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Development and Characterization Of B-Cyclodextrin-Based Nanosponge Gel Containing Herbal Extract using a Quality by Design (Qbd) Approach and its Antimicrobial Activity

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KEYWORDS

Nanosponges, Antimicrobial activity, *Bryophyllum pinnatum,* Beta-cyclodextrin, polymer, Diphenyl carbonate

ABSTRACT:

This research explores the development of a β-cyclodextrin-based nanosponge gel incorporating an extract of *Bryophyllum pinnatum* to enhance its antibacterial properties. The extract is rich in bioactive compounds, including tannins, phenols, steroids, glycosides, alkaloids, and flavonoids. Nanosponges were synthesized using β-cyclodextrin and diphenyl carbonate as a crosslinking agent, resulting in spherical, porous structures. The nanosponges exhibited stable particle characteristics, with an average particle size of 192.01 nm and a zeta potential of -1.2 mV. The formulated gel showed high permeability and controlled release properties. The nanosponge formulation demonstrated improved antimicrobial activity against *E.coli,* compared to the *Bryophyllum pinnatum* extract. The zone of inhibition increased with concentration, reaching up to 13 mm at 1.5 mg/mL (Table 19), compared to only 7 mm for the extract. The findings support the potential of nanosponge-based delivery systems to enhance the therapeutic efficacy of plant extracts, highlighting their promise for future biomedical applications.

1. Introduction

Nanotechnology offers diverse applications in the medical and nutraceutical sectors, where it is utilized in various forms such as nanoparticles, nanotubes, nanofibers, and nanocomposites[1]**.** Among these, nanosponges (NSs) represent an advanced class of nanocarriers, defined by their compact, cross-linked, and porous polymeric structures[2]**.** These nanocarriers serve multiple functions, including the delivery of catalysts and gases, inhibition of enzymatic activity, and adsorption of harmful substances. Their advantages include high biodegradability, thermal and pH stability, biocompatibility, and low cytotoxicity[3]**.** Various types of nanosponges have been explored, such as those based on metals, β-cyclodextrin (CD), silicon, ethylcellulose, and DNAzymes[4]**.** Among these, β-cyclodextrin-based nanosponges (CDNSs) are the most extensively researched due to their unique three-dimensional network, selective delivery capabilities, low toxicity, and ability to provide controlled release[5]**.**

Many molecules have been studied concerning their potential uses as anti-tumour drugs or antimicrobials. With this precedent, natural products have shown promising results as bactericide agents. *Bryophyllum pinnatum* is extensively used to treat the various ailments in folk medicine. The plant is enriched with a diverse range of active therapeutic constituents which are responsible for various significant pharmacological effects. It is consuming for the treatment and management of various pathologies such as conjunctivitis, edema, piles, cuts, eczema, constipation, epilepsy, cholera, asthma, chest colds, menstrual disorders, chicken pox and fever[6]**.** *Bryophyllum pinnatum* is widely utilized in ayurvedic medicines for the treatment of numerous conditions such as menorrhagia, hemorrhoids, vomiting, corns, ophthalmia

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and hematemesis[7]. *Bryophyllum pinnatum* different crude extracts were analyzed for their anti- microbial effect and it was determined that the extracts have broad spectrum anti-bacterial activity[8].

Due to the potential benefits of β-cyclodextrin nanosponges and *Bryophyllum pinnatum* extract, the present study aims to formulate a β-cyclodextrin-based nanosponge gel loaded with *Bryophyllum pinnatum* extract for the evaluation of antimicrobial activity. The Quality by Design (QbD) approach was employed to optimize the nanosponge formulation.

2. Material and methods

2.1 Chemical

Petroleum ether and copper sulfate were obtained from Rankem, while methanol and ethanol were sourced from Molychem. Beta-cyclodextrin and magnesium were purchased from Himedia, and diphenyl carbonate (DPC) from Spectrochem. Concentrated HCl, 95% ethanol, and chloroform were supplied by Clorofiltind.

2.2 Collection of plant

Fresh leaves of *Bryophyllum pinnatum* (300 g) were collected and thoroughly washed with tap water to remove adhered debris and contaminants. The cleaned plant material was shade-dried at room temperature for three days, followed by oven-drying at 45 °C until a constant weight was achieved. The plant material was then powdered using a mechanical grinder and stored in an airtight container for further use. The botanical identity of the plant was authenticated by a qualified plant taxonomist, and a voucher specimen was deposited for future reference.

2.3 Extraction

Powdered *Bryophyllum pinnatum* leaves were sequentially extracted using Soxhlet apparatus. Initially, extraction was performed with petroleum ether at 60°C until the solvent in the siphon tube turned colorless. The marc was dried and re-extracted with methanol under the same conditions. Extracts were concentrated using a Buchi rotary evaporator at 40°C[9]. The percentage yield was calculated as:

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

The obtained extracts were evaluated for organoleptic properties (color, odor, and percentage yield), labeled accordingly, and stored in sealed containers for future use.

2.4 Phyto-chemical Screening

Qualitative phytochemical analysis was performed to detect the presence or absence of various phytoconstituents. Standard procedures were followed, and the results were interpreted based on the formation of precipitates or changes in color intensity as indicators of specific compounds[10].

2.5 Quantitative Phytochemical Estimation

2.5.1 Total Phenolic Content

The total phenolic content of *Bryophyllum pinnatum* extract was determined using the Folin-Ciocalteu assay. Briefly, 0.2 mL of the extract (from stock solution) was mixed with 2.5 mL of 2.5% sodium carbonate solution and 2.5 mL of Folin-Ciocalteu reagent. The mixture was diluted to a final volume of 7 mL with distilled water and incubated at room temperature for 2 hours. Absorbance was measured at 760 nm using a spectrophotometer. Gallic acid standard solutions (20–100 µg/mL) were used to generate a calibration curve. The Folin-Ciocalteu reagent reacts with phenolic compounds producing a blue color, the intensity of which is proportional to the phenolic content, expressed as gallic acid equivalents (GAE)[11].

2.5.2 Total Flavonoid Content

Total flavonoid content of *Bryophyllum pinnatum* extract was measured using the Aluminum chloride colorimetric method. Briefly, 0.5 mL of the extract was mixed with 2 mL of distilled water, followed by the addition of 0.15 mL of 5% sodium nitrite solution. After 6 minutes, 0.15 mL of 10% aluminum chloride solution was added, and the mixture was allowed to stand for another 6 minutes. Subsequently, 2 mL of 4% sodium hydroxide was added, and the solution was thoroughly mixed. The absorbance was measured at 510 nm using a UV spectrophotometer. A calibration curve was prepared using rutin standard solutions ranging from 20 to 100µg/mL. The total flavonoid content was calculated from the calibration curve and expressed as milligrams of rutin equivalents per gram of dry extract[12].

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

2.6 Formulation of β-Cyclodextrin Nanosponges

β-Cyclodextrin-based nanosponges were synthesized using a cross-linking method. Briefly, β-cyclodextrin and a suitable cross-linker (e.g., diphenyl carbonate or carbonyldiimidazole) were mixed in a specific molar ratio and heated at an elevated temperature under stirring for several hours to promote cross-linking and form the nanosponge network. The resultant solid was washed thoroughly with distilled water and organic solvents to remove unreacted materials, and then dried under vacuum. The dried nanosponges were ground into a fine powder and stored in airtight containers for further characterization and drug loading[13].

2.7 Extract loading into nanosponges

Freeze-drying was employed to load the extract into the prepared nanosponges. Briefly, 1 g of placebo nanosponges was dispersed in 50 mL of double-distilled water using magnetic stirring. Approximately 100 mg of the plant extract was added to the dispersion and mixed thoroughly. The suspension was then centrifuged at 2000 rpm for 10 minutes to remove any free, unentrapped extract present in the supernatant. The resulting supernatant containing the drug-loaded nanosponges was freeze-dried at –81°C under a vacuum of 0.0010 mbar. The dried powder of extract-loaded nanosponges was collected and stored in airtight containers for further use[14].

2.8 Composition of nanosponges formulation

Table 1: Composition

S. No	Formulations Code	Beta-Cyclodextrin: Polymer (mg) X1	Diphenyl carbonate-DPC (cross linker) (mg) X2	Stirring duration (hours) X3	Drug (1:1 Ratio) (mg)	Temperature (°C)
1	F1	300	125	1	100	90 to 100
2	F2	100	125	3	100	90 to 100
3	F3	200	50	1	100	90 to 100
4	F4	300	50	2	100	90 to 100
5	F5	100	50	2	100	90 to 100
6	F6	200	200	1	100	90 to 100
7	F7	100	125	1	100	90 to 100
8	F8	100	200	2	100	90 to 100
9	F9	300	200	2	100	90 to 100
10	F10	300	125	3	100	90 to 100
11	F11	200	200	3	100	90 to 100
12	F12	200	50	3	100	90 to 100

2.9 Design of experiment

The formulation of nanosponges was optimized using Expert Design software (version 12.0.1.0). A second-order polynomial model was employed to generate quadratic response surface models, facilitating the analysis of the effects of formulation variables on nanosponge characteristics.

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2.9.1 Variables that is independent along with dependent

Table 2: variables that are independent along with dependent

Independent variables	Dependent variables
(X1) Polymer (mg)	(Y1) (nm) Particle size
(X2) Cross linker (mg)	(Y2) EE (%)
(X3) Stirring time(hrs)	

2.9.2 Variable values

Table 3: Values of variables

Factor	Name	Units	Type	Minimum	Maximum	Low Coded	High Coded	Mean	Std. Dev.
A	Polymer	Mg	Numeric	100.00	300.00	-1 ↔ 100.00	+1 ↔ 300.00	200.00	85.28
B	Cross-linker	Mg	Numeric	50.00	200.00	-1 ↔ 50.00	+1 ↔ 200.00	125.00	63.96
C	Time to stir	Hrs	Numeric	1.0000	3.00	-1 ↔ 1.00	+1 ↔ 3.00	2.00	0.8528

2.10Parameters for evaluating nanosponge

2.10.1 Zeta potential together with size of particle

The particle size of the nanosponges was measured using a Malvern Zetasizer [15].

2.10.2 Scanning Electron Microscopic or SEM

The morphology of drug-loaded nanosponges was examined using scanning electron microscopy (SEM). Samples were coated with a thin layer of metal (such as gold or platinum) to enhance conductivity. Secondary electron imaging was employed to capture surface details. Electron dispersion was analyzed in accordance with Rutherford and Kramer's laws[16].

2.11Formulation of Nanosponges gel

Carbopol-934 was dispersed in warm water and allowed to hydrate for 2 hours. Separately, carboxymethyl cellulose and methylparaben were dissolved in distilled water at room temperature. The hydrated Carbopol dispersion was then mixed with this solution. The pH of the mixture was adjusted to neutral using triethanolamine. Nanosponges dispersion was incorporated into the gel base, followed by the addition of propylene glycol as a penetration enhancer. The final formulation was mixed thoroughly to obtain a uniform gel [17].

2.11.1 Contents of gel's composition

Table 4: Gel composition

S. No	Excipients	Quantity in gm
1.	Carbopol 934	1.00 gm
2.	Carboxymethyl cellulose	1.00 gm

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

3.	Propylene glycol	0.5 ml
4.	Methyl paraben	0.2 ml
5.	Nanosponges	1.0 gm
6.	Tri-ethanolamine	q.s
7.	Water	100 ml

2.12 Evaluation of gel containing nanosponges

2.12.1 Physical appearance

The prepared gel formulation was evaluated visually for appearance, color, odor, and homogeneity[18].

2.12.2 pH

The pH of the gel formulation was determined using a digital pH meter (Model: EI Instruments)[19].

2.12.3 Viscosity

The viscosity of the gel formulation was measured using a Brookfield viscometer (spindle no. 61) at 100 rpm and ambient temperature[20].

2.12.4 Spreadability

An ideal topical gel should exhibit a high coefficient of spreadability for effective application. Spreadability was evaluated by placing approximately 1 g of the gel between two glass slides. A 50 g weight was placed on the upper slide, and the time taken for the gel to spread a fixed distance was recorded. Spreadability (S) was calculated using the following formula[21]:

S= M*L/T

3. Results

3.1 Yields as Percentage

Table 5: Percentage yield of crude extract from *Bryophyllum pinnatum*

S. No	Name of the Plant	Solvent	Weight of theory	Yield in gm	Percentage yield
1	*Bryophyllum pinnatum*	Pet ether	298	1.47	0.49%
2		Methanol	286.21	6.60	2.30%

3.2 Phytochemical screening

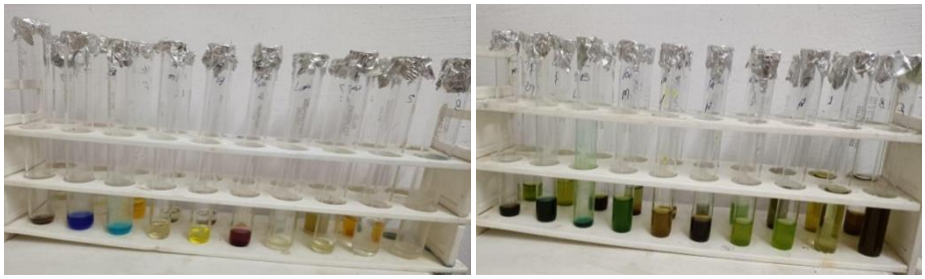

Figure 1: Phytochemical screening of petroleum ether and methanolic *Bryophyllum pinnatum* extract

Journal of Chemical Health Risks
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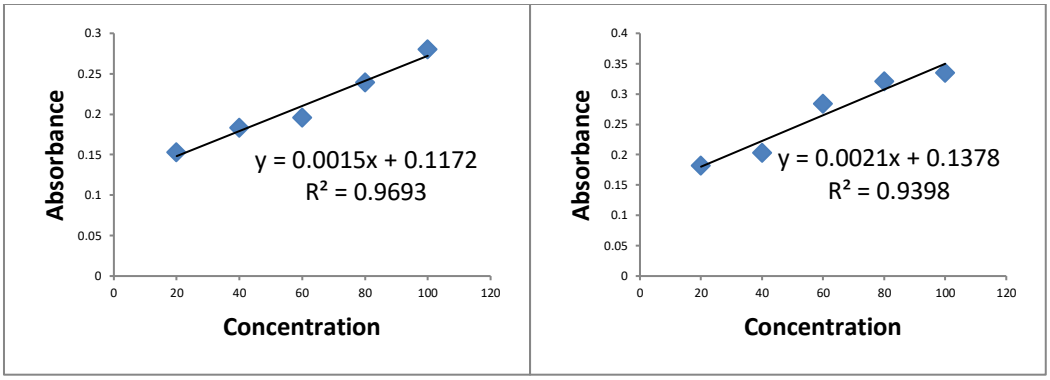
JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

3.3 Quantitative TPC and TFC Analysis



Graph 1 : Standard curve for gallic acid and rutin

Table 6: TPC and TFA

TPC of methanolic extract of *Bryophyllum pinnatum* (in mg GAE/g)	54.66 mg/gm
TFC of methanolic extract of *Bryophyllum pinnatum* (in mg RE/g)	17.66 mg/gm

3.4 Design of expert (DOE) software to optimize formulation

Table 7: Build information of DOE software

File Version	**12.0.1.0**		
Type of Study	Surface of Response	**Subtype**	Randomized
Type of Design	Box-Behnken	**Runs**	12
Model of Design	Quadratic	**Blocks**	No Blocks

Table 8: Independent variables

File Version	**12.0.1.0**		
Type of Study	Response Surface	**Subtype**	Randomized
Type of Design	Box-Behnken	**Runs**	12
Model of Design	Quadratic	**Blocks**	No Blocks
Build Time (ms)	2.00		

Table 9: Dependent and Independent variables

S. No	Coding	Dependent Variables
1.	Y1	(nm) Particle size

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

2.	Y2	(mV) Zeta potential
		Independent Variables
1.	X1	Polymer (mg)
2.	X2	Cross-linker (mg)
3.	X3	Stirring time (Hrs)

Table 10: Formulation Tests Based on Box-Behnken Design and Evaluation Parameters

S. No	Formulation trials					Evaluation parameter	
	Beta-cyclodextrin - Polymer (mg) X1	Diphenyl carbonate-Cross linker (DPC) (mg) X2	Time stirring (hours) X3	Extract (milligrams)	Temperature (ºC)	Particle size (in nm)	Zeta potential
1	200	200	3	200	90 to 100	124.64	-1
2	300	125	3	200	90 to 100	254.36	-1.2
3	300	125	1	200	90 to 100	860.96	-0.8
4	100	125	3	200	90 to 100	178.25	-0.7
5	300	50	2	200	90 to 100	492.83	-0.9
6	200	50	3	200	90 to 100	274.89	-1.3
7	200	50	1	200	90 to 100	775.21	-0.5
8	300	200	2	200	90 to 100	406.75	-1.5
9	100	50	2	200	90 to 100	492.18	-0.4
10	100	200	2	200	90 to 100	509.79	-0.3
11	200	200	1	200	90 to 100	924.04	-0.1
12	100	125	1	200	90 to 100	810.11	-0.2

3.4.1 Fit Summary

Table 11: Response 1: Size of particles

Source	P-value in sequence	Adjusted R^2	Predicted R^2	
Linear	**< 0.0001**	**0.9358**	**0.8950**	**Suggested**
2FI	0.1374	0.9629	0.9029	
Quadratic	0.3898	0.9670	0.8561	Aliased

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

3.5 Analysis of variance (ANOVA) for linear models was used to assess the impact of formulation variables on particle size

3.5.1 Response 1:-size of particle

Table 12: Response 1:-size of particle (For linear models, ANOVA)

Source	Total Squares	Mean Square	F-value	p-value	
Model	8.060E+05	2.687E+05	54.46	< 0.0001	significant
A-Polymer	75.46	75.46	0.0153	0.9046	
B-Cross linker	610.58	610.58	0.1238	0.7341	
C-Stirring time	8.053E+05	8.053E+05	163.25	< 0.0001	
Residual	39464.22	4933.03			
Cor Total	8.454E+05				

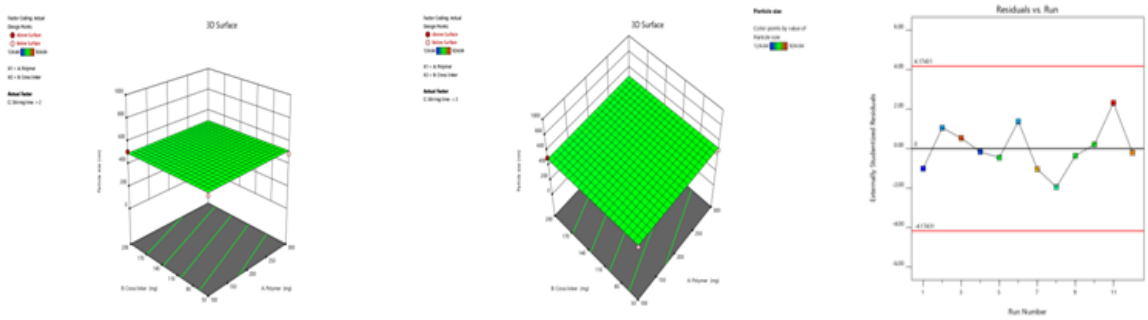

Figure 2: Response surface plot showing the combined effect of cross-linker and polymer on the particle size of nanosponges

3.5.2 Effect of formulation factors on zeta potential

Table 13: Reaction 2: Fit Summary Zeta potential

Source	P-value in sequence	Adjusted R^2	Predicted R^2	
Linear	**0.0032**	**0.7339**	**0.5645**	**Suggested**
2FI	0.6002	0.6978	0.2089	
Quadratic	0.9681	0.5072	-1.1506	Aliased

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

3.6 For linear models, ANOVA

3.6.1 Response 2 (linear model of ANOVA) Zeta potential

Table 14: Response 2: ANOVA linear model zeta potential

Source	Total Squares	Mean Square	F-value	p-value	
Model	1.83	0.6100	11.11	0.0032	significant
A-Polymer	0.9800	0.9800	17.85	0.0029	
B-Cross linker	0.0050	0.0050	0.0911	0.7705	
C-Stirring time	0.8450	0.8450	15.39	0.0044	
Residual	0.4392	0.0549			
Cor Total	2.27				

Table 15: Predicted and actual zeta potential values of formulations along with particle size

Formulations	Size of particle		Zeta potential	
	Real Value	Value Prediction	Real Value	Value Prediction
F1	124.64	182.66	-1.0000	-1.04
F2	254.36	194.47	-1.20	-1.42
F3	860.96	829.01	-0.8000	-0.7667
F4	178.25	188.32	-0.7000	-0.7167
F5	492.83	520.48	-0.9000	-1.12
F6	274.89	200.13	-1.30	-1.09
F7	775.21	834.68	-0.5000	-0.4417
F8	406.75	503.00	-1.50	-1.07
F9	492.18	514.33	-0.4000	-0.4167
F10	509.79	496.86	-0.3000	-0.3667
F11	924.04	817.20	-0.1000	-0.3917
F12	810.11	822.87	-0.2000	-0.0667

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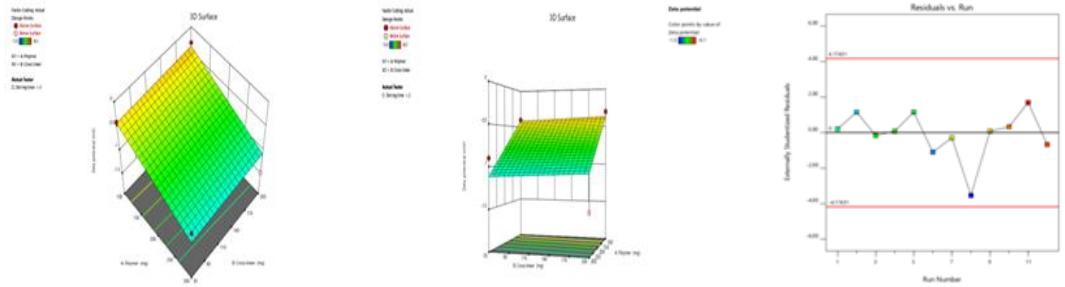

Figure 3: Response surface plot displaying cross linker and impact polymer combined on entrapment the efficiency of Nanosponges

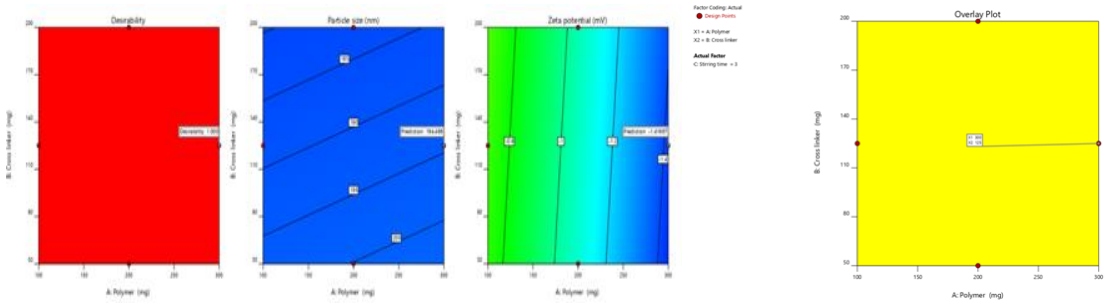

Figure 4: Response surface plot showing prediction data for efficiency and plot overlay formulation of optimization

3.7 Optimization of Nanosponge formulation

Table 16: Formulation of Nano Sponge optimized

S.No	Polymer	Cross-linker	Stirring time	Size of particle	Zeta potential	Extract (mg)	
1	**300.000**	**125.000**	**3.000**	**194.466**	**-1.417**	**200**	**Selected**
2	111.320	116.378	2.882	227.095	-0.721	200	
3	142.019	108.049	1.613	631.621	-0.419	200	

Figure 5: Optimized Nanosponges formulation

Journal of Chemical Health Risks

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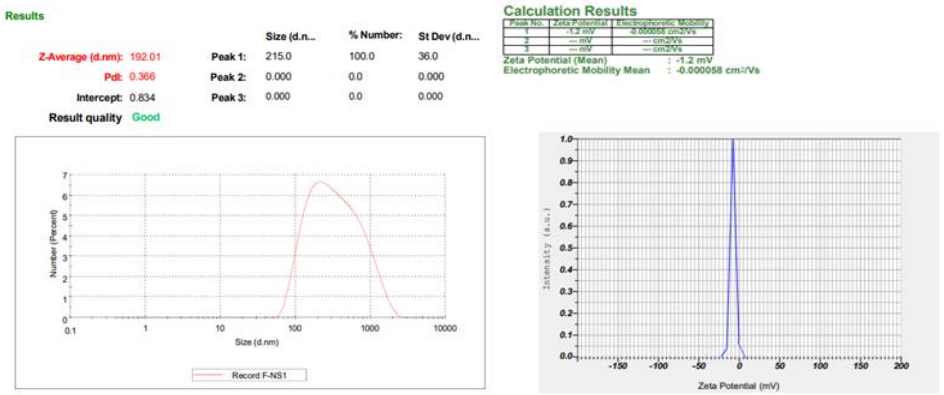
JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

3.8 Characterization of an optimized formulation

3.8.1 Zeta Potential and Particle Size



Graph 2: Zeta Potential and Particle Size of optimized formulation

Table 17: Particle size

Formulation	Size of particle		Zeta potential	
	(Value Prediction)	(Actual value)	(Value Prediction)	(Actual value)
Nanosponges	194.4	192.01 nm	- 1.4 mV	-1.2mV

3.9 Scanning electron microscope

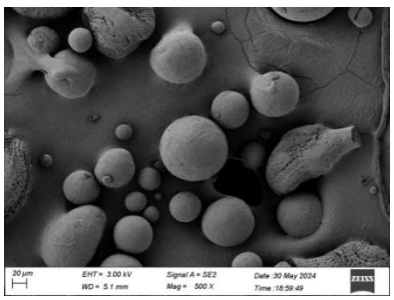


Figure 6:- Scanning Electron Microscope of optimized formulation

3.10 Characterization of Nano sponges loaded gel

3.10.1 Physical appearance

Table 18 Physical appearance of gel formulation

Parameter	Result
Colour	White to brown
Odour	Odorless

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

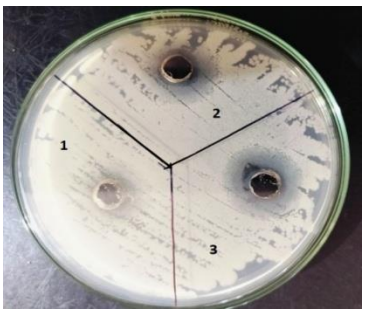
Appearance	Transparent
Homogeneity	Homogeneous

Table 19: Viscosity, pH and Spredability

Formulation	Viscosity	pH	Spredability
Gel	6107±0.42	6.1	11.02

3.11 Results of antibacterial activity against *E.coli*

Table 19: Anti-microbial activity against *E. coli*



S. No	Name of sample	Zone of Inhibition (in mm)
1	Extract (1mg/ml)	7 mm
2	Nano gel Formulation (1.0mg/ml)	10 mm
3	Nano gel Formulation (1.5mg/ml)	13 mm

Figure 7: Antimicrobial Activity against *E. Coli* bacteria

4. Discussion

The extraction of *Bryophyllum pinnatum* using petroleum ether and methanol yielded 0.49% and 2.30%, respectively, indicating a higher efficiency of methanol in extracted phytoconstituents from the plant matrix (Table 5). Phytochemical screening further validated the solvent-based difference in composition, with methanolic extracts showing more prominent presence of secondary metabolites, including phenols and flavonoids.

The total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extract were quantified as 54.66 mg GAE/g and 17.66 mg RE/g respectively (Table 6). These values suggest that *Bryophyllum pinnatum* possesses considerable antioxidant potential, which is beneficial in nanoparticle stabilization and could enhance the therapeutic value of the formulated nanosponges.

Design of Experiments (DOE) software, employing the Box-Behnken design, was used to optimize the formulation parameters of the nanosponges. A quadratic model was selected to study the effects of three independent variables—polymer concentration (X1), cross-linker concentration (X2), and stirring time (X3)—on two responses: particle size (Y1) and zeta potential (Y2) (Tables 9).

The predicted values of particle size and zeta potential were closely aligned with their respective actual values (Table 17), indicating the accuracy of the DOE model. The optimized formulation (polymer: 300 mg, cross-linker: 125 mg, stirring time: 3 hours) achieved a predicted particle size of 194.4 nm and a zeta potential

Journal of Chemical Health Risks

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JCHR (2025) 15(3), 1843-1856 | ISSN:2251-6727

of -1.4 mV, while actual values were 192.01 nm and -1.2 mV respectively, confirming the model's reliability.

SEM analysis (Figure 6) confirmed the spherical morphology and uniform distribution of the optimized nanosponges. The gel loaded with nanosponges showed acceptable physicochemical characteristics including a transparent appearance, odor less nature, and homogeneity. The gel exhibited good viscosity (6107 ± 0.42 cps), neutral pH (6.1), and satisfactory spreadability (11.02 cm) (Table 18), making it suitable for topical application.

The nanosponge formulation demonstrated improved antimicrobial activity against *E.coli,* compared to the *Bryophyllum pinnatum* extract. The zone of inhibition increased with concentration, reaching up to 13 mm at 1.5 mg/mL (Table 19), compared to only 7 mm for the extract. This enhancement could be attributed to the improved bioavailability and surface interaction of nanosponges with microbial cells.

5. Conclusion

Overall, the study highlights the feasibility of developing a stable and effective nanosponge-based gel formulation using *Bryophyllum pinnatum* extract, with promising applications in pharmaceutical or cosmeceutical fields. *In vivo* studies are needed to evaluate therapeutic efficacy and safety. Mechanistic investigations into drug release and skin permeation, along with long-term stability studies, will further establish the formulation's reliability.

6. References

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