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Original Research Article

DEVELOPMENT AND EVALUATION OF *GMELINA ARBOREA* BASED PHYTOSOMAL DRUG DELIVERY SYSTEM

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ABSTRACT

The present study was undertaken to develop and evaluate phytosomes of the ethanolic extract of *Gmelina arborea* in order to improve the encapsulation, stability, and release characteristics of its bioactive phytoconstituents. The leaves were extracted using ethanol, yielding 9.50% w/w extract. Preliminary phytochemical screening confirmed the presence of alkaloids, glycosides, flavonoids, phenolic compounds, carbohydrates, saponins, and tannins, while quantitative analysis revealed total phenolic and flavonoid contents of 0.856 mg/100 mg and 0.921 mg/100 mg, respectively. Twelve phytosomal formulations (F1–F12) were prepared using the solvent evaporation method by optimizing phospholipid, cholesterol, extract concentration, and solvent volume. The prepared phytosomes were evaluated for particle size, entrapment efficiency, morphology, *in vitro* drug release, release kinetics, and stability. Among all formulations, F10 was identified as the optimized formulation, exhibiting the smallest particle size (198.6 nm) and the highest entrapment efficiency (91.82%). Transmission Electron Microscopy confirmed the formation of discrete, spherical phytosomal vesicles with smooth surfaces. The optimized formulation showed sustained drug release, achieving 98.12% cumulative release within 12 hours. Drug release kinetics followed the first-order model (R^2 = 0.9944), indicating concentration-dependent release with diffusion-controlled behavior. Stability studies conducted under long-term and accelerated storage conditions for three months demonstrated no significant changes in physical appearance, particle size, entrapment efficiency, or drug content, confirming the stability of the optimized formulation. The findings indicate that phytosomal encapsulation is an effective strategy for enhancing the physicochemical properties, stability, and sustained release of *Gmelina arborea* phytoconstituents, making it a promising herbal drug delivery system for future therapeutic applications.

Keywords: *Gmelina arborea*, Phytosomes, Ethanolic extract, Phospholipids, Cholesterol, Particle size, Entrapment efficiency, Transmission Electron Microscopy, *In vitro* drug release, Release kinetics, Stability studies.

INTRODUCTION

Medicinal plants have been extensively used in traditional systems of medicine for the prevention and treatment of various diseases owing to their rich content of bioactive phytochemicals (Latif *et al.*, 2026). Herbal medicines are gaining increasing global acceptance because they are considered safer, cost-effective, and possess fewer adverse effects than many synthetic drugs. However,

despite their therapeutic potential, many plant-derived bioactive compounds exhibit poor aqueous solubility, low permeability, and limited oral bioavailability, which restrict their clinical efficacy. Therefore, the development of novel drug delivery systems has become an important strategy to improve the therapeutic performance of herbal medicines (Sen *et al.,* 2011).

Gmelina arborea Roxb., belonging to the family Lamiaceae (formerly Verbenaceae), is a well-known medicinal plant widely distributed throughout India and Southeast Asia. Different parts of the plant, including the leaves, bark, roots, fruits, and flowers, have been traditionally used in Ayurveda for the management of inflammation, fever, liver disorders, diabetes, microbial infections, and various other ailments (Bhattacharyya *et al.*, 2025). The leaves are particularly rich in flavonoids, phenolic compounds, glycosides, tannins, and other secondary metabolites responsible for antioxidant, anti-inflammatory, hepatoprotective, antimicrobial, and wound-healing activities. Despite these pharmacological properties, the therapeutic application of *Gmelina arborea* extract is often limited because of poor stability and inadequate absorption of its bioactive constituents (Cedillo-Cortezano *et al.*, 2024).

Phytosomes are advanced lipid-based vesicular drug delivery systems specifically developed to enhance the delivery of plant-derived bioactive compounds. In phytosomes, phytoconstituents form molecular complexes with phospholipids through hydrogen bonding and other intermolecular interactions, resulting in improved lipid compatibility and membrane permeability (Kothapalli *et al.*, 2024). Unlike conventional herbal extracts, phytosomes exhibit enhanced solubility, greater gastrointestinal absorption, improved bioavailability, prolonged systemic circulation, and better therapeutic efficacy. In addition, phytosomal formulations provide protection against degradation of sensitive phytochemicals and facilitate sustained drug release (Barani *et al.,* 2021).

Among the various methods available for phytosome preparation, the solvent evaporation technique is widely employed because of its simplicity, reproducibility, and ability to produce stable phospholipid complexes with high entrapment efficiency. Optimization of formulation variables such as phospholipid concentration, cholesterol content, extract concentration, and solvent volume plays a significant role in determining the particle size, encapsulation efficiency, stability, and release behavior of phytosomes (Jacob *et al.,* 2025).

In the present study, phytosomes of the ethanolic extract of *Gmelina arborea* were developed using the solvent evaporation method. The prepared formulations were evaluated for particle size, entrapment efficiency, vesicular morphology by Transmission Electron Microscopy (TEM), in vitro drug release, release kinetics, and stability under ICH storage conditions. The study aimed to optimize a stable phytosomal formulation capable of enhancing the physicochemical characteristics and controlled release of *Gmelina arborea* phytoconstituents, thereby improving their potential therapeutic effectiveness and pharmaceutical applicability.

MATERIALS AND METHODS

Material

The materials used in the present study included ethanolic extract of *Gmelina arborea* leaves as the active phytoconstituent, soya phospholipid (lecithin) and cholesterol for the preparation of phytosomes. Dichloromethane was used as the reaction solvent, while n-hexane served as the precipitation solvent. Ethanol and distilled water were used during extraction and analytical procedures. All chemicals and reagents employed in the study were of analytical reagent (AR) grade. The formulation and evaluation studies were carried out using standard laboratory equipment, including a rotary vacuum evaporator, magnetic stirrer, analytical balance, centrifuge, UV–Visible spectrophotometer, FTIR spectrophotometer, particle size analyzer (DLS), Transmission Electron Microscope (TEM), and an ICH stability chamber.

Methods

Extraction by maceration process

Leaves of *Gmelina arborea* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction. 50 gm of dried powdered of *Gmelina arborea* has been extracted with ethanol solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40ºC (Mukherjee, 2007).

Determination of percentage yield

The percentage yield of extract was calculated by using following formula:

Percentage yield = Weight of Extract/ Weight of powder drug Taken×100

Phytochemical Screening of Ethanolic Extract of *Gmelina arborea*

Preliminary phytochemical screening of the ethanolic extract of *Gmelina arborea* was carried out using standard qualitative methods to identify the major classes of phytoconstituents (Kokate, 1994; Audu *et al.,* 2007).

Estimation of total phenol content

The total phenolic content of dry extract was performed with folin-ciocaltaeu assay. 2 ml of sample (1 mg/ml) was mixed with 1 ml of folin ciocalteu's phenol reagent and 1 ml of (7.5 g/L) sodium carbonate solution was added and mixed thoroughly (Gaur Mishra *et al.*, 2017). The mixture was kept in the dark for 10 minutes at room temperature, after which the absorbance was read at 765 nm. The total phenolic content was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution (5-25µg/ml). The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as 100 milligrams of Gallic acid equivalents (GAE)/100mg of dried sample.

Estimation of total flavonoids content

Preparation of standard solution 10mg quercetin was weighed and made up to 10ml with Methanol in a 10ml volumetric flask. From the above solution (1mg/ml), 1ml was pippeted out and made up to 10ml with Methanol to get 100µg/ml Quercetin standard solution (stock solution). From the stock solution, solutions of concentration 5, 10, 15, 20 and 25 µg/ml were prepared (Gaur Mishra *et al.,* 2017). 3 ml of each standard and test was mixed with 1 ml of 2% Aluminium chloride solution. The solutions were mixed well and the absorbance was measured against

the blank at 420nm using UV-Visible spectrophotometer. A standard graph was plotted using various concentrations of Quercetin and their corresponding absorbance.

Formulation development of phytosomes

Phytosomes of *Gmelina arborea* extract were prepared by the solvent evaporation technique. Predetermined quantities of phospholipid (phosphatidylcholine), cholesterol, and *Gmelina arborea* extract were accurately weighed according to the formulation composition. The weighed materials were transferred into a 100 mL round-bottom flask containing the required volume of dichloromethane as the reaction solvent. The mixture was stirred until complete dissolution and then refluxed at 50 ± 2°C for 3 hours to facilitate the formation of the phytosomal complex.

After completion of the reaction, the organic solvent was removed under reduced pressure using a rotary vacuum evaporator until a thin film was formed. The resulting residue was treated with 20 mL of n-hexane under continuous stirring to precipitate the phytosomal complex. The precipitated phytosomes were collected by filtration and dried under vacuum to remove residual organic solvents. The dried phytosomal powder was further kept in a desiccator for 24 hours and stored in amber-coloured glass containers at room temperature until further characterization (Singh and Narke, 2015).

Characterization of phytosomes

Entrapment efficiency

Phytosome preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 4.

The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped *Gmelina arborea* extract was recorded at λ_{max} 420.0 nm using UV/visible spectrophotometer. Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 420.0 nm (Dubey and Shirsat, 2020). Amount of quercetin in supernatant and sediment gave a total amount of *Gmelina arborea* extract in 1 ml dispersion. The percent entrapment was calculated by following formula:

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X } 100$$

Particle size and size distribution

The particle size, size distribution and zeta potential of optimized phytosomes formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK)]. The electric potential of the phytosomes, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell (Rani *et al.*, 2022).

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid (Jagtap *et al.*, 2023). The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).

In vitro dissolution rate studies

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus. The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5°C and 75 rpm. 10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37ºC) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy (Jain *et al.*, 2023).

RESULTS AND DISCUSSION

The present study was carried out to develop and evaluate phytosomes of the ethanolic extract of *Gmelina arborea* with the objective of improving the physicochemical characteristics and release behavior of the plant bioactive constituents. The findings demonstrated that the selected formulation variables significantly influenced phytosome formation, particle size, entrapment efficiency, and stability.

The ethanolic extraction of *Gmelina arborea* leaves produced a black-colored extract with a percentage yield of 9.50% w/w (Table 2). The satisfactory extraction yield indicates efficient recovery of phytoconstituents using ethanol as the extraction solvent. Ethanol is known to extract both polar and moderately non-polar phytochemicals effectively, thereby producing an extract rich in biologically active compounds suitable for phytosomal formulation.

Preliminary phytochemical screening (Table 3) confirmed the presence of several important secondary metabolites, including alkaloids, glycosides, flavonoids, phenolic compounds, carbohydrates, saponins, and tannins, whereas proteins and diterpenes were absent. The presence of these phytoconstituents suggests that the extract possesses significant pharmacological potential, particularly antioxidant activity attributed to phenolic compounds and flavonoids. The absence of proteins and diterpenes indicates the selectivity of ethanol in extracting desired phytochemicals while minimizing unwanted constituents.

The quantitative estimation of phytoconstituents (Table 4) revealed that the ethanolic extract contained 0.856 mg/100 mg of total phenolic content and 0.921 mg/100 mg of total flavonoid content. The relatively high flavonoid content compared to total phenols suggests that flavonoids constitute a major class of bioactive compounds in *Gmelina arborea*. These compounds are well recognized for their antioxidant, anti-inflammatory, hepatoprotective, and free radical scavenging activities, thereby supporting the selection of the extract for phytosomal drug delivery.

Particle size analysis and entrapment efficiency studies (Table 5) demonstrated that formulation variables markedly influenced the characteristics of the phytosomes. Particle size ranged from 198.6 to 286.4 nm, while entrapment efficiency varied between 72.45% and 91.82%. The optimized formulation F10 exhibited the smallest particle size (198.6 nm) together with the highest entrapment efficiency (91.82%), indicating efficient interaction between phospholipids and the

phytoconstituents. The nanosized vesicles provide a larger surface area for absorption and are expected to enhance dissolution, membrane permeation, and oral bioavailability of the encapsulated phytochemicals.

The particle size distribution of the optimized formulation (Figure 1) confirmed the formation of nanosized vesicles with a narrow size distribution, indicating uniformity and good physical stability. Smaller particle size reduces sedimentation and aggregation while improving dispersion characteristics, making the phytosomal system more suitable for pharmaceutical applications.

Transmission Electron Microscopy (Figure 2) further confirmed the successful formation of phytosomes. The TEM image revealed predominantly spherical vesicles with smooth surfaces and well-defined boundaries. The vesicles appeared discrete without significant aggregation, demonstrating the structural integrity and stability of the optimized formulation. The morphology observed by TEM was consistent with the particle size obtained through dynamic light scattering analysis.

The *in vitro* drug release study (Table 6) demonstrated that the optimized formulation F10 exhibited a biphasic release profile characterized by an initial burst release followed by sustained release. Approximately 22.15% of the drug was released within the first 30 minutes, followed by a gradual increase to 98.12% cumulative drug release after 12 hours. The initial release may be attributed to phytoconstituents present near the surface of the vesicles, whereas the subsequent sustained release resulted from gradual diffusion through the phospholipid matrix. Such a controlled release profile is advantageous for maintaining prolonged therapeutic activity while reducing fluctuations in drug concentration.

The release kinetic analysis (Table 7) showed that the optimized formulation exhibited the highest correlation with the first-order kinetic model (R^2 = 0.9944), followed by the Korsmeyer–Peppas model (R^2 = 0.9707) and the Higuchi model (R^2 = 0.9382). These findings indicate that drug release from the phytosomes was predominantly concentration dependent and mainly governed by diffusion through the phospholipid matrix. The lower correlation with the zero-order model (R^2 = 0.8272) suggests that the formulation did not release the drug at a constant rate but rather followed controlled diffusion kinetics.

Stability studies of the optimized formulation (Table 8) demonstrated excellent physical and chemical stability under both long-term (25 ± 2°C/60 ± 5% RH) and accelerated (40 ± 2°C/75 ± 5% RH) storage conditions over a period of three months. No significant changes in physical appearance were observed under long-term storage, while only slight aggregation was noted under accelerated conditions after three months. The particle size increased only marginally from 198.6 nm to 209.7 nm, entrapment efficiency decreased slightly from 91.82% to 88.94%, and drug content remained above 97% throughout the study. These minor variations are within acceptable pharmaceutical limits and indicate that the phospholipid bilayer effectively protected the encapsulated phytoconstituents against degradation during storage.

Table 1: Different formulations of phytosomes

Formulation Code	Phospholipid (mg)	Cholesterol (mg)	*Gmelina arborea* Extract (mg)	Dichloromethane (ml)
F1	100	50	100	25
F2	100	100	100	25
F3	100	150	100	25
F4	100	200	100	25
F5	100	100	50	25
F6	100	100	100	25
F7	100	100	150	25
F8	100	100	200	25
F9	100	100	100	10
F10	100	100	100	25
F11	100	100	100	50
F12	100	100	100	75

Table 2: % Yield of leaves extract of *Gmelina arborea*

S. No.	Colour	Extract	% Yield (w/w)
1	Black	Ethanolic	9.50

Table 3: Phytochemical screening of extract *of Gmelina arborea*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Wagner's Test	+ve
	Hager's test	+ve
2.	**Glycosides**	
	Legal's test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	-ve
4.	**Phenol**	
	Ferric Chloride Test	+ve
5.	**Proteins**	
	Xanthoproteic test	-ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpenes**	

	Copper acetate test	-ve
9.	**Tannins** Gelatin Test	 +ve

Table 4: Total phenol and total flavonoid content of *Gmelina arborea* extract

S. No.	Extract	Total Phenol content (mg/100mg)	Total Flavonoids content (mg/100mg)
1.	Ethanolic extract	0.856	0.921

Table 5: Particle Size and Entrapment Efficiency of *Gmelina arborea* loaded Phytosomes

Formulation Code	Particle Size (nm)	Entrapment Efficiency (%)
F1	286.4 ± 4.8	72.45 ± 1.26
F2	241.8 ± 3.6	80.32 ± 1.14
F3	218.6 ± 3.1	87.65 ± 0.98
F4	229.5 ± 4.2	84.18 ± 1.22
F5	205.7 ± 2.8	74.96 ± 1.05
F6	241.8 ± 3.6	80.32 ± 1.14
F7	258.9 ± 4.1	85.73 ± 0.94
F8	281.3 ± 4.9	82.56 ± 1.31
F9	264.5 ± 5.2	76.84 ± 1.43
F10	**198.6 ± 2.5**	**91.82 ± 0.86**
F11	214.7 ± 3.3	89.16 ± 0.92
F12	232.9 ± 3.8	85.94 ± 1.08

Average of three determinations (n=3)

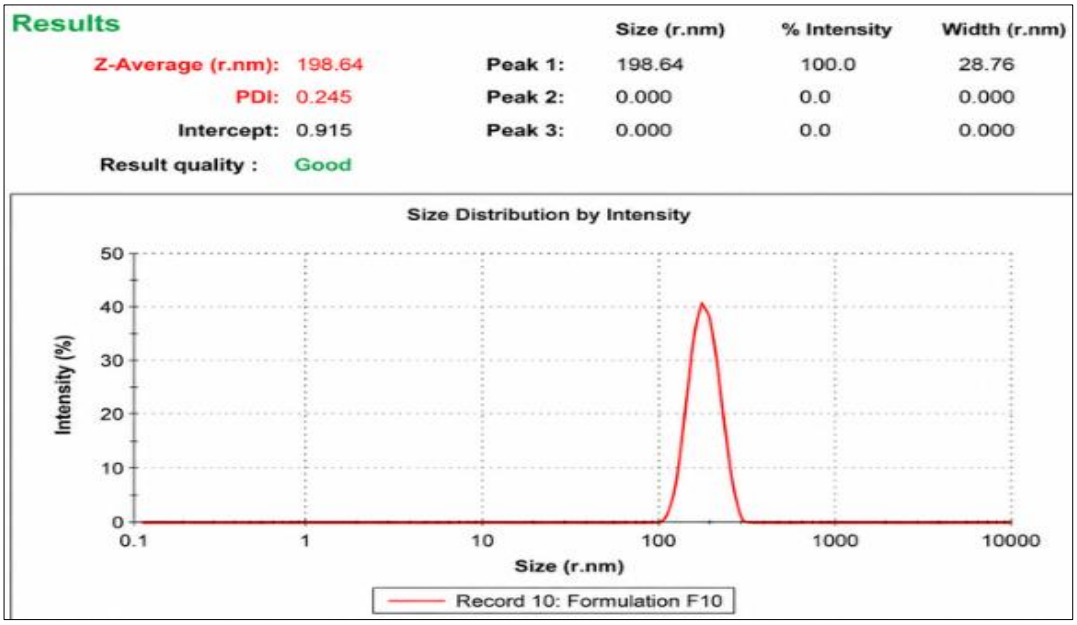

Figure 1: Particle size of optimized formulation F12

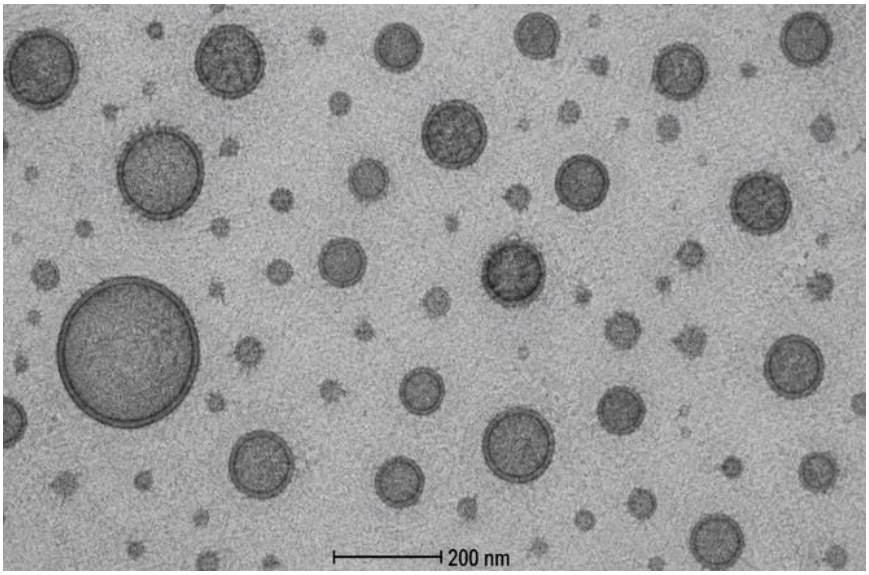

Figure 2: TEM image of phytosomes

Table 6: *In-vitro* drug release data for optimized formulation F10

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	22.15	1.345	77.85	1.891
1	1	0.000	36.12	1.558	63.88	1.805
2	1.414	0.301	45.63	1.659	54.37	1.735
4	2	0.602	75.45	1.878	24.55	1.390
6	2.449	0.778	88.12	1.945	11.88	1.075
8	2.828	0.903	94.65	1.976	5.35	0.728
12	3.464	1.079	98.12	1.992	1.88	0.274

Table 7: Regression analysis data of optimized formulation F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.8272	0.9944	0.9382	0.9707

Table 8: Results of stability studies of optimized formulation F10

Storage Condition	Time	Physical Appearance	Particle Size (nm)	Entrapment Efficiency (%)	Drug Content (%)
Initial	0 Month	Light brown, free-flowing powder	198.6	91.82	99.45
25 ± 2°C / 60 ± 5% RH	1 Month	No change	199.8	91.34	99.18
25 ± 2°C / 60 ± 5% RH	2 Months	No change	201.2	90.92	98.94
25 ± 2°C / 60 ± 5% RH	3 Months	No significant change	202.8	90.46	98.67
40 ± 2°C / 75 ± 5% RH	1 Month	No significant change	203.6	90.18	98.52
40 ± 2°C / 75 ± 5% RH	2 Months	Slight increase in particle size	206.4	89.56	98.18
40 ± 2°C / 75 ± 5% RH	3 Months	Slight aggregation observed	209.7	88.94	97.86

CONCLUSION

The present study successfully developed and optimized phytosomes of the ethanolic extract of *Gmelina arborea* using the solvent evaporation method. The optimized formulation (F10) exhibited nanosized vesicles, high entrapment efficiency, sustained drug release, and excellent physical stability under both long-term and accelerated storage conditions. TEM analysis confirmed the formation of spherical phytosomal vesicles, while release kinetic studies indicated that drug release followed first-order kinetics. Overall, the developed phytosomal formulation demonstrated improved physicochemical properties and controlled release behavior, suggesting its potential as an effective herbal drug delivery system for enhancing the bioavailability and therapeutic efficacy of *Gmelina arborea* phytoconstituents.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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