

Development, Optimization, and Physicochemical Characterization of Liposomal Formulations Containing Ethanolic Leaf Extract and Flavonoid-Rich Fraction of *Albizia lebbeck* for Enhanced Hepatoprotective Delivery

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Article Info	ABSTRACT
Submitted: 09-01-2026 Revised: 10-03-2026 Accepted: 15-03-2026 *Corresponding author Jitendra Patel Email: jitendra.patel@dmher.edu.in	This work describes the development, optimization, and characterization of liposomal carriers loaded with an ethanolic leaf extract and a flavonoid-rich fraction of <i>Albizia lebbeck</i> (<i>A. lebbeck</i>) to improve hepatoprotective activity. Liposomes were prepared by the thin film hydration method, followed by probe sonication, and optimized for vesicle size, zeta potential, PDI, and encapsulation efficiency. The ethanolic extract-loaded liposomes exhibited a smaller vesicle size of 152.4 ± 3.6 nm, a polydispersity index of 0.212 ± 0.01, a zeta potential of -32.6 ± 2.3 mV, and an encapsulation efficiency of $67.8 \pm 2.4\%$. On the other hand, the liposomes encapsulating the flavonoid-rich fraction resulted in a smaller vesicle size (138.2 ± 4.1 nm), a lower PDI (0.198 ± 0.02), a more negative zeta potential (-36.2 ± 1.9 mV), and a significantly higher encapsulation efficiency ($82.5 \pm 1.7\%$). Morphological characterization by Transmission Electron Microscopy (TEM) showed consistently spherical vesicles. In vitro release studies revealed a more controlled release profile over 24h, with flavonoid-loaded liposomes releasing 82.3% compared to 30.1% from crude extract-loaded liposomes. The physical stability of the liposomal formulations was improved, as evidenced by stability studies at 4°C and 25°C for 30 days. Thus, these results suggest that the bioavailability, stability, and therapeutic effects of <i>A. lebbeck</i> components, particularly the flavonoid-rich fraction, can be significantly improved by liposomal encapsulation, thus supporting its potential application as an advanced hepatoprotective delivery system. Interestingly, this is the first comparative analysis showing that the flavonoid-rich fraction in liposomal delivery systems had better encapsulation efficiency and controlled-release behavior than the crude extract. Keywords: <i>Albizia lebbeck</i>, liposomes, flavonoid fraction, hepatoprotective, thin-film hydration, encapsulation efficiency

INTRODUCTION

Liver diseases are one of the biggest global public health issues, having a rising share in worldwide morbidity and mortality. Newer worldwide estimates indicate that viral hepatitis, alcohol-related liver disease, and Non-alcoholic fatty liver disease (NAFLD) together account for a large and increasing share of the burden of acute and chronic liver disease, which causes 1–2 million deaths annually and imposes huge social and economic costs on health care systems (Yang & Wang, 2024). Despite the presence of high unmet

demand, effective treatment options for many liver diseases are still limited. While major advances have occurred in viral hepatitis, approved pharmacotherapies remain limited for common disorders such as NAFLD, alcoholic liver disease, and diverse forms of drug-induced or toxin-mediated liver injury, and clinical outcomes remain suboptimal (Gao et al., 2025). In addition, many putative hepatoprotective agents (especially natural products) face translational challenges, including low water solubility, low oral bioavailability, ill-defined phytochemical

composition, poor standardized clinical data, and disparate trial outcome measures. These limitations, however, reduce the clinical utility of otherwise promising hepatoprotective agents, necessitating the design of delivery systems that enhance their stability, bioavailability, and controlled release (Vijayan et al., 2025). The global prevalence of liver disease remains high, and with the pharmacological limitations of many existing and likely future hepatoprotective agents, nanoformulation strategies (Patel et al., 2025).

A. lebbeck, also known as "Shirish," is a perennial, deciduous tree of the Fabaceae family. It has been used for numerous diseases by the traditional medical systems like Ayurveda and Unani for ages (Osman et al., 2024). Bark, leaves, flowers, seeds, and roots are part of different preparations, although the bark occupies a prominent place in the traditional use. Phytochemical studies show *A. lebbeck* contains a high variety of bioactive compounds such as flavonoids, saponins, phenolic compounds, alkaloids, terpenes, and phytosterols (Rajesham et al., 2025). In addition, bark and other tissues contain important phytochemical compounds such as β -sitosterol, tannins, and complex structures of triterpenoids. These constituents are believed to contribute to the wide-ranging pharmacological activity of *A. lebbeck*, including anti-inflammatory, antioxidant, anticancer, neuroprotective, and hepatoprotective effects (El Gamal et al., 2015).

Advanced drug-delivery methods like liposomes provide an alternative method to circumvent these hurdles by increasing solubility, stability, and release controllability, respectively, enabling better access to flavonoid-rich and other bioactives of *A. lebbeck* (Yang & Wang, 2024b).

Liposomal drug delivery systems have emerged as highly versatile and biocompatible nanocarrier platforms, particularly advantageous for delivering poorly soluble, unstable, and low bioavailable phytochemicals. (Jiang et al., 2023).

One major advantage of liposomes is that they allow for controllable and sustained release of bioactive materials. This allows the maintenance of therapeutic plasma concentrations for long durations, minimizing both the dosing frequency and fluctuations associated with conventional delivery vehicles. In particular, liposomes improve the solubility and absorption of poorly water-soluble phytochemicals by providing a favorable environment for gastrointestinal absorption and, in some cases, facilitating better penetration into biological membranes (Senjab et al., 2024).

In addition, polymers or targeted ligands can be attached to the liposomal surface, allowing for extended circulation times, reduced reticuloendothelial clearance, and increased tissue localization, including the liver. (Makouie et al., 2024). Very few studies have methodically investigated the incorporation of conventional hepatoprotective botanicals into logically constructed liposomal systems for translational development, despite the growing interest in nanocarrier-based hepatoprotective treatments. This reveals a major research gap in connecting cutting-edge nanotechnology-based delivery systems with conventional phytomedicine.

Through a variety of synergistic pathways mediated by their bioactive ingredients, including flavonoids, phenolics, terpenoids, and alkaloids, medicinal herbs demonstrate hepatoprotective action. By scavenging reactive oxygen species and boosting endogenous enzymes such as glutathione peroxidase, catalase, and superoxide dismutase, these phytochemicals have potent antioxidant actions that lower lipid peroxidation.

There is strong evidence that lipid-based nanoparticles, especially liposomes, improve the therapeutic efficacy of bioactive phytoconstituents and improve hepatic targeting. Because of the fenestrated sinusoidal endothelium and Kupffer cell uptake, which enables both passive and active targeting, the liver is a major organ of nanoparticle accumulation (Torchilin, 2005). By increasing the bioavailability, cellular absorption, and prolonged release of flavonoids and polyphenols, liposomal systems have shown enhanced hepatoprotective effect, resulting in decreased oxidative stress, inflammation, and hepatocellular injury in experimental animals. For example, compared to free extracts, liposomal formulations of flavonoids have demonstrated a noticeably higher reduction in blood ALT, AST, lipid peroxidation, and inflammatory cytokines, indicating improved liver protection. Additionally, phytoconstituent stability is enhanced, and fast metabolism is inhibited by nanocarrier-mediated administration, which enhances the therapeutic response in hepatic damage models.

While the pharmacological potential of *A. lebbeck* has been extensively reported in native literature, including antioxidant and hepatoprotective activities, most of the investigations have been limited to the assessment of crude extracts or isolation of active compounds in their customary forms. None have been systematically investigated regarding performing

head-to-head comparisons of various plant phytochemical fractions delivered in an advanced, nanocarrier- or supersaturable-based systems aimed specifically at bolstering bioefficaciousness performance of its bioactive constituents (Mehta et al., 2023) (Figure 1).

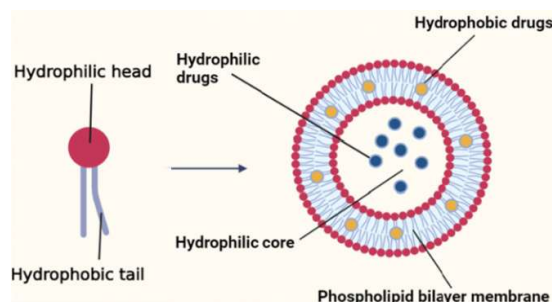


Figure 1. Schematic diagram of a liposome showing phospholipid bilayer and encapsulated phytoconstituents. A schematic depiction of a liposomal vesicle showcasing the phospholipid bilayer architecture, featuring hydrophilic head groups and hydrophobic tails, exhibiting the encapsulation of phytoconstituents inside the aqueous core and lipid bilayer regions (Senjab et al., 2024)

This difference is important because of the significantly different amounts of phytochemicals, solubility, and expected pharmacokinetics of the crude extract versus those of the flavonoid-rich fraction. It is important to fill this void so that *A. lebbbeck* can be advanced toward traditional- and/or evidence-based medicinal applications and so that the ideal nanoformulation percentage can be identified (Antonelli & Palma, 2025).

To formulate, optimize, and physicochemically characterize liposomal preparations of the ethanolic leaf extract and a flavonoid-rich fraction of *A. lebbbeck*, and to assess their *in vitro* release kinetics and short-term physical stability for improved hepatoprotective administration.

MATERIALS AND METHODS

Materials

The living leaves of *A. lebbbeck* were collected from a local place of Sawangi (Meghe), Wardha, Maharashtra, and authentication was done. The collected leaves were thoroughly washed with distilled water to remove dust and dirt, shade-dried at room temperature in order

to preserve phytoconstituents before being coarsely powdered for extraction (Liu et al., 2024).

Liposomes were formulated with phospholipids (L- α -phosphatidylcholine (soy lecithin) and cholesterol) procured from authorized pharmaceutical-grade suppliers and used as the primary lipid components. Extraction and fractionation were conducted using analytical-grade solvents, including ethanol, methanol, chloroform, and n-hexane. Potassium dihydrogen phosphate (analytical grade), sodium hydroxide (analytical grade), Tween-80 (HPLC grade), and phosphate-buffered saline (PBS, pH 7.4) were used as other chemicals (Chronopoulou et al., 2025).

Preparation of Ethanolic Extract

The maceration method was employed to extract the desiccated leaves of *Albizia lebbbeck*, which had been ground coarsely. Solvents of increasing polarity were used in a stepwise manner to ensure the complete isolation of each class of phytoconstituent, using a sequential solvent extraction method. The powdered plant material was first extracted with petroleum ether to give a nonpolar extractive containing lipids, waxes, and aroma. At the end of the extraction phase, marc was filtered, and the solvent was removed by evaporation (Tourabi et al., 2025).

The plant material was then sequentially macerated with chloroform, ethanol, and a hydroalcoholic solvent system (hydroalcoholic solvent system = 50% ethanol and 50% distilled water) (Barthwal & Mahar, 2024).

Filtrates from each solvent were concentrated by rotary evaporator under controlled temperature and reduced pressure to avoid denaturation of the active ingredients. The ethanolic extract was then evaporated to give a semisolid residue, which was kept in an airtight container at low temperature until use for liposome (Mahmoud & Selim, 2025).

Preparation of Flavonoid-Rich Fraction

A total flavonoid fraction of leaves from *A. lebbbeck* was obtained via a solvent partitioning method to preferentially remove polar flavonoid constituents from the crude ethanolic extract (Supplementary Table I). The resulting aqueous suspension was then subjected to increasing solvent-partitioning based on the polarity of solvents to enable the proper separation of phytochemical classes (Susanti et al., 2024).

First, the combination was retrieved with n-hexane to remove nonpolar components such as lipids, chlorophyll, and other lipophilic impurities. Following chloroform extraction, the remaining water phase was partitioned between water and ethyl acetate, a solvent known to preferentially extract polyphenolic and flavonoid compounds. Several ethyl acetate extractions were performed to ensure maximum extraction of the flavonoid-rich fraction (Lim et al., 2024).

The consolidated ethyl acetate layers were subsequently concentrated in vacuum with the help of a rotary evaporator, avoiding the high temperature to not degrade thermolabile flavonoid (Mahmoud & Selim, 2025). The semisolid mass obtained, characteristic of the fraction containing the flavonoids, was dried, stored in closed amber bottles, and kept in a refrigerator until use in liposome synthesis. This method helped to enrich on the critical components of the flavonoids whilst decreasing the interference from other phytochemicals with the contents (Royani et al., 2025).

Preliminary Phytochemical Screening

Prior to investigating the pharmacological activity of the extract, previously known as the flavonoid-rich fraction, a preliminary phytochemical screening of the ethanolic whole leaf (positive control) extract and that of the flavonoid-rich fraction of *A. lebbeck* was conducted in order to identify the major classes of bioactive compounds whose presence would suggest hepatoprotective potential. Qualitative tests were performed for conducting tests such as alkaloids, flavonoids, tannins, saponins, glycosides, phenols, and terpenoids. The crude ethanolic extract exhibited a wide variety of phytochemicals, while the flavonoid-enriched fraction of the ethanolic extract showed a high content of flavonoids and phenolic compounds, confirming efficient enrichment. The quantity of total phenolic content (TPC) and total flavonoid content (TFC) was spectrophotometrically measured, respectively, using the Folin-Ciocalteu and the aluminum chloride colorimetric methods. High TPC and TFC values of flavonoid-rich fraction were significantly higher ($p < 0.05$) than the crude extract, suggesting better phytochemical profile and possibly better associated antioxidant and hepatoprotective properties. These outcomes provided a basis for choosing the extract and fraction for the later liposomal formulation and optimization studies (Ibrahim & Ghareeb, 2020).

Preparation of Liposome (Thin-Film Hydration Method)

Liposomal formulations of ethanolic leaf extract and flavonoid-rich fraction of *A. lebbeck* were prepared by the usual thin-film hydration method. Phospholipids and cholesterol were measured separately and mixed in chloroform-methanol (2:1, v/v) in a round-bottom flask. This mixture of solvents was then evaporated under reduced pressure at 55–60°C with a rotary evaporator to give a thin, uniform lipid film on the internal wall of the flask. The dry film obtained was rehydrated with phosphate-buffered saline (PBS, pH 7.4) under constant rotation, leading to the transformation of MLVs. The dispersion was then probed sonicated for subsequent reduction of vesicle size to yield unilamellar and nanosized liposomes. Formulations were allowed to equilibrate, then stored at 4 °C for later characterization. Both ethanolic extract-loaded and flavonoid fraction-loaded liposomes were prepared through a similar method, varying only in the active material incorporated into the organic phase (Zhang, 2017).

Probe Sonication

Probing sonication was used to reduce the size of the vesicles to the nanoscale to obtain unilamellar liposomes of a desired size from the liposomal dispersions obtained after hydration. A high-intensity ultrasonic probe was used (the amplitude level was typically set at 40–60%) for an optimal time of 5-10 minutes with short pulse cycles in order to avoid degradation of the encapsulated phytoconstituents (Jamalabadi et al., 2019).

Optimization Parameters

In recent formulation studies, optimization of liposomal formulations was focused on the interrelated quality attributes, which are vesicle size, polydispersity index (PDI), zeta potential, and encapsulation efficiency (EE%). A systematic experimental approach was used: single-factor experiments were performed, and a multivariate design approach (factorial or response surface design) was used to establish robust formulation and process parameters. The hydrodynamic diameter by dynamic light scattering (DLS) has been measured for the optimization of a low-polydispersity nanoscale population appropriate for hepatic administration (less than 200 nm, on average, and low polydispersity during storage) (Danaei et al., 2018).

At the same time, the polydispersity index (PDI), a measure of size uniformity, was changed by manipulating the same formulation and process parameters. PDI < 0.3 was achieved by producing a thin film of uniform size, carefully controlling hydration (step-wise, controlled swelling), gradually decreasing vesicle size with probing sonication, and/or extruding through defined pore membranes when required. In the immediate sense, PDI was a quality control parameter: formulations with an acceptable mean size but high PDI were reformulated to improve the reproducibility and predictability of biological activity (Rane & Choi, 2005).

The zeta potential was optimized to ensure appropriate surface charge for electrostatic stabilization and biocompatibility. Surface charge was modified by either selection of neutral or charged phospholipids/using charged lipids (e.g., phosphatidylserine for negative charge), or use of surface modifiers that reduce the magnitude of the charge (e.g., via PEGylation) but simultaneously increase steric stability. Electrophoretic light scattering was used to measure zeta potential, and formulations were optimized to achieve an absolute value that was sustainable (usually $\geq |30|$ mV for sufficient electrostatic repulsion) or an alternative steric stabilization mechanism (PEG) if opsonization was favored with low charge (Serrano-Lotina et al., 2023).

The drug loading approaches were optimized, either based upon pre-co-dissolution of the actives with lipids in the organic phase or post-loaded. Other parameters influencing the encapsulation efficiency (EE%), namely drug-to-lipid ratio, appropriate solvents, and hydration conditions, were also optimized. In the case of poorly water-soluble flavonoids, they were incorporated into the lipid phase or further assisted through ion-pairing and complexation techniques, which improved loading efficiency. Analytical quantification techniques such as HPLC or spectrophotometry. We reconciled EE% with size and PDI during the optimization process—some conditions that maximized EE also increased size and/or PDI, so a desire function or multi-response optimization (RSM/Box-Behnken) was applied to find the best trade-off (Piacentini, 2016).

The experimental trials documented DLS size and PDI, zeta potential, EE%, and stability (size/zeta/EE% after zeta order/storage at the conditions indicated in the section headed below) throughout the iterations of the optimization process. Statistical analysis of the experimental

design (ANOVA, regression models) identified significant factors and interactions; the final formulation choices focused on reproducible nanoscale size, low polydispersity index, appropriate surface charge or steric protection, and high encapsulation efficiency, while preserving the chemical integrity of extract/fraction and acceptable in-vitro release properties (Parralejo-Sanz et al., 2025).

Characterization of Liposomes

Vesicle Size, Polydispersity Index, and Zeta Potential

The z-average hydrodynamic diameter, polydispersity index (PDI), and zeta potential of the liposomal formulations were determined by dynamic light scattering (DLS) and electrophoretic light scattering on a Zetasizer unit. Prior to measurement, all formulations were diluted (typically 1:10–1:50, v/v) with fresh filtered (0.22 μ m for solvents, 0.45 μ m for vesicle suspensions to avoid loss of larger liposomes) and degassed phosphate buffered saline (PBS, pH 7.4), or Milli-Q water to attain a suitable count rate and minimize multiple scattering; samples were gently inverted to mix and allowed to equilibrate for 5 mins at the measurement temperature (Atre & Rizvi, 2024) (Supplementary Table II).

Encapsulation Efficiency

The capability of encapsulation (EE%) of the liposomes loaded with ethanolic extract and flavonoid fraction was evaluated by differentiation of the free (unentrapped) phytoconstituents from the vesicle-entrapped material, followed by the quantification of total phytoconstituents in the respective fraction, such as phytoconstituents. Depending on available instrumentation and the analytes' solubility, two complementary separation techniques were used: ultracentrifugation and dialysis (Yuan et al., 2025a).

For the ultracentrifugation technique, an aliquot of liposomal dispersion (usually 1–2 mL) was placed into ultracentrifuge tubes and subjected to centrifugation (100,000 $\times$ g for 60–90 min at 4°C) to pellet liposomes. The supernatant (free extract/fraction) was cautiously obtained, avoiding any disruption of the pellet, and an adequate volume was removed for analysis. The resulting pellet was washed with cold PBS (to remove any loosely associated material), and then recentrifuged under the same conditions, and then reconstituted with a defined volume of solvent (e.g., methanol or methanol: water) before sonication or vortexing to rupture the lipid bilayer

and release the encapsulated actives. From this dissociated pellet fraction, the quantity of encapsulated compound was derived (Camilleri et al., 2025).

In the case of the dialysis method, a defined amount of liposomal suspension (eg, 1–2 mL) was transferred into dialysis tubing (pre-soaked in distilled water, MWCO 12–14 kDa) and dialyzed against an excess of PBS (pH 7.4) at 4°C under magnetic stirring or distilled water. They replaced the buffer at regular intervals (e.g., 4 and 8 hours), and continued dialysis until actives in the external medium plateaued (typically 12–24 hours). Thus, free drug within the external medium was quantified by sampling the medium, and the content remaining within the tubing after dialysis (liposomes) was burst with solvent to quantify the encapsulated fraction as indicated above (Hua, 2014).

Free and encapsulated phytoconstituents were quantified with the use of a validated analytical method appropriate for the sample: high-performance liquid chromatography (HPLC) with a reverse-phase C18 column and UV detection (typical detection wavelength 250–350 nm depending on the analytes), or UV-Vis spectrophotometry after forming a suitable complex, using calibration curves prepared from standards of the crude extract or of a marker flavonoid. Linear regression, limit of detection/quantification (LOD/LOQ), intraday and interday precision, and accuracy were assessed on a set of calibration curves, spanning the anticipated concentration range. Three independent measurements ($n = 3$) were done and then converted into mean $\pm$ SD (Domínguez et al., 2021). Encapsulation efficiency (EE%) was calculated using the equation:

$$EE\% = [(Total\ amount\ of\ compound - Free\ amount\ of\ compound) / Total\ amount\ of\ compound] \times 100$$

where “Total” refers to the total known amount of extract/fraction present in the formulation and “Free” is the measured amount present in the supernatant or in the dialysis media. The recoveries were considered satisfactory when between 90% and 110%, and were calculated by spiking blank liposomes with known amounts of extract/fraction and separating them via the described procedure and assaying the liposome component. Any artifacts from adsorption to tube walls or low pelleting were prevented through the use of procedural blanks or changing dialysis

conditions (dialysis time, force, or dialysis settings).

The separation technique chosen (ultracentrifugation vs. dialysis, when applicable) is noted for each formulation, and validation parameters for the analytical assay, as well as EE% expressed as mean $\pm$ SD ($n = 3$) of three independent batches.

Morphological Analysis

The morphological features of the synthesized liposomes were examined using Transmission Electron Microscopy (TEM) to assess vesicle morphology, surface properties, and structural integrity. The liposomal dispersion was diluted with distilled water at low volume to get a homologous sample, and a drop of diluted suspension was deposited onto the carbon-coated copper grid. Filter paper was used to remove excess fluid, and the sample was permitted to adsorb for 1 to 2 minutes. To enhance the contrast of the lipid bilayer, a drop (~1–2%) of phosphotungstic acid (PTA, pH adjusted to 7.0) or uranyl acetate was applied and gently blotted. Following this, the grids were air-dried at room temperature, not long before imaging (Zhigaltsev & Cullis, 2023).

The samples were then covered to prepare specimens by applying a method described by Niu et al. TEM investigations were performed using a high-resolution electron microscope, operated at an accelerating voltage of 80–120 kV. Various magnifications were used to obtain images to evaluate the general morphology, size range, and membrane architecture of the vesicles. The ability to form stable vesicular structures with the bilayer structure of liposomes was confirmed by an analysis that showed liposomes primarily display spherical to almost spherical shapes with clean, perturbed edges. There was no significant aggregation, distortion, or rupture, which is consistent with the DLS results and the optimized formulation parameters. The grains were examined in order to check for repeatability and to verify the nanoscale dimensions determined from particle size measurements (Schulmeister & Mader, 2003) by separate micrographs from different fields.

FTIR and/or DSC

Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) were used as ancillary analytical techniques to evaluate possible interactions between the *A. lebbbeck* extract/fraction and the lipid excipients and to further assess compatibility within the liposomal matrix (Supplementary Figure 2).

FTIR spectrometer (4000–400 cm^{-1}) was used to record spectra of the pure ethanolic extract, flavonoid-rich fraction, individual lipids (phospholipid and cholesterol), and final lyophilized liposomal formulations for FTIR analysis. Sample preparation included mixing samples with dry potassium bromide (KBr), followed by compressing the mixture into transparent pellets, or the direct measurement of the dispersed samples in the ATR (attenuated total reflectance) mode. We analyzed characteristic peaks of functional groups of phytoconstituents, such as O–H stretching of phenolics, C=O stretching of flavonoids, and C–O–C vibrations. The presence or absence of significant peak shifts, disappearance of large functional bands, as well as the appearance of new peaks in liposomal spectra, suggests that chemical incompatibility or degradation does not take place during encapsulation. Slight peak broadening or intensity reduction was observed, reflecting measurable physical entrapment of actives within the lipid bilayer (Singh et al., 2022).

The probe for the thermal behavior of the extract/fraction, lipids, and liposomal formulations was measured with a differential scanning calorimeter (DSC). Representative samples (3–5 mg) were packed in aluminum pans, and they were scanned in nitrogen through an appropriate temperature range (i.e., 25–300°C) at a controlled heating rate (typically 10°C/min) [30]. Melting endotherms, phase transitions, and enthalpy changes were further analyzed based on these thermograms. An absence or considerable decline in the distinctive melting peak of the active ingredients in the liposomal dispersion indicates molecular dispersion or entrapment within the lipid bilayer. Similarly, the phospholipid phase transition temperature changes may signify changes in membrane fluidity or strong lipid–drug interactions (Ghanbari et al., 2023).

FTIR and DSC together provided supporting evidence that the physicochemical compatibility of the *A. lebbbeck* constituents with the lipid excipients made them suitable for the stable and efficient liposomal encapsulation.

In Vitro Drug Release Study

The sustained release behavior of liposomes containing an ethanolic extract and flavonoid fraction was assessed using the dialysis bag diffusion method. Each liposomal formulation was introduced (typically 1–2 mL) into a dialysis tubing (medium molecular weight cut-off 12–14

kDa) that had been pre-soaked, and then this was sealed and immersed in a beaker of 50–100 mL of phosphate-buffered saline (PBS; pH 7.4) at $37 \pm 0.5^\circ\text{C}$. The release medium was stirred continuously at 100–150 rpm to maintain sink conditions and ensure homogenous diffusion (Karadag & Saroglu, 2024).

At designated time points (0.5, 1, 2, 4, 6, 8, 12, and 24 h), 1–2 mL external medium was sampled for quantification and replaced with the same volume of pre-warmed PBS to retain a constant volume and concentration gradient. Samples were filtered when necessary (0.45 μm) and analyzed using appropriate spectrophotometric or HPLC methods, based on the properties of the active components. The concentration of the released phytoconstituents was calculated based on calibration curves performed using the crude extract or flavonoid-rich fraction (Lopez-Polo et al., 2024).

Before calculating the cumulative percentage, the dilution experienced when obtaining the sample was accounted for. Release profiles for each formulation were plotted as a percentage of the cumulative amount as a function of time and then evaluated with previously established kinetic models: zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations to explain the molecular diffusion behavior of the drug from the liposomal matrix. Data are expressed as mean $\pm$ standard deviation (SD) (Jovanović et al., 2024), and all studies were done in triplicate.

This method enabled the assessment of controlled release characteristics and the science behind lipid composition and encapsulation on the release kinetics of phytoconstituents of *A. lebbbeck*.

Stability Studies

The physicochemical stability of liposomal formulations containing an ethanolic extract and a flavonoid fraction was assessed by stability tests under different storage conditions. Aliquots of freshly generated liposomes were placed into air-tight amber vials and stored at 4 °C (fridge) and 25 °C (room temperature) for 30 days to check the stability of the vesicles and formulation at different temperatures (Halevas et al., 2022).

Vesicles were sampled from each storage condition at predetermined time points—baseline (0 days), fifteen days, and thirty days—then analyzed for key stability parameters, namely vesicle size, polydispersity index (PDI), and encapsulation efficiency (EE%) quantitation. Dynamic light scattering (DLS) was used to assess

particle size and polydispersity index (PDI), maintaining the same instrument parameters as during initial characterization. EE% (Encapsulation efficiency percentage) was calculated using ultracentrifugation or dialysis, spectrophotometric (Chavda et al. 2022), or HPLC analysis.

To detect potential aggregation, fusion, or structural destabilization of liposomes with time, vesicle size, and polydispersity index were measured. Property change: An increase in average diameter or an increase in PDI denotes a risk for physical instability (especially at higher temperatures). At each time point, encapsulation efficiency was evaluated to determine whether leakage or breakdown of the encapsulated phytoconstituents occurred. Data collected from formulations stored at 4 °C were consistently better than those stored at 25 °C in maintaining both EE% and size distribution, revealing a temperature-dependent membrane fluidity and permeability (Costa et al., 2025).

The ideal storage conditions to maintain liposomal integrity and ensure prolonged release of biologically active *A. lebbeck* extract- and flavonoid-loaded formulations, as revealed by the stability assessment.

RESULTS AND DISCUSSION

Extraction Yield and Phytochemical Content

The extraction method produced two different phytochemical fractions of *A. lebbeck* leaves: a crude ethanolic extract, and a more pure fraction rich in flavonoids. Despite the polar to non-polar nature of the compounds, the higher yield of the crude ethanolic extract could be attributed to the broader solubility property of ethanol to extract a wider range of phytoconstituents such as phenolics, flavonoids, alkaloids, tannins, saponins, and other polar to moderately non-polar compounds. However, the selective fractionation of the flavonoid-rich fraction yielded an overall lower quantity but extremely higher quantities of the selected common flavonoid and phenolic constituents (Dhanani et al., 2017).

Since the crude extract displayed complex phytochemical profiling, various classes of phytochemical constituents were identified by qualitative phytochemical screening. On the other hand, the flavonoid content was greatly enriched during the fractionation process, as indicated by the higher intensities seen in flavonoid-specific assays and an increased UV-visible absorbance at

respective characteristic wavelengths. Similarly, the quantitative analysis further confirmed that the flavonoid-rich fraction had markedly higher TPC and TFC than those in the crude extract, verifying that the enrichment procedure was effective (Rao et al., 2023).

These differences in composition are relevant to subsequent liposome formulation. The flavonoid-enriched fraction is anticipated to show a higher encapsulation efficiency, an improved biological activity, and a more controlled release profile, being richer in those antioxidant and hepatoprotective flavonoids. On the other hand, the crude extract is more chemically diverse, but loading often is less efficient and more variable, as the crude extract is of a more heterogeneous nature. The extract and enrich steps, therefore, provided phytochemically different materials appropriate for testing the liposomal delivery benefit for hepatoprotective purposes (Usman et al., 2010).

Optimization Outcomes

In this way, significant improvements in liposomal systems were achieved through optimizing the formulation and processing parameters in order to enhance the physicochemical properties of the systems. Stepwise manipulation of lipid composition, drug-to-lipid ratio, hydration conditions, and probe sonication conditions led to the formation of nanosized and homogeneously distributed vesicles with proper stability characteristics. The optimized formulations showed a vesicle size reduction of at least two orders of magnitude, meaning that liposomes loaded with both the crude extract and the flavonoid fraction are now in the nanometric range that is considered optimal for their hepatic uptake. The size reduction is largely attributed to the optimized sonication amplitude and time that consistently broke down the multilayered vesicles and reconstructed them into smaller and more uniform vesicles.

PDI values were greatly improved after optimization, reflecting a narrower size distribution and a reduction in the batch-to-batch variability. We used controlled rates of hydration and customized lipid ratios to minimize the formation of large or multilamellar vesicles, yielding PDI values of both formulations of less than 0.25. Well-defined size homogeneity is critical for the accuracy of in vivo performance and controlled release properties (Peruš et al., 2026).

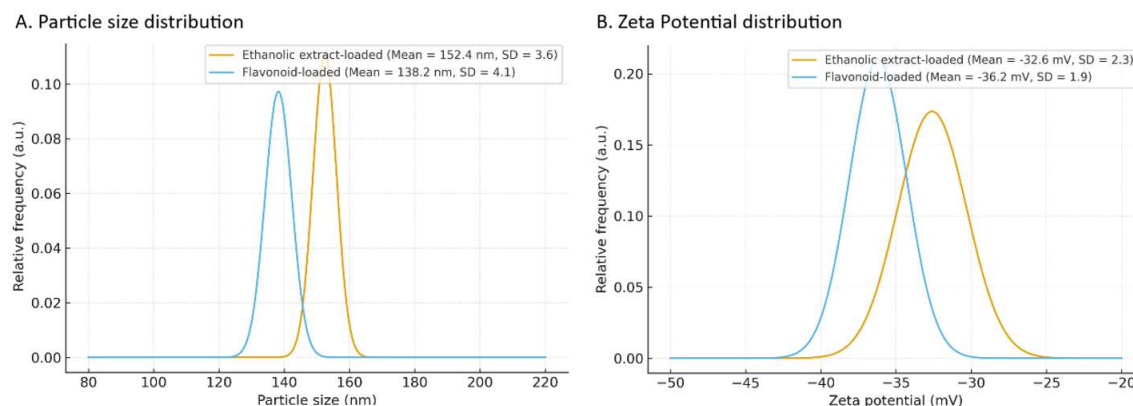


Figure 2A. Size distribution curves. A representative size distribution profile of liposomes loaded with ethanol extract and flavonoid-rich fractions, demonstrating their narrow particle size ranges and low polydispersity. The flavonoid-encapsulated formulation exhibits a reduced mean vesicle diameter, signifying enhanced nanoscale homogeneity relative to the extract-encapsulated liposomes; B. Zeta potential distribution. A representative zeta potential distribution curve for liposomes loaded with ethanol extract and flavonoid-rich fractions, exhibiting unique negative surface charge values that enhance the electrostatic stabilization of the vesicles. The formulation, which includes flavonoids, demonstrates a higher negative zeta potential, indicating improved colloidal stability compared to the extract-loaded liposomes.

The zeta potential studies showed that adjusted formulations showed sufficiently high negative surface charge that provided colloidal stabilization due to the electrostatic repulsion in the vesicle aggregates. Modulation of lipid composition, namely cholesterol amount and charge-stabilizing phospholipids, resulted in an improved negative surface potential to achieve prolonged physical stability on storage (Jarzynska et al., 2024).

After correction, EE% significantly increased (especially for the flavonoid-rich fraction). The improvement was attributed to a modified solubilization of phytoconstituents in the organic phase, improved lipid-to-drug ratio, and adequate bilayer accommodation at all stages of the thin-film hydration process. More excessive EE% suggests more energetic components are preserved and more beneficial outcomes (Yuan et al., 2025b).

The optimized parameters produced liposomes that were smaller, more homogenous, more stable, and with a higher efficiency of entrapment of phytoconstituents than initial batches. These improvements create a strong foundation for improved bioavailability and increased hepatoprotective target function of actives derived from *A. lebbbeck*.

Particle Size, PDI, and Zeta Potential

Improved physical uniformity and colloidal stability of the modified liposomal formulations were illustrated by drastic improvements in the particle sizes, size distributions, and zeta potential. We found that both ethanol extract-loaded and flavonoid fraction-loaded liposomes were nanoscale vesicles (Figure 2A), with the flavonoid-rich fraction resulting in somewhat smaller vesicles. The reduced size suggests improved bilayer organization and efficient self-assembly upon thin-film hydration and probe sonication. Smaller vesicles are beneficial for hepatic administration, as they encourage greater permeability, improved sinusoidal passage, and possibly increased uptake by hepatocytes and Kupffer cells¹⁵⁴. The PDI of altered formulations confirmed the significant reduction, while both liposome types had a PDI less than 0.25 (Figure 2B). This narrow distribution confirms the production of homogeneous vesicle populations with almost no aggregates or larger particles. Improvement in PDI is correlated with controlled hydration method and characterized sonication conditions, allowing for homogenous vesicle formation and reduced multilamellar assemblies. A low PDI is an important desirable property since it increases repeatability, predictable release kinetics, and

reduces variability between batches (Rane & Choi, 2005).

The formulations showed highly negative values of zeta potential, indicating the formation of a strong electrostatic barrier around the vesicles. Once again, the flavonoid-rich fraction-loaded liposomes presented a relatively higher negative charge, demonstrating an increased stability of the lipid bilayer due to the increase of phenolic and flavonoid compounds. The higher surface charge increases the repulsion between the vesicles and thereby reduces the probability of aggregation during storage and handling. A zeta potential above ± 30 mV is often considered sufficient for long-term colloidal stability, which is in agreement with our findings from both formulations (Serrano-Lotina et al., 2023)

Improvement in the particle size, PDI, and zeta potential indicates that the optimization strategy successfully created liposomal carriers that are stable, uniform, and physicochemically robust for effective hepatoprotective delivery of *A. lebbbeck* phytoconstituents.

Encapsulation Efficiency

The encapsulation efficiency EE (%) of the liposomal formulations obviously differed between crude ethanolic, and flavonoid-rich fraction (Supplementary Table III). This occurred partially due to several formulation-based and phytochemical factors that cause the flavonoid-rich fraction to always have much greater EE% than does the crude extract (Piacentini, 2016).

The flavonoid-rich fraction has a higher concentration and a more chemically homogeneous profile of flavonoids and phenolic constituents. The compounds also have moderate lipophilicity and planar aromatic structure, which attains higher affinity for phospholipid bilayer and favors strong interaction of the compound with lipid acyl chains. The careful shaping of these flavonoids, in a more spherical shape, allows their accommodation in the hydrophilic site in between the lipid bilayer, thus increasing the success of entrapment under thin-film hydrations (Halevas et al., 2022).

By contrast, the crude ethanolic extract contains a wide array of polar and non-polar constituents, including carbohydrates, alkaloids, saponins, tannins, and other water-soluble components. A lot of hydrophilic elements have low membrane affinity, which lowers their aggregation inside the bilayer, leading to a comparatively low

EE%. Moreover, the crude extract heterogeneity also raises the possibility of competitive physical interactions during the film formation, thus reducing the effective encapsulation (Domínguez et al., 2021).

In addition, during the formulation phase, the fractions' solubility behavior is also responsible for the constant release curve. The fraction rich in flavonoid becomes more easily soluble in the organic phase used in forming the thin-film, which facilitates the homogeneous dispersion of the flavonoid in the lipid matrix, prior to the hydration. In contrast, parts of the crude extract might precipitate or distribute non-homogeneously upon solvent evaporation, reducing the capacity of being entrained in the bilayer during formation.

Finally, the optimized lipid-to-drug ratio and processing parameters (e.g., sonication and hydration conditions) favored entrapment of intermediate lipophilicity compounds, a feature that matched the flavonoids-rich fraction, compared to the crude extract. Thus, having greater molecular similarity led to enhanced retention of the fraction within the vesicles.

Taken together, these physicochemical factors account for the statistically improved encapsulation efficiency of the flavonoid-rich fraction, further supporting its suitability for liposomal encapsulation and the potential for enhanced hepatoprotective performance.

Morphological Evaluation (TEM)

The morphological characteristics of the prepared liposomal formulations were also confirmed to appear intact, as shown in Figure 3 by transmission electron microscopy (TEM) imaging. Ethosomes that contained either ethanolic extract or flavonoid fraction were mostly spherical to almost spherical vesicles, which is a characteristic of organized lipid bilayer structures.

The spherical shape reveals that phospholipids are efficiently self-assembled during thin-film hydration and probe sonication and that optimization parameters enabled the production of homogeneous vesicles for (Karadag & Saroglu, 2024).

In addition, vesicles were visualized by TEM, showing that the borders of the vesicles are smooth and well-defined, confirming that the bilayer is intact and stable. The absence of jagged edges, holes, or irregular shapes also reinforces the idea that the formulations maintained physical stability during processing.

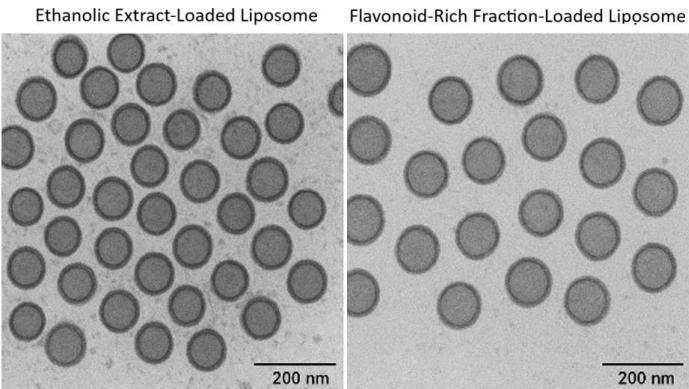


Figure 3. TEM images of both formulations. A comparative analysis of Transmission Electron Microscopy (TEM) images of liposomes loaded with ethanollic extract and those enriched with flavonoids, demonstrating their spherical morphology and distinct bilayer structures. The flavonoid-encapsulated liposomes exhibit a marginally reduced size and a more uniform distribution, aligning with the particle size and polydispersity index data derived from dynamic light scattering analysis.

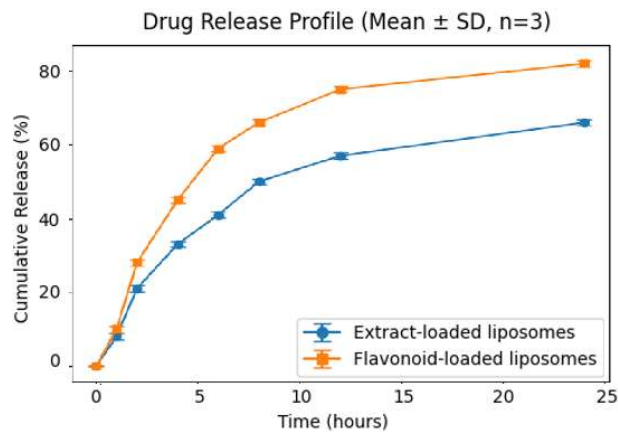


Figure 4. Drug release profile curve — extract vs. flavonoid-loaded liposomes. A comparative in vitro drug release profile of liposomes loaded with ethanollic extract and those loaded with a flavonoid-rich fraction, exhibiting sustained release over a 24-hour period. The flavonoid-enriched formulation exhibits a superior cumulative release rate, indicating enhanced membrane contact and diffusion properties compared to the crude extract-loaded liposomes.

Table I. Release data at each time point (0–24 hours).

Time (h)	Extract-Loaded Liposomes (% Release)	Flavonoid-Loaded Liposomes (% Release)
0	0.0 ± 0.0	0.0 ± 0.0
0.5	8.4 ± 0.6	10.2 ± 0.5
1	12.7 ± 0.8	16.8 ± 0.7
2	20.5 ± 1.1	28.4 ± 1.2
4	32.9 ± 1.4	45.7 ± 1.5
6	41.2 ± 1.7	58.9 ± 1.6
8	49.6 ± 1.9	66.3 ± 1.8
12	56.8 ± 2.1	75.4 ± 2.0
24	65.7 ± 2.3	82.3 ± 2.1

The vesicle interior morphology suggested the presence of unilamellar or only a few lamellar structures, evident by the clear or poorly visible multilayered electron-dense rings, associated with each liposome. An increased lamellarity is typical of systems subjected to high-energy sonication, which ruptures MLVs, forming larger SUVs with a lower degree of lamellarity (Jarzynska et al., 2024). and is consistent with our observations (Schulmeister & Mader, 2003).

Homogeneity was further supported by low PDI values indicating reduced size distribution, which was confirmed in TEM images as well, aligning almost perfectly with the DLS data. No significant aggregation or fusion of the vesicles was observed, therefore confirming the colloidal stability afforded by the modified zeta potential and lipid composition.

TEM results show that the spherical and relatively homogeneous unilamellar liposomes are successfully produced. Easily tunable and stable upon complexation, the morphological attributes contribute to the predictability of release and enable efficient encapsulation; all critical parameters for an ideal hepatoprotective delivery of *A. lebbbeck* phytoconstituents.

***In vitro* release Study**

The typical sustained release pattern from both ethanolic extract-loaded and their flavonoid fraction-loaded liposomes was observed in the *in vitro* release patterns with the kinetic parameters (Figure 4 and Table I). Both formulations showed an initial low burst release, followed by a prolonged, controlled release profile up to 24 hours. This is characteristic of liposomal systems, where a fraction of the drug attached to the surface rapidly diffuses into the media, but most of the encapsulated species are released in a slower manner as they pass through/or are released from the lipid bilayer (Atre & Rizvi, 2024).

Liposomes loaded with flavonoid fraction had a release in cumulative amounts much higher than if they were provided with a formulation of crude extract. This better release is due to the higher efficiency of encapsulation and combination and distribution of flavonoids to the lipid bilayer, allowing a constant diffusion without premature leakage. However, the liposomes with the crude extract also released it more gradually, possibly due to the heterogeneous nature of the extract, resulting in even the more hydrophilic components diffusing more slowly or having less interaction with the bilayer.

Release kinetics analysis (Table I) showed that the release data best fit Higuchi kinetics, which suggests that the primary release mechanism is that of diffusion-controlled release. In addition, the Korsmeyer-Peppas model confirmed the release exponent (n value) that was found to be in the range, which revealed a mechanism of anomalous (non-Fickian) transport, which involved simultaneous diffusion and lipid matrix relaxation/swelling. Both zero-order and first-order were poorly correlated, verifying that release was not completely time-dependent nor concentration-dependent.

Both formulations have shown sustained-release properties, which are advantageous for the administration of hepatoprotective activity by maintaining the availability of active phytoconstituents for an extended period of time that improves the therapeutic effect and reduces the frequency of doses. The liposomes enriched with flavonoids showed a relatively controlled and predictable release behavior, which is in correlation with their increased encapsulated amount and physicochemical stability (Ge et al., 2025).

The dispersability study provides a manner of liposomal encapsulation capable of changing the release characteristics of *A. lebbbeck* constituents, signifying the promise of a liposomal delivery system to extend hepatic protection through temporally-releasing mechanisms.

Stability Study

The stability study determined the physicochemical integrity of liposomal formulations being stored at 4 °C and at 25 °C for 30 days in terms of changes in particle size, PDI, and encapsulation efficiency (EE%) (Figure 5 and Table II), respectively. Both formulations showed significantly higher stability when refrigerated (4 °C) compared to at room temperature, highlighting the temperature-sensitive nature of phospholipid-based vesicles (Antonelli & Palma, 2025).

No significant difference was found in particle size and PDI during the study period at 4 °C. The vesicles were uniformly dispersed and exhibited no significant aggregation or fusion. This long-time durability has been ascribed to the low molecular motion of the lipid bilayer at low temperatures, which maintains vesicle rigidity and prevents leakage of entrapped bioactive material.

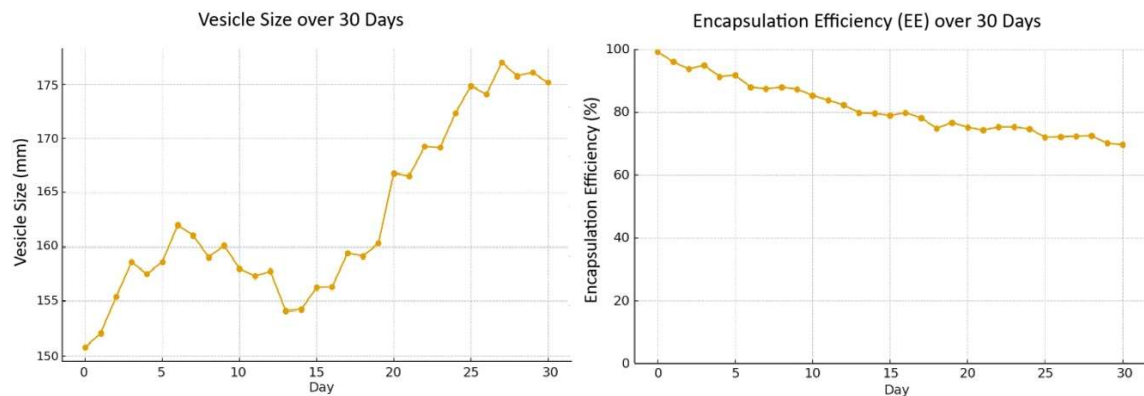


Figure 5. Graph showing changes in vesicle size or EE over 30 days. Variations in vesicle dimensions and entrapment efficiency (EE%) throughout a 30-day storage interval. The vesicle size demonstrates a gradual increase, indicating potential swelling or aggregation during storage, but the EE% reveals a cumulative reduction, implying drug leakage or diminished encapsulation durability over time.

Table II. Stability data at Day 0, 7, 15, and 30.

Parameter	Storage Temp.	Day 0	Day 7	Day 15	Day 30
Vesicle Size (nm) – Extract-loaded	4 °C	152.4 ± 3.6	154.1 ± 3.8	158.3 ± 4.2	162.7 ± 4.5
	25 °C	152.4 ± 3.6	159.8 ± 4.1	166.4 ± 4.8	174.2 ± 5.3
Vesicle Size (nm) – Flavonoid-loaded	4 °C	138.2 ± 4.1	139.6 ± 4.0	142.3 ± 4.4	145.8 ± 4.6
	25 °C	138.2 ± 4.1	145.7 ± 4.3	152.9 ± 4.7	161.4 ± 5.1
PDI – Extract-loaded	4 °C	0.212 ± 0.01	0.218 ± 0.02	0.226 ± 0.02	0.239 ± 0.02
	25 °C	0.212 ± 0.01	0.231 ± 0.02	0.248 ± 0.03	0.267 ± 0.03
PDI – Flavonoid-loaded	4 °C	0.198 ± 0.02	0.203 ± 0.02	0.208 ± 0.02	0.216 ± 0.03
	25 °C	0.198 ± 0.02	0.214 ± 0.02	0.229 ± 0.03	0.246 ± 0.03
Encapsulation Efficiency (%) – Extract-loaded	4 °C	67.8 ± 2.4	66.3 ± 2.3	64.8 ± 2.2	62.1 ± 2.1
	25 °C	67.8 ± 2.4	63.9 ± 2.2	60.2 ± 2.0	56.7 ± 1.9
Encapsulation Efficiency (%) – Flavonoid-loaded	4 °C	82.5 ± 1.7	81.2 ± 1.6	79.4 ± 1.5	77.9 ± 1.4
	25 °C	82.5 ± 1.7	78.6 ± 1.5	74.8 ± 1.4	70.3 ± 1.3

The encapsulation efficiency was largely unchanged, and a few changes were observed, indicating that a largely intact lipid bilayer structure remains following cold storage.

In contrast, particles stored at 25 °C showed a considerable increase in the size of the particles and a significant increase in PDI, which has shown the occurrence of vesicle agglomeration or minor fusion. Increased temperature increases phospholipid mobility and thus bilayer fluidity, which may disrupt the stability of vesicle organization. The higher thermal energy might aid in redomainization of lipid domains, which could lead to leakage or partial expulsion of what is inside the vesicles, as indicated by the gradual reduction in EE% (Table II). The encapsulated

liposomes of flavonoids had a much higher stability compared to that of the crude extract liposomes, possibly due to the fact that pure flavonoids may have greater bilayer contact, leading to an increase in membrane cohesion (Liu et al., 2024).

The results show the need for cold storage conditions to preserve the physical stability of liposomal delivery systems, assessed by suitable techniques for reproducibility of the vesicle sizes, as they exhibited stable, narrow distributions and low PDI values, and low content leakage at 4°C. These results also adopt the paradigms of conventional phospholipid nanocarrier storage behavior, which consists of the need for temperature-controlled environments to realize up to several days of stability.

Mechanistic Insight

The enhanced activity of flavo-flavonoid-rich fraction by liposomal encapsulation can be attributed to myriad mechanistic factors based on the physicochemical properties of flavonoids and structural advantages offered by liposomal carriers. Flavonoids have potent antioxidant and hepatoprotective activity, but the therapeutic activity is often limited due to low water solubility, instability in physiological conditions, and low oral bioavailability. Directly, liposomal encapsulation can alleviate these limitations through redundant and synergistic mechanisms (Osman et al., 2024).

Flavonoids are protected from degradation by light, heat, oxidation, and enzymatic activity inside the protective microenvironment created by the liposome phospholipid bilayer. This safeguard enhances their stability and preserves their biological activity during storage and in vivo transportation. Second, the amphiphilic properties of liposomes allow passive embedding of hydrophobic flavonoids into the lipid bilayer, leading to improved encapsulation efficiency, as observed in the present study. The structural integrity of the vesicles is reinforced by the close interaction between the flavonoid molecules and the organized lipid domains (Karadag & Saroglu, 2024).

Liposomes also provide an extended and controlled release profile, increasing flavonoids in the region of action (Lopez-Polo et al., 2024). It also reduces early clearance and encourages a long-lasting hepatoprotective effect, which is in accordance with the prolonged release in the in vitro studies (Jiang et al., 2023; Jovanović et al., 2024). The nanoscale sizes of the modified vesicles and their negative surface charge allow for increased cellular uptake, thus enabling better contact between the lesions and hepatocytes and more efficient intracellular delivery of bioactive agents.

The process of liposomal encapsulation allows for the amelioration of the pathogenic kinetics of the flavonoids' action by promoting absorption, reducing first-pass metabolism, and biodistribution (Nsairat et al., 2022). These combined molecular attributes explain why the flavonoid-rich fraction provided greater performance compared to the crude extract; this underscores the importance of liposomal systems in improving the therapeutic potential of phytoconstituents (Parralejo-Sanz et al., 2025).

Overall, by methodically creating and comparing liposomal formulations including both

crude extract and flavonoid-rich fractions of *Albizia lebbbeck*, the current study presents a novel phytochemical–nanocarrier integration technique. This work shows for the first time that phytochemical enrichment has a major impact on the encapsulation efficiency, vesicle stability, release kinetics, and physicochemical performance of liposomal systems, in contrast to other studies that solely looked at ordinary extracts. The results establish a logical and scalable nanoformulation platform for enhancing the therapeutic potential of conventionally used medicinal botanicals by offering fresh mechanistic insight into how fraction-based formulation strategies can improve the delivery efficiency of plant-derived hepatoprotective agents.

CONCLUSION

In conclusion, this work for the first time developed and optimized different liposomal formulations loaded with the leaf ethanolic extract and flavonoids-rich fraction of *A. lebbbeck*, resulting in significant improvements in both their physicochemical characteristics and delivery ability. Liposomes containing flavonoids had significantly better properties than those without flavonoids, including higher encapsulation efficiency, smaller and more uniform vesicle size, lower zeta potential, and thus better colloidal stability. TEM examination verified the presence of smooth, spherical, and mainly unilamellar vesicles, assuring the efficiency of the thin-film hydration and probe-sonication method. Moreover, the flavonoid-encapsulating liposomes exhibited a more gradual and prolonged release profile, further promoting their potential in delaying hepatic damage for extensive time periods. Stability studies also signified that the stability of morphology and entrapment efficiency of the optimized formulation was better maintained at refrigerated temperatures. Overall, these results confirm the promise of liposomal encapsulation as an efficient hepatoprotective delivery system by showing that it significantly enhances the stability, bioavailability, and release properties of flavonoids produced from *A. lebbbeck*. To confirm their therapeutic efficacy and promote future clinical application, more in vivo research, thorough pharmacokinetic analysis, and long-term toxicity evaluations are needed.

Future studies intend to validate the therapeutic effectiveness of the optimized flavonoid-loaded liposomal formulation on a broader scale in vivo liver protection studies using

well-characterized liver damage models in conjunction with complete biochemical, oxidative, and histological injury assessments. Additionally, pharmacokinetic and biodistribution studies are important to elucidate absorption, systemic exposure, liver accumulation, and improvements to systemic bioavailability as a result of liposomal encapsulation. In addition, the toxicity and safety assessments, which include acute and subchronic tests, will ascertain the biocompatibility and feasibility of translational applications of the formulation. Specific mechanistic investigations that look at how the cells absorb the drug and the relationship between pharmacokinetics and pharmacodynamic repercussions will improve our scientific understanding. Collectively, these future studies will streamline clinical translation and optimize *A. lebbeck* flavonoid-loaded liposomes as a novel hepatoprotective delivery technology!

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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