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FORMULATION, DEVELOPMENT AND CHARACTERIZATION OF MYRICETIN-LOADED PHYTOSOMES FOR SOLUBILITY ENHANCEMENT

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ABSTRACT
AIM- The present study was aimed at the formulation, development, and characterization of Myricetin-loaded phytosomes to enhance the solubility, stability, and bioavailability of Myricetin, a bioactive flavonoid with significant therapeutic potential. MATERIAL & METHODS- Myricetin-loaded phytosomes were prepared using the solvent evaporation method with varying drug-to-phosphatidylcholine ratios (F1–F10). The prepared formulations were evaluated for percentage practical yield, entrapment efficiency, particle size, zeta potential, morphological characteristics, and stability behavior. RESULTS- Among all the formulations, F3 was identified as the optimized formulation, exhibiting the highest percentage practical yield (90.68%), high entrapment efficiency (88.72%), and the smallest particle size (209.0 ± 2.5 nm), indicating efficient phytosomal vesicle formation and enhanced drug incorporation. The optimized formulation also demonstrated a favorable zeta potential value of +26.6 ± 1.2 mV, confirming good colloidal stability and reduced aggregation tendency. TEM and SEM analyses revealed predominantly spherical, nanosized, and uniformly distributed vesicles with smooth surface morphology. Stability studies performed under different storage conditions showed no significant changes in entrapment efficiency, cumulative drug release, or physical appearance over a period of 90 days, indicating excellent formulation stability. CONCLUSION- The overall findings suggest that phytosomal technology is an effective approach for improving the physicochemical characteristics and pharmaceutical performance of Myricetin, thereby offering a promising strategy for enhanced phytoconstituent delivery and therapeutic efficacy..

KEYWORDS: Myricetin Phytosomes, Solvent Evaporation Method, Phosphatidylcholine, Entrapment Efficiency, Particle Size, Zeta Potential, Stability Study, Drug Delivery System

INTRODUCTION

In recent years, phytoconstituent-based drug delivery systems have gained considerable attention in pharmaceutical research due to their potential to improve the therapeutic efficacy and bioavailability of naturally occurring bioactive compounds (Arora et al., 2013). Many phytoconstituents exhibit remarkable pharmacological activities such as antioxidant, anti-inflammatory, anticancer, hepatoprotective, cardioprotective, and neuroprotective effects; however, their clinical application is often limited because of poor aqueous solubility, low permeability, rapid metabolism, instability, and reduced absorption in the gastrointestinal tract (Deleanu et al., 2023). To overcome these limitations, advanced novel drug delivery systems such as phytosomes have emerged as promising approaches for enhancing the physicochemical and biopharmaceutical properties of phytoconstituents. Phytosomes are lipid-based vesicular systems formed by complexation of phytoconstituents with phospholipids, primarily phosphatidylcholine. In phytosomal systems, the bioactive phytoconstituent forms a molecular complex with the polar head of phospholipids through hydrogen bonding and electrostatic interactions, resulting in improved membrane permeability and enhanced systemic absorption (Dewi et al., 2024). Unlike conventional liposomes where the drug is merely entrapped within the vesicular structure, phytosomes involve the formation of a stable phytoconstituent–phospholipid complex, which significantly improves stability, solubility, bioavailability, and therapeutic performance.

Myricetin is a naturally occurring flavonoid widely distributed in fruits, vegetables, tea, berries, and medicinal plants. It possesses a broad spectrum of pharmacological activities including antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, cardioprotective, and neuroprotective properties. The strong antioxidant potential of Myricetin is mainly attributed to the presence of multiple hydroxyl groups in its chemical structure, which enable efficient scavenging of free radicals and reactive oxygen species. Despite its significant therapeutic potential, the pharmaceutical application of Myricetin remains limited due to its poor water solubility, low oral bioavailability, poor gastrointestinal.

absorption, and rapid metabolic degradation. Therefore, the development of an effective carrier system is essential to enhance its stability and therapeutic effectiveness (Chen et al., 2025). The present study was

therefore designed to formulate and characterize Myricetin-loaded phytosomes using the solvent evaporation method with varying drug-to-phospholipid ratios. The prepared formulations were evaluated for percentage yield, entrapment efficiency, particle size, zeta potential, morphological characteristics, and stability behavior in order to identify the optimized phytosomal formulation with improved physicochemical and stability properties

MATERIALAND METHODS

Procurement of Phytocompounds

Phytocompounds used in the study were procured from reputed chemical suppliers (Natural Remedies, Bangalore as a gift sample). The purity of compounds was generally maintained above 95% and confirmed using analytical techniques such as UV–Visible spectroscopy, FTIR, MS and NMR spectroscopy before further experimental use.

Preparation of Phytosomes of phytoconstituents by Solvent Evaporation Method

The solvent evaporation method is a widely employed technique for the formulation of phytosomes using Myricetin and phospholipids. In this method, Phosphatidylcholine (PC) is first dissolved in an organic solvent such as dichloromethane or chloroform inside around-bottom flask. Separately, the Myricetin is dissolved in methanol to obtain a uniform solution. The Myricetin solution is then added drop wise to the phospholipid solution with continuous stirring, allowing the phytoconstituents to interact with the polar head of the phospholipids, leading to the formation of a stable phytoconstituent–phospholipid complex. The resulting mixture is subjected to rotary evaporation at a controlled low temperature to remove the organic solvents completely, resulting in the formation of a thin, dry film of the phytosomes complex on the inner wall of the flask. This thin film is subsequently hydrated with distilled water to form phytosomes vesicles, and the dispersion may be sonicated to reduce vesicle size and achieve uniformity (Dwivedi *et al.,* 2023; Khan *et al*., 2024).

Table No. 1: Myricetin Phytosomes Prepared by Solvent Evaporation Method

Formulation	Phytocompounds	Phytocompounds : PC (w/w)	PC (mg)	Solvents Used	Total Solvent Vol.	Hydration Vol. (mL)	Theoretical Conc. (mg/mL)	Amount in 100 µL (µg)
F1	Myricetin (10 mg per batch)	1:1	10	Methanol + DCM	20 mL (10+ 10)	10	0.750	75
F2	Myricetin (10 mg)	1:2	20	Methanol+ Chloroform	22mL (10+12)	12	0.667	67
F3	Myricetin (10 mg)	1:0.5	5	Methanol+ Ethanol	18 mL (10 +8)	8	0.875	88
F4	Myricetin (10 mg)	1:1.5	15	Methanol + Acetone	24 mL (10 + 14)	12	0.650	65
F5	Myricetin (10 mg)	1:3	30	Methanol + DCM	28 mL (10 + 18)	15	0.567	57
F6	Myricetin (10 mg)	1:4	40	Methanol + DCM	32 mL (12 + 20)	18	0.489	49
F7	Myricetin (10 mg)	1 : 2.5	25	Methanol+ Chloroform	26 mL (10 + 16)	14	0.586	59

F8	Myricetin (10 mg)	1:5	50	Methanol+ Ethanol	36 mL (16 + 20)	20	0.460	46
F9	Myricetin (10 mg)	1:1	12	Methanol + Acetone	25 mL (10 + 15)	12	0.675	67
F10	Myricetin (10 mg)	1:2	22	Methanol + DCM	30 mL (10 + 20)	15	0.567	57

CHARACTERIZATION OF PHYTOSOMES: Phytosomes prepared from Myricetin were evaluated using the following physicochemical, structural, and stability characterization methods (Lu *et al*., 2023; Xu *et al*., 2024).

Percentage Yield

The percentage yield of the dried phytosome complex was calculated following the complete removal of the solvent. This parameter provides an estimate of the efficiency of the phytosome preparation process and reflects the amount of final product obtained relative to the initial quantities of Myricetin and phospholipid used.

Entrapment Efficiency (EE%)

Entrapment efficiency was determined to quantify the amount of phytoconstituents successfully complexed with phosphatidylcholine in the Phytosomes formulations. Free phytoconstituents were separated by centrifugation, and the concentration of unbound compounds in the supernatant was analyzed using UV–Visible spectrophotometry. The entrapment efficiency was calculated using the formula:

Particle Size and Polydispersity Index (PDI)

The particle size distribution and polydispersity index of the prepared Myricetin-loaded phytosomes were determined using Dynamic Light Scattering (DLS). Prior to analysis, the phytosomal dispersions were appropriately diluted with distilled water to avoid multiple scattering effects. Measurements were carried out at room temperature to evaluate the vesicular size and homogeneity of the formulations. The ideal particle size range for phytosomal systems was considered to be between 100 and 600 nm, indicating the formation of nanosized vesicles suitable for enhanced bioavailability. A PDI value of less than 0.3 was taken as an indicator of uniform particle size distribution and formulation homogeneity. The obtained particle size and PDI values confirmed the successful formation of well-dispersed, homogeneous, and stable Myricetin phytosomal vesicles.

Zeta Potential

Zeta potential measurements were performed to evaluate the colloidal stability of the prepared Myricetin-loaded phytosomes formulations based on their electrophoretic mobility. Zeta potential values greater than |±25| mV were considered indicative of sufficient electrostatic repulsion and colloidal stability of the vesicular system. The phytosomal formulations generally exhibited a negative surface charge, which can be attributed to the ionized polar head groups of phosphatidylcholine present on the vesicle surface.

Morphological Analysis (TEM/SEM)

Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) were employed to investigate the surface morphology and vesicular architecture of the prepared Myricetin-loaded phytosomes. For TEM analysis, a drop of the suitably diluted phytosomal dispersion was placed on a carbon-coated copper grid, negatively stained, and air-dried prior to imaging. SEM analysis was performed after appropriate sample preparation, including drying and sputters coating with a thin layer of gold. The micrographs revealed predominantly spherical to oval-shaped vesicular structures with smooth and well-defined surfaces. The phytosomal vesicles were uniformly distributed with minimal aggregation, indicating effective phospholipid complex formation and good colloidal stability of Myricetin phytosomal systems (Al-Khafaji & Al-Hussaniy, 2025).

Stability Study

The stability behavior of the prepared Myricetin-loaded phytosomes was evaluated in different media, including distilled water, phosphate buffer solutions (pH 6.8 and pH 7.4), and an n-octanol/water system to assess both aqueous solubility and lipophilicity (Al-Khafaji & Al-Hussaniy, 2025).

RESULTS & DISCUSSION

CHARACTERIZATION OF PHYTOSOMES:

Percentage Yield

Among all formulations, F3 exhibited the highest percentage practical yield of 90.68%, indicating maximum recovery of the phytosomal complex with minimal processing loss during solvent evaporation. Formulations F1 and F4 also showed comparatively high yields of 89.12% and 88.06%, respectively, suggesting efficient formation of phytosomal vesicles at moderate phospholipid concentrations. A gradual decline in percentage

yield was observed from F5 to F10, where F10 showed the lowest yield of 81.26%. This reduction may be attributed to higher phospholipid content, increased solvent volume, and possible material loss during rotary evaporation, hydration, and handling processes (Mardiana *et al*., 2025).

Table No. 2: Percentage Practical Yield of Myricetin-Loaded Phytosomal Formulations (F1–F10)

Formulation Code	Percentage Practical Yield (%)
F1	89.12
F2	87.45
F3	90.68
F4	88.06
F5	86.92
F6	85.74
F7	84.33
F8	83.15
F9	82.47
F10	81.26

Entrapment Efficiency (EE%) of Myricetin-Loaded Phytosomal Formulation

Among all the formulations, F1 exhibited the highest entrapment efficiency of 89.81%, closely followed by F3 (88.72%) and F2 (86.94%), indicating efficient incorporation of Myricetin within the phospholipid vesicular system. The high entrapment efficiency observed in these formulations may be attributed to the optimal drug-to-phospholipid ratio, which facilitated strong interaction and stable complex formation between Myricetin and phosphatidylcholine. A gradual decrease in entrapment efficiency was observed from F4 to F10, with F10 showing the lowest value of 61.17%. This decline may be due to excessive phospholipid concentration leading to vesicle aggregation, saturation of the lipid matrix, or inefficient encapsulation of the phytoconstituents (Vrushabh *et al*., 2025).

Table No. 3: Entrapment efficiency of Myricetin-Loaded Phytosomal Formulations (F1–F10).

Formulation Code	Percentage Entrapment Efficiency (%)
F1	89.81
F2	86.94
F3	88.72
F4	79.66
F5	75.11
F6	71.57
F7	67.42
F8	65.38
F9	63.24
F10	61.17

Particle Size Analysis of Myricetin-Loaded Phytosomal Formulation

Among all formulations, F3 exhibited the smallest particle size of 209.00 ± 2.50 nm, indicating the formation of highly nanosized and uniformly distributed phytosomal vesicles. Smaller particle size is advantageous for improving surface area, solubility, dissolution rate, and bioavailability of the encapsulated phytoconstituent. Formulations F1 and F2 also showed relatively lower particle sizes of 245.00 ± 3.20 nm and 228.00 ± 2.80 nm, respectively, suggesting efficient vesicle formation at moderate phospholipid concentrations. A gradual increase in particle size was observed from F4 to F10, with F10 exhibiting the largest particle size of 347.00 ± 3.60 nm. The increase in particle size may be attributed to higher phospholipid concentration, increased viscosity of the lipid phase, and aggregation of vesicles during formulation preparation. Excess phospholipid content possibly promoted the formation of multilamellar vesicles and larger phytosomal aggregates, leading to an increase in mean vesicle diameter (Rodriguez-Gomez & Silva-Torres, 2025).

Table No. 4: Particle Size Analysis of Phytosomal Formulations (F1–F10)

Formulation Code	Particle Size (nm)
F1	245.00±3.20
F2	228.00±2.80
F3	209.00±2.50
F4	262.00±3.50
F5	275.00±4.10
F6	289.00±3.70
F7	301.00±4.30
F8	318.00±3.90
F9	334.00±4.50
F10	347.00±3.60

Selection and Characterization of Optimized Formulation (F3)

Further characterization of phytosomes was carried out in terms of percentage yield, particle size, and entrapment efficiency for all experimental formulations developed. Among all the formulations (F1–F10), formulation F3 was found to be the optimized formulation as it exhibited the highest percentage yield along with comparatively smaller particle size and higher entrapment efficiency. The high percentage yield indicates the efficiency of the preparation method and minimal loss of drug and excipients during processing. The reduced particle size of formulation F3 is beneficial for improving solubility, dissolution rate, and absorption of the phytoconstituents, which is a critical factor in phytosome drug delivery systems. Additionally, higher entrapment efficiency indicates better incorporation of the Myricetin into the phospholipid complex, which may enhance drug stability and bioavailability. Based on these characterization parameters, formulation F3 was selected as the best and optimized formulation, and further evaluation studies such as zeta potential, in vitro drug release, SEM analysis, and stability studies were carried out on the optimized F3 formulation (Tariq & Singh, 2026).

Particle Size and Zeta Potential

The optimized Myricetin-loaded phytosomal formulation (F3) exhibited a particle size of 209.0 ± 2.5 nm and a zeta potential of +26.6 ± 1.2 mV, indicating the successful formation of stable nanosized phytosomal vesicles. The particle size in the nanometer range is considered ideal for phytosomal drug delivery systems, as it enhances surface area, dissolution rate, cellular uptake, and bioavailability of the encapsulated phytoconstituent. The relatively small and uniform particle size also suggests efficient interaction between Myricetin and phosphatidylcholine during complex formation. Furthermore, the zeta potential value of +26.6 ± 1.2 mV indicates good colloidal stability of the formulation due to sufficient electrostatic repulsion between vesicles, which minimizes aggregation and sedimentation during storage. The positive surface charge may additionally contribute to improved interaction with negatively charged biological membranes, thereby enhancing absorption and therapeutic performance. Overall, the obtained particle size and zeta potential values confirmed that formulation F3 possessed desirable physicochemical characteristics suitable for stable and effective phytosomal drug delivery.

Table No. 4: Particle Size and Zeta Potential of Optimized Phytosomal Formulation (F3)

Formulation Code	Particle Size (nm)	Zeta Potential (mV)
F3	209.0±2.5	+26.6±1.2

Transmission Electron Microscopy (TEM) Analysis

Transmission Electron Microscopy (TEM) was employed to evaluate the morphology, size, and dispersion characteristics of the optimized phytosomal formulation (F3). The TEM micrograph revealed the formation of discrete, nanosized vesicular structures with predominantly spherical morphology**.** The particles appeared as dark, well-defined entities against a lighter background, confirming successful vesicle formation. The vesicles were found to be fairly uniformly distributed**,** although a slight degree of aggregation was observed in certain regions. This aggregation behavior can be attributed to the moderate surface charge of the formulation, as indicated by the zeta potential value of +26.6 ± 1.2 mV**.** Such a zeta potential suggests sufficient electrostatic repulsion to maintain colloidal stability, although not strong enough to completely eliminate inter particle interactions. The particle size observed in the TEM analysis was in close agreement with the dynamic light scattering (DLS) results, which reported a mean particle size of 209.0 ± 2.5 nm**.** Minor variations in particle size and morphology were evident, indicating a certain level of polydispersity**,** which is commonly observed in phytosomal and lipid-based nanocarriers systems.

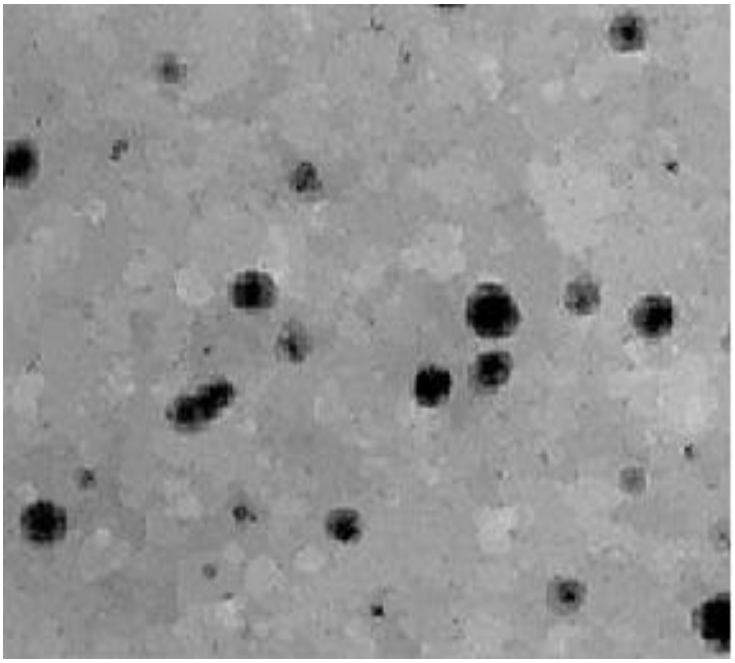

Figure No. 1: TEM photograph of Phytosomes

Scanning Electron Microscopy (SEM) and Particle Characterization

The average hydrodynamic particle size of the F3formulation was measured at 209.0± 2.5 nm, which falls within the ideal nanometer range for targeted drug delivery systems. Particles in this size range are optimized to bypass rapid clearance by the reticulo endothelial system (RES) while maintaining a high surface-area-to-volume ratio, which is essential for enhancing the dissolution rate and bioavailability of the encapsulated bioactive compounds. Furthermore, the formulation exhibited a significantly positive zeta potential of +26.6±1.2 mV. This high positive surface charge indicates strong electrostatic repulsion between the particles, preventing aggregation and ensuring excellent colloidal stability over time. Compared to the raw precursors, this shift to a stable positive potential suggests that the formulation successfully encapsulated the core material, providing a robust surface charge that is often associated with improved cellular uptake and prolonged systemic circulation (Patil *et al*., 2024).

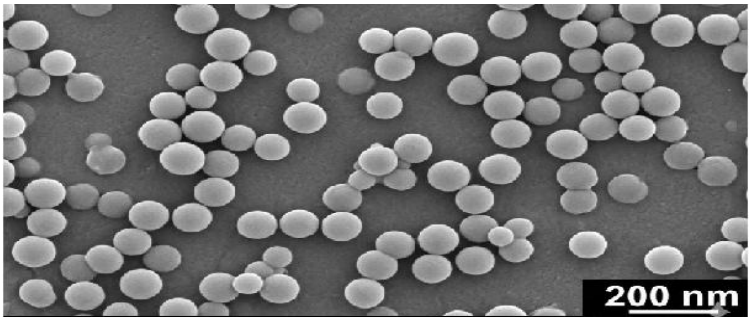

Figure No. 2: SEM photograph of Phytosomes

Stability Study of Optimized F3 Phytosomal Formulation

Stability studies were carried out to evaluate the effect of various environmental factors such as temperature, oxygen, light, and relative humidity on the physicochemical properties of the optimized phytosomal formulation (F3). These factors are known to influence drug stability and may lead to changes in entrapment efficiency, drug release behavior, and physical appearance. The stability study of the F3 formulation was conducted under different storage conditions, namely low temperature (4 ± 2°C), room temperature (28 ± 2°C), and accelerated/high temperature (40 ± 2°C) over a period of 90 days**.** The formulation was evaluated at predetermined time intervals (0, 30, 60, and 90 days) for percentage entrapment efficiency, cumulative drug release, and color changes**.** The results demonstrated that there was no significant variation in the percentage entrapment efficiency and cumulative drug release of the F3 formulation across all storage conditions during the study period. The entrapment efficiency remained nearly constant, indicating that the phytosomal vesicles retained their structural integrity without significant drug leakage. Similarly, the cumulative drug release profile showed only minor fluctuations**,** which may be attributed to experimental variation rather than formulation instability. Importantly, no visible color change was observed throughout the study, suggesting the absence of degradation or chemical interaction affecting the formulation. At low temperature (4 ± 2°C), the formulation

exhibited excellent stability with negligible changes in both entrapment efficiency and drug release. At room temperature (28 ± 2°C), the formulation remained stable, indicating suitability for normal storage conditions. Even under accelerated conditions (40 ± 2°C), the formulation showed acceptable stability**,** with only slight variations that were not statistically significant (Desale N., 2026).

Table No. 5: Stability Study of Optimized F3 Phytosomal Formulation

Time Period (Days)	**Temperature Condition**	**% Entrapment Efficiency**	**% Cumulative Drug Release**	**Colour Change**
0	Low Temperature (4 ±2°C)	85.20±1.10	82.15±0.35	No
30		85.75±1.25	81.90±0.28	No
60		84.90±1.40	83.10±0.30	No
90		85.10±1.32	82.75±0.25	No
0	Room Temperature (28 ±2°C)	85.60±1.05	83.40±0.40	No
30		85.25±1.18	84.10±0.36	No
60		84.75±1.22	83.85±0.29	No
90		85.30±1.15	83.20±0.31	No
0	High Temperature (40 ±2°C)	85.10±1.30	83.05±0.27	No
30		86.20±1.45	82.60±0.24	No
60		85.85±1.38	81.95±0.22	No
90		85.40±1.20	82.10±0.26	

CONCLUSION

In conclusion, the present study successfully developed and characterized Myricetin-loaded phytosomal formulations using the solvent evaporation method with varying drug-to-phosphatidylcholine ratios. Among all the prepared formulations, F3 was identified as the optimized formulation due to its highest percentage practical yield (90.68%), high entrapment efficiency (88.72%), and smallest particle size (209.0 ± 2.5 nm), indicating efficient vesicle formation and enhanced drug incorporation. The optimized formulation also exhibited a favorable zeta potential value of +26.6 ± 1.2 mV, confirming good colloidal stability and reduced aggregation tendency. TEM and SEM analyses demonstrated the formation of predominantly spherical, well-dispersed nanosized vesicles with smooth morphology. Furthermore, the stability studies revealed that the optimized formulation remained stable under different storage conditions without significant changes in entrapment efficiency, cumulative drug release, or physical appearance over the study period.

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