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Original Article

Berberine Chloride Phytosomes: Development and Evaluation of a Novel Phospholipid Based Delivery System

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ABSTRACT

Background: Phytosomes are an advanced drug delivery system designed to improve the solubility, stability, and bioavailability of plant-based compounds. **Objectives:** This study aimed to formulate and characterize berberine chloride-loaded phytosomes using soya lecithin. **Materials and Methods:** Berberine chloride was isolated from the ethanolic extract of *Berberis aristata* roots and characterized by UV, FTIR, TLC, SEM, mass spectroscopy, and ^{1}H NMR. Phytosomes were prepared in two different ratios, 1:1.5 (Batch A) and 1:2 (Batch B), using the rotary evaporation method. Batch A (1:1.5) was selected based on compound microscopy and physical characters and subjected further to characterization techniques including SEM, FTIR, DSC, particle size analysis, zeta potential, entrapment efficiency, and drug loading. **Results:** Compound microscopy of phytosomes showed uniform particle distribution and loss of crystallinity of berberine chloride. SEM confirmed spherical, aggregated phytosomal particles, while FTIR indicated drug-phospholipid interaction through peak shifts. DSC thermograms exhibited endothermic peaks at 214.19ºC, supporting successful complexation and thermal stability. Particle size and zeta potential were found to be 125.7 nm and -24.3 mV, suggesting nanoscale particles with good colloidal stability. Entrapment efficiency and drug loading were 65% and 103.93%, respectively. **Conclusion:** The study concluded that berberine chloride phytosomes were successfully developed and characterized, despite lacking a hydroxyl group, complexation occurs through hydrophobic interaction and Van der Waals force with the methoxy group and electrostatic interactions between its quaternary nitrogen and the phospholipid's phosphate group.

Keywords: Berberine chloride phytosomes, *Berberis aristata*, Entrapment efficiency, Phospholipid, Zeta potential.

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INTRODUCTION

Phytosomes are a novel drug delivery system utilized to increase the bioavailability of phytoconstituents. It includes conjugating plant materials or extracts with phospholipids. Phospholipids are comprised of a hydrophilic head and a hydrophobic tail. The water-soluble free drug is entrapped inside the hydrophilic head. This technology has gathered interest within the pharmaceutical community because of its ability to improve the solubility of poorly soluble plant extracts and increase absorption (Nandhini and Ilango, 2020). Phytosome complexes shaped between a phytoconstituent and phospholipid contain a liposomal structure but are stable and have favorable pharmacokinetics, hence increasing the absorption of phytoconstituents and improving therapeutic effects (Kapse and Mulla, 2024).

B. aristata (family *Berberidaceae*) is an important medicinal plant, and nearly each part of the plant has a few therapeutic values. Its root, stem, bark, and fruits have been utilized in numerous Ayurvedic and Unani preparations (CSIR, 1988; Awari *et al*., 2024; Basera, *et al*., 2021). Berberine is an isoquinoline alkaloid with a wide run of pharmacological and biochemical effect (Sood *et al*., 2019). It occurs in the roots, rhizomes, and stem bark of *B. aristata*. It shows antimicrobial, antidiarrheal, antihypertensive, antiarrhythmic, anti-inflammatory, and antineoplastic activities are well established. *B. aristata* is utilized in the indigenous system of medicine as a tonic, demulcent, diaphoretic, diuretic, and alternative and is used to cure different illnesses such as wound healing, skin infections, rheumatism, snakebites, menorrhagia, jaundice, and ophthalmic diseases (Kirtikar, 1992; Nadkarnis,1982).

The therapeutic potential of berberine chloride was challenged due to its limited aqueous solubility and naturally occurring hydrophobic characteristic (Fan and Zhang, 2019).

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Usually, phytosomes are made through phytomolecules, which are anchored through chemical bonds to the polar choline head and the lipid-soluble hydrophobic phosphatidyl portion comprising the body and tail, which envelops the choline-bound material.

The formation mechanism of berberine chloride phytosomes differs from that of conventional phytosomes because berberine lacks a free hydroxyl group and instead contains a methoxy group, which interacts primarily through hydrophobic interactions and Van der Waals forces to stabilize the complex. Additionally, the quaternary nitrogen in berberine carries a positive charge that electrostatically interacts with the negatively charged phosphate group of the phospholipid head, further contributing to complex stability. The main objective of this research is to develop and characterize berberine chloride phytosomes with soy lecithin.

MATERIALS AND METHODS

Chemicals and Reagents

Soya lecithin, n-hexane, Dichloromethane, Ethanol (95% pure), Methanol, Ethyl acetate, Formic acid, Glacial acetic acid, Distilled water, Conc. HCl, Dragendorff's spray reagent.

Instruments

Rotary evaporator (Roteva), UV-visible spectrophotometer (UV-3200 Lab India), FTIR (Agilent Cary 630), DSC (Shimadzu), SEM (JEOL Ltd.,) Particle size analyzer (HORIBA SZ-100), Zeta potential analyzer (HORIBA SZ-100), NMR (Bruker), and HR-LCMS (Agilent LCMS)

Preparation of *B. aristata* Ethanolic Extract

B. aristata root powder (purchased from Dagduteli Kashtaushadhi shop, local market, Nashik) was extracted using alcohol by the Soxhlet extraction method. The solution was filtered and concentrated to obtain the *B. aristata* ethanolic extract.

Isolation of Berberine Chloride

B. aristata ethanolic extract was treated with concentrated hydrochloric acid till precipitation ceased and kept the solution overnight, which resulted in the formation of crystals of berberine chloride (Kokate CK, 1994). The solution was then filtered using vacuum filtration, and the yellow mass obtained was dried, recrystallized, and subjected to characterization through chemical tests for general alkaloids, TLC, UV in methanol (5 mg in 5 mL), compound microscopy, SEM, DSC, FTIR, mass spectrometry, and NMR.

TLC parameters followed as Stationary phase-Silica gel GF254, mobile phase- Ethyl acetate: Formic acid: Glacial acetic acid: Water (8:1:1:2), Track-01, Sample- isolated berberine chloride in methanol (10 mg/mL), Spray reagent- Dragendorff's (Mukherjee PK, 2005; Wagner H and Bladt S, 2007).

Preparation of Phytosomes

Isolated berberine chloride and soya lecithin were taken in two different ratios: 1:1.5 (Batch A) and 1:2 (Batch B), respectively. Each batch was treated separately and mixed with 20 mL of dichloromethane, followed by stirring at 40°C in a rotary evaporator for 1 hr to form a thin film. A few milliliters of n-hexane were added, and the mixture was centrifuged. The sediments were collected and dried (Barani *et al.*,2021; Gungor, 2024).

Both batches were examined physically and subjected to compound microscopy and batch A was selected as the optimized batch for further analysis of phytosomes based on its result. It was further characterized by SEM, FTIR, DSC, particle size analysis, zeta potential, entrapment efficiency, and drug loading (Tripathy, 2013; Patil, 2023; Dewi, 2024; Vrushabh, *et al.*, 2023).

Characterization of Berberine Chloride Phytosomes

(Das and Kalita, 2014; Sharma and Sahu, 2016; Metkari, 2023).

Compound Microscope

A few milligrams of berberine chloride and berberine chloride phytosomes dry powder, both batches were mounted individually and examined under the compound microscope at 10x and 45x magnifications. The images helped visualize the development of phytosomes at the laboratory level.

SEM

The surface morphology of the phytosomes was analyzed using Scanning Electron Microscopy (SEM). For sample preparation, the phytosome powder was placed on double-sided adhesive carbon tape and coated uniformly with a thin layer of gold in a vacuum to improve conductivity. The examination was conducted at a voltage of 20 kV, and images were captured at magnifications of 500× and 2000×. Berberine chloride was also analyzed to observe its development and uniform structure.

FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was employed to identify the functional groups of the phytosomes and berberine chloride. About 2 mg of each sample was carefully ground with 200 mg of dry Potassium Bromide (KBr) at a 1:100 ratio and pressed into a thin, clear pellet using a hydraulic press. FTIR spectra were recorded over a scanning range of 4000-400 cm^{-1} to identify distinctive absorption peaks of functional groups. Overlay studies of phytosomes and berberine chloride were also carried out as shown in Figure 3 (Pavia *et al.*, 2009; Singh *et al.*, 2023).

DSC

The thermal properties of the phytosomes were determined using DSC. Approximately 5-10 mg of each sample was precisely weighed

and sealed in an aluminum pan, while an empty pan served as the reference. The analysis was carried out under nitrogen gas at a flow rate of 50 mL/min, with a heating rate of 10°C/min, across a temperature range of 30°C to 300°C. The obtained thermogram was used to determine melting point, crystallinity, and potential interactions. Overlay studies of phytosomes and berberine chloride were performed as shown in Figure 4.

Particle Size and Zeta Potential

The particle size and zeta potential of the phytosomes were determined using Dynamic Light Scattering (DLS) with a Zetasizer. A diluted sample of the phytosome formulation was prepared in distilled water and analyzed at 25°C. The results were presented as average particle size distribution and surface charge, indicating the formulation's stability.

Entrapment Efficiency and Drug Loading

A sample of berberine chloride phytosomes (5 mg) was dissolved in 5 mL of methanol and centrifuged at 6000 rpm. The supernatant was subjected to UV absorbance measurement. A prior linearity curve was developed at 345 nm (λ_{max}) for standard berberine chloride (10 ppm), and entrapment efficiency was calculated using the following formulas:

$$\text{Entrapment efficiency} = \text{Total amount of drug} - \frac{\text{Amount of free drug}}{\text{Total amount of drug}} \times 100$$

$$\text{Drug loading} = \frac{\text{Total weight of phytosomes}}{\text{Amount of drug in phytosomes}} \times 100$$

RESULTS

B. aristata ethanolic extract yield was obtained as 29.60% w/w with a reddish-brown sticky semisolid mass.

Characterization of isolated Berberine chloride

Its crystals are obtained as a bright yellow color. The general chemical test of alkaloids by Mayer's reagent, Dragendorff's reagent, Wagner's reagent, and Hager's reagent gave buff, yellowish-orange, reddish-brown, and yellow ppt, respectively for it.

For TLC condition a single-track sample application gave a single yellow spot in daylight before spray and, after spray with Dragendorff's reagent, gave an orange color, at R_f 0.60.

Compound microscopy evaluation shows the yellow needle shaped crystal-like structure at 10x and 45x for it. SEM shows the sharp, angular appearance shape, which demonstrates crystalline structure.

The FTIR spectrum of pure isolated berberine chloride showed characteristic peaks at 2846 cm^{-1} for the methoxyl group, 1606.92 cm^{-1} for aromatic C=C stretching, and 1566.20 cm^{-1} for the isoquinoline alkaloid (C=N stretch), as shown in the FTIR overlay with phytosomes as the upper spectrum line.

The DSC thermogram of pure isolated berberine chloride showed a sharp endothermic peak around 190-195°C, corresponding to its melting point, indicating its crystalline nature as shown in DSC overlay with phytosomes as the upper blue line.

The mass spectrum of isolated berberine chloride showed a major peak at m/z 336.39, corresponding to its molecular ion, while minor peaks at m/z 337.43 and 338.44 represented isotopic forms, confirming its molecular weight and purity.

The ^{1}H NMR spectrum of isolated berberine chloride showed a peak at 3.99 ppm corresponding to the methoxy group (-OCH_3), signals between 7.18-7.60 ppm for aromatic protons of the isoquinoline ring, and a peak at 4.92 ppm for the methylene proton, confirming the isoquinoline skeleton.

Berberine chloride phytosomes Batch A (1:1.5) and Batch B (1:2), examined physically shows a yellowish brown color, more darkening of brown tinge and sticky masses are observed for Batch B.

Microscopic images of berberine chloride phytosomal batches as shown in Figure 1 (Batch A: a to d, Batch B: e to h) observed under 10× and 45× magnification in both bright and dark phases

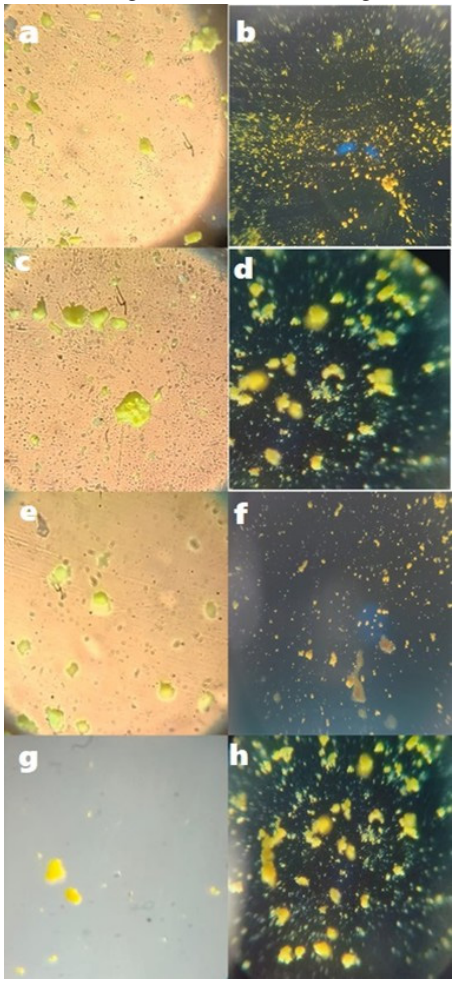

Figure 1: Compound Microscopy of Berberine chloride phytosomes, for Batch A (1:1.5): a,to d ; Batch B (1:2): e to h; 10 x : a, b, e, f ; 45 x : c, d, g, h; at Bright light : a, c, e, g ; at Dark phase : b, d, f, h.

showed well-developed, small, yellowish-green sub-spherical shape particles. No needle-shaped crystalline forms of berberine chloride were visible. Secondly, among the observed batches, Batch A showed fine, well-dispersed, yellowish-green particles with fewer aggregates under both bright and dark phase microscopy, compared to Batch B.

Batch A shows the following characterization results.

The SEM image of isolated berberine chloride is presented in Figure 2a**,** where the particles exhibit sharp edges and a distinctly angular appearance, consistent with a crystalline texture. In contrast, the SEM image of the phytosome formulation (Batch A), shown in Figure 2b, revealed predominantly spherical to slightly irregular particles, indicating a non-crystalline or semi-amorphous morphology. The surface appeared mildly rough, suggesting aggregation of smaller subunits. The scale bar (10 µm and 50 µm) indicated that most particles were within the 150-800 nm range, confirming a nano- to submicron size. The uniform contrast and absence of sharp edges further support the loss of crystallinity after complexation. A few particles appeared fused at contact points, likely due to phospholipid coating or partial coalescence during drying.

The overlay FTIR spectrum in Figure 3 shows, two lines: the upper line represents isolated berberine chloride and the lower line represents the berberine-phytosome complex. In berberine chloride, characteristic peaks are observed near 1605 cm^{-1} (aromatic C=C stretching) and 1250-1030 cm^{-1} (C-O-C stretching). In the phytosome spectrum, additional bands appear at around 1735 cm^{-1} and 1230 cm^{-1}, corresponding to the ester (C=O) and phosphate (P=O) groups of the phospholipid.

The DSC thermogram of berberine chloride phytosomes gave the endothermic peak at 214.19°C, which indicates the crystallinity with successful formation of a stable, amorphous phytosomal complex. The overlay matches the compatibility between berberine chloride and its phytosomes, as shown in Figure 4.

The average particle size of the formulated phytosomes was 125.7 nm with a PDI of 0.828, indicating a relatively narrow and uniform size distribution. The zeta potential was -24.3 mV, confirming negatively charged and stable particles.

The entrapment efficiency of the berberine chloride phytosome was found to be 65%**,** while the drug loading capacity was 103.93%, indicating efficient incorporation of berberine within the phospholipid complex.

DISCUSSION

All four basic general chemical tests for alkaloids are positive for isolated crystals confirming alkaloidal nature of it. TLC gave a single spot indicating purity and again positive spray test for alkaloids. Compound microscopy and SEM reveal pure crystalline shape structure of it.

FTIR characteristic absorption bands confirm the functional groups present in berberine chloride, indicating its purity and structural stability before complex formation.

The sharp endothermic peak in DSC confirms the crystalline and thermally stable form of berberine chloride, supporting its purity prior to complex formation.

The observed molecular ion peak and its isotopic pattern agree with the expected mass of berberine chloride, validating its structural identity and purity.

The characteristic ^{1}H-NMR peaks confirm the structural framework of berberine chloride, consistent with its isoquinoline alkaloid nature.

The absence of crystalline structures and the presence of uniformly shaped sub spherical particles in lab level compound microscopy suggest that berberine chloride was successfully complexed with phospholipids, confirming effective phytosome formation.

The reduced aggregation in Batch A indicates better dispersion and stable phytosome formation, suggesting an optimal

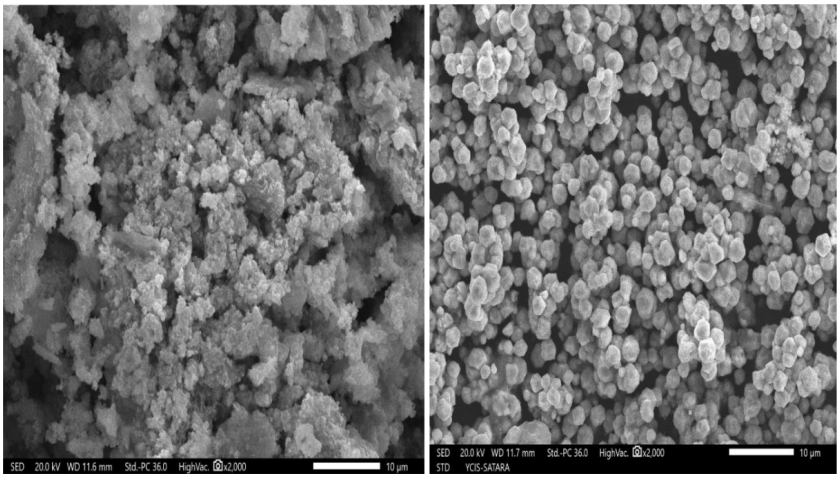

Figure 2: SEM image of (a) isolated Berberine chloride and (b) phytosomes (1:1.5).

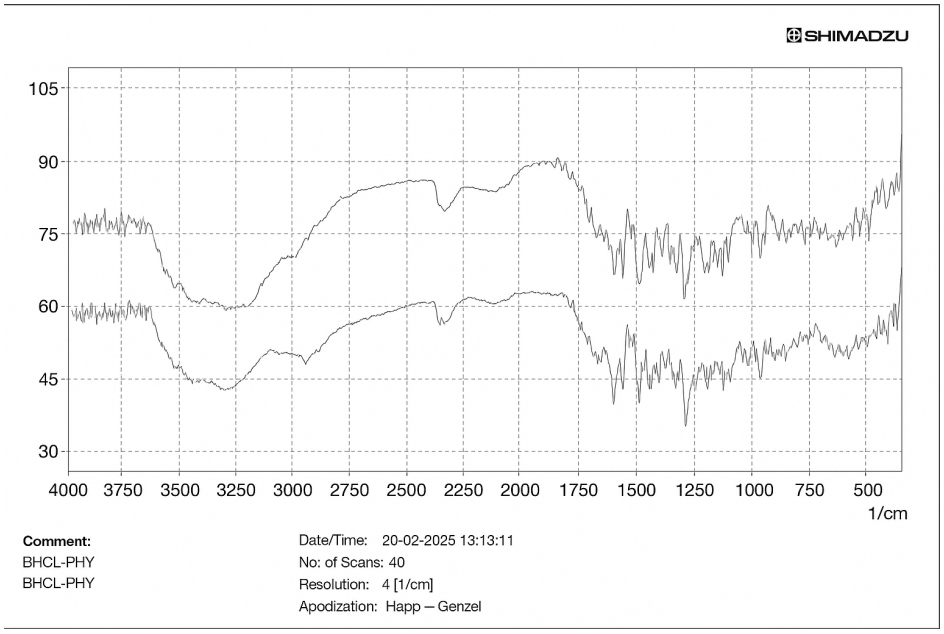

Figure 3: FTIR Overlay of isolated Berberine chloride and Berberine chloride phytosomes.

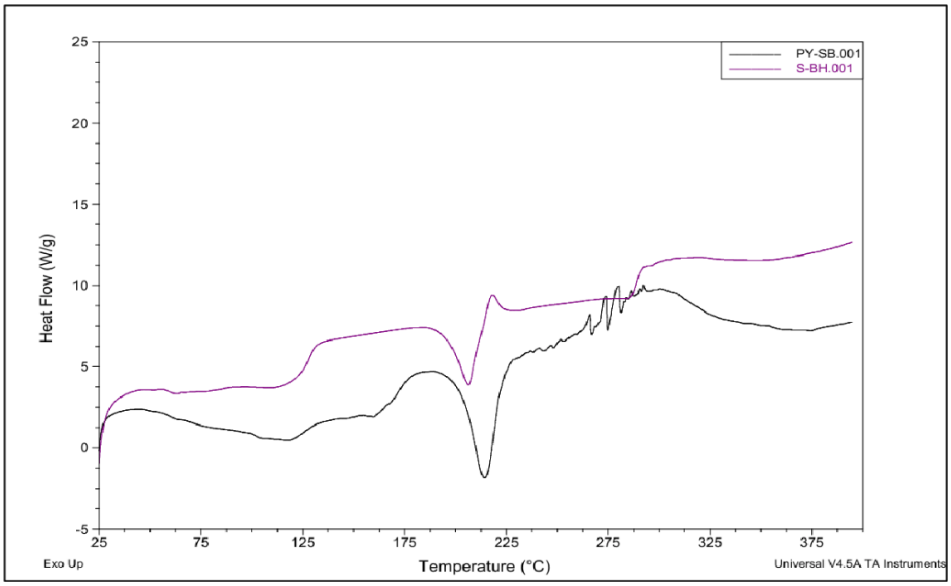

Figure 4: DSC Overlay of isolated Berberine chloride and Berberine chloride phytosomes.

drug-phospholipid interaction and uniform complexation. Hence, Batch A was selected for further evaluation.

The observed spherical morphology and reduced crystallinity confirm successful phytosome formation, in SEM. The semi-amorphous nature indicates effective complexation between berberine chloride and phospholipids. Minor aggregation and fusion of particles are typical of dried phytosomal systems and reflect good compatibility between components. The overall uniformity and nano-sized range suggest improved stability and potential for enhanced bioavailability.

In the FTIR overlay spectrum, the shifts and broadening of peaks in the phytosome spectrum compared with pure berberine chloride indicate interaction between the drug and the phospholipid. The displacement of the C=O and P=O bands suggests electrostatic bonding between the positively charged quaternary nitrogen of berberine and the negatively charged phosphate group of the phospholipid. These spectral changes confirm the successful formation of a stable berberine-phospholipid complex through hydrophobic and Van der Waals interactions.

The nanoscale size suggests successful phytosome formation with good dispersion uniformity. The negative surface charge in zeta potential likely originates from the phosphate head groups of phospholipids, which contribute to electrostatic repulsion and prevent aggregation. Overall, the results indicate satisfactory colloidal stability of the formulation.

The moderate entrapment efficiency suggests that a substantial amount of berberine was successfully complexed with phospholipids despite its quaternary nature and limited solubility.

The high drug loading value indicates good compatibility between berberine and the lipid matrix. These results confirm that the formulation method effectively improved berberine's association with phospholipids, which may enhance its stability and permeability in further applications.

CONCLUSION

The developed berberine chloride phytosomes exhibited nanosized, uniform, and stable particles with a negative zeta potential, confirming good dispersion stability. Spectroscopic and thermal analyses confirmed successful complexation without structural degradation. The formulation also showed satisfactory entrapment efficiency and drug loading, indicating efficient incorporation within the lipid matrix. Overall, these findings confirm that the phytosomal approach effectively enhances the physicochemical stability and potential skin permeation of berberine chloride for topical therapeutic applications.

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ABBREVIATIONS

***B. aristata*:** *Berberis aristata*; **Conc.:** Concentrated; **^{o}C:** Degree Celsius; **DLS**: Dynamic Light Scattering; **DSC:** Differential Scanning Calorimetry; **FTIR:** Fourier Transform Infrared Spectroscopy; **HCl:** Hydrochloric Acid; **^{1}H-NMR:** Hydrogen Proton Nuclear Magnetic Resonance; **HR-LCMS:** High-Resolution Liquid Chromatography-Mass Spectrometry; **KBr:** Potassium Bromide; ***m/z*:** Mass-to-Charge Ratio; **mV:** Millivolt; **nm:** Nanometer; **PDI:** Polydispersity Index; **ppm:** Parts Per Million; **R_f:** Retention Factor (in TLC); **rpm**: Revolutions Per Minute; **SEM:** Scanning Electron Microscopy; **TLC:** Thin Layer Chromatography; **UV:** Ultraviolet Spectroscopy; **w/w**: Weight by Weight;; **λ_{max}:** Maximum Wavelength of Absorbance; **µm:** Micrometer.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

The authors declare the project is self-funded.

ETHICAL STATEMENT

The research work did not involve human participants or animal subjects. Therefore, approval from the Institutional Ethics Committee was not required.

STATISTICAL ANALYSIS

The particle size, Polydispersity Index (PDI), and zeta potential values were obtained as direct instrumental readings for the prepared sample. Since only a single formulation was evaluated and no comparative groups were involved, statistical analysis was not required. The results are presented as observed values.

AUTHOR CONTRIBUTIONS

Dr. Daksha Attarde and Neeraj Lohagaonkar contributed to the study design, laboratory work, data analysis, and manuscript writing. Anushka Deore and Bhagyashree Borde contributed to laboratory work and manuscript writing. The final version of the manuscript was reviewed and approved by Dr. Daksha Attarde.

SUMMARY

Phytosomes serve as a promising lipid-based system to enhance the delivery of poorly soluble phytoconstituents such as berberine chloride. In this study, berberine chloride-loaded phytosomes were successfully formulated using soya lecithin by the rotary evaporation method and optimized at a 1:1.5 ratio. Microscopic and SEM analyses revealed spherical, well-dispersed particles with reduced crystallinity, while FTIR and DSC confirmed complex formation through hydrophobic, Van der Waals, and electrostatic interactions between berberine and phospholipids. The nanosized particles (125.7 nm) with a zeta potential of -24.3 mV indicated good stability, and entrapment efficiency (65%) along with drug loading (103.93%) demonstrated efficient incorporation. Collectively, these findings confirm that phytosome formation significantly improved the physicochemical characteristics and compatibility of berberine chloride, supporting its potential for enhanced formulation stability and bioavailability.

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