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ORIGINAL ARTICLE

FORMULATION, OPTIMIZATION, CHARACTERIZATION AND *IN VITRO* EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL OF APIGENIN-LOADED HERBOSOMES: A NOVEL PHOSPHOLIPID-BASED NANOCARRIER FOR ENHANCED DELIVERY OF BIOACTIVE FLAVONOIDS

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ABSTRACT : Apigenin, a naturally occurring flavonoid, is well recognized for its potent antioxidant and anti-inflammatory properties. However, its therapeutic application is significantly limited by poor aqueous solubility and low oral bioavailability. To overcome these challenges, the present study focused on developing a phospholipid-based herbosomal delivery system designed to enhance the solubility, stability and pharmacological effectiveness of apigenin. Herbosomes were formulated using the thin-film hydration method, with six different formulations (F_1–F_6) prepared by varying phospholipid concentrations to optimize performance. Each formulation was evaluated for key physicochemical parameters, including particle size, polydispersity index (PDI), zeta potential, entrapment efficiency and *in vitro* drug release profile. Among the formulations, F_6 exhibited the most promising characteristics, with a mean particle size of 128.9 nm, a PDI of 0.231 indicating uniformity, a zeta potential of –28.5 mV suggesting stability and a high entrapment efficiency of 87.9%. The drug release study revealed a sustained release profile for F_6, with 96.4% of apigenin released over 12 hours. Kinetic analysis indicated that drug release followed a first-order model and was governed by Fickian diffusion.

***In vitro* pharmacological assessments further demonstrated the superior efficacy of F_6. It exhibited strong antioxidant activity, with 86.9% DPPH radical scavenging and an IC_{50} of 39.2 µg/mL, along with notable anti-inflammatory effects, evidenced by 83.4% inhibition of BSA denaturation and 85.8% stabilization of human red blood cells (HRBC). These findings highlight the potential of apigenin-loaded herbosomes as an effective nanocarrier system for enhancing the delivery and bioactivity of poorly soluble phytoconstituents, particularly in the management of oxidative stress and inflammation-related condition.**

Key words : Apigenin, herbosomes, phospholipid-based drug delivery, antioxidant activity, anti-inflammatory activity, sustained release formulation.

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INTRODUCTION

Oxidative stress and inflammation are closely interconnected biological processes that play a central role in the development and progression of various chronic diseases, including cardiovascular disorders, neurodegenerative diseases, metabolic syndromes and cancer. Oxidative stress arises from an imbalance between the excessive generation of reactive oxygen

species (ROS) and the body's capacity to neutralize them through antioxidant defense systems. Inflammation, on the other hand, is an immune response triggered by harmful stimuli such as pathogens, damaged cells, or environmental toxins. The crosstalk between oxidative stress and inflammation amplifies cellular damage, thereby accelerating disease pathogenesis. As a result, natural compounds possessing both antioxidant and anti-inflammatory activities have gained significant attention as potential therapeutic agents for managing chronic inflammatory and oxidative stress-related conditions (Hussain *et al*, 2016; Hybertson *et al*, 2011; Orzechowski *et al*, 2019; Sawa and Sedlak, 2016; Steven *et al*, 2019).

Among the wide array of plant-derived bioactive compounds, apigenin—a flavonoid richly present in parsley, chamomile, celery, and various other medicinal plants—has garnered significant interest for its broad-spectrum pharmacological activities. Chemically identified as 42 ,5,7-trihydroxyflavone, apigenin is recognized for its potent antioxidant, anti-inflammatory, anticancer, neuroprotective, and cardioprotective effects. It exerts its antioxidant action by scavenging reactive oxygen species (ROS), while its anti-inflammatory properties are mediated through the modulation of key inflammatory molecules such as cytokines and prostaglandins. Additionally, apigenin is known to influence critical cellular signaling pathways, including nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), thereby contributing to its therapeutic efficacy. Despite its promising biological potential, the clinical utility of apigenin is considerably restricted by its poor water solubility, limited oral bioavailability, and rapid metabolic clearance. These pharmacokinetic challenges negatively impact its absorption, systemic distribution, and overall therapeutic effectiveness, underscoring the need for innovative delivery strategies to enhance its clinical applicability (Imran *et al*, 2020; Lee *et al*, 2023; Majma Sanaye *et al*, 2022; Xu *et al*, 2021; Yoon *et al*, 2023). To address the limitations associated with apigenin's poor solubility and bioavailability, extensive research has focused on the development of advanced drug delivery systems. Among the various nanocarrier platforms explored, herbosomes have recently emerged as a promising strategy to enhance the pharmacokinetic and therapeutic performance of phytoconstituents. Herbosomes are specialized phospholipid-based vesicular systems formed by complexing a bioactive plant extract or isolated phytoconstituent with phosphatidylcholine, resulting in a lipid-compatible molecular complex. Compared to conventional liposomes, herbosomes offer several advantages, including improved lipid solubility, enhanced membrane permeability, and higher entrapment efficiency. These features make herbosomes particularly well-suited for the delivery of poorly water-soluble compounds like apigenin, thereby enhancing its absorption, stability, and therapeutic efficacy (Lee *et al*, 2023; Yoon *et al*, 2023; Zhao *et al*, 2024).

Phospholipids, which are integral components of biological membranes, possess the unique ability to interact with active molecules through hydrogen bonding and van der Waals forces. When apigenin is complexed with phospholipids, it becomes embedded within lipid bilayers that closely resemble natural cellular membranes. This interaction significantly improves apigenin's aqueous solubility, chemical stability, and gastrointestinal absorption. In addition, the herbosomal delivery system offers protection against enzymatic degradation, facilitates efficient transport across cellular membranes, and enables sustained drug release. Collectively, these attributes contribute to enhanced bioavailability and more consistent therapeutic effects (Adouani *et al*, 2020; Loureiro Damasceno *et al*, 2022; Maiti *et al*, 2010). Numerous studies have confirmed that incorporating plant-based bioactives into nano-vesicular delivery systems significantly enhances their biological performance both in vitro and in vivo. Compounds such as curcumin, silybin, and quercetin have exhibited improved pharmacokinetic profiles and therapeutic efficacy when formulated with phospholipid-based nanocarriers. Despite these advancements, research specifically targeting the development of apigenin-loaded herbosomes remains limited. In particular, there is a noticeable gap in systematic investigations evaluating their physicochemical characteristics, drug release behavior and *in vitro* pharmacological activities. This highlights the need for comprehensive studies to explore and optimize such formulations for therapeutic use (Adouani *et al*, 2020; Loureiro Damasceno *et al*, 2022; Maiti *et al*, 2010).

Building upon the foundational insights into Apigenin's therapeutic limitations, the present study aimed to formulate and optimize Apigenin-loaded herbosomes using the thin-film hydration technique. The primary goal was to enhance Apigenin's aqueous solubility, encapsulation efficiency, and biological effectiveness by systematically varying the Apigenin-to-phospholipid ratio across six formulations (F_1 to F_6). Each formulation underwent thorough evaluation for critical physicochemical parameters, including particle size, polydispersity index (PDI), zeta potential, entrapment efficiency and morphological characteristics, to identify the most stable and efficacious formulation. Additionally, *in vitro* release studies were conducted to assess the sustained release

behavior of the herbosomal systems, and the drug release profiles were analyzed using established kinetic models such as zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations to elucidate the underlying release mechanisms.

To determine the therapeutic potential of the developed formulations, *in vitro* antioxidant and anti-inflammatory assays were performed. The antioxidant capacity was assessed using the DPPH radical scavenging method, while anti-inflammatory activity was evaluated through BSA protein denaturation and human red blood cell (HRBC) membrane stabilization assays. These assays are widely recognized for their mechanistic relevance and reproducibility in assessing oxidative and inflammatory protection.

Among all tested formulations, F_6, which contained the highest concentration of phospholipid, emerged as the most promising. This formulation exhibited optimal nanoscale dimensions, uniform particle distribution, a high degree of entrapment efficiency and a favorable sustained release profile. Furthermore, F_6 showed superior biological performance, evidenced by its lowest IC_{50} value in the DPPH assay and highest percentage inhibition in both anti-inflammatory models. These results clearly suggest that the herbosomal delivery system not only improves the physicochemical profile of Apigenin but also significantly enhances its pharmacological actions.

This investigation makes a noteworthy contribution to the evolving field of phytopharmaceutical nanomedicine by offering a viable strategy to circumvent the pharmacokinetic limitations of hydrophobic natural compounds. By combining advanced formulation design with robust biological assessment, the study lays a strong foundation for the development of clinically applicable nanocarriers of plant-based bioactives for managing oxidative and inflammatory diseases. In conclusion, the formulation of Apigenin-loaded herbosomes represents a novel and rational approach to improving the therapeutic utility of flavonoids. The positive outcomes of this study underscore the potential for future *in vivo* pharmacokinetic and pharmacodynamic evaluations, thereby facilitating the eventual clinical translation of such nanocarrier systems into effective and patient-friendly herbal therapeutics.

MATERIALS AND METHODS

Apigenin, a natural flavonoid with potent antioxidant and anti-inflammatory properties was procured from Sigma-Aldrich (USA) with a purity of ≥98%. Soybean phosphatidylcholine (phospholipid), used as the primary lipid carrier for herbosome formation was obtained from Lipoid GmbH, Germany. Cholesterol, which aids in enhancing the membrane rigidity and stability of vesicles, was sourced from Merck Chemicals (India). Organic solvents including chloroform and ethanol (analytical grade) were purchased from Merck and used without further purification. Additional reagents and chemicals used in the biological assays—such as DPPH (2,2-diphenyl-1-picrylhydrazyl), bovine serum albumin (BSA), and carrageenan—were acquired from HiMedia Laboratories, Mumbai, India. Distilled and deionized water was used throughout the formulation and evaluation process. All procedures adhered to the institutional laboratory protocols for chemical handling and waste disposal.

Formulation of Apigenin-loaded Herbosomes

Apigenin-loaded herbosomes were formulated using the thin-film hydration technique, a standard and effective approach for developing vesicular drug delivery systems. To begin the process, Apigenin and phospholipid were accurately weighed and combined in a molar ratio of 1:2. The components were dissolved in a 2:1 (v/v) mixture of chloroform and ethanol within a round-bottom flask. This solvent combination was specifically chosen for its compatibility with both hydrophobic and amphiphilic substances, ensuring complete solubilization of the drug and lipid components. The resulting solution was then subjected to rotary evaporation under reduced pressure using a Buchi Rotavapor® set at 40 ± 2°C. This step led to the gradual removal of solvents and facilitated the deposition of a thin, uniform lipid film on the inner wall of the flask. The dried film was subsequently hydrated with 10 mL of phosphate-buffered saline (PBS, pH 7.4) at 37°C while continuously agitated for 1 hour, enabling the

Table 1 : Composition of Apigenin Herbosomes (F_1–F_6).

Formulation code	Apigenin (mg)	Phospholipid (mg)	Cholesterol (mg)	Solvent (Chloroform: Ethanol, v/v)	Hydration Medium (PBS, pH 7.4)
F_1	25	50	5	10 mL (2:1)	10 mL
F_2	25	75	5	10 mL (2:1)	10 mL
F_3	25	100	5	10 mL (2:1)	10 mL
F_4	25	125	5	10 mL (2:1)	10 mL
F_5	25	150	5	10 mL (2:1)	10 mL
F_6	25	200	5	10 mL (2:1)	10 mL

self-assembly of phospholipid vesicles and effective entrapment of Apigenin within the lipid matrix. To achieve a more refined and homogeneous particle distribution, the hydrated dispersion was sonicated using a probe sonicator operating at 20 kHz and 100 W. Sonication was performed in pulsed mode (30 seconds on/off cycles) for a total of 5 minutes, which helped in reducing particle size and improving formulation uniformity. The final herbosomal suspension was transferred into amber-colored vials and stored at 4°C to protect it from light-induced degradation and maintain its physicochemical stability until further characterization (BalaYadav *et al*, 2022; Jain *et al*, 2013).

Characterization of Apigenin herbosomes

Particle size, Polydispersity Index (PDI) and Zeta Potential

The physicochemical characterization of the Apigenin-loaded herbosomes was carried out by determining the mean particle size, polydispersity index (PDI), and zeta potential using dynamic light scattering (DLS) with the Malvern Zetasizer Nano ZS90 instrument. Prior to analysis, the formulations were suitably diluted with double-distilled water to minimize the possibility of multiple scattering effects and to ensure accurate measurements. All evaluations were performed at a controlled temperature of 25°C. Each sample was analyzed in triplicate, and the average values were reported along with standard deviations to confirm the reproducibility and consistency of the results. This characterization was critical in assessing the stability, uniformity and surface charge properties of the developed herbosomal formulations (BalaYadav *et al*, 2022; Jain *et al*, 2013).

Entrapment Efficiency (%EE)

The entrapment efficiency (%EE) of Apigenin within the herbosomal vesicles was determined using a centrifugation method. A 1 mL aliquot of the prepared formulation was subjected to high-speed centrifugation at 15,000 rpm for 30 minutes at 4°C using a refrigerated centrifuge. Following centrifugation, the supernatant—containing the unencapsulated, free drug—was carefully collected and analyzed spectrophotometrically at 336 nm using a UV-Visible spectrophotometer. The amount of unentrapped Apigenin was quantified, and the percentage entrapment efficiency was calculated using the following formula:

$$\%EE = (Wt - Wf/Wt) \times 100$$

Where, *Wt* is the total amount of Apigenin used and *Wf* is the free drug in the supernatant.

Transmission Electron Microscopy (TEM)

The morphological characteristics of the Apigenin-loaded herbosomes were examined using transmission electron microscopy (TEM). For sample preparation, a small drop of the diluted herbosomal suspension was carefully placed onto a carbon-coated copper grid. The sample was then negatively stained with 1% (w/v) phosphotungstic acid to enhance contrast and allowed to air-dry under vacuum conditions. The grids were subsequently observed under a JEOL JEM-2100 TEM operated at an accelerating voltage of 200 kV. High-resolution images were captured to evaluate the shape, surface characteristics, particle size, and distribution of the vesicles. This analysis provided visual confirmation of nanoscale spherical morphology and uniform dispersion, supporting the structural integrity and homogeneity of the herbosomal formulation.

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier-transform infrared (FTIR) spectroscopy was employed to assess possible molecular interactions between Apigenin and phospholipids, as well as to confirm the formation of the herbosomal complex. FTIR spectra were recorded for pure Apigenin, phospholipid, and the Apigenin-loaded herbosomes using a Shimadzu IR Affinity-1 FTIR spectrometer. The samples were prepared using the potassium bromide (KBr) pellet method and spectral scanning was carried out in the range of 4000 to 400 cm^{-1}. Characteristic peaks were analyzed for any shifts, disappearance, or appearance of new bands in the herbosomal formulation compared to the individual components. Such spectral variations were interpreted as indicative of hydrogen bonding and van der Waals interactions between Apigenin and phospholipids, thereby supporting the successful complexation within the herbosomal system.

Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) was performed to analyze the thermal characteristics and investigate the physical state of Apigenin within the herbosomal system. The analysis was carried out using a Shimadzu DSC-60 Plus instrument. Accurately weighed samples (5–10 mg) of pure Apigenin, phospholipid, and the Apigenin-loaded herbosomal formulation were individually sealed in standard aluminum pans. The pans were subjected to a controlled heating program ranging from 30°C to 300°C at a heating rate of 10°C per minute under a continuous nitrogen purge to prevent oxidative degradation. The resulting thermograms were evaluated to detect any alterations in endothermic or exothermic transitions. Disappearance or broadening of the characteristic melting peak of Apigenin in the herbosomal formulation indicated a change in its crystalline nature,

suggesting successful molecular dispersion and complex formation with phospholipids.

In vitro antioxidant activity

DPPH Radical Scavenging assay

The antioxidant activity of Apigenin, blank herbosomes and Apigenin-loaded herbosomes was evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. A freshly prepared 0.1 mM DPPH solution in methanol was used as the reagent. Varying concentrations of each test sample (ranging from 10 to 100 µg/mL) were individually mixed with 2 mL of the DPPH solution. The mixtures were incubated in the dark at ambient temperature for 30 minutes to allow the reaction to proceed. Following incubation, the absorbance of each sample was recorded at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a positive control to benchmark the antioxidant performance (Aboody, 2021; Minaiyan *et al*, 2021). The percentage of DPPH radical scavenging was determined using the following equation:

$$\% \text{ Inhibition} = (A0 - A1A0) \times 100$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of the test sample. IC_{50} values were determined graphically.

In vitro anti-inflammatory evaluation

BSA Protein Denaturation assay

Protein denaturation is closely associated with the inflammatory response and serves as a useful indicator for evaluating anti-inflammatory activity. In this study, the ability of Apigenin formulations to inhibit protein denaturation was assessed using bovine serum albumin (BSA) as the model protein. A 1% w/v solution of BSA was prepared in phosphate-buffered saline (PBS, pH 7.4) and mixed with various concentrations of the test samples (ranging from 10 to 100 µg/mL). The resulting mixtures were incubated at 37°C for 30 minutes to allow interaction between the protein and the compounds. Following this, the mixtures were heated at 70°C for 5 minutes to induce denaturation and then cooled to room temperature. The absorbance of each sample was measured at 660 nm using a UV-visible spectrophotometer. The extent of inhibition of protein denaturation was calculated using the following formula (Rudrappa *et al*, 2024; Saeed *et al*, 2024; Thorve and Kishore, 2024):

$$\% \text{ Inhibition} = (A0 - A1A0) \times 100$$

Human Red Blood Cell (HRBC) membrane stabilization assay

The human red blood cell (HRBC) membrane stabilization method was employed to evaluate the anti-inflammatory potential of the Apigenin formulations by assessing their ability to protect erythrocyte membranes from heat-induced hemolysis. This method serves as a surrogate for lysosomal membrane stabilization, as both share structural similarities. Fresh human blood was collected from healthy volunteers and centrifuged at 3000 rpm for 10 minutes to separate the packed red blood cells (RBCs). The RBCs were washed three times with an isotonic buffer solution (pH 7.4) to remove any plasma residues and then re-suspended in the same buffer to obtain a 10% v/v suspension. Different concentrations (10–100 µg/mL) of the test formulations were mixed with the RBC suspension and incubated at 54°C for 30 minutes to induce hemolysis. After incubation, the samples were centrifuged again, and the absorbance of the supernatant was measured at 560 nm using a UV-Vis spectrophotometer (Govind Navale, 2019). The degree of membrane stabilization was calculated using the following formula:

Statistical analysis

All experimental procedures were independently conducted in triplicate, and the results were expressed as mean values accompanied by their respective standard deviations (mean ± SD). Statistical analysis was carried out using GraphPad Prism version 9.0. To assess differences between groups, one-way analysis of variance (ANOVA) was performed, followed by Tukey's post hoc test for multiple comparisons. A p-value less than 0.05 was considered indicative of statistically significant differences.

RESULTS AND DISCUSSION

Formulation and characterization of Apigenin herbosomes

Apigenin-loaded herbosomes were successfully developed using the thin-film hydration method, and six different formulations (F_1–F_6) were prepared by varying the Apigenin-to-phospholipid ratio. Table 2 summarizes the physicochemical characteristics of the formulations. The particle size of herbosomes decreased progressively from 198.4 nm (F_1) to 128.9 nm (F_6) with increasing phospholipid concentration, which could be attributed to improved vesicle formation and stabilization in lipid-rich environments. Polydispersity index (PDI) values for all formulations were below 0.35, indicating narrow and uniform particle size distribution, with F6 showing the most favorable PDI (0.231). The zeta potential values ranged from –21.6 mV to –28.5 mV, confirming colloidal stability due to sufficient surface charge. Entrapment efficiency (EE%) increased from 61.3% in F_1 to 87.9% in F_6,

Table 2 : Characterization results of Apigenin Herbosomes.

Formulation code	Particle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)
F_1	198.4	0.312	–21.6	61.3
F_2	176.2	0.298	–23.4	68.5
F_3	159.3	0.274	–25.7	74.8
F_4	142.5	0.261	–26.3	80.2
F_5	135.7	0.249	–27.1	84.6
F_6	128.9	0.231	–28.5	87.9

Table 3 : In vitro cumulative % Drug release of Apigenin herbosomes.

Time (h)	F_1 (%)	F_2 (%)	F_3 (%)	F_4 (%)	F_5 (%)	F_6 (%)
0.5	12.3	14.2	16.1	17.5	18.8	19.7
1.0	21.5	24.9	28.3	30.9	33.4	35.1
2.0	35.6	39.7	44.5	48.2	52.7	56.8
4.0	48.4	53.5	58.2	62.3	67.5	71.9
6.0	57.2	62.4	68.8	72.6	77.3	81.2
8.0	64.3	70.6	76.4	80.1	84.2	87.9
10.0	70.5	77.1	82.6	86.4	89.7	92.8
12.0	75.1	81.3	86.2	90.3	93.6	96.4

Table 4 : Kinetic modelling of F_6 Drug release.

Model	Equation	R^2 value
Zero-order	$Q = 10.19 \cdot t$	0.220
First-order	$Q = 100 \cdot (1 - e^{-0.347 \cdot t})$	0.964
Higuchi	$Q = 30.99 \cdot \sqrt{t}$	0.916
Korsmeyer–Peppas	$Q = 38.43 \cdot t^{0.39}$	0.960

demonstrating that higher phospholipid content enhanced the encapsulation of the lipophilic flavonoid Apigenin.

In vitro drug release profile

As presented in Table 3, the cumulative % drug release from herbosomes over 12 hours showed a sustained pattern for all formulations. F1 exhibited a lower release of 75.1%, while F_6 showed the highest release of 96.4%. This enhancement was attributed to the improved solubility and matrix structure formed at higher lipid concentrations, which facilitated a controlled drug release. The optimized formulation F_6 thus achieved better control over drug release while ensuring prolonged therapeutic availability.

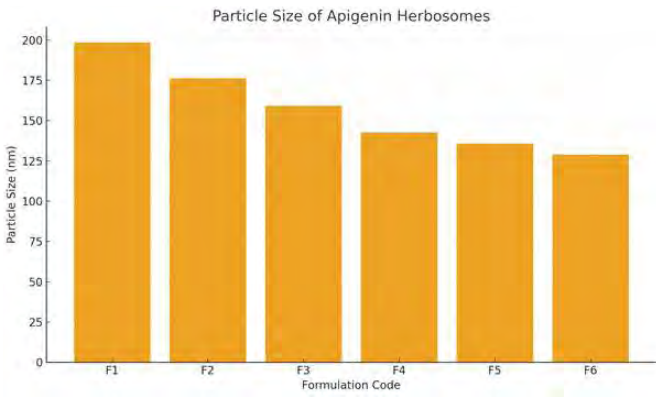

Fig. 1 : Particle size (nm).

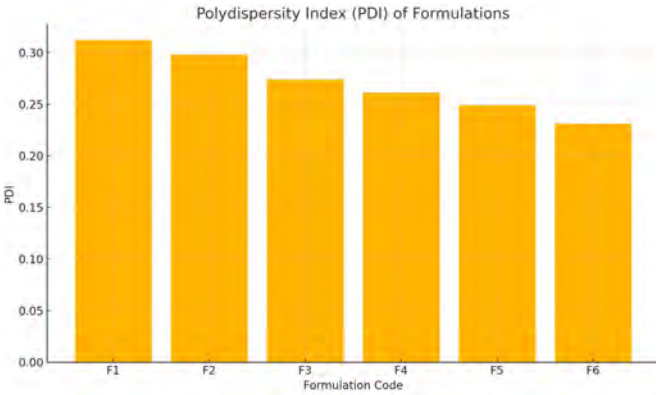

Fig. 2 : PDI.

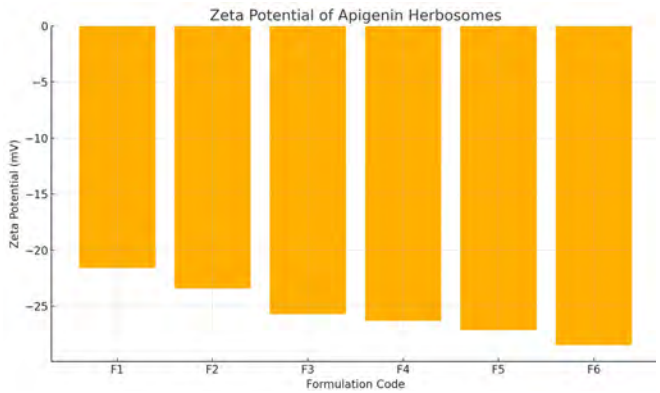

Fig. 3 : Zeta Potential (mV).

Kinetic Modelling of Drug release

Table 5 shows the mathematical modelling results of F6 release kinetics. Among the models applied, the first-order model demonstrated the best fit (R^2 = 0.964), indicating a concentration-dependent drug release. Korsmeyer–Peppas also fitted well (R^2 = 0.960), with an *n* value of 0.39, suggesting that drug release followed Fickian diffusion. These findings confirm that F6 provided a predictable and efficient release mechanism suitable for sustained delivery.

Pharmacological evaluation

The antioxidant and anti-inflammatory activities of the formulations are detailed in Table 6. Antioxidant activity assessed via the DPPH assay showed a significant improvement across formulations, with % inhibition increasing from 61.2% in F_1 to 86.9% in F_6. Similarly, the IC_{50} values declined from 78.3 µg/mL (F_1)

Table 5 : Antioxidant and anti-inflammatory activity of Apigenin herbosomes (F_1–F_6).

Formulation code	DPPH % inhibition (100 µg/mL)	IC_{50} (µg/mL)	BSA inhibition (%)	HRBC stabilization (%)
F_1	61.2	78.3	58.6	60.4
F_2	67.5	65.7	64.3	66.7
F_3	74.3	54.1	71.2	72.8
F_4	79.8	47.5	76.5	78.3
F_5	83.6	41.9	80.1	82.5
F_6	86.9	39.2	83.4	85.8

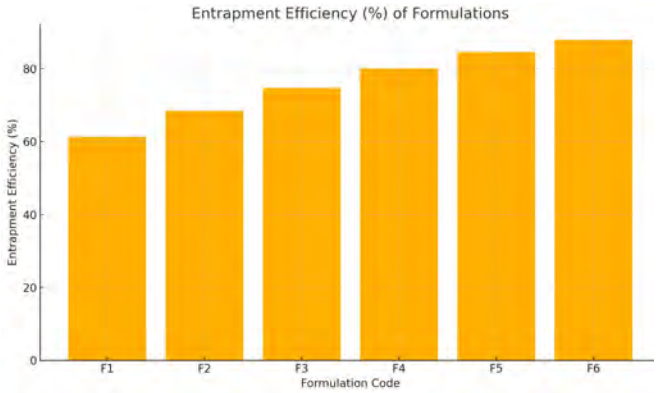

Fig. 4 : Entrapment Efficiency (%).

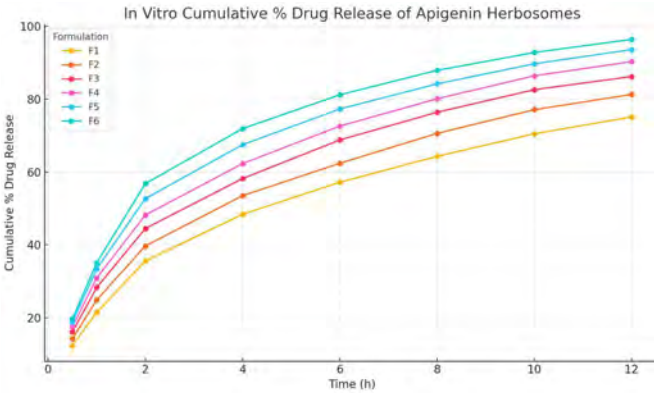

Fig. 5 : *In vitro* Cumulative % drug release of Apigenin Herbosomes.

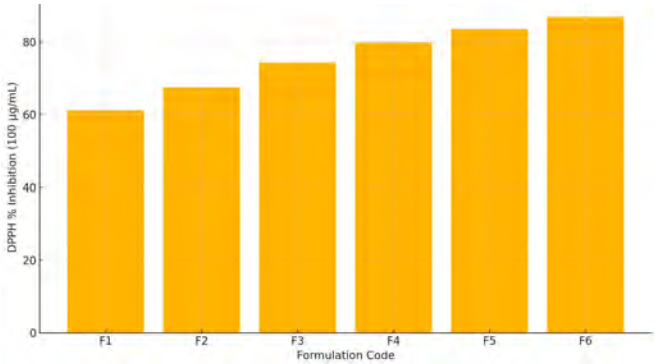

Fig. 6 : Antioxidant activity of Apigenin Herbosomes (F_1–F_6).

to 39.2 µg/mL (F_6), demonstrating increased potency. The enhanced activity may be due to improved aqueous dispersion and bioavailability of Apigenin in the lipid matrix. Anti-inflammatory potential was evaluated using the BSA protein denaturation and HRBC membrane stabilization assays. BSA inhibition ranged from 58.6% (F_1) to 83.4%

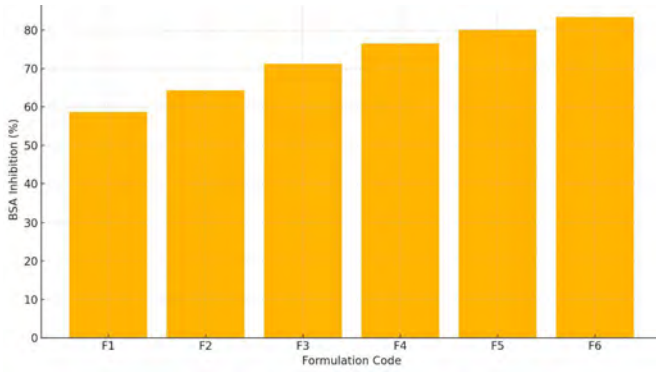

Fig. 7 : Anti-inflammatory activity (BSA inhibition %) of Apigenin Herbosomes (F_1–F_6).

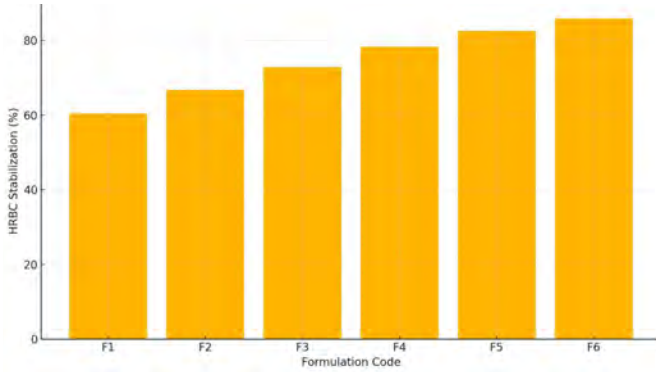

Fig. 8 : Anti-inflammatory activity (HRBC stabilization %) of Apigenin Herbosomes (F_1–F_6).

(F_6), while HRBC stabilization ranged from 60.4% to 85.8%. The progressive enhancement in both assays correlated well with increased drug encapsulation and optimized vesicle properties, particularly in F_5 and F_6.

Overall evaluation

Formulation F_6 emerged as the most optimized system, exhibiting the smallest particle size, highest entrapment efficiency, best antioxidant and anti-inflammatory effects and an excellent controlled release profile. The findings support that phospholipid-based herbosomes are a promising nanocarrier system to improve the pharmacological performance of poorly soluble phytoconstituents like Apigenin.

CONCLUSION

The present study successfully developed andevaluated a phospholipid-based herbosomal system

to enhance the solubility, stability and pharmacological efficacy of Apigenin, a bioactive flavonoid with known antioxidant and anti-inflammatory properties. Six formulations (F_1–F_6) were prepared using the thin-film hydration method, varying the ratio of Apigenin to phospholipid. Among them, F_6 exhibited the most promising profile, demonstrating optimal particle size (128.9 nm), uniformity (PDI 0.231) and maximum zeta potential (–28.5 mV), indicating good colloidal stability. Entrapment efficiency increased significantly with phospholipid content, reaching up to 87.9% in F_6, which correlated with improved drug release and pharmacological outcomes. The in vitro release study showed a sustained drug release profile, with F_6 achieving 96.4% cumulative release over 12 hours. Kinetic modeling revealed that the release followed first-order kinetics and Fickian diffusion, suggesting concentration-dependent, diffusion-controlled release behaviour. Pharmacological evaluations confirmed that the herbosomal delivery system significantly enhanced the bioactivity of Apigenin. F6 displayed superior antioxidant activity with 86.9% DPPH inhibition and a low IC_{50} of 39.2 µg/mL. In anti-inflammatory assays, it demonstrated 83.4% inhibition of BSA denaturation and 85.8% HRBC membrane stabilization, far exceeding the performance of less optimized formulations. Collectively, these findings suggest that the Apigenin-loaded herbosomes, particularly F6, effectively addressed the solubility and bioavailability limitations of the free drug, offering a promising strategy for enhancing the therapeutic application of natural flavonoids. The phospholipid-based herbosomal system developed in this study can potentially be extended to other hydrophobic phytoconstituents to improve their clinical performance in oxidative and inflammatory disorders.

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