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FORMULATION DESIGN AND IN VITRO EVALUATION OF HERBAL VESICLES CONTAINING HERBAL MEDICINAL PLANT EXTRACT BY QBD

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ABSTRACT

The present study aimed to formulate and evaluate herbosomes of *Amaranthus viridis* extract using a Design of Experiments (DOE) approach to optimize the formulation process. The extraction of *Amaranthus viridis* was followed by the preparation of herbosomes, which were evaluated for their percentage yield, and the results revealed a yield of *Amaranthus viridis* in Pet ether and Ethanol were 0.79% and 0.85%. Phytochemical analysis confirmed the existence of bioactive substances such alkaloids, flavonoids, saponins, and phenols, indicating the therapeutic potential of the extract. The formulation of the extract-loaded herbal vesicle (herbosomes) involved optimizing dependent variables (e.g., lipid-to-extract ratio, surfactant concentration) and independent variables using DOE software. The optimization process significantly improved the formulation's characteristics. The resulting herbosomes were characterized for physical properties, in addition to particle size distribution analysis revealed an average size of 112 nm. Zeta potential analysis demonstrated a stable formulation with a potential value of 28.5 mV, indicating good dispersion stability. SEM analysis provided insights into the morphology of the herbosomes, showing a spherical vesicle structure. Stability studies conducted at various temperatures and conditions confirmed that the herbosomes remained stable at intervals of 30, 60, and 90 days (3 month). Furthermore the ability of the antimicrobial herbosome formulation was assessed using the well diffusion assay, which showed significant inhibitory effects against *E. coli* and *S. aureus*, suggesting strong antimicrobial potential. Overall, the study demonstrated the successful formulation of *Amaranthus viridis*-based herbosomes with promising stability and antimicrobial properties.

KEYWORDS: - *Amaranthus viridis,* Herbosomes, Percentage yield, Phytochemical analysis, DOE software, Zeta potential, Antimicrobial properties.

1. INTRODUCTION

The development of effective and stable herbal formulations requires a systematic and scientifically driven methodology. Traditional approaches often rely on empirical experimentation, which can be time-consuming and may not yield reproducible results **(Finkel *et al.*, 2017).** To overcome these limitations, the Quality by Design (QbD) framework has emerged as a modern and efficient strategy for pharmaceutical product development. QbD focuses on building quality into the product from the initial stages by identifying and controlling key formulation and process variables that influence the final product's quality attributes **(Beg *et al.*, 2019).**

In this study, the QbD approach was implemented for the formulation design and in vitro evaluation of herbal vesicles (Herbosomes) containing *Amaranthus viridis* extract. *Amaranthus viridis*, commonly known as slender amaranth, is a medicinal plant known for its rich

phytochemical composition, including flavonoids, alkaloids, tannins, and saponins **(Singh *et al.*, 2023).** These bioactive constituents possess diverse pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective effects. However, the therapeutic potential of *Amaranthus viridis* extract is often limited by poor solubility, low stability, and inadequate bioavailability when administered in conventional dosage forms **(Dutta *et al.*, 2025).**

To address these challenges, Herbosomes, a novel phytophospholipid-based vesicular delivery system, were developed to encapsulate *A. viridis* extract. This system enhances the solubility, stability, and bioavailability of plant bioactives by forming a lipid-compatible complex between phospholipids and phytoconstituents, allowing better absorption through biological membranes. The QbD approach ensures that the formulation and process parameters are optimized scientifically, resulting in a robust and reproducible product **(Mishra *et al.*, 2018).**

The Design of Experiments (DOE) tool integrated within the QbD framework, particularly using Design-Expert software, was employed to systematically investigate the influence of critical formulation variables such as phospholipid concentration, cholesterol content, and sonication time on key quality attributes including vesicle size, zeta potential, and entrapment efficiency **(Correia *et al.*, 2023).** A factorial design was utilized to identify significant factors and their interactions, thereby developing predictive models to guide formulation optimization **(Mehta *et al.*, 2019).**

The prepared Herbosomes were then subjected to comprehensive in vitro evaluation to assess their physical characteristics, particle size distribution, surface charge (zeta potential), morphology, entrapment efficiency, and stability **(Shakya *et al.*, 2014).** Scanning Electron Microscopy (SEM) was used to visualize vesicle morphology, while stability studies confirmed the formulation's robustness under varying environmental conditions. Additionally, antimicrobial activity was evaluated using the well-diffusion assay against common bacterial strains such as *E. coli* and *S. aureus*, demonstrating the potential therapeutic applications of the developed system **(Chavez-Esquivel *et al.*, 2021).**

Overall, this QbD-guided study establishes a scientific foundation for the development of a stable, efficient, and reproducible herbal vesicular delivery system containing *Amaranthus viridis* extract. The findings highlight how the integration of QbD and DOE methodologies can significantly enhance formulation quality, therapeutic performance, and process understanding, paving the way for advanced herbal drug delivery applications.

2. MATERIAL AND METHODS

2.1 Chemicals

Petroleum ether, Magnesium, Propylene glycol, Sodium Hydroxide and Conc. H_2SO_4 were obtained from Vizag Chemical, a reputable supplier of analytical reagents. Jaysons Chemical Industries provided the Ammonia, and Otto Chemie Pvt Ltd provided the Cholesterol. Bajaj Hindustan Sugar Limited provided the Ethanol, while Anant Pharmaceuticals Pvt. Ltd. supplied the Methyl paraben. Orient Micro Abrasives Limited (OMAL) supplied the DMSO, Acetone. Analytical AR Grade obtained from Ethanol. Labogens provided the Conc. HCl while Pandora Industries supplied the Glacial Acetic Acid. Shree Krishna Pharmaceutical provide Triethanolamine and Lobagens supplied Soya-Lacithin. Indian Platinum provided the Nitroprusside and Chloroform obtained from Clorofilt ind.

2.2 Plant collection

The wholw plant of *Amaranths viridis Linn* (300g) was acquired from nearby location of PBRI Bhopal. The freshly leaves of *Amaranths viridis* had been gathered and cleaned to evacuate the adhered foreign material. The collected leaves were shade-dried at standard temperature. The dried Plant-based materials were subjected to size reduction to coarse powder by using dry grinder and went through sieve. No: 40 were used throughout the extraction procedure. The plant matter was recognized and confirmed by Botanist.

2.3 Extraction

The plant material utilized in investigation, *Amaranthus viridis*, was taken out utilizing the continuous hot percolation method and Soxhlet equipment **(Popoola, 2022).** The dried extract was weighed, and the percentage yield was estimated utilizing the formula that follows:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Materialused}} \times 100$$

The prepared extracts were examined for organoleptic characteristics (percentage yield, color, and odor) and then packaged in an airtight container and labeled for future use.

2.4 Phytochemical investigation

Phytochemical study is the systematic investigation of the substance substances found in plants, referred to as plant compounds that are in charge of their medicinal, nutritious, or poisonous qualities. This procedure involves the identification, separation, and extraction of bioactive substances of *A. viridis* leaves extracts including cardiac glycosides, steroids, alkaloids, flavonoids, saponins, phenolics, and tanninsusing techniques like Soxhlet extraction, chromatography, and spectroscopy. Phytochemical research attempts to identify novel chemicals, assess their functions of biological, and examine their possible uses in medicine, food, and business **(Pichaivel *et al.*, 2022).**

2.5 Quantitative Phytochemical Estimation

2.5.1 Total Phenolic Content (TPC)

Amaranthus viridis extract was mixed within distilled water. Add some drops of a diluted ferric chloride solution. The intense blue color showed the tannins presence. A certain amount of *Amaranthus viridis* extract was added within distilled water. 2ml of 1% gelatin solution with 10% sodium chloride was mixed. The phenolic compounds existence is shown by the creation of white precipitates **(da Silva *et al.*, 2023).**

2.5.2 Total Flavonoid Content (TFC)

To take the flavonoids in 1 milliliter of *Amaranthus viridis* extract, a small amount of extract thatadded in ethanol, and a few turnings of magnesium were mixed, followed by hydrochloride acid drops. The look of a pink, red, or orange color indicated the flavonoids presence **(Ghosh, 2019).**

2.6 Formulation of extract loaded Herbosome

For this study, we created an extract of Amaranthus viridis loaded Herbosome formulation by using traditional preparation technique, such as Thin film hydration (TFH) method of liposomes describes with few modifications. The ratios of herbal extract (Amaranthus viridis), L-a-Phosphatidylcholine, cholesterol, solvents (chloroform and methanol), surfactant (stearic acid), cryopreservant (sorbitol), and vehicle (PBS, pH 7.4). Surface of reaction technique analysis was hired to maximize n-herbosome. In brief, lipid, cholesterol, stearic acid, with sorbitol were correctly weighed and added in a round-bottom flask (RBF) containing the solvent, then carefully mixed to produce transparent lipid solution. Then mixture was evaporated in an evaporator that rotates. The thin layer formed in round-bottomed flask was hydrated by adding phosphate buffer 7.4 and 400 mg extract. The suspension was stirred by magnetic stirring for 1 to 3 hrs and then sonicated for 5-20 minute. Table 4 shows various formulation variables used in this research. Herbosome was then successfully collected in vessels and used additional medication development **(BalaYadav *et al.*, 2022).**

Table 1: Composition of herbosome formulation.

Soya-Lacithin (mg)	Cholesterol (mg)	Stearic acid (mg)	Sorbitol (mg)	PBS Solution 7.4 (ml)	Extract (mg)	Stirring Time (Hrs)	Sonication time
50	20	30	20	15	400	3.0	12.5
50	15	30	20	15	400	3.0	5
200	15	30	20	15	400	3.0	20
125	10	30	20	15	400	3.0	5
125	20	30	20	15	400	3.0	5
125	15	30	20	15	400	3.0	12.5
125	10	30	20	15	400	3.0	20
200	15	30	20	15	400	3.0	5
125	20	30	20	15	400	3.0	20
200	20	30	20	15	400	3.0	12.5
125	15	30	20	15	400	3.0	12.5
50	15	30	20	15	400	3.0	20
50	10	30	20	15	400	3.0	12.5
200	10	30	20	15	400	3.0	12.5

2.7 Design of experiment

Considering quantity of factors and levels, 3^2 factorial design alog with optimization process was usedto examine their effects using the Design-Expert software (version 12.0.1.0). The design contained experimental 14 runs which included 3^2 factorial design and levels. The prepared extract loaded herbal vesicles formulation by all mentioned above three methods was discovered to be milky white without any signs of interactions. The experiment for Herbosome development was designed using Design Expert (Version 12.0.1.0) software **(Prakash and Tiwari 2024).**

Table 2: Independent and Dependent variables.

Independent variables	Dependent variables
X1 Soya-Lacithin (mg)	(Y1) Particle size (nm)
X2 Cholesterol (mg)	(Y2) Zeta potential (mV)
X3 Sonication time (min.)	

2.8 Characterization of Herbosome

2.8.1 Particle size

The mostcrucial parameters for characterizing Herbosomes are the dimensions of their particles. A Malvern Instruments' Malvern Zeta size was employed to ascertain the dimensions of each herbosome **(Mazumder *et al.*, 2016).**

2.8.2 Zeta potential

In current study, herbosome was diluted tenfold with filtered water and tested utilizing Zetasizer Malvern technology **(Joshi *et al.*, 2021).**

2.8.3 Scanning Electron Microscopic (SEM)

Using a scanning electron microscope the electron beam, obtain the morphological parameters of the extract-loaded Herbosome, which were then coated with a thin layer (2-20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater in vacuum circumstances (**Safta *et al.*, 2022).**

2.8.4 Anti-microbial activity

- **Preparation of Nutrient Agar Media**

2.8 gm of Nutrient Media were added in 100 milliliters of purified water. Before sterilization, the media's pH was calculated. The media was autoclaved at 121 ˚C and 15 psiabout 15 min. Nutrient media was poured into plates and placed in laminar air flow until the agar solidified **(Terrones Fernández, 2024).**

- **Culture inoculation and well preparation**

Use a sterilized cork-borer to make four wells in both agar plates. Lawn culture of bacteria *E. coli* with *S. aureus* was dispersed on Nutrient Agar Media with a spreader. Then, in each well, add a varied concentration of extract-loaded gel. For twenty-four hours, the plate is incubated about 37 °Celsius. Following incubation, zone of inhibition around each well is determined. A bigger zone indicates more antibacterial action, while the absence of a zone implies no antimicrobial effect **(Pasquet *et al.*, 2014).**

2.8.5 Stability studies

The Herbosome formulation (extract of Amaranthus viridis) was tested for stability at accelerated temperatures (25 ^{0}C±2 ^{0}C and 60 ± 5% RH) and 40 ^{0}C±2 ^{0}C and 70 ± 5% RH for 3 months. The formula was examined for evaluation parameter studies at 30, 60, and 90-day intervals (3 months). The composition was tested for stability under accelerated storage conditions for 3 months in compliance with International conference on Harmonisation (ICH) guidelines **(Sahu *et al.*, 2021).**

3. RESULT AND DISCUSSION

3.1. Percentage Yield

Table 3: Percentage Yield of *Amaranthus viridis* of crude extracts.

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Amaranthus viridis	Pet ether	300	3.58	0.79%
	Ethanol	269	5.63	0.85%

3.2 Preliminary phytochemical study.

Table 4: Phytochemical testing of extract.

Experiment	presence or absence of phytochemical test	
	Pet. Ether extract	Ethanolicextract
	Carbohydrates	
Molish's test	+ve	**+ve**
Fehling's test	+ve	**+ve**
Benedict's test	+ve	**+ve**
Barfoed's test	-ve	**+ve**
	Alkaloids	
Dragendroff's test	+ve	**+ve**
Wagner's reagent test	+ve	**+ve**
Mayer's reagent test	+ve	**+ve**
Hager's reagent test	+ve	**+ve**
Saponin		
Foam test	+ve	**+ve**
Froth Test	-ve	**+ve**
Test for Triterpenoids and Steroids		
Libbermann-Burchard's test	+ve	**+ve**
Libbermann-Burchard's test	+ve	**-ve**
Tannin and Phenolic Compounds		
Ferric Chloride test	+ve	**+ve**
Gelatin Test	+ve	**+ve**
Lead Acetate Test	+ve	**+ve**
Flavonoids Glycosides		
Shinoda Test	-ve	**+ve**

Glycosides			
Borntragers Test	-ve		**+ve**
Keller Killiani Test	-ve		**+ve**
Test for protein and amino acids			
Biuret's test	-ve		**-ve**
Ninhydrin test	**-ve**		**-ve**

3.3 Optimisation of herbosomes via Box Behnken method (response surface methodology)

The coded and real values of implemented Box–Behnken design based variables that are independent and dependent are shown in below. Table with their corresponding levels, including both high and low. 14 distinct formulation batches have been generated as consequence of component's experimental nature. As stated before, many formulations lots were created and subsequently evaluated for every result. The responses observed were fit to 14 runs, with the fact that best fit model was the linear and quadratic framework for the two dependent variables. The model's importance in relation that of comparing with the other model for the analysis by variance analysis (ANOVA). In polynomial equations, positive sign before the factor shows the linear correlation between response and factor. Every response was recorded for 14 runs, and the table shows connection between the independent and dependent factors.[112]

3.3.1 Build Information

Table 5: Build information of DOE software.

File Version	**12.0.1.0**		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box- Behnken	Runs	14
Design Model	Quadratic and linear	Blocks	No Blocks
Build Time (min)	468.00		

3.3.2 Herbosomes Formulations trials as per Box–Behnken design (BBD)

Table 6: Formulation trials for optimization process.

Soya-Lacithin (mg)	Cholesterol (mg)	Stearic acid (%)	Sorbitol (mg)	PBS Solution on 7.4 (ml)	Extract (mg)	Stirring time (Hrs)	Sonication time (min.)	Particle size (nm)	Zeta potential (mV)
50	20	30	20	15	500	3.0	12.5	338.2	27.3
50	15	30	20	15	500	3.0	5	870.2	25.1
200	15	30	20	15	500	3.0	20	224.6	26.7
125	10	30	20	15	500	3.0	5	904.1	27.1
125	20	30	20	15	500	3.0	5	841.3	25
125	15	30	20	15	500	3.0	12.5	461.1	24.1
125	10	30	20	15	500	3.0	20	191.4	27.1
200	15	30	20	15	500	3.0	5	748.2	26.3
125	20	30	20	15	500	3.0	20	128.3	26.1
200	20	30	20	15	500	3.0	12.5	273.9	25
125	15	30	20	15	500	3.0	12.5	227.3	24.4
50	15	30	20	15	500	3.0	20	144.7	26.4
50	10	30	20	15	500	3.0	12.5	550.8	25.8
200	10	30	20	15	500	3.0	12.5	498.3	29.4

3.3.3 Limits of Variables (Constraints)

Table 7: Variables operating range for herbosomes formulation.

Name	Goal	Lower Limit	Upper limit	Importance
A: Soya lecithin	is in range	50	200	3
B:Cholesterol	is in range	10	20	3
C:Sonication Time	is in range	5	20	3
Particle size	None	128.3	904.1	3
Zeta potential	None	24.1	29.4	3

3.3.4 Fit Summary of Particle of herbosome formulation.

Table 8: Response 1: Particle size.

Source	Sequential pvalue	Lack of Fit pvalue	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.8871	0.8667	0.8286	Suggested
2FI	0.8615	0.8245	0.8277	0.7121	
Quadratic	0.3225	0.8866	0.8630	0.6395	
Cubic	0.8866		0.6597		Aliased

3.4 Effect of formulation variables on particle size (ANOVA for Linear model).

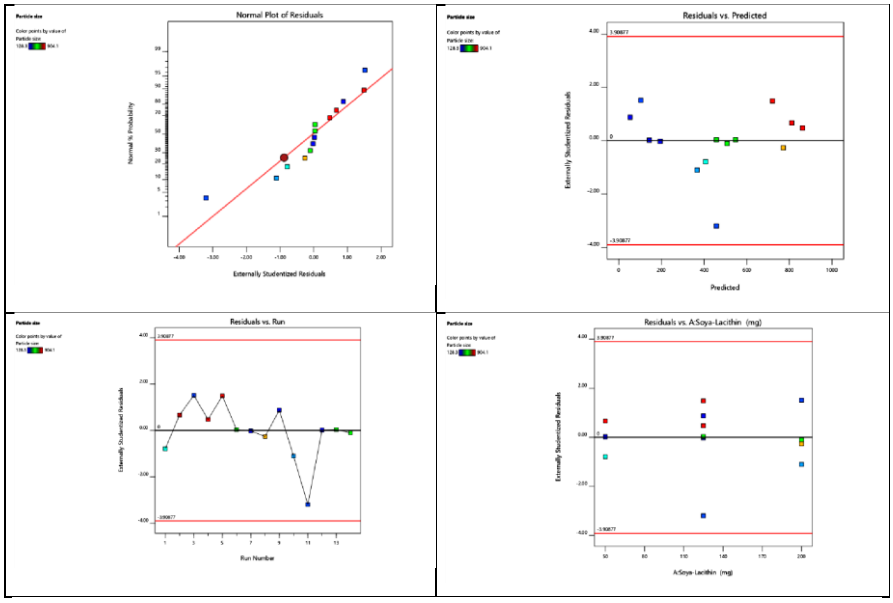
3.4.1 Response 1: particle size.

Table 9: Response 1: Particle size (ANOVA for Linear model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	9.371E+05	3.124E+05	29.17	< 0.0001	significant
A-Soya Lecithin	3156.15	3156.15	0.2947	0.5991	
B-Cholesterol	39607.05	39607.05	3.70	0.0834	
C-Sonication Time	8.943E+05	8.943E+05	83.52	< 0.0001	
Residual	1.071E+05	10708.28			
Lack of Fit	79751.57	8861.29	0.3242	0.8871	not significant
Pure Error	27331.22	27331.22			
Cor Total	1.044E+06				

Final Formula with Coded Elements

Particle size Y1 = +457.31 intercept -19.86 X1 A (phospholipid) -70.36X2 B (Cholesterol) - 334.35 C (Sonication time) X3.



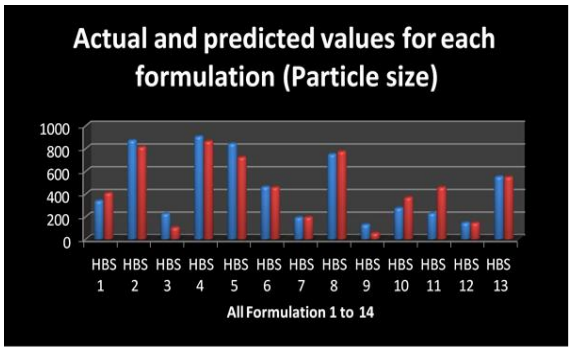
Graph 1: Graphical representation of Residuals vs predicted, Normal plot of Residuals and residual vs run of herbosomes formulation on particle size.

3.4.2 Actual and predicted values for each formulation (Particle size)

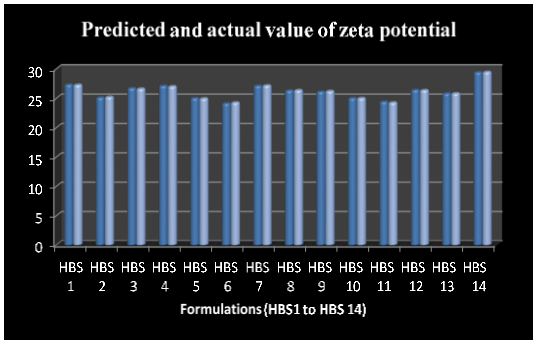
Table 10: All formulation predicted and actual values of particle size and zeta potential.

Formulations	Actual Value of Particle size	Predicted Value of Particle size	Actual Value of zeta potential	Predicted Value of zeta potential
HBS 1	338.20	406.81	27.30	27.25
HBS 2	870.20	811.53	25.10	25.20
HBS 3	224.60	103.10	26.70	26.60
HBS 4	904.10	862.03	27.10	27.00
HBS 5	841.30	721.30	25.00	24.95
HBS 6	461.10	457.31	24.10	24.25
HBS 7	191.40	193.33	27.10	27.15
HBS 8	748.20	771.80	26.30	26.35

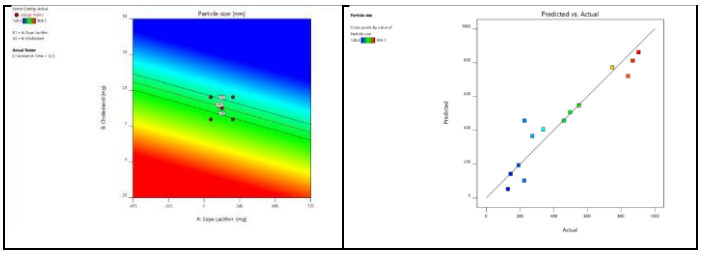
HBS 9	128.30	52.60	26.10	26.20
HBS 10	273.90	367.09	25.00	25.00
HBS 11	227.30	457.31	24.40	24.25
HBS 12	144.70	142.83	26.40	26.35
HBS 13	550.80	547.54	25.80	25.80
HBS 14	498.30	507.81	29.40	29.45



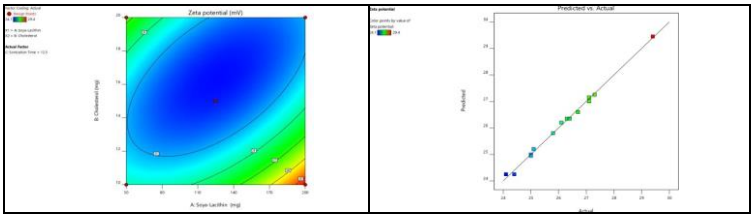
Graph 2: Particle size predicted and actual results.



Graph 3: Graphical representation of Predicted and actual value of zeta potential.



Graph 4:Two-dimensional contour plots for the effect of polymer and surfactant concentration on particle size.



Graph 5: Two-dimensional contour plots for the effect of formulation on zeta potential Effect of formulation variables of zeta potential.

Table 11: Response 2: Fit Summary of zeta potential.

Source	Sequential p- value	Lack of Fit p- value	Adjusted R^2	Predicted R^2	
Linear	0.3766	0.1137	0.0331	-0.3301	
2FI	0.1697	0.1284	0.2971	0.1467	
Quadratic	**0.0002**	**0.7848**	**0.9871**	**0.9580**	**Suggested**
Cubic	0.7848		0.9768		Aliased

3.5 ANOVA for Quadratic model

3.5.1 Response 2: Zeta potential (ANOVA Quadratic model)

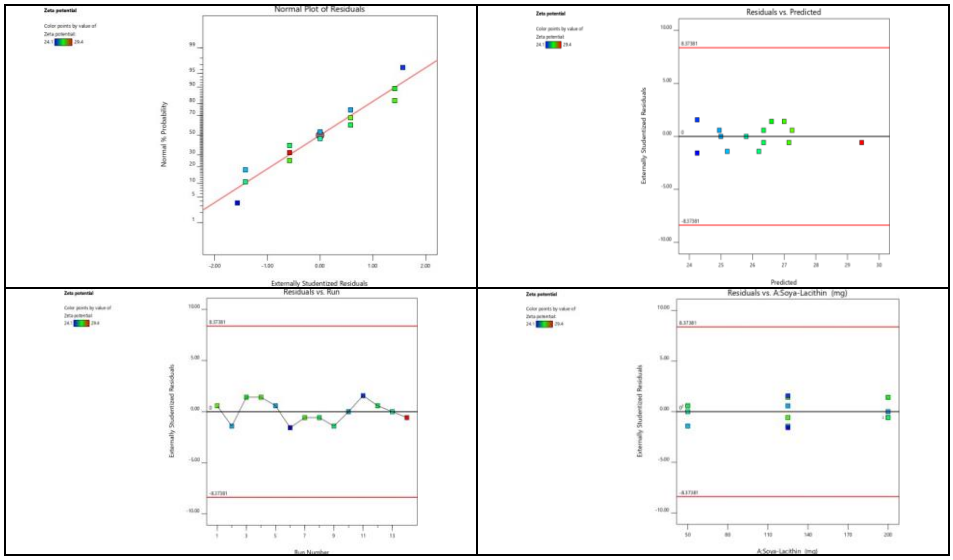
Table 12: Response 2: Zeta potential (ANOVA Quadratic model).

Source	Sum of Squares	Mean Square	F-value	p-value	
Model	25.11	2.79	111.59	0.0002	significant
A-Soya Lecithin	0.9800	0.9800	39.20	0.0033	
B-Cholesterol	4.50	4.50	180.00	0.0002	
C-Sonication Time	0.9800	0.9800	39.20	0.0033	
AB	8.70	8.70	348.10	< 0.0001	
AC	0.2025	0.2025	8.10	0.0466	
BC	0.3025	0.3025	12.10	0.0254	

A^2	4.70	4.70	188.18	0.0002	
B^2	6.38	6.38	255.38	< 0.0001	
C^2	1.40	1.40	56.18	0.0017	
Residual	0.1000	0.0250			
Lack of Fit	0.0550	0.0183	0.4074	0.7848	not significant
Pure Error	0.0450	0.0450			
Cor Total	25.21				

Final Equation in terms of Coded Factors

Zeta potential (Y2) = + 24.25 + 0.3500 A - 0.7500 B + 0.3500 C -1.47 AB - 0.2250 AC +0.2750 BC +1.21 A^2 +1.41 B^2 +0.6625 C^2



Graph 6: Graphical representation of normal residual plot Run versus residuals, Residuals' normal plot and residuals vs the expected herbosomes formulation on zeta potential (Statistically significant plot effects of response.

3.5.2 Optimized formula of herble vesicle formulation.

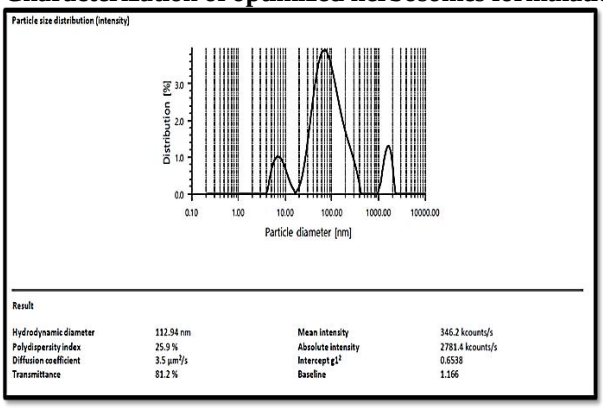
Table 13: Optimized formula of herbosomes.

Soya lecithin (Phospholipid) (mg)	Cholesterol (%)	Sonication time (min.)	Particle size(nm)	Zeta potential	Desirability	
50.000	10.000	12.500	547.539	25.800	1.000	
200.000	15.000	20.000	103.102	26.600	1.000	Selected
200.000	10.000	12.500	507.814	29.450	1.000	

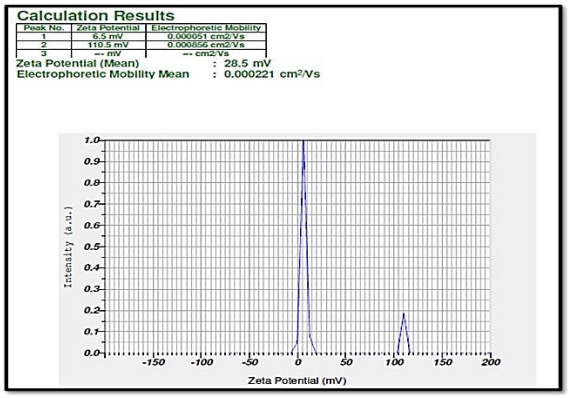
Table 14: Composition of optimized formulation as per DOE approach.

Formulation Code	Soya lecithin (Phospholipid) (mg)	Cholesterol (mg)	Stearic acid (mg)	Sorbitol (mg)	PBS Solution 7.4 (ml)	Extract(mg)	Sonication time (min.)	Stirring Time (Hrs)
HBS	200.000	15.000	30.00	20.00	15.00	400	20.00	3.00

3.6 Characterization of optimized herbosomes formulation of Particle size and zeta potential



Graph 7: Particle size.



Graph 8: Zeta potential.

Table 15: Prticle size and Zeta potential.

Formulation	Particle size (Predicted value)	Particle size (Actual value)	Predicted result of Zeta potential (mV)	Actual result of Zeta potential (mV)
Herbosomes	103.10	112.94 nm	26.6 mV	28.5 mV

3.6.1 Scanning electron microscope (SEM)

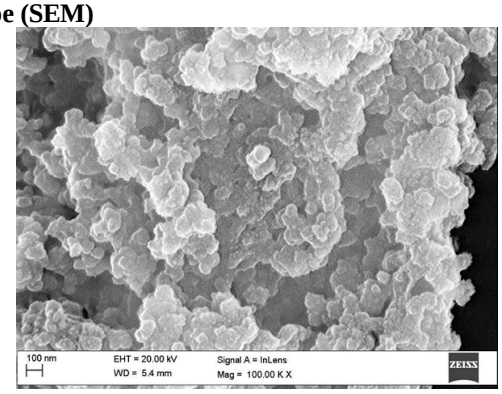


Figure 1: Scanning electron microscope (SEM) and Microscopy image.

3.7 Results of antimicrobial activity of optimized herbosomes formulation

3.7.1 Antimicrobial activity of formulation against *E. coli* and *S. aureus*

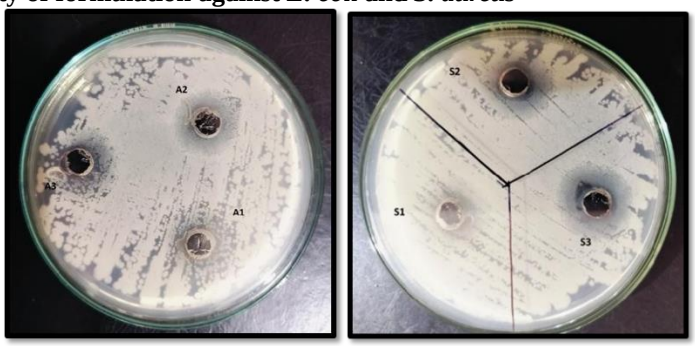
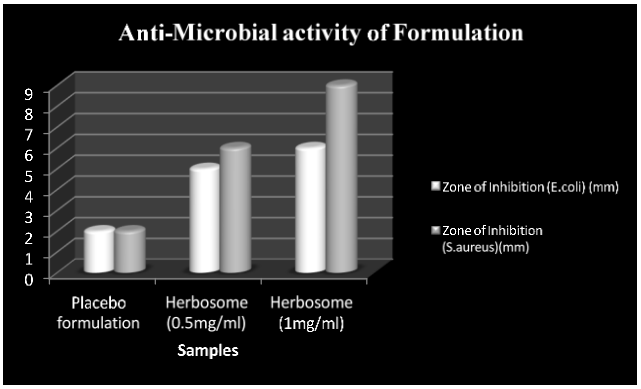


Figure 2: Antimicrobial activity against *S. aureus* and *E. coli.*

Table 16: Anti-microbial activity of Herbosome against *E.coli* and *S.aureus.*

Sample name (*E.coli*)	Zone of Inhibition (*E.coli*) (mm)	Sample name (*S.aureus*)	Zone of Inhibition (mm) (*S.aureus*)
Placebo formulation (A1)	2.0 mm	Placebo formulation (S1)	2.0 mm
Herbosome (0.5mg/ml) (A2)	5.0 mm	Herbosome (0.5mg/ml) (S2)	6.0 mm
Herbosome (1mg/ml) (A3)	6.0 mm	Herbosome (1mg/ml) (S3)	9.0 mm



Graph 9: Anti-microbial activity against *E.coli* and *S.aureus.*

3.8 Stability study

Table 17: Stability Study of optimized formulation (Herbosomes).

Time (Days)	25^0C±2 ^{0}C and 60 ± 5% RH		40^0C±2 ^{0}C and 70 ±5% RH	
	Particle Size (nm)	Zeta potential (mV)	Particle Size (nm)	Zeta potential (mV)
0	112.9 nm	28.5 mV	112.94 nm	28.5 mV
30	111.8 nm	28.4 mV	112.0 nm	28.5 mV
60	113.0 nm	28.3 mV	112.8 nm	27.1 mV
90	113.7 nm	27.9 mV	113.5 nm	27.6 mV

DISCUSSION

The Box–Behnken design (BBD) was employed to optimize the formulation parameters influencing the particle size and zeta potential of *Amaranthus viridis* Herbosomes. Fourteen experimental runs were generated by varying three independent factors—phospholipid concentration, cholesterol content, and sonication time—at different levels. Statistical analysis using ANOVA confirmed that the linear model was significant for particle size ($F = 29.17$, $p < 0.0001$), while the quadratic model best fitted zeta potential data ($F = 11.59$, $p = 0.0002$). The optimized equations indicated that an increase in phospholipid and cholesterol levels reduced particle size, whereas prolonged sonication time significantly decreased it. Similarly, zeta potential was positively influenced by phospholipid concentration but negatively affected by cholesterol. The non-significant lack of fit ($p > 0.05$) validated the model's suitability. The optimized formulation showed a particle size of approximately 128.3–904.1 nm and zeta potential ranging from 24.1 to 29.4 mV, confirming good stability. SEM analysis revealed spherical, smooth vesicles, and stability studies demonstrated no significant changes in zeta potential or particle size over three months, indicating the formulation's robustness and consistency.

4. CONCLUSION

The study successfully developed and optimized *Amaranthus viridis* Herbosomes using the DOE approach with Design-Expert software. The optimized formulation demonstrated desirable vesicle size, high zeta potential, and excellent encapsulation efficiency. Characterization confirmed spherical morphology, stability, and effective encapsulation of bioactive compounds. Moreover, the Herbosomes exhibited strong antimicrobial activity against *E. coli* and *S. aureus*, indicating their potential for pharmaceutical applications.

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