

Characterization of Optimized Granules

The Angle of Repose: The fixed funnel method was employed to measure the angle of repose. A funnel was secured with its tip at a given height (h) above a graph paper placed on a flat horizontal surface. The blend carefully pored through the funnel until the apex of the conical pile just touched the tip of the funnel. Next, the radius of the base of the conical pile was measured. The angle of repose (θ) was calculated using the following formula: Tan θ = h/r Where, θ = Angle of repose, h = Height of the cone, r = Radius of the cone base. Values for the angle of repose ≤30° usually indicate a free-flowing material and angles ≥40° suggest a poorly flowing material, 25-30 show excellent flow properties, 31-35 show good flow properties, 36-40 show fair flow properties, and 41-45 showing passable flow properties.

Bulk Density: 15 g powder blend introduced into a dry 100 ml cylinder, without compacting. The powder was carefully leveled without compacting, and Vo's apparent unsettled volume was read. The bulk density was calculated using the following formula. ρb = M/Vo Where, ρb = Apparent bulk density, M = Weight of sample, V = Apparent volume of powder.

Tapped Density: After carrying out the procedure as given in the measurement of bulk density, the cylinder containing the sample was tapped 500 times initially followed by an additional tap of 750 times until the difference between succeeding measurement is less than 2% and then tapped volume, Vf was measured, to the nearest graduated unit. The tapped density was calculated using the following formula in gm per ml. ρtap = M/Vf Where, ρtap = Tapped density, M = weight of the sample, Vf = Tapped volume of powder.

Compressibility Index: The Compressibility index (Carr's index) measures the propensity of a powder to be compressed. It is determined from the bulk and tapped densities. In theory, the less compressible material, the more flowable it is. As such, it measures the relative importance of inter particulate interactions. Such interactions are generally less significant in a free-flowing powder, and the bulk and tapped densities will be closer in value. There are frequently greater interparticle interactions for poorer flowing materials, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the Carr's Index which is calculated using the following formulas: Compressibility index = [(ρtap – ρb)/ρtap]/×100 Where, ρb = Bulk Density, ρtap = Tapped Density.

Hausner's Ratio: Hausner's ratio is an indirect index of ease of powder flow. The following formula calculates it. Hausner's Ratio = Tapped Density (ρT)/bulk density (ρB) Where ρT tapped Density and ρB is Bulk Density. Lower Hausner's ratio (<1.25) indicates better flow properties than higher ones, between 1.25 to 1.5 showing moderate flow properties and more than 1.5 poor flow.

Table 4: Compressibility Index Values Carr's Index

Compressibility	Index Properties
≤10	Excellent
11 – 15	Good
16 – 20	Fair
21 – 25	Passable
26 – 31	Poor
32 – 37	Very Poor
>38	Very Very Poor

Granules Friability

Granule friability was subsequently measured in triplicate for the produced granules, as this measurement illustrates the granule strength. First, the granule fraction >250 µm was separated by sieving before analysis. Then, 10 g (m_1) of this fraction was added to a plexiglass drum with baffles together with 200 glass beads (mean diameter of 4 mm) (Carl Roth, Karlsruhe, Germany) and attached to a friability. The drum was rotated at a speed of 25 rpm for 10 min. Afterward, the granules and glass beads were separated. The granular mass >250 µm was again determined (m_2). Finally, the granule friability (%) was calculated according to the below Equation:

$$\text{Granule Friability (\%)} = \frac{(m1 - m2)}{m1} \text{ X } 100$$

Disintegration Test for Granules

In-vitro disintegration time of formulated herbal granules was determined by using digital disintegration test apparatus. In-vitro disintegration test was carried out at 37±0.5 °C in 0.1 N HCl, 10 mg of granules were placed in each of the six tubes of disintegration test apparatus. The time required for the complete disintegration of granules in each tube was noted.(22-25)

Stability

The accelerated stability study was carried out for 6 months. The temperature was regulated at 40 °C ± 2 °C with relative humidity (RH) 75%±5% using stability chamber (Frontline). The granules were packed in the sachet, and after 3 months, organoleptic and physicochemical parameters were evaluated.(26, 27)

RESULTS AND DISCUSSION

Organoleptic Evaluation

Organoleptic parameters like appearance, color, taste, and odor were performed in Table 5. From the data, it was observed that herbal powders contain their original characteristic.

Phytochemical Evaluation

Phytochemical Analyses were carried out to evaluate the suitability of the formulation for nutritional purposes. As per the data shown in Table 6, negative and positive signs indicate the absence or presence of respective components.

Physicochemical Evaluation

The extractive Value of Moringa powder and beetroot powder using water and Methanol is contained in Table 7. The result showed that the extractive Value of Moringa powder and beetroot powder was 8.543±0.32 %W/W and 14.38±0.17 in water which was higher than in Methanol which was 7.932±0.124 %W/W and 11.23±0.44, respectively. The choice of solvent solution is primarily determined by the type of bioactive substances being studied. Also accessible are various solvent solutions for extracting bioactive components from natural materials. The chemical composition of phytochemicals, the extraction method employed, sample particle size, the solvent utilized, and the presence of interfering compounds all affect extraction efficiency. The solvent and sample composition are the most relevant parameters under the same extraction time and temperature.

Table 5: Organoleptic Parameters of Herbal Powders

Parameters	Moringa Oleifera Leaf Powder	Beta Vulgaris Root Powder
Appearance	Fine powder	Fine powder
Colour	Dark green	Reddish pink
Taste	Mucilaginous	Sweet
Odor	Odorless	Characteristic

Table 6: Phytochemical Screening of Drugs

S. No.	Components	Test	Beta Vulgaris Root Powder		Moringa Oleifera Leaf Powder	
			Methanolic Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract
1	Alkaloids	Wagners test	-VE	+VE	-VE	+VE
2	Flavanoids	Shinoda test	+VE	+VE	+VE	+VE
3	Protein	Biuret test	+VE	+VE	+ VE	+VE
4	Carbohydrates	Benedicts test	+ VE	+VE	+ VE	+VE
5	Phenol	Lead acetate test	+ VE	+VE	-VE	-VE
6	Fat	Filter paper test	-VE	-VE	+ VE	+VE

Table 7: Physicochemical Parameters

Parameters Evaluated	Moringa Oleifera Leaf Powder	Beta Vulgaris Root Powder
	Value (%W/W)	Value (%W/W)
Moisture content	2.5±0.34	2.9±0.98
Ash value	4.9±0.34	7.65±0.28
Water-soluble Extractive value	8.543±0.320	14.38±0.17
Alcohol soluble Extractive value	7.932±0.124	11.23±0.44

Note: (Mean±SD, n = 3).

Determination of Total Phenolics

Total phenolics content was measured by the Folin-Ciocalteu method, according to procedure liner calibration curve of gallic acid with an R2 value of 0.9853 was determined. Figure 1 shows mean TPC of the beta vulgaris powder measured using the GAE equation of Y= 1.9071x+0.1727, whereby Y= absorbance at 765 nm and X = total phenolic compounds (TPC) in mg/ml. The extract showed an absorbance of 0.524 with TPC 18.4±0.034 mg/gm.

Optimization

The statistical significance of the difference in Y1 and Y2 was tested by ANOVA using a polynomial equation.

Y1 (DT time) = +3.71-0.93*A + 1.33*B – 0.30*AB

Y2 (Friability) = + 8.11-1.67*A – 7.17*B + 0.75*AB + 0.33*A^2 + 1.83*B^2

For (DT time) response Y1, The Model F-value of 487.60 implies the model is significant. Values of "Prob > F" less

Figure 1: Standard Calibration Curve for Gallic Acid (Absorbance vs. Concentration)

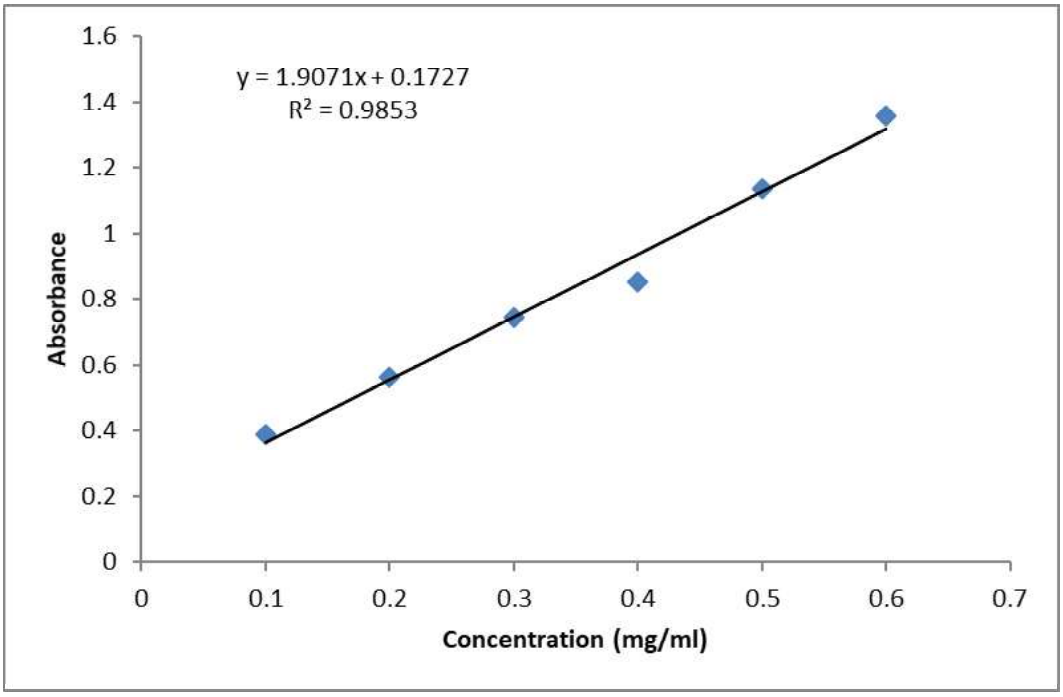


Table 8: Batches for Optimization of Granules by 32 Complete Factorial Design

Formulation Code	Concentration of Disintegrants (A) (%)	Concentration of Binder (B) (%)	Disintegration Time (Y1) (min)	Friability (Y2) %
F1	6	10	05±0.05	03±0.12
F2	6	6	3.8±0.12	08±0.69
F3	4	10	6.3±0.25	04±0.36
F4	8	6	2.6±0.06	07±0.89
F5	4	6	4.6±0.35	10±0.47
F6	4	2	03±0.25	20±0.56
F7	6	2	2.4±0.08	07±0.78
F8	8	2	1.8±0.48	15±0.61
F9	8	10	3.9±0.09	02±0.92

Note: (Mean±SD, n = 3).

than 0.0500 indicate model terms are significant. In this case, A, B, and AB are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Predicted R-Squared" of 0.9966 is in reasonable agreement with the "Adjusted R-Squared" of 0.9945. The difference is less than 0.2. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 64.510 indicates an adequate signal; thus, the proposed model can be used to navigate the design space.

For (Friability) response Y2, The Model F-value of 1030.71 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case, A, B, AB, and B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The "Predicted R-Squared" of 0.9994 is in reasonable agreement with the "Adjusted R-Squared" of 0.99984. It means the difference is less than 0.2. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. The ratio of 84.98 indicates an adequate signal; thus, the proposed model can be used to navigate the design space.

Effect of Disintegrant and Binder Concentration on DT Time

Response surface plot for DT time (Y1) is shown in Figure 2 from the graph, it can be observed that concentration of disintegrant and binder had a significant effect on DT time. As the concentration of sodium starch glycolate increases, there are decreases in DT time, which indicates faster breakdown of granules; on the other hand, as the concentration of starch paste increases, DT time increases, which is due to the increases in hardness of granules. Contour plots for a concentration of independent variables are shown in Figure 2.

Effect of Disintegrant and Binder Concentration on Friability

Response surface plot for Friability (Y2) is shown in Figure 3; from the graph, it can be observed that the concentration of disintegrant and binder had a significant effect on friability. As an increase in the concentration of starch paste, there are decreases in friability, which indicates more complex the

Figure 2: Three-Dimensional Response Surface Plots and Contour Plot for DT Time of Granules

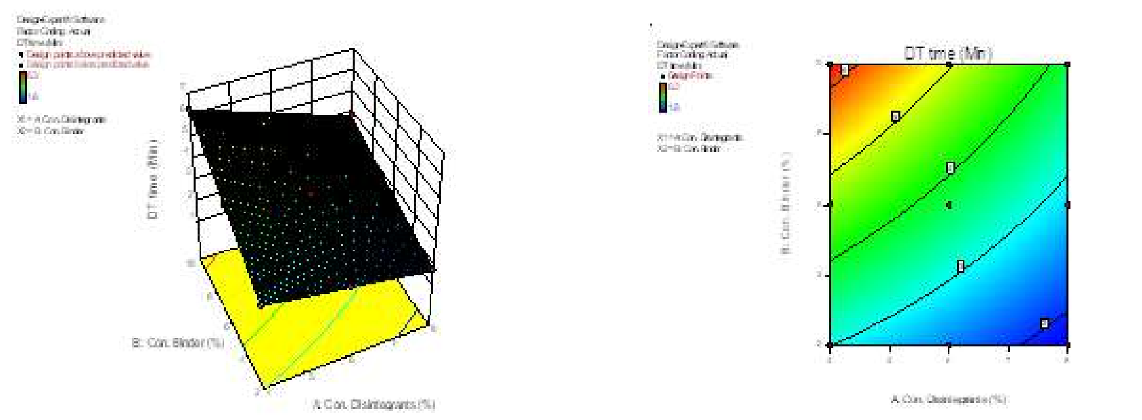
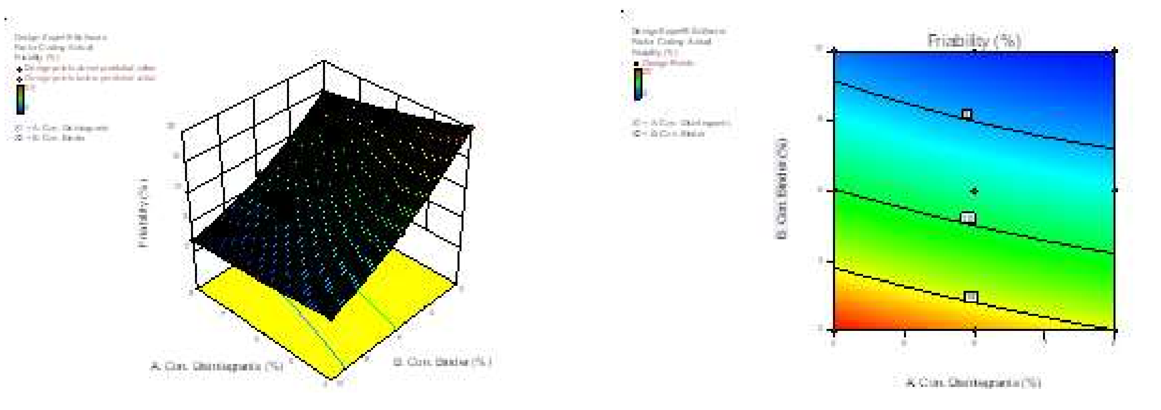


Figure 3: Three-Dimensional Response Surface Plots and Contour Plot for Friability of Granules



granules; on the other hand, as the concentration of sodium starch glycolate increases, friability decreases. Contour plots for the concentration of independent variables are shown in Figure 3.

Checkpoint Analysis

Two checkpoint batches were prepared (F10 and F11). Table 9 shows the actual and predicted value of independent parameters.

Optimization Using Desirability Function

The desirability function was utilized to optimize the best batch. After studying the effect of the independent variables on the responses, the levels of the variables that give the optimum responses were determined. The optimized batch with different factors, results, and desirability are shown in Table 10.

Table 9: Observation of Checkpoint Batch

Check Point Batch	Concentration of Factors		Measured Value*		Predicted Value	
	Conc. Disintegrants	Conc. Binders	DT Time (min)	Friability (%)	DT Time (min)	Friability (%)
F10	5	4	3.80±0.13	13.86±0.45	3.43	13.25
F11	7	8	4.34±0.18	4.98±0.75	3.83	4.42

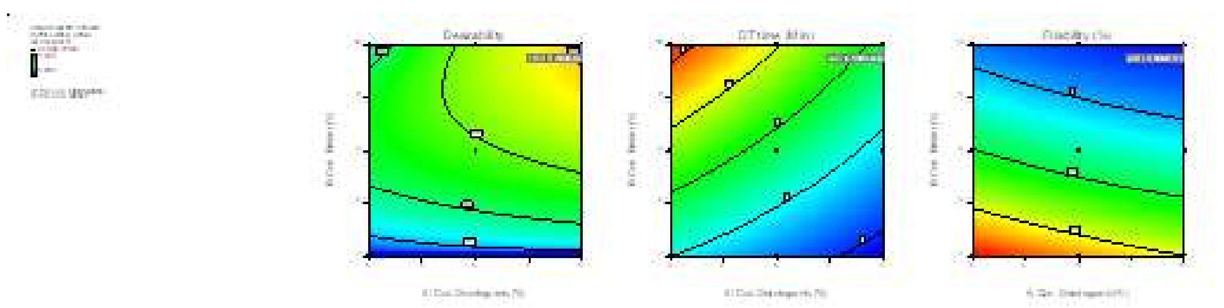
Note: (Mean±SD, n = 3).

Table 10: Optimized Batch Using Desirability Function

Optimized Batch	Concentration of Factors		Measured Value*		Predicted Value		Desirability
	Conc. Disintegrants	Conc. Binders	DT Time (min)	Friability (%)	DT Time (min)	Friability (%)	
F12	8	10	4.1±0.24	2.83±0.58	3.8	2.19	0.818

Note: (Mean±SD, n = 3).

Figure 4: Optimization Based on the Desirability



Evaluation of Optimized Granules

Sensory Evaluation of Optimized Granules

Sensory evaluation parameters observed in Herbal Energy booster powder drink. The observed parameters like color, taste, flavor, texture, overall acceptability at room temperature of the formulation are presented in Table 11. The drink has exquisite taste, flavor, and overall acceptability based on paired comparison evaluation. Change in sensory characters during storage was also analyzed.

Table 11: Sensory Score of Powder

Parameter	Score
Colour	7
Odour	6
Taste	6
Overall	7

Characterization of Optimized Granules

Optimized was evaluated to determine the flow behavior of granules. The data are shown in Table 12 concluded that prepared granules have good flow properties as all the parameters were in the range of good flow behavior. Therefore,

Table 12: Characterization of Optimized Batch

Formulation Code	Bulk Density	Tapped Density	Hausner's	Carr's Compressibility	Angle of Repose (θ)
	Gm/cm^3	Gm/cm^3	Ratio	Index %	
F12	0.307±0.003	0.378±0.007	1.23±0.09	18.78±0.18	32°.95'±0.19

Note: (Mean±SD, n = 3).

Table 13: Stability Data of Optimized Batch F9

Formulation F9	Disintegration Time (min)	Friability (%)	Hausner's	Carr's Compressibility	The Angle of Repose (θ)
			Ratio	Index %	
Initial	4.1±0.24	2.83±0.58	1.23±0.09	18.78±0.18	32°.95'±0.19
After 3 months	4.3±0.67	3.04±0.12	1.24±0.07	18.98±0.13	33°.12'±0.12
After 6 months	4.4±0.98	3.13±0.29	1.26±0.04	19.02±0.27	33°.34'±0.16

Note: (Mean±SD, n = 3).

as the granules have good flow properties, they will be easy for packaging.

Stability

The stability study was carried out for 6 months. As per the data shown in Table 13, no significant changes were observed at the end of each 3 months. Finally, it was concluded that prepared granules were stable for a more extended period.

Determination of ICP-OES[28-30]

As shown in Table 14, elements like Fe, Zn, Cu and Ca were estimated 812.61, 1637.76, 525.33 and 1.61 mg per 100 g respectively. Therefore, the result shows that this formulation might fulfill daily recommended values (D.R.V.) as per the government guidelines; the continuous use of this formulation under medical supervision can supply the above minerals.

Table 14: Estimation of elements of Moringa tablet by ICP-OES

S. No.	Element	Result (per 100 gm)
1	Fe	21.2 mg
2	Ca	758 mg
3	Zn	525.33 mg
4	Cu	1.61 mg

CONCLUSION

Herbal granules of Moringa oleifera leaf powder, Beta vulgaris root powder, Lauha bhasma, and Yashad bhasma were prepared using the wet granulation method. Optimization of granules was done by selecting the disintegrant concentration and concentration of starch paste as an independent parameter by 3^2 complete factorial designs. The dependent parameters angel of repose and friability were evaluated. The desirability function optimized batch was confirmed, and its physicochemical parameters were evaluated, which were matched with the standard limit. Total phenolic content was found in satisfactory. Element detection by ICP-OES gave promising results in granule formulation.

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