

DESIGN AND DEVELOPMENT OF PREVENTIVE EPIGENETICS AS REMEDY FOR THALASSEMIA MANAGEMENT

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ABSTRACT:

Thalassemia is an inherited hemoglobin disorder associated with impaired globin synthesis, chronic anemia, oxidative stress, and long-term complications, necessitating supportive strategies alongside established medical management. The present study aimed to design and develop a polyherbal nutraceutical powder as a potential supportive approach for thalassemia management. Four medicinal plants, namely *Vitex negundo* (Nirgundi), *Moringa oleifera* (Moringa), *Curcuma longa* (Turmeric), and *Withania somnifera* (Ashwagandha), were selected based on their reported nutritional and pharmacological properties. The herbal materials were subjected to organoleptic evaluation, micromeritic characterization, solubility studies, preliminary phytochemical screening, FTIR characterization, compatibility assessment, and process optimization. Phytochemical screening demonstrated the presence of several bioactive constituents, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and saponins. FTIR analysis confirmed characteristic functional groups in the selected herbal materials and showed no major spectral changes following combination, indicating satisfactory compatibility. Process optimization was performed by varying herbal mixture concentration and blending speed, with particle-size distribution considered as the principal response. The optimized formulation exhibited uniform appearance, acceptable flow characteristics, and satisfactory particle-size distribution. The developed formulation provides a feasible polyherbal nutraceutical platform for further investigation in the context of thalassemia. However, the present findings are limited to preliminary formulation and physicochemical characterization and do not establish clinical efficacy or a definitive epigenetic effect. Further biological, toxicological, molecular, epigenetic, and clinical studies are required to determine its safety, mechanism, and therapeutic relevance. Overall, the study provides a foundation for future development of a standardized herbal nutraceutical formulation as a potential adjunctive supportive intervention in thalassemia management.

Keywords: Thalassemia; Polyherbal nutraceutical; *Vitex negundo*; *Moringa oleifera*; *Curcuma longa*; *Withania somnifera*; Phytochemical screening; FTIR; Process optimization; Epigenetics.

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INTRODUCTION:

Thalassemia is a group of inherited hematological disorders characterized by reduced or absent synthesis of one or more globin chains, resulting in ineffective erythropoiesis and varying degrees of chronic anemia. The disorder is broadly classified into α -thalassemia and β -thalassemia, depending on the affected globin chain. The clinical severity ranges from asymptomatic carrier states to severe transfusion-dependent disease requiring lifelong medical care. The thesis source identifies thalassemia as an important health burden, particularly in South Asia, the Mediterranean region, the Middle East, and parts of Africa and Southeast Asia.

The underlying pathology of thalassemia is associated with an imbalance in globin-chain synthesis, ineffective erythropoiesis, and premature destruction of erythroid cells, chronic anemia, and progressive iron accumulation. In transfusion-dependent patients, repeated blood transfusions further contribute to iron overload, which may subsequently affect organs such as the liver, heart, and endocrine system. These complications emphasize the importance of maintaining adequate supportive care and developing complementary approaches that can be investigated alongside established treatment.

Current management of clinically significant thalassemia includes regular blood transfusion, iron-chelation therapy, hematopoietic stem-cell transplantation, and, in selected circumstances, emerging gene-based approaches. Transfusion therapy remains an important component of management for patients with severe and

transfusion-dependent disease, while iron chelation is required to control transfusional iron accumulation. Stem-cell transplantation and gene therapy may provide potentially curative approaches for selected patients, but their application can be limited by donor availability, treatment-related complications, technical requirements, and cost.

The molecular basis of thalassemia also provides an opportunity to investigate mechanisms beyond conventional replacement therapy. Globin production is regulated at the genetic and epigenetic levels, and mechanisms such as DNA methylation, histone modification, and regulation of gene expression can influence cellular phenotype. The research hypothesis in the source thesis proposes that selected herbal agents may potentially influence gene-regulatory mechanisms associated with fetal hemoglobin production, oxidative stress, and erythropoietic pathways. However, this proposed epigenetic activity requires experimental validation and should not be assumed solely from the presence of bioactive phytochemicals.

Natural products and medicinal plants have attracted considerable interest as sources of bioactive compounds with antioxidant, anti-inflammatory, nutritional, and regulatory properties. The source thesis emphasizes the long-standing use of medicinal plants and the increasing interest in scientifically evaluating traditional herbal preparations. At the same time, variability in botanical materials, extraction procedures, composition, and quality control highlights the need for systematic formulation development and characterization of herbal products.

A polyherbal nutraceutical approach may provide a practical means of combining multiple botanical ingredients with complementary nutritional and pharmacological characteristics. In the present study, Nirgundi (*Vitex negundo*), Moringa (*Moringa oleifera*), Turmeric (*Curcuma longa*), and Ashwagandha (*Withania somnifera*) were selected for development of the herbal nutraceutical powder. The source thesis identifies these plants as the selected herbal agents for investigating the proposed preventive-epigenetic concept and reports that the formulation was intended to explore their potential contribution to gene regulation, antioxidant activity, and supportive management of thalassemia.

Moringa oleifera is recognized as a nutritionally valuable plant containing various vitamins, minerals, polyphenols, and other bioactive constituents. *Withania somnifera* contains withanolides and has been investigated for antioxidant, anti-inflammatory, adaptogenic, and cellular regulatory activities. *Curcuma longa*, particularly its curcuminoid constituents, has been widely studied for antioxidant and anti-inflammatory effects and for interactions with molecular pathways involved in gene regulation. *Vitex negundo* contains diverse phytoconstituents and has been investigated for antioxidant and anti-inflammatory activities. The thesis further discusses experimental evidence suggesting that constituents of these plants may influence cellular and gene-regulatory pathways; however, direct evidence for a clinically meaningful epigenetic effect in thalassemia remains to be established.

Nutritional considerations are also relevant to thalassemia because chronic disease, increased physiological requirements, and treatment-related factors can influence nutritional status. The literature included in the source thesis specifically identifies nutritional disorders among patients with β -thalassemia major, supporting the need for continued investigation of appropriate nutritional and nutraceutical strategies.

In this context, the present research was designed to develop and characterize a herbal nutraceutical powder for thalassemia management using a systematic pharmaceutical formulation approach. The study involved evaluation of the selected herbal materials, preliminary phytochemical screening, micromeritic characterization, FTIR-based compatibility assessment, formulation development, particle-size evaluation, and process optimization. The research objectives specifically included investigation of the potential of natural herbal nutraceutical plants, evaluation of their properties, FTIR compatibility assessment, formulation of the herbal powder, and characterization of the developed formulation.

The formulation was further approached through Quality by Design (QbD) and Design of Experiments (DoE) principles to investigate the influence of selected processing variables on the quality attributes of the powder. The source thesis identifies formulation optimization and characterization as central components of the research, while the experimental work evaluated blending conditions and particle-size distribution.

Therefore, the present study represents a preliminary formulation-development investigation into a polyherbal nutraceutical powder comprising *Vitex negundo*, *Moringa oleifera*, *Curcuma longa*, and *Withania somnifera*. The work provides a pharmaceutical basis for further investigation of the formulation's nutritional, antioxidant, molecular, and potential epigenetic properties. Importantly, the developed preparation is intended to be investigated as a potential supportive or adjunctive nutraceutical approach, rather than as a replacement for established transfusion, chelation, or other evidence-based thalassemia therapies. The thesis itself identifies the need for further in-vivo and clinical evaluation to establish long-term safety and efficacy.

MATERIALS AND METHODS:

MATERIALS:

The herbal ingredients selected for the development of the nutraceutical powder were Nirgundi (*Vitex negundo*), Ashwagandha (*Withania somnifera*), Turmeric (*Curcuma longa*), and Moringa (*Moringa oleifera*). The plant materials were procured from Labwear Chemicals Pvt. Ltd. and used as the principal botanical components of

the formulation. Ethanol, ferric chloride, sulfuric acid, glacial acetic acid, and hydrochloric acid (HCl) were obtained from the same supplier and used for extraction, phytochemical screening, and other analytical investigations. All reagents and chemicals employed during the study were of appropriate analytical grade and were used as received unless otherwise specified.

Preformulation Studies

Preformulation studies were carried out to characterize the selected herbal powders and assess their physicochemical properties before formulation development. The studies included organoleptic evaluation, micromeritic properties, solubility assessment, and preliminary phytochemical screening. These investigations were performed to establish the suitability and uniformity of the selected herbal ingredients for preparation of the nutraceutical powder.

Organoleptic Evaluation

The selected herbal powders were evaluated for their colour, odour, taste, texture, and general appearance by appropriate organoleptic methods. The observations were recorded and compared among the individual herbal materials to assess their preliminary quality characteristics.

Micromeritic Properties

The micromeritic properties of the selected herbal powders were evaluated to determine their flowability and packing characteristics. Bulk density, tapped density, Carr's compressibility index, Hausner's ratio, and angle of repose were determined using standard procedures. Bulk and tapped densities were calculated from the respective powder volumes before and after tapping. Carr's index and Hausner's ratio were derived from the density values, while the angle of repose was determined by allowing the powder to flow through a funnel and measuring the height and radius of the resulting powder pile. The obtained parameters were used to assess the suitability and flow characteristics of the herbal powders for formulation development.

Solubility Studies

The solubility behaviour of the selected herbal powders was investigated in different solvents, including distilled water, ethanol, and chloroform. An excess quantity of each herbal powder was added to the respective solvent and allowed to equilibrate with intermittent shaking. The samples were subsequently observed for dissolution behaviour, and the solubility characteristics were recorded.

Preparation of Extract

5-10g of powdered form of herbal drug were boiled with solvent (Water/Ethanol) for 15 min with the help of heating mantle. The extract was filter with the Whatman filter paper. After filtration this filtered extract were used for the phytochemical tastings.

Phytochemical Analysis

The prepared herbal extracts were subjected to preliminary qualitative phytochemical screening to identify major classes of bioactive constituents. The extracts were tested for alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolic compounds, steroids, and other relevant phytoconstituents using standard qualitative chemical tests. The presence of tannins was indicated by the development of a blue-green or black colour following treatment with ferric chloride, while saponins were detected by persistent foam formation after vigorous shaking with distilled water. Terpenoids were assessed using a concentrated sulfuric acid reaction, and glycosides were evaluated by acid hydrolysis followed by neutralization and Fehling's reagent. The observations were recorded based on characteristic colour changes, precipitate formation, or foam development.



Figure 1: Terpenoids test

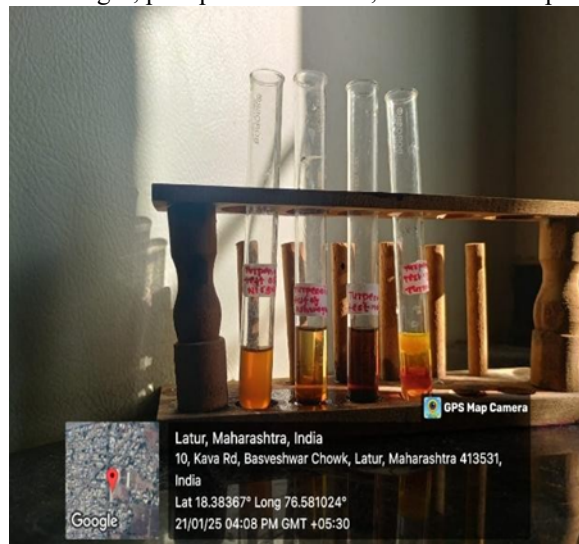


Figure 2: Tannins test

Drug-Excipient Compatibility Study

The compatibility of the selected herbal ingredients was investigated using Fourier-transform infrared spectroscopy (FTIR). Individual herbal powders and their physical mixture were analyzed over the spectral range of 4000–400 cm^{-1} . The obtained spectra were compared with those of the individual components to identify characteristic functional groups and to determine any substantial changes or interactions between the formulation components.

Formulation of Herbal Nutraceutical Powder

The selected herbal ingredients, namely Nirgundi, Ashwagandha, Turmeric, and Moringa, were obtained in powdered form and used for formulation development. The ingredients were accurately weighed according to the predetermined formulation ratio and subjected to uniform blending. The powders were initially passed through a suitable sieve and subsequently mixed using a mixer-grinder at approximately 1000–1200 rpm for a predetermined period to obtain a homogeneous blend. The resulting formulation was evaluated for its physical characteristics and particle-size distribution to ensure adequate uniformity and suitability as a herbal nutraceutical powder. The final blended product was stored in a suitable well-closed container until further evaluation.

RESULTS:

Preformulation and Physicochemical Evaluation

The developed herbal nutraceutical powder was evaluated for its organoleptic, micromeritic, and solubility characteristics. The formulation showed a uniform dark greenish colour, characteristic herbal odour, and amorphous nature. The micromeritic evaluation indicated satisfactory packing and moderate flow properties, with an angle of repose of $36 \pm 1^\circ$, Carr's index of $23 \pm 2\%$, and Hausner's ratio of 1.29 ± 0.03 . The powder was soluble in water and showed comparatively higher solubility in the organic solvents evaluated. Overall, the observed properties indicated that the developed herbal powder possessed suitable physical characteristics for further evaluation.

Table 1: Preformulation and Physicochemical Evaluation of Herbal Nutraceutical Powder

Sr. No.	Evaluation Parameter	Result
1	Colour	Dark greenish, uniform
2	Odour	Pleasant herbal odour
3	Nature	Amorphous
4	Bulk density	$0.31 \pm 0.01 \text{ g/mL}$
5	Tapped density	$0.59 \pm 0.02 \text{ g/mL}$
6	Hausner's ratio	1.29 ± 0.03
7	Carr's compressibility index	$23 \pm 2\%$
8	Angle of repose	$36 \pm 1^\circ$
9	Solubility in ethanol	More soluble
10	Solubility in water	Soluble
11	Solubility in methanol	Easily soluble

Phytochemical Studies

Preliminary phytochemical screening of the selected herbal extracts revealed the presence of several important classes of phytoconstituents. Alkaloids, terpenoids, and tannins were detected in all four extracts, while flavonoids were present in Nirgundi, Turmeric, and Ashwagandha. Glycosides were detected in Moringa and Turmeric, whereas saponins were observed only in Moringa.

Table 2: Phytochemical Study

Sr. No	Test	Nirgundi Extract	Moringa Extract	Turmeric Extract	Ashwagandha Extract
1	Alkaloid (Meyer's Test)	+	+	+	+
2	Flavonoid (Alkaline Test)	+	–	+	+

3	Terpenoid (Salkowski Test)	+	+	+	+
4	Glycoside (Killer. K Test)	–	+	+	–
5	Tannins (Ferric Chloride Test)	+	+	+	+
6	Saponins	–	+	–	+

Determination of λ_{max} of Nirgundi Extract

The absorption maximum of the Nirgundi (*Vitex negundo*) extract was determined using a UV–Visible spectrophotometer (Shimadzu UV-1800). A 50 $\mu\text{g/mL}$ ethanolic solution was scanned over 200–400 nm, and the maximum absorbance was observed at 210 nm.

Calibration Curve of Nirgundi Extract

A calibration curve was prepared by measuring the absorbance of Nirgundi extract solutions at different concentrations (1–5 $\mu\text{g/mL}$) at the predetermined wavelength of 210 nm. The absorbance values were plotted against the corresponding concentrations to establish the concentration–absorbance relationship.

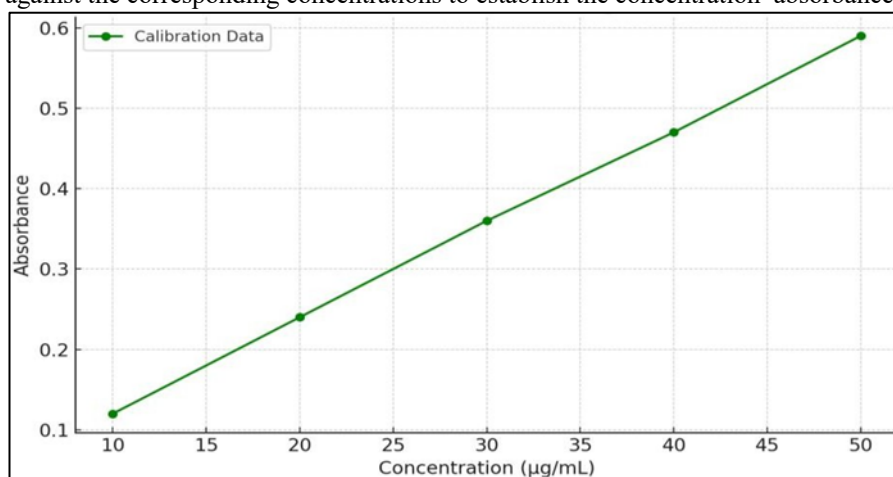


Figure 3: Graphical representation of calibration curve

FTIR Characterization of Herbal Ingredients

FTIR analysis was performed to identify the characteristic functional groups present in the selected herbal ingredients. The spectra showed characteristic absorption bands corresponding to major functional groups, confirming the chemical identity of the herbal materials. The observed bands were consistent with functional groups commonly associated with the phytochemical constituents of the respective plants.

Nirgundi

The FTIR spectrum of Nirgundi (*Vitex negundo*) exhibited a characteristic absorption band at 1027.51 cm^{-1} , corresponding to C–O stretching associated with alcohol and ether groups. This confirmed the presence of oxygen-containing functional groups in the herbal material.



Figure 4: FTIR Spectrum of Nirgundi (*Vitex negundo*)

Moringa

The FTIR spectrum of Moringa (*Moringa oleifera*) showed characteristic bands at 2916.23, 1626.57, and 1049.24 cm⁻¹, corresponding mainly to C–H, C=C, and C–O vibrations. These findings indicated the presence of hydrocarbon, aromatic/unsaturated, and oxygen-containing functional groups.

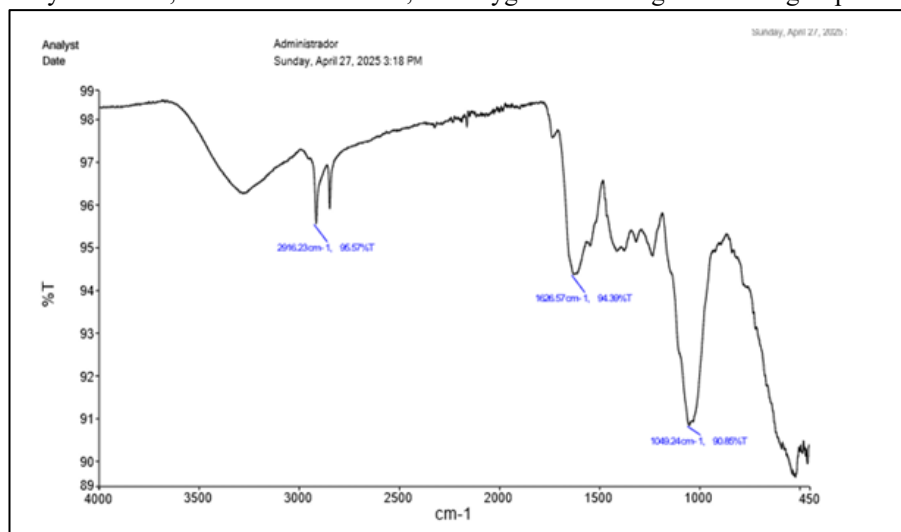


Figure 5: FTIR Spectrum of Moringa (*Moringa oleifera*)

Turmeric

The FTIR spectrum of Turmeric (*Curcuma longa*) exhibited characteristic absorption bands at 3275.57, 1510.59, and 1023.62 cm⁻¹, attributable to O–H, C=C, and C–O vibrations, respectively. The spectrum demonstrated the presence of hydroxyl, aromatic, and oxygen-containing functional groups characteristic of the herbal material.

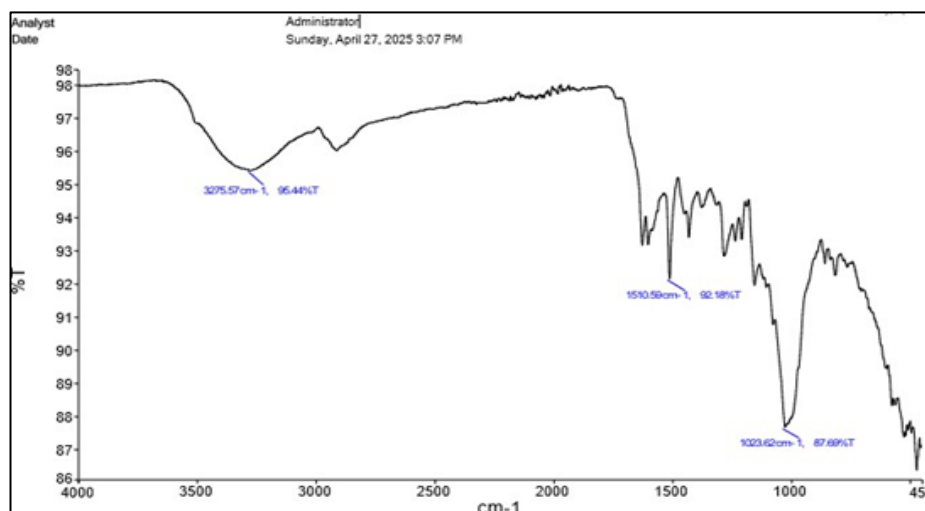


Figure 6: FTIR Spectrum of Turmeric (*Curcuma longa*)

Ashwagandha

Ashwagandha (*Withania somnifera*) showed characteristic FTIR bands at 1618.91, 1317.36, 1014.96, and 518.18 cm⁻¹, corresponding to aromatic, C=N, C-O, and ring deformation vibrations, respectively. These characteristic bands confirmed the presence of diverse functional groups in the herbal material.

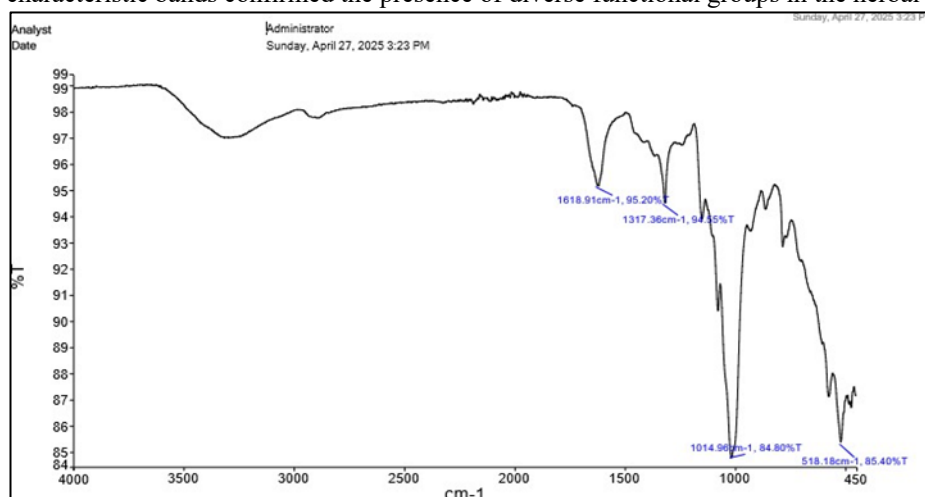


Figure 7: FTIR Spectrum of Ashwagandha (*Withania somnifera*)

Compatibility Study

The compatibility of the selected herbal ingredients was evaluated by FTIR spectroscopy over the range of 4000–400 cm⁻¹. The individual ingredients and their combined formulation showed retention of their characteristic absorption bands without major peak shifts or disappearance of prominent peaks. The absence of substantial spectral changes suggested no evident chemical incompatibility among the selected herbal ingredients, supporting their suitability for incorporation into the nutraceutical powder formulation.

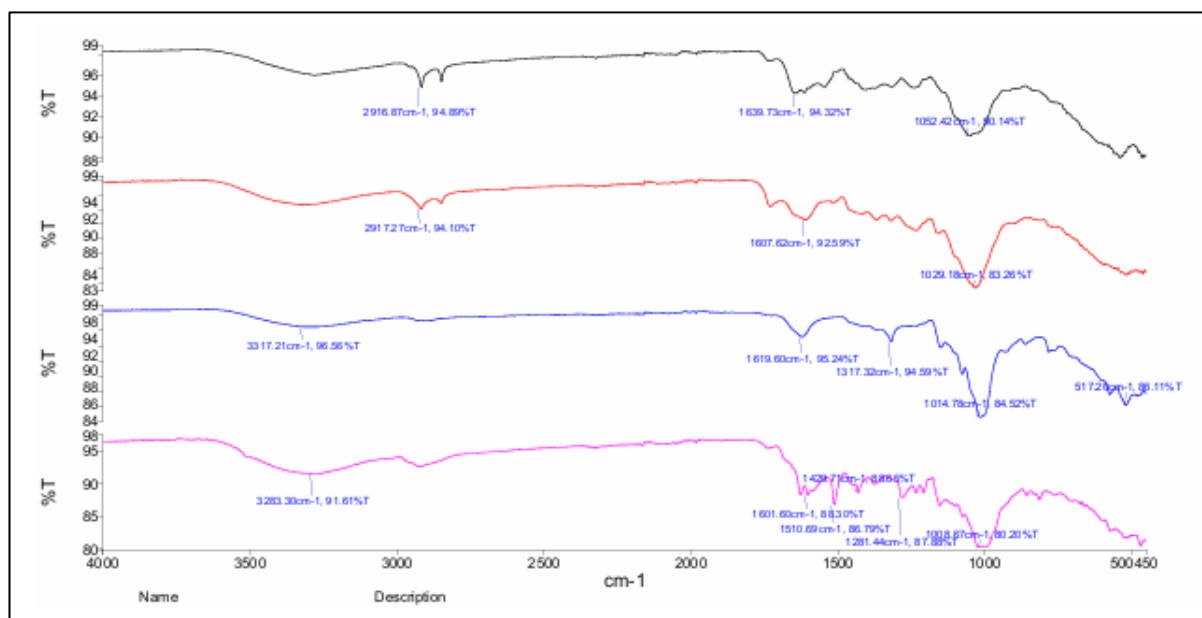


Figure 8: FTIR Spectrum of the Herbal Nutraceutical Powder and Selected Herbal Ingredients

Process Optimization of Herbal Nutraceutical Powder

Process optimization was performed to identify suitable herbal powder concentration and blending speed for obtaining a uniform nutraceutical powder with desirable particle-size characteristics. Ten experimental batches were prepared by varying the herbal drug concentration and blending speed. Among the investigated batches, B5, containing 30 g herbal mixture blended at 800 rpm for 2 min, showed satisfactory mixing, uniformity, particle-size characteristics, and overall formulation performance. Therefore, these conditions were selected as the optimized processing parameters.

Table 3: Optimization of Process Parameters for the Powder Formulation

Sr. No.	Batch Sample Id	Herbal drug mixture (gm)	Mixing & Blending speed (Rpm)	Time (min)	Observation
1	B1	10	1000	2	Mixing & Blending was observed
2	B2	20	800	2	Not properly mixed
3	B3	10	800	2	Less therapeutic activity
4	B4	20	1000	2	Herbs not blend uniformly
5	B5	30	800	2	Properly mixed and blend also uniform particle size gives good therapeutic activity all satisfied
6	B6	30	1200	2	Particle size not satisfied

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7	B7	30	1000	2	Particle size very fine
8	B8	20	1000	2	Not properly mix
9	B9	10	1200	2	Very fine powder obtained
10	B10	20	1200	2	Less therapeutic activity



Figure 9: Formulation Batches of Herbal Nutraceutical Powder

Characterization:

Particle Size Distribution

Particle-size distribution was evaluated using a sieve-shaker method. A 25 g sample was subjected to sieving for 15 min, and the percentage weight retained on each sieve was determined. The results demonstrated distribution of the powder across different sieve sizes, indicating variation in particle dimensions within the formulation. The particle-size profile was subsequently used as the principal response for optimization of the blending process.

Table 4: Particle Size Distribution

Sr. No	Sieve No (mm)	Avg. Aperture Size (um)	Weight Retained on Sieve (gm)	% Wt. Retained on Sieve	Cumulative % Wt. Retained	
					Under Size	Out Size
1	20	850	0	0	13.2	0
2	40	425	0.44	2.2	41.2	13.64
3	60	250	1.14	7.2	36.9	43.14
4	80	180	12.33	61.65	52.2	38.2
5	100	150	1.74	8.7	70.2	53.34
6	120	125	2.34	11.7	93.2	72.54

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7	140	45	3.12	15.6	0	96.72
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Design of Experiment and Statistical Optimization

A Design of Experiments (DoE) approach was employed to systematically investigate the influence of processing variables on particle-size distribution. Herbal drug concentration and blending speed were selected as independent variables at three levels, while particle-size distribution was considered the dependent response. Design-Expert® software (Version 13.0.1) was used for experimental design and statistical analysis. The experimental design comprised 10 formulation runs, allowing assessment of the effect of the selected process variables and identification of suitable processing conditions.

Table 5: Factorial design with upper, middle & lower limits of all factor

Sr. No.	Independent factors	Level		
		-1	0	+1
1	Herbal drug concentration	10	20	30
2	Speed	800	1000	1200

Table 6: Box Behnken Experimental design

Std	Run	Factor I Herbal drug concentration(gm)	Factor II Speed (rpm)	Particle size distribution
5	1	10	1200	545.79
7	2	20	800	534.41
2	3	30	800	525.02
9	4	20	1000	466.87
1	5	10	800	515.64
4	6	30	1200	561.01
6	7	30	1000	579.78
10	8	20	1000	476.25
3	9	10	1000	585.21
8	10	20	1200	512.56

Statistical Analysis and Model Evaluation

The effect of the selected process variables, herbal mixture concentration (A) and blending speed (B), on particle-size distribution was evaluated using ANOVA. The linear model showed an overall F-value of 2.14 with a p-value of 0.1880, indicating that the model was not statistically significant at the 5% significance level. Among the investigated factors, blending speed showed a greater contribution to the observed response than herbal mixture concentration, although it did not reach statistical significance ($p = 0.0861$). The model showed an R^2 value of 0.3797, with an adjusted R^2 of 0.2024, indicating limited explanatory capability of the fitted linear model.

The lack-of-fit test was not statistically significant ($p = 0.1602$), suggesting that the deviation between the model and experimental observations was not statistically significant relative to the experimental error. However, the negative predicted R^2 (-0.0138) indicates limited predictive performance of the model with the available experimental data. Therefore, the statistical results should be interpreted cautiously, and the selected formulation conditions should primarily be supported by the experimental performance and predefined formulation criteria.

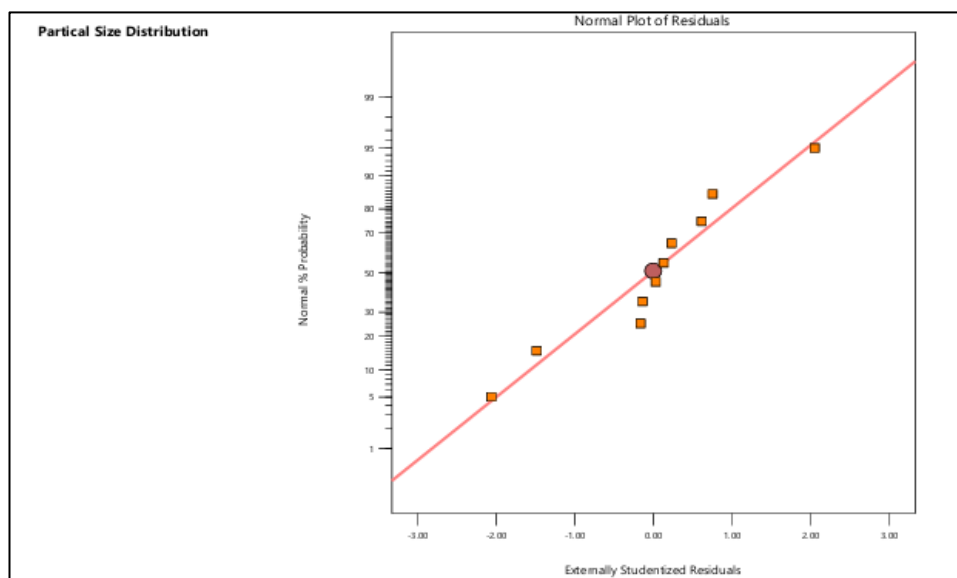


Figure 10: Normal Probability Plot of Externally Studentized Residuals

The normal probability plot showed the distribution of the externally studentized residuals around the expected linear trend. This plot was used to assess the normality assumption of the experimental errors.

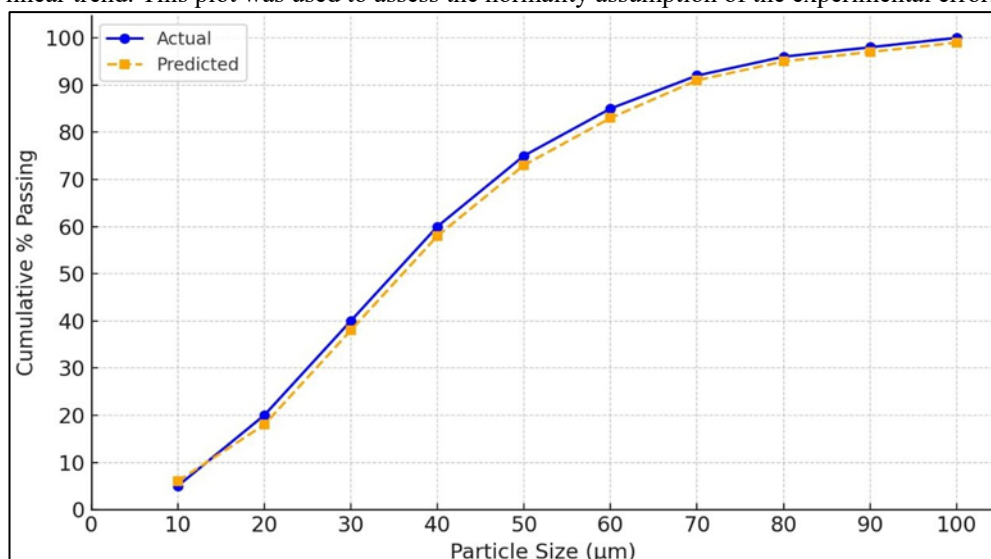


Figure 11: Predicted versus Actual Response Plot for Particle-Size Distribution

The predicted-versus-actual plot was used to visualize the agreement between experimentally observed and model-predicted particle-size values. The distribution of the observations indicates the extent to which the fitted model represents the experimental response.

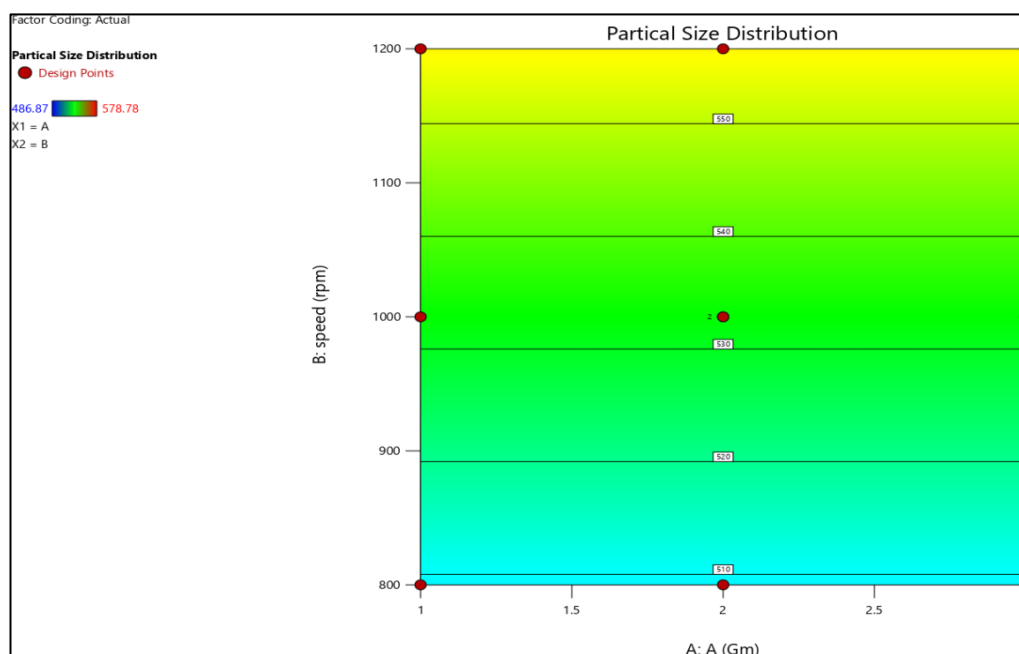


Figure 12: Counter plot showing combined effect of drug concentration and Speed

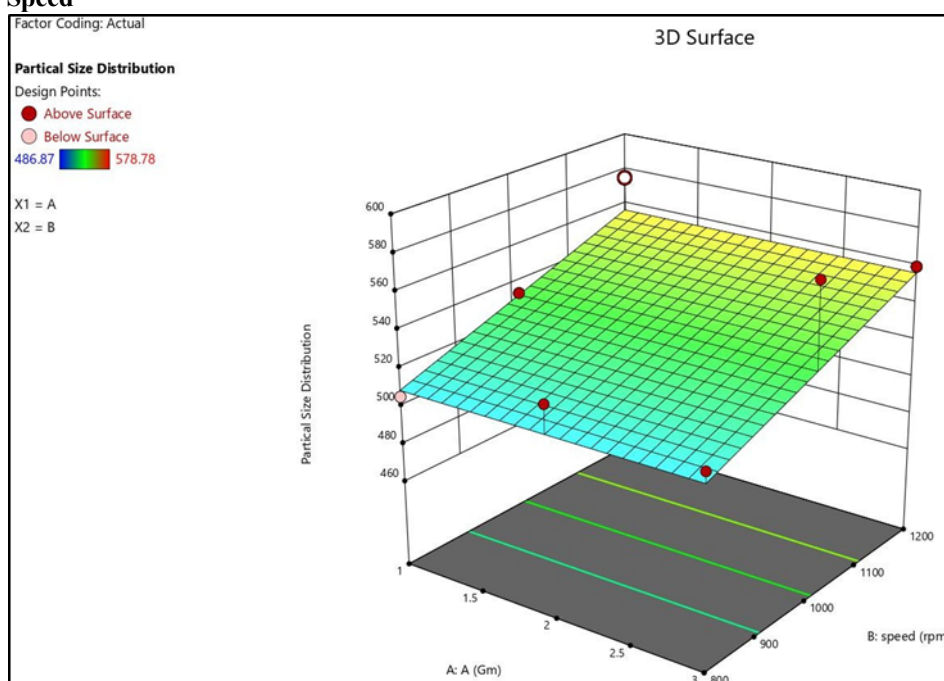


Figure 13: 2D- 3D Response surface plot showing combined effect counter plot showing combined effect of drug concentration and speed when mixing & blending powder

DISCUSSION:

Thalassemia is an inherited hemoglobin disorder characterized by impaired globin-chain synthesis and chronic anemia. Current management primarily involves transfusion support, iron-chelation therapy, and, in selected patients, hematopoietic stem-cell transplantation. Therefore, there is interest in supportive nutritional and phytochemical approaches that may help address oxidative stress and other complications associated with the disorder. The present study focused on the development and preliminary evaluation of a polyherbal nutraceutical powder containing Moringa (*Moringa oleifera*), Ashwagandha (*Withania somnifera*), Turmeric (*Curcuma longa*), and Nirgundi (*Vitex negundo*).

The selected herbs contain diverse phytoconstituents with reported antioxidant, anti-inflammatory, and other pharmacological activities. Preliminary phytochemical screening in the present study demonstrated the presence of several secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and saponins, depending on the individual extract. These constituents may contribute to the overall biological activity of the formulation. However, the present phytochemical findings establish the chemical profile of the extracts and

should not by themselves be interpreted as evidence of therapeutic efficacy in thalassemia.

Moringa (*Moringa oleifera*) is a nutrient-rich plant containing various vitamins, minerals, polyphenols, flavonoids, and other bioactive constituents. Its antioxidant and anti-inflammatory properties have been investigated in several experimental models. The presence of flavonoid and phenolic constituents may be relevant to oxidative-stress management; however, the therapeutic role of Moringa specifically in thalassemia requires further experimental and clinical validation.

Ashwagandha (*Withania somnifera*) contains withanolides and other bioactive compounds and has been investigated for antioxidant, anti-inflammatory, adaptogenic, and immunomodulatory activities. Experimental evidence also suggests that some constituents may influence cellular signaling and gene-regulatory pathways. These observations provide a rationale for further investigation of Ashwagandha in nutraceutical formulations, although a direct epigenetic or therapeutic effect in thalassemia cannot be concluded from the present formulation study.

Turmeric (*Curcuma longa*), particularly its major constituent curcumin, has been extensively investigated for antioxidant and anti-inflammatory activities. Curcumin has also been reported in experimental studies to interact with molecular mechanisms involved in DNA methylation, histone modification, and microRNA regulation. Such findings provide a scientific basis for investigating curcumin-containing formulations in the broader context of epigenetic research. Nevertheless, these molecular observations should not be considered evidence that the present formulation produces clinically relevant epigenetic modification in thalassemia.

Nirgundi (*Vitex negundo*) contains flavonoids, terpenoids, and other phytoconstituents and has traditionally been investigated for anti-inflammatory and antioxidant properties. Experimental studies have reported effects on inflammatory and cellular signaling pathways. However, evidence directly establishing its epigenetic activity or therapeutic efficacy in thalassemia remains limited, and further mechanistic studies are required.

The formulation development studies demonstrated acceptable physical characteristics of the polyherbal powder. The formulation showed a uniform appearance and characteristic herbal odour, while the micromeritic evaluation indicated suitable handling characteristics. FTIR characterization demonstrated characteristic functional groups in the individual herbal materials, and comparison of the spectra of the combined formulation did not reveal major spectral changes suggestive of obvious incompatibility. Process optimization further demonstrated that variations in herbal mixture concentration and blending speed affected the particle-size characteristics of the formulation.

The DoE analysis provided a systematic approach for investigating the influence of formulation-processing variables on particle-size distribution. Although the fitted statistical model did not demonstrate statistical significance at the 5% level, the experimental results allowed identification of processing conditions that produced a satisfactorily blended powder. Thus, the optimized formulation represents a suitable basis for subsequent physicochemical, phytochemical, biological, and mechanistic investigations.

Importantly, the present study should be considered a formulation-development and preliminary characterization study, rather than evidence that the herbal powder is a treatment or cure for thalassemia. The proposed preventive-epigenetic concept requires further investigation through quantitative phytochemical characterization, antioxidant and cellular assays, molecular studies of relevant epigenetic pathways, appropriate preclinical models, and ultimately well-designed clinical studies. In particular, the formulation should be evaluated for its safety and suitability in thalassemia patients, where iron status and existing transfusion and chelation therapies require careful consideration.

Overall, the study provides a preliminary scientific basis for the development of a polyherbal nutraceutical powder containing *M. oleifera*, *W. somnifera*, *C. longa*, and *V. negundo*. The physicochemical characterization, phytochemical screening, FTIR evaluation, and process optimization support the feasibility of the developed formulation. Further mechanistic and biological investigations are necessary to determine whether the formulation can provide clinically meaningful supportive benefits and whether its proposed epigenetic mechanisms are experimentally substantiated.

CONCLUSION:

The present study successfully focused on the design and development of a polyherbal nutraceutical powder as a potential supportive approach for thalassemia management. The formulation was developed using a systematic optimization approach incorporating Nirgundi (*Vitex negundo*), Moringa (*Moringa oleifera*), Turmeric (*Curcuma longa*), and Ashwagandha (*Withania somnifera*). The selected herbal ingredients demonstrated satisfactory organoleptic characteristics and the presence of several important phytoconstituents, including alkaloids, flavonoids, terpenoids, tannins, glycosides, and saponins. FTIR characterization confirmed the characteristic functional groups of the individual herbal materials, while compatibility assessment of the combined formulation showed no major spectral changes indicative of obvious incompatibility. Optimization of the formulation process by varying herbal mixture concentration and blending speed resulted in a homogeneous powder with satisfactory particle-size characteristics and acceptable micromeritic properties. The developed formulation therefore provides a feasible basis for further investigation as a herbal nutraceutical supplement intended for supportive management of thalassemia. The presence of antioxidant and other bioactive

phytoconstituents provides a rationale for further exploration of its potential role in addressing oxidative stress and related complications associated with thalassemia. However, the present findings are limited to formulation development and preliminary physicochemical and phytochemical evaluation and should not be interpreted as evidence of a cure or established clinical efficacy. Further comprehensive in-vitro, preclinical, toxicological, molecular, epigenetic, and well-controlled clinical studies are necessary to establish the safety, biological effectiveness, mechanism of action, and clinical relevance of the developed polyherbal nutraceutical powder in thalassemia management.

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All authors have declared no conflict of interest.

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