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Original Article

Formulation, Optimization and Characterization of *Pongamia pinnata* Phytosomes for Therapeutic Potential

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ABSTRACT

Background: Phytosomes are complex structures formed by the combination of natural phospholipids with phytoconstituents. The purpose could be to enhance the bioavailability of active compounds, such as flavonoids and polyphenols present in *Pongamia pinnata* by forming phytosomes, thus improving their absorption and efficacy. **Materials and Methods:** The preparation process was optimized by Box Behnken design to attain desirable particle size and encapsulation efficiency. Independent variables included Phospholipid, Cholesterol and dichloromethane. Evaluation of the *Pongamia pinnata* phytosomes included, particle size, entrapment efficiency, zeta potential and *in vitro* drug release. Optimized phytosomes was then subjected to *in vivo* diabetic potential by using streptozotocin induced antidiabetic model. **Results:** Optimised phytosomes formulation showed an entrapment effectiveness of 73.12%, Particle size 138.95 nm and zeta potential was -45.65 mV. The scanning electron micrograph revealed uniformly sized and shaped particles. *In vitro* drug release study of optimized phytosomes exhibits a sustained or prolonged drug release pattern, which can be advantageous for certain therapeutic applications. The findings on release kinetic study suggest that the release of the active ingredient from the optimized phytosomal formulation follows a diffusion-based mechanism, as indicated by the higher regression coefficient (r^2) values obtained for the Higuchi (0.995) and Korsmeyer-Peppas models (0.992). *In vivo* antidiabetic study reveals that, the phytosomes of *Pongamia pinnata* demonstrated better antidiabetic effects than hydroalcoholic extract. **Conclusion:** The phytosome formulation of *Pongamia pinnata* significantly boosts the bioavailability and efficacy of its bioactive compounds, indicating superior therapeutic potential in managing diabetes and its concomitant complications compared to the hydroalcoholic extract.

Keywords: Antidiabetic effect, Box Behnken design, Phytosomes, *Pongamia pinnata*.

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INTRODUCTION

Hyperglycaemia occurs when glucose builds up in the bloodstream due to an inability of the body to either use or create adequate insulin.[1] Diabetic symptoms include increased thirst, urination, exhaustion, impaired vision, poor wound healing, recurring infections and unexplained weight loss. Diabetic consequences include cardiovascular disease, renal failure, neuropathy, retinopathy and foot ulcers.[2] In order to prevent complications and maintain optimal health; people with diabetes mellitus usually need to make lifestyle changes like changing their diet, exercising regularly, monitoring blood glucose level regularly and may also need to take medication like insulin or oral hypoglycaemic agents.[3]

The leguminous tree species *Pongamia pinnata*, whose common names include Indian Beech and Pongam Tree, is indigenous to Southeast Asia and South Asia.[4] It grows well in both dry and moist soil conditions[5]. Traditional medicine makes use of the tree's seeds, leaves and bark to cure an extensive range of conditions, including skin illnesses,[6] rheumatism,[7] wound healing,[8] inflammation,[9] and antioxidant.[10] Research has shown that its extracts can help manage blood sugar levels, which could make them anti-diabetic.[11,12] Nevertheless, additional studies are required to validate its effectiveness and safety in managing of diabetes.[13]

Phytosomes are a kind of specialized delivery system used in pharmaceutical and nutraceutical industries to advance bioavailability and effectiveness of plant-derived bioactive compounds, particularly phytochemicals such as flavonoids, polyphenols and other plant extracts.[14] The complexation process forms a molecular structure where the bioactive compound is surrounded and bound by a phospholipid bilayer, like the structure of cell membranes.[15,16] The phytosome enhances the solubility of the bioactive compound in both water and lipid

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environments, which can improve its absorption across biological membranes and also increase bioavailability. However, like any delivery system, the formulation and manufacturing process of phytosomes must be carefully optimized to ensure stability, safety and efficacy.[17-20]

MATERIALS AND METHODS

Plant collection and authentication

Pongamia pinnata leaves were harvested in June 2021 from Vindhya Herbals (MFPPARC), Bhopal and authenticated by the Department of Botany, Career College, Bhopal.

Extraction from Leaves of *Pongamia pinnata*

A hydroalcoholic solvent (Ethanol: Aqueous; 80:20 v/v) was used to extract 150 g of powdered *Pongamia pinnata* leaves by a maceration procedure. Heating the extract to levels higher than its boiling point caused it to evaporate. Lastly, % yields of dried extracts were resolute.[21]

Design of Experiment

The Box-Behnken Design (BBD) is a cost-effective and efficient optimization method that requires fewer runs, avoids extreme conditions and provides accurate quadratic modeling with balanced exploration of the design space.[22] The components that were selected for experimental design are as revealed in Table 1.

Preparation of phytosomes

Cholesterol and an aerial *Pongamia pinnata* extract were mixed in varying proportions to create the complex as given in Table 1. The reaction media, dichloromethane, was introduced to round-bottom flask (100 mL) containing a weight amount of extract, phospholipids and cholesterol. The complex's reaction temperature was maintained at 50°C though mixture was refluxed for 3 hr. 20 mL of n-hexane was added to evaporate clear fluid while stirring. To remove any residual solvents, the precipitate underwent filtration and vacuum drying. The remnants that had dried were gathered and kept in a glass container with an amber hue for future reference. Desiccators had been used the night before.[23,24]

Evaluation of Prepared Phytosomes

Entrapment efficiency and Particle size: After preparing the phytosomes, they were centrifuged at 12000 rpm for 1 hr at 4°C in a Remi cooling centrifuge.[25] The transparent liquid above the sediment was delicately removed to isolate the flavonoids that were not trapped. The absorbance of this liquid at λ_{max} 420.0 nm, measured with a UV/visible spectrophotometer was used to identify non-entrapped *Pongamia pinnata*. The total amount of *Pongamia pinnata* in a 1 mL dispersion was determined by the number of flavonoids in the supernatant and sediment. Following calculation was used to compute % of entrapment:

$$\% \text{ Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{x100}$$

Particle size of improved phytosome formulations was determined using DLS with use of a computerised examination system (Malvern Zetamaster ZEM 5002, Malvern, UK).[26] After two rounds of mixing with distilled water and 5 min of vortexing, the formulation was analysed for phytosome particle size.

Zeta potential analysis

The Malvern zetasizer was used to determine Zeta potential.[27] To find phytosomes' electric potential, which includes zeta potential of its Stern layer, diluted system was injected into a zeta potential measurement cell.

Scanning electron microscopy

An optimised formulation was selected and analysed for superficial morphology and shape using a SEM (JEOL Japan 6000). For surface morphology, microphotographs were obtained at a greater magnification (200X).

In vitro drug release study

To measure the *in vitro* drug release of the sample, a USP-type I (basket) dissolution apparatus was employed.[28] Add 900 mL of 0.1 N HCl to the dissolved flask and maintain a temperature of 37±0.5°C with the speed 75 rpm. The prepared phytosomes amounted to 10 mg in each of the dissolving equipment baskets. For 12 hr the gadget remained switched on. Using a 10 mL pipette, 3 mL of sample was removed at 30 min, 1, 2, 4, 6, 8 and 12 hr intermissions up to 12 hr. Every time, the identical amount of the sample was added to the fresh dissolution media (37°C) and the absorbance was measured using spectroscopy.

Release Kinetics data for *in vitro* drug release

Mathematical treatment for release kinetics of prepared Phytosomes formulation can be analyzed by using zero order, first order kinetic, higuchi and korsmeyer-peppas model. These models help in understanding and predicting the release behavior of active compounds from the Phytosomes over time.[29,30]

In vivo anti-diabetic potential of herbal extract and prepared Phytosomes on STZ induced anti-diabetic model

The Wistar rats, weighing between 150 and 200 g, were kept in groups of six under regulated environments of temperature (25±2°C) and humidity (55-65%). Subjected to a regular 12 hr light/dark cycle. Standard rat feed and water were provided to rats without restriction. After a week of acclimation to the lab, the rats were ready to participate in the trials. Methods described in the literature were used to decide antidiabetic action in living organisms.[31-34]

The experiments were conducted from 8:00 to 15:00 in a quiet setting. Rats were divided into six groups for each series of studies. The experiments were approved by the IAEC.

Induction of Experimental Diabetes in Rats

After rats were starved overnight, they were injected intraperitoneally with a Streptozotocin solution containing 60 mg/kg in 100 mM citrate buffer at a pH of 4.5. Glibenclamide is used as standard. After 48 hr, the researchers checked blood glucose levels of rats; those animals were deemed diabetic if their readings were more than 250 mg/dL.[35]

Blood sampling and glucose assessment

Blood was obtained for glucose determination through tail snipping and for total protein, lipid profile and biochemical param estimation through retro-orbital bleeding from the ophthalmic venous plexus.

Statistical analysis

The data was analysed using GraphPad Instant 8.0.1 once the relevant variables were input. The values (n=6) are expressed as mean±S.E.M. The one-way ANOVA values, followed by Tukey's *post hoc* test, indicate that the following values are statistically significant: # p<0.01 vs. normal group; ***p<0.001, **p<0.01, *p<0.05 compared. diabetic control group, respectively.

RESULTS

Preparation and optimization of *Pongamia pinnata* Phytosome and Model Investigation, the Box-Behnken design matrix with the actual values, entrapment efficiency and particle size are summarized in Table 1. The 2D contour plot and 3D surface plot for entrapment efficiency and particle size are as displayed in Figures 1 and 2 respectively.

The results of ANOVA for a regression model for entrapment efficiency are summarized in Table 2, which helps to understand how different factors contribute to variability in response variable. The overall regression model is significant, meaning combined factors enlighten a major portion of variability in response variable (F-value=7.54, p-value=0.0072). Specifically, cholesterol (B) and dichloromethane (C) have significant main effects, by p-values of 0.0239 and 0.0008, respectively, suggesting that changes in these factors significantly influence the response. Additionally, dichloromethane exhibits a significant quadratic effect (F-value=12.14, p-value=0.0102), indicating a non-linear relationship with response variable. Interaction effects are mostly not significant, except for a marginally significant interaction between phospholipids and dichloromethane (F-value=4.94, p-value=0.0617), hinting at a potential combined influence worth further exploration. Lack of fit test (F-value=0.2577, p-value=0.8884) is not significant, confirming that model fit data well. Overall, models effectively capture the key influences on response variable, with cholesterol and dichloromethane being primary significant factors.

Table 1: Formulation optimisation and results of Particle size and Entrapment efficiency.

F. Code	Factor 1: Phospholipids %	Factor 2: Cholesterol %	Factor 3: Dichloromethane (mL)	Entrapment efficiency (%)	Particle size (nm)
F1	1.25	1	10	70.12	174.45
F2	1.25	0.5	20	68.45	172.54
F3	1.25	1.5	20	65.15	185.65
F4	1.25	0.5	30	72.25	145.85
F5	0.5	1	20	67.85	183.32
F6	1.25	0.5	20	69.05	159.98
F7	2	1.5	30	66.36	175.45
F8	1.25	1	10	68.85	158.95
F9	0.5	1	10	65.58	145.85
F10	1.25	1.5	30	71.45	165.74
F11	0.5	0.5	30	69.85	168.95
F12	2	1	20	69.85	184.74
F13	0.5	1.5	20	65.12	198.78
F14	2	0.5	20	67.78	181.25
F15	0.5	1	10	74.45	183.32
F16	1.25	1.5	20	67.85	160.25
F17	2	1	20	73.12	138.95

Table 2 summarizes results of ANOVA for a regression model for particle size, which helps to understand how different factors contribute to variability in response variable. ANOVA analysis reveals that overall model is highly significant (F-value: 132.58, *p*-value: <0.0001), indicating it effectively explains the variability in the response variable. Significant main effects were found for phospholipids (*p*-value: 0.0141), cholesterol (*p*-value: <0.0001) and dichloromethane (*p*-value: 0.0161). Significant interactions include phospholipids and cholesterol (*p*-value: <0.0001), phospholipids and dichloromethane (*p*-value: <0.0001) and cholesterol and dichloromethane (*p*-value: 0.0013). Additionally, quadratic effects for phospholipids (*p*-value: 0.0021) and dichloromethane (*p*-value: <0.0001) were significant, indicating non-linear relationships, while quadratic effect for cholesterol was not significant (*p*-value: 0.5702). Model fits data well, as evidenced by a non-significant lack of fit (*p*-value: 0.7919). These results highlight cholesterol and dichloromethane as crucial predictors with significant main and interaction effects, alongside notable non-linear effects for phospholipids and dichloromethane.

Results of entrapment efficiency and particle size

Evaluation of entrapment efficiency and particle size of prepared Phytosomes of *Pongamia pinnata* yielded entrapment efficiency values ranging from 65.15% to 74.45% and particle size values ranging from 138.95 to 198.78 nm.

For run order 4, the entrapment efficiency was found to be 72.25 (Actual Value) and 73.41 (Predicted Value) and the particle size was 145.85 (Actual Value) and 145.73 (Predicted Value), similarly, for run order 17, the entrapment efficiency was 73.12 (Actual Value) and 72.18 (Predicted Value) and the particle size was 138.95 (Actual Value) and 137.67 (Predicted Value) as shown in Table 3. In all 17 runs obtained by DOE, run order 4 and 17 consistently exhibited high entrapment efficiency as well as particle size values that were within the desired range. Based on these results, run order 4 and 17 were selected as the optimized formulations for *Pongamia pinnata* extract-containing Phytosomes.

Table 2: ANOVA for a regression model for entrapment efficiency and Particle Size.

Source	Entrapment efficiency		Particle Size		
	F-Value	*p*-Value	F-Value	*p*-Value	
Model	7.54	0.0072	132.58	<0.0001	Significant
A-Phospholipids	0.5203	0.4941	10.53	0.0141	
B-Cholesterol	8.25	0.0239	91.79	<0.0001	
C-Dichloromethane	31.38	0.0008	9.94	0.0161	
AB	1.72	0.2305	88.73	<0.0001	
AC	4.94	0.0617	484.53	<0.0001	
BC	0.0252	0.8784	26.91	0.0013	
A^2	0.6341	0.4520	22.48	0.0021	
B^2	4.08	0.0831	0.3546	0.5702	
C^2	12.14	0.0102	329.12	<0.0001	
Residual					
Lack of Fit	0.2577	0.8884	0.3510	0.7919	Not significant
Pure Error					
Cor Total					

Table 3: Actual responses vs. predicted responses.

Formulation	Run Order	Composition (%) Phospholipids/Cholesterol/ Dichloromethane	Response	Actual Value	Predicted value
OPPPF1	4	1.25/0.5/30	Particle Size	145.85	145.73
			Entrapment Efficiency	72.25	73.41
OPPPF2	17	2.0/1.0/20	Particle Size	138.95	137.67
			Entrapment Efficiency	73.12	72.18

Zeta potential

The zeta potential values of the optimized *Pongamia pinnata* phytosomes formulations, OPPPF1 and OPPPF2, were found to be -42.25 mV (Figure 3) and -45.65 mV (Figure 4), respectively. Higher value of OPPPF2 leads to better stability than OPPPF1 and is considered as optimized formulation for further study.

Scanning electron microscopy

In the study of the optimized formulation OPPPF2, SEM analysis was performed to examine microstructure and surface characteristics of formulation. During SEM analysis, images of the optimized formulation were captured using an electron beam that scans the sample's surface. SEM image of the Optimized *Pongamia pinnata* Phytosomes Formulation (OPPPF2) reveals the particle morphology of the phytosomes. The SEM image

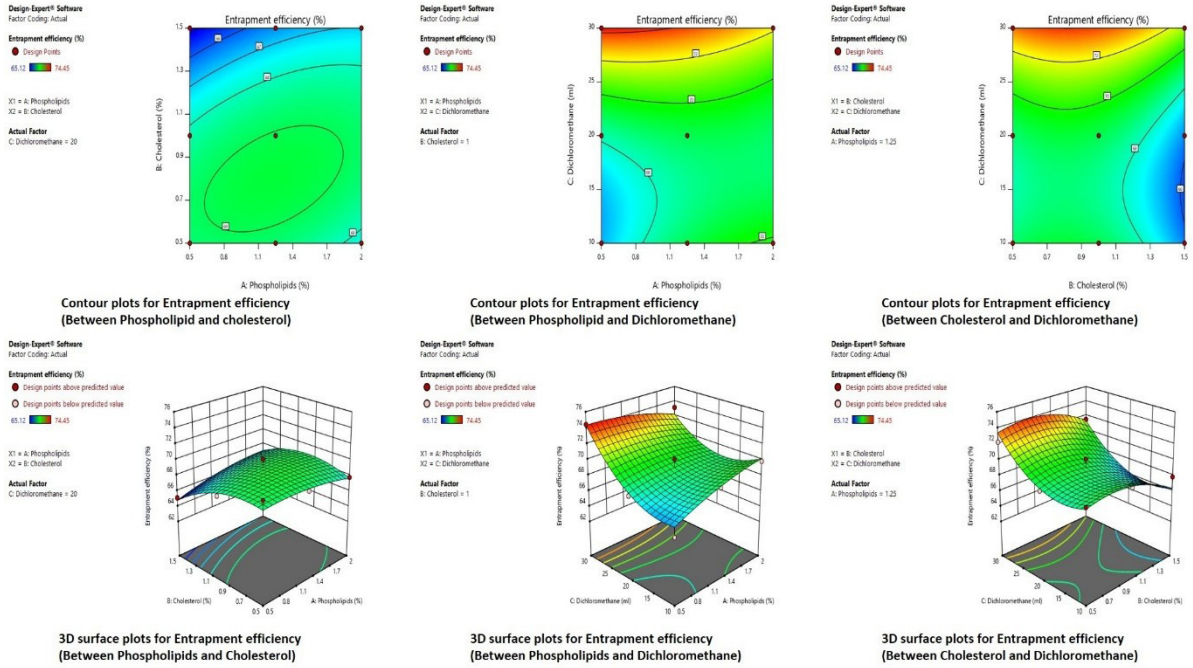

Figure 1: 2D contour plots and 3D surface plot for entrapment efficiency.

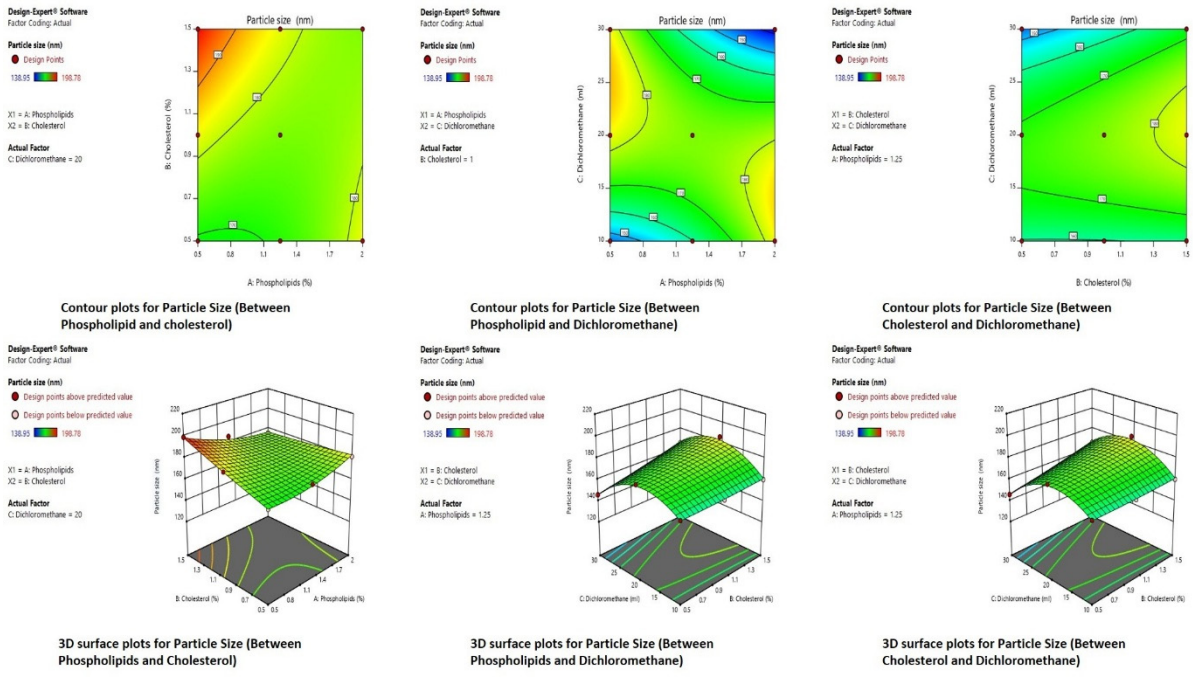

Figure 2: 2D contour plots and 3D surface plot for particle size.

(Figure 5) may show the particles are spherical in shape and well-dispersed.

Percentage Drug release from Phytosomes formulation

Outcomes of *in vitro* drug release of optimised formulation were revealed in Figure 6. In the case of OPPPF2, cumulative drug release percentages steadily increase over time. Initially, the drug release is relatively slower, with 18.85% and 29.98% released after 0.5 and 1 hr, respectively. As the release progresses, the percentages increase, reaching 36.65% and 49.98% after 2 and 3 hr, respectively. This indicates an increasing rate of drug release over time. After 4 hr, the drug release becomes more pronounced, with 59.98% of the drug released. The release continues to progress, reaching 69.98%, 78.85% and 89.98% after 6, 8 and 10 hr, respectively. Finally, after 12 hr, the cumulative drug release reaches 98.78%, indicating that a significant portion of the drug has been released from OPPPF2 within the given time frame.

Release Kinetics of *Pongamia pinnata* optimized Phytosomal formulations

The results show that the Higuchi model was best fit for release kinetics of phytosomal formulation OPPPF2, since it had the highest regression coefficient (r^2=0.995). The Korsmeyer-Peppas model also exhibited a good fit (r^2=0.992). However, the zero-order and first-order models showed relatively lower regression coefficients (r^2=0.958 and 0.858, respectively), indicating a less accurate fit to the experimental data.

Antidiabetic conclusion of Hydroalcoholic Extract of *Pongamia pinnata* (HEPP) and its Phytosomes (OPPPF2)

All results of in vivo antidiabetic activity are shown in Table 4. The animals in each group had their blood glucose levels measured at 21 days. The standard drug glibenclamide significantly reduces blood glucose levels by Day 21 (p<0.05). This demonstrates its efficacy in managing hyperglycemia. By conclusion of trial, serum glucose levels of all treatment groups, which were administered 100 mg/kg and 200 mg/kg of HEPP, respectively, fell significantly (p<0.05). This statement is reporting the results of an experiment in which the effects of different doses of OPPPF2 on serum glucose levels in rats were evaluated. Results showed that both doses significantly decreased (p<0.05) the serum glucose levels. The specific values for the different treatment groups are also given (165.6±7.4 and 142.3±7.9) for OPPPF2. The 200 mg/kg phytosome formulation (Group VII) shows a comparable effect to glibenclamide by Day 21, suggesting it might be a viable alternative. The hydroalcoholic extract (Groups IV and V) shows glucose-lowering effects but is less effective than glibenclamide and the phytosome formulation.

Results on body weight and total protein indicate that treatment with *Pongamia pinnata* extracts, as well as its phytosomes, had a positive effect on preventing excessive weight loss in STZ-induced diabetic rats. Phytosome formulations appeared to be more effective in improving body weight than hydroalcoholic extract and slightly lower than glibenclamide.

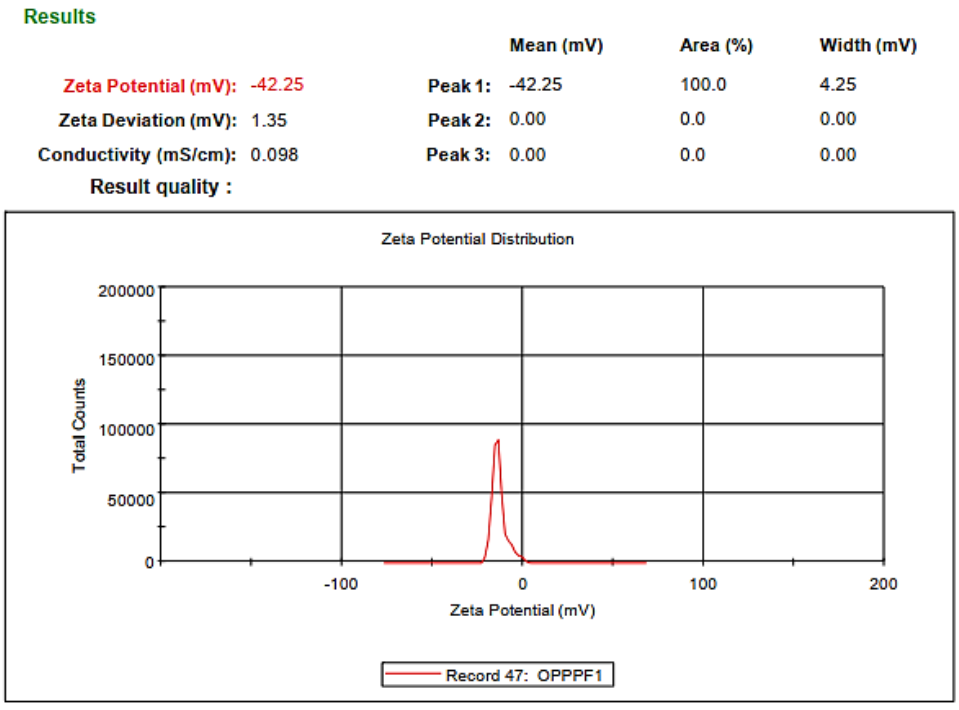

Figure 3: Zeta potential of optimized phytosomes formulations OPPPF1.

Table 4: **Antidiabetic effect of Hydroalcoholic extract *Pongamia pinnata* (HEPP) and its Phytosomes (OPPPF2) in STZ induced diabetic rats.**

Groups	Treatment	Glucose level	Final weight	TP (g/dL)	HDL (mg/dL)	LDL (mg/dL)	SGPT (U/L)	SGOT (U/L)	SOD (U/mg protein)	GSH (U/mg protein)	Lipid peroxidation (nM of MDA/min×mg protein)
I	Normal	80.1 ±6.8	207.5 ±9.18	7.8 ±1.9	65.5 ±3.8	93.3 ±6.8	67.4 ±4.5	55.7 ±4.6	18.4 ±2.6	77.4 ±2.4	20.4 ±1.7
II	Diabetic Control	423.3±11.8#	130.1 ±7.9#	6.5 ±2.4#	35.6 ±3.4#	184.6 ±7.9#	144.2 ±6.3#	148.2 ±4.8#	8.4 ±1.9#	28.1 ±1.6#	62.6 ±6.7#
III	Diabetic + Glibenclamide (600 µg/kg).	134.7±7.8*	223.3 ±7.4*	10.5 ±2.4**	66.2 ±3.8***	98.7 ±6.8***	75.1 ±4.3***	67.3 ±4.9***	20.8 ±1.7***	65.8 ±1.3***	28.3 ±2.9***
IV	Diabetic +HEPP (100mg/kg)	188.3±9.2*	161.1 ±7.8	7.9 ±2.5	48.1 ±3.2*	144. 6±7.9	98.8 ±5.8	107.5 ±7.5*	10.1 ±1.7*	49.6 ±1.4**	48.3 ±5.5*
V	Diabetic +HEPP (200mg/kg).	172.3±7.9*	171.7 ±8.2	8.5 ±2.7	55.6 ±3.4**	136.8 ±7.8	107.6 ±6.4	99.6 ±6.7***	11.5 ±1.2*	53.5 ±1.2**	41.5 ±5.1*
VI	Diabetic +OPPPF2 (100mg/kg).	165.6±7.4*	180.1 ±7.8*	13.6 ±2.8*	58.1 ±3.4*	110.6 ±6.9*	100.4 ±6.3*	96.5 ±7.2**	165.6 ±7.4*	55.8 ±1.3***	42.3 ±5.32*
VII	Diabetic + OPPPF2 (200mg/kg).	142.3±7.9*	171.7±7.2*	13.5 ±2.7*	59.6 ±3.7**	106.8 ±7.8*	91.6 ±6.5**	89.2 ±6.5**	142.3±7.9*	60.8 ±1.3***	37.8 ±5.8**

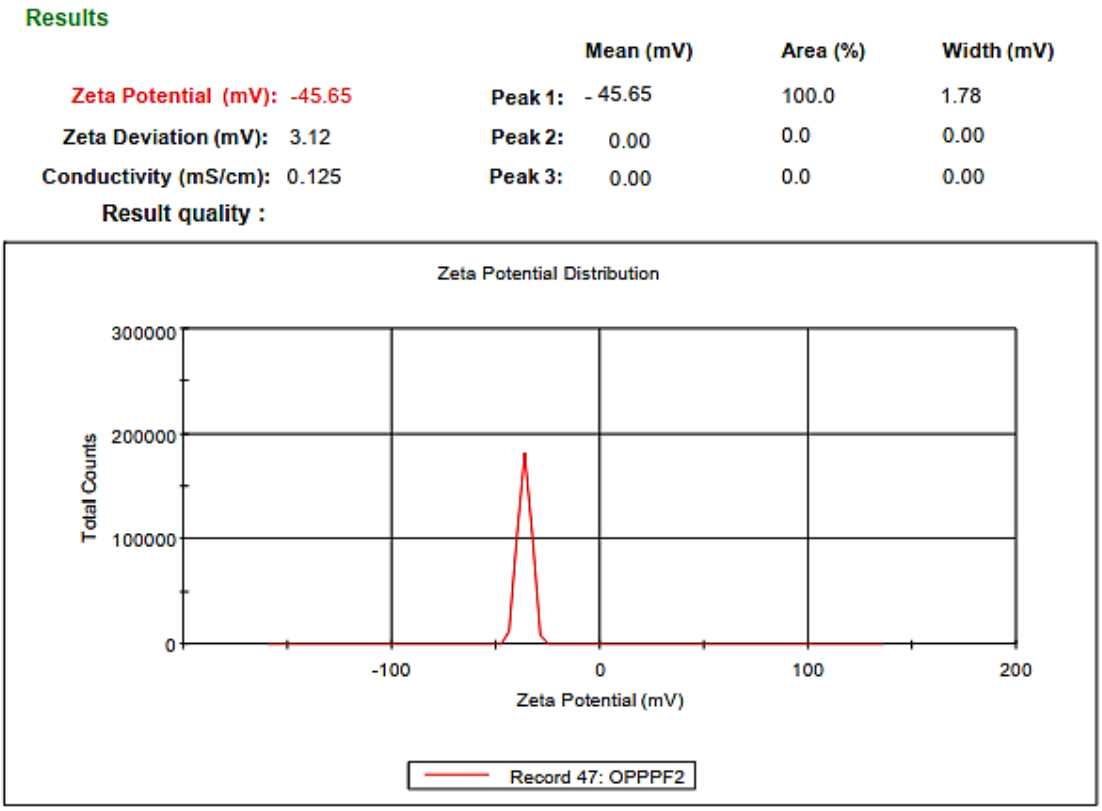

Figure 4: Zeta potential of optimized phytosomes formulations OPPPF2.

The results suggest that treatment with *Pongamia pinnata* extracts, especially in form of phytosomes, led to an increase in total protein levels in STZ-induced diabetic rats. Phytosome formulations, both at lower and higher doses, were particularly more effective in raising TP levels than glibenclamide and both hydroalcoholic extract.

Phytosomes were found to high significantly increase (p<0.001) HDL cholesterol levels and knowingly decrease (p<0.01) LDL cholesterol levels as compared to HEPP. The OPPPF2, especially at 200 mg/kg, closely approach the HDL and LDL levels seen with glibenclamide, demonstrating comparable efficacy.

Phytosomes were found to satisfactorily significant decrease (p<0.001) SGOT and SGPT levels as associated to HEPP. The OPPPF2, especially at 200 mg/kg, demonstrate significant hepatoprotective effects, comparable to glibenclamide in reducing SGPT and SGOT levels.

Phytosomes were found to high pointedly increase (p<0.001) SOD levels as compared to HEPP. The OPPPF2, at both lower and higher doses, appeared to be particularly effective in restoring SOD levels, which can help reduce oxidative stress associated with diabetes. The 200 mg/kg phytosome formulation (Group VII) approaches glibenclamide's efficacy in increasing SOD levels. Phytosomes were found to favorable significantly increase (p<0.001) GSH levels as compared to HEPP. HEPP and its Phytosome formulations, at both lower and higher doses, were effective in restoring GSH levels. The phytosome formulations, particularly at 200 mg/kg, achieve comparable results, with GSH levels significantly higher than those in the HEPP groups. Phytosomes were found to favorable significantly decrease (p<0.01) lipid peroxidation levels as compared to HEPP. Phytosome formulations, at higher doses, were particularly effective in reducing MDA levels. The 200 mg/kg phytosome formulation closely approaches this effect with that of glibenclamide, outperforming both the hydroalcoholic extract groups.

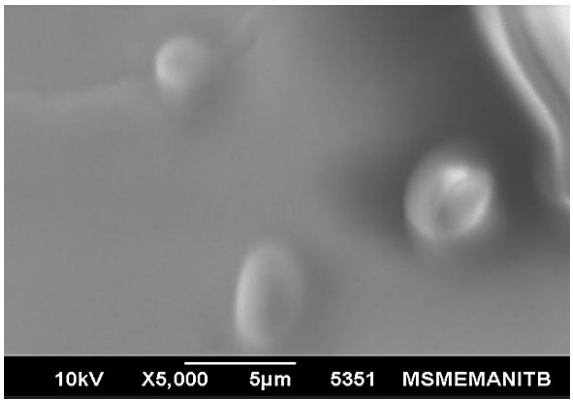

Figure 5: SEM Image of Optimized formulation OPPPF2.

DISCUSSION

This research mainly focused on developing Phytosomes of *Pongamia pinnata* extract to enhance bioavailability. The phytosomal approach ensures better stability and absorption, making it a promising strategy for antidiabetic therapy. The Phytosomes were optimised using a Box-Behnken experimental design[36]. The dependent variables were particle size as well as entrapment efficiency and independent variables were phospholipids, cholesterol and dichloromethane. Characterization of the seventeen formulations showed that their entrapment efficiencies varied from 65.15 to 74.4% and that their particle sizes ranged from 138.95 to 198.78 nm. Phytosomes' efficacy as medication delivery devices is strongly influenced by two crucial param: particle size and entrapment efficiency.[37] Based on the results, run orders 4 (OPPPF1) and 17 (OPPPF2) were selected as the optimized formulations for *Pongamia pinnata*. These

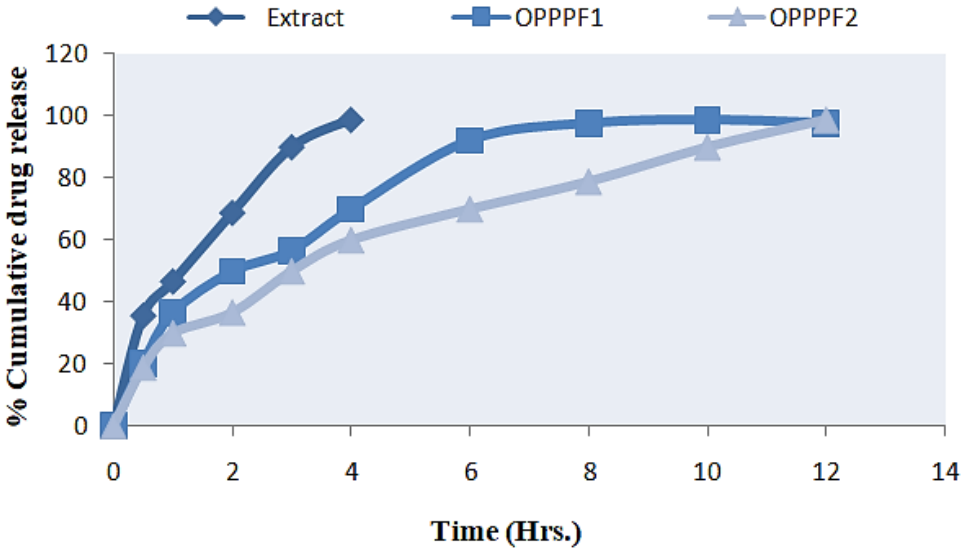

Figure 6: *In vitro* drug release of optimized formulation.

formulations are expected to have a high drug-loading capacity and proper particle size, making them potentially suitable for drug delivery and other biomedical claims.

Zeta potential values of the optimized *Pongamia pinnata* phytosome formulations, OPPPF1 and OPPPF2, were determined to be -42.25 mV and -45.65 mV, respectively. Zeta potential serves as a measure of surface charge of colloidal particles or nanoparticles, such as phytosomes, providing crucial insights into their stability and behaviour in formulations.[38] A negative zeta potential indicates a net negative charge on the particle surface, which in turn suggests sufficient stability and prevention of undesired interactions or aggregation among particles. Furthermore, these negative zeta potential values imply that the optimized phytosome formulations OPPPF2 have potential advantages for enhancing bioavailability and drug delivery.[39] Zeta potential values between ±30 mV to ±60 mV typically indicate that the formulation has sufficient electrostatic repulsion to maintain a stable dispersion. In this case, the values are within this range, signalling that the formulations are likely to be stable and well-dispersed.

The observed smooth and spherical surface morphology aligns with typical phytosome characteristics. Smooth and spherical phytosomes are less prone to degradation, ensuring a longer shelf life with enhanced stability. The SEM image confirms that the *Pongamia pinnata* phytosomes possess an optimal surface morphology for drug delivery applications, with uniformity, smoothness and minimal aggregation supporting their potential in enhancing therapeutic efficacy.

A sustained or protracted drug release pattern is indicated by the fact that the cumulative drug release percentages for OPPPF2 continue to progressively grow over longer periods of time. It is possible that certain medicinal applications could benefit from this property. According to the findings of the comparative research of regression coefficients, the Higuchi model had the greatest regression coefficient (r^2=0.995), which indicates that the model provides a better fit to the release kinetics of OPPPF2. In addition, the Korsmeyer-Peppas model demonstrated a satisfactory level of fit (r^2=0.992). Dependent on this data, it appears that the release of the active component from the OPPPF2 is facilitated by a process that is dependent on diffusion. Based on the data, it appears that OPPPF2 demonstrates a drug release profile that is both regulated and sustained. The capability of the formulation to release the drug gradually over an extended period is advantageous for the purpose of preserving therapeutic drug levels and improving the efficiency of the drug delivery system.[40]

In the treatment groups, the serum glucose levels were significantly reduced (p<0.05) at both dose of HEPP and OPPPF2. When it came to antidiabetic testing, phytosomes derived from *Pongamia pinnata* performed better than hydroalcoholic extract. The greater doses of extracts and the phytosomes that were associated with them led to more significant reductions in the levels of glucose in the blood. The OPPPF2, particularly at 200 mg/kg, demonstrates promising glucose-lowering effects, approaching the efficacy of the standard drug glibenclamide, while the hydroalcoholic extract shows moderate but dose-dependent efficacy. The results suggest the phytosome formulation may enhance the bioavailability and therapeutic potential of *Pongamia pinnata* for diabetes management.

There was a greater impression that the phytosome formulations were more successful in enhancing body weight. Based on the data, it appears that these medications may have the ability to act as anti-diabetic medicines, which would help alleviate the catabolic effects that diabetes has on body weight. Additionally, an increase in TP level suggests that phytosomes may have a beneficial effect on the functioning of the protein metabolism in diabetes situations. The OPPPF2, particularly at 200 mg/kg, show promising effects in improving protein levels and body weight, rivaling and in some respects surpassing the standard drug glibenclamide. While the hydroalcoholic extract demonstrates dose-dependent efficacy, it is less effective than both the phytosome formulation and glibenclamide, emphasizing the value of enhanced formulations for diabetes management.

Phytosomes significantly amplified HDL cholesterol levels (p<0.001) and significantly reduced LDL cholesterol levels (p<0.01) compared to the HEPP. These results suggest that phytosome treatments may positively impact the lipid profile, particularly by improving HDL levels and reducing LDL levels in diabetic conditions. The OPPPF2 especially at 200 mg/kg, demonstrate significant lipid-modulating effects, approaching the efficacy of glibenclamide in improving HDL and reducing LDL levels. This will help managing diabetes-associated dyslipidemia.

Phytosomes were found to significantly reduction SGOT and SGPT levels (p<0.001) compared to the HEPP. The OPPPF2, especially at 200 mg/kg, demonstrate significant hepatoprotective effects, comparable to glibenclamide in reducing SGPT and SGOT levels. These findings suggest that phytosomes may positively impact liver function in diabetic conditions, as lower SGPT and SGOT levels indicate a potential reduction in liver damage.

Phytosomes significantly increased SOD (p<0.001) and GSH levels (p<0.001) while significantly decreasing lipid peroxidation levels (p<0.01) compared to the HEPP. Both lower and higher doses of phytosome formulation effectively restored SOD and GSH levels, reducing oxidative stress associated with diabetes. Additionally, higher doses were particularly effective in lowering MDA levels, indicating a reduction in lipid peroxidation. The OPPPF2 particularly at 200 mg/kg, demonstrate significant antioxidant activity, rivalling the efficacy of glibenclamide in restoring SOD and GSH levels and reducing lipid peroxidation. These findings suggest that phytosome treatments positively

impact antioxidant defence mechanisms and oxidative stress in diabetic conditions.

The findings collectively suggest that *Pongamia pinnata*, particularly in phytosome form and at higher doses, holds promise as a potent antidiabetic agent, warranting further exploration for therapeutic applications in diabetes management. Further clinical studies are recommended to elucidate the full extent of their therapeutic potential and to establish their efficacy and safety in diverse patient populations.

CONCLUSION

It represents a significant advancement in harnessing the medicinal properties of this versatile plant species. Through the utilization of phytosome technology, which involves complexing bioactive compounds with phospholipids, researchers have sought to improve bioavailability and efficacy of *Pongamia pinnata*-derived phytochemicals. The process of preparing *Pongamia pinnata* phytosomes involves the encapsulation of bioactive compounds from *Pongamia pinnata*, such as flavonoids and other polyphenols, within a phospholipid bilayer. This encapsulation improves the solubility of the bioactive compounds in both water and lipid environments, thereby facilitating their absorption across biological membranes. The evaluation of *Pongamia pinnata* phytosomes typically involves assessing param to ensure quality, stability and effectiveness of the formulation. Studies investigating the therapeutic potential of *Pongamia pinnata* phytosomes have shown promising results. Enhanced bioavailability and targeted delivery of bioactive compounds to specific tissues or organs may improve the efficacy of *Pongamia pinnata*-derived phytochemicals in managing various health conditions. The antidiabetic effect of phytosomes were studied by considering the different biochemical markers and which were found promising as well. The phytosome formulation of *Pongamia pinnata* at 200 mg/kg consistently demonstrates efficacy comparable to glibenclamide across all tested param, surpassing the hydroalcoholic extract in managing diabetes, associated dyslipidemia, liver dysfunction and oxidative stress. This highlights the superior therapeutic potential of the phytosome formulation in treating diabetes and its complications.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Required approval and authorisation were obtained from IAEC and CPCSEA, under the Reg. No. 650/Po/Re/S/2002/2022/CPCSEA/18 during their meeting on 24/09/2022.

ABBREVIATIONS

DOE: Design of Experiments; **nm:** Nanometer; **mV:** Millivolts; **mg:** Milligram; **mL:** Milliliter; **rpm:** Revolutions per minute; **HEPP:** Hydroalcoholic extract of *Pongamia pinnata*; **OPPPF2:** Optimised phytosome formulation of *Pongamia pinnata* extract; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **STZ:** Streptozotocin; **ANOVA:** Analysis of Variance; **SEM:** Scanning electron microscopy; **HDL:** High density lipoprotein; **LDL:** Low density lipoprotein; **TP:** Total Protein; **SGPT:** Serum glutamic pyruvic transaminase; **SGOT:** Serum glutamic-oxaloacetic transaminase; **SOD:** Superoxide dismutase; **GSH:** Glutathione; **MDA:** Malondialdehyde.

SUMMARY

Pongamia pinnata phytosomes were developed by solvent evaporation technique. Box Behnken design was used to optimise the process to achieve the desired encapsulation efficiency and particle size. Particle size and entrapment efficiency are the dependent variables and phospholipid, cholesterol and dichloromethane were the independent factors. Zeta potential, particle size, entrapment efficiency and *in vitro* drug release of the produced phytosomes were evaluated. The projected values were compared to the experimental data and an analysis of the ANOVA model revealed that the lack of fit was not statistically significant; indicating that the model was ideal and the quadradic equation was the best match. The *in vivo* diabetic potential of optimised phytosomes was then tested using an antidiabetic model produced by streptozotocin. Overall, the results from studies conducted on animals indicate that *Pongamia pinnata* has potential as an effective antidiabetic agent, especially when taken in phytosome form and at larger doses. This justifies more research into potential therapeutic uses for managing diabetes. To assess their efficacy and safety in a range of patient populations, as well as to clarify the entire scope of their therapeutic potential, more clinical research is advised.

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