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Formulation and Evaluation of *Tinospora Cordifolia* - Cholesterol Phytophospholipid Complex for Enhanced Anti-Inflammatory Activity

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Abstract

Tinospora cordifolia is widely recognized for its anti-inflammatory and immunomodulatory properties, offering therapeutic potential for managing inflammatory disorders such as arthritis and chronic autoimmune diseases. To develop and evaluate a *Tinospora cordifolia*-cholesterol phytophospholipid complex to enhance entrapment efficiency and optimize drug release profiles, facilitating its application in inflammatory disorder management. To compare the drug entrapment efficiency and release profiles of nine formulations, identify the impact of cholesterol-to-extract ratios, and determine the most effective formulation. Nine formulations (F1–F9) of the *Tinospora cordifolia*-cholesterol phytophospholipid complex were prepared and evaluated for drug entrapment efficiency and *in vitro* release over eight hours. Burst and sustained release characteristics were analyzed, and the influence of varying cholesterol ratios was assessed to determine optimal formulation parameters. Formulation F7 showed the highest entrapment efficiency (92.91%), followed closely by F4 (92.31%), both indicating superior drug retention. F4 displayed an optimal release profile, with a burst release of 36.9% in the first hour and sustained release of 92.3% by the eighth hour. Higher cholesterol ratios, as seen in F4 and F2, enhanced stability and controlled release, while lower ratios in formulations such as F1 and F7 resulted in reduced performance. This research demonstrates the feasibility of a cholesterol-based phytophospholipid complex for enhancing the delivery of *Tinospora cordifolia*. Formulation F4 emerged as the most promising candidate due to its balanced burst and sustained release properties, offering potential for clinical applications in inflammatory disorder management. Future studies should validate its pharmacokinetic and in vivo therapeutic efficacy.

Keywords: *Tinospora cordifolia*, Phytophospholipid, Anti-inflammatory activity, Sustained release, Drug entrapment

Introduction

Inflammation is a complex biological response of the body's immune system to harmful stimuli, such as pathogens, damaged cells, or irritants (1). It is a protective mechanism aimed at removing the harmful stimuli and initiating the healing process. Inflammation can be classified as either acute or chronic, depending on its duration and the underlying cause. Acute inflammation is a short-term response to injury or infection, characterized by redness, heat, swelling, and pain. It usually resolves once the threat has been eliminated, and

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tissue repair is underway. Chronic inflammation is a prolonged and persistent form of inflammation that can last for months or even years. It often occurs when the immune system fails to eliminate the causative agents or when it mistakenly targets healthy tissue, leading to diseases like arthritis, asthma, cardiovascular disease, and even cancer (2-5).

Tinospora cordifolia, commonly known as Guduchi or Giloy, is a climbing shrub widely used in traditional Indian medicine, particularly in Ayurveda. It is known for its broad spectrum of medicinal properties, including anti-inflammatory, antioxidant, immunomodulatory, and hepatoprotective effects. Due to these attributes, *Tinospora cordifolia* has been extensively studied for its therapeutic potential in the treatment of various inflammatory diseases. The bioactive compounds responsible for the medicinal properties of *Tinospora cordifolia* include Alkaloids as they known for their anti-inflammatory and immune-boosting effects, Glycosides that contribute to the plant's anti-inflammatory and hepatoprotective activities, Steroids which have an anti-inflammatory effect similar to corticosteroids, diterpenoids Known to exhibit anti-cancer, anti-inflammatory, and antimicrobial properties, Phenolic compounds and flavonoids compounds exert antioxidant and anti-inflammatory effects by neutralizing free radicals and reducing oxidative stress.

Research has shown that *Tinospora cordifolia* extracts (6, 7, 8, 9) can inhibit the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), which are key mediators of inflammation. The plant has also been demonstrated to inhibit the activity of cyclooxygenase (COX) enzymes, thus reducing the production of inflammatory prostaglandins. These mechanisms underline its efficacy as an anti-inflammatory agent. Due to its potent anti-inflammatory properties, *Tinospora cordifolia* is used in the treatment of several inflammatory disorders, including Rheumatoid arthritis, Gout**,** Allergic disorders, Gastrointestinal disorders however, despite its promising therapeutic potential, the poor water solubility and bioavailability of certain phytochemicals in *Tinospora cordifolia* limit its effectiveness in some applications. This challenge has led to the exploration of novel drug delivery systems, including the development of phytosomes, to enhance the bioavailability and therapeutic efficacy of the plant's bioactive compounds.

Phytosomes are a novel drug delivery system designed to improve the bioavailability and absorption of plant-based compounds. The term "phytosome" is derived from "phyto," meaning plant, and "some," referring to a vesicle-like structure. Phytosomes are essentially complexes of natural plant extracts (phytochemicals) and phospholipids, which form vesicles that encapsulate the bioactive compounds, enhancing their stability and absorption in the body.

The incorporation of *Tinospora cordifolia* extracts into a phytosome complex has been investigated as a strategy to improve the bioavailability and anti-inflammatory efficacy of the plant's bioactive compounds. By forming a complex with phospholipids, the solubility, absorption, and stability of the extract are significantly enhanced, leading to improved therapeutic outcomes. The bioactive compounds from *Tinospora cordifolia* are extracted using ethanol. The extract is then complexed with phospholipids, typically phosphatidylcholine, through the formation of hydrogen bonds between the hydroxyl groups of the plant extract and the polar head of the phospholipid. The optimized phytosomal formulation of *Tinospora cordifolia* demonstrated several advantages over conventional extracts:

- Enhanced bioavailability**:** The phytosome complex improved the solubility and absorption of the bioactive compounds, leading to higher plasma concentrations and prolonged therapeutic action.

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

- Increased anti-inflammatory activity: The phytosome formulation showed enhanced anti-inflammatory activity *in vitro* and in vivo, reducing the production of pro-inflammatory cytokines and inhibiting the activity of COX enzymes more effectively than the non-phytosomal extract (10-12).
- Improved entrapment efficiency: The phytosome formulation exhibited high entrapment efficiency, with over 90% of the bioactive compounds being successfully encapsulated within the phytosome vesicles.
- Sustained release: The phytosome complex provided a controlled release of the bioactive compounds, ensuring a prolonged anti-inflammatory effect and reducing the frequency of administration.

Materials and Methods

Methods

Collection and authentication of plant materials

Fresh stems of *Tinospora cordifolia* (13-18) were collected from fully grown plants in Nellore, Andhra Pradesh. The plant material was authenticated and identified by the faculty of the Botany Department at DRW College, Gudur, Nellore District. The voucher specimen (V. No. 72) has been retained in the Department of Biology at DRW College for future reference.

The collected stems were thoroughly washed with water to remove any impurities and dried in a shaded, well-ventilated area to prevent degradation of active compounds. Once completely dried, the plant material was stored in air-tight containers to protect it from moisture and contamination, ensuring its suitability for further experimental studies.

Preparation of plant extract

Fifty grams of air-dried, powdered stems of *Tinospora cordifolia* were extracted using 95% alcohol through cold maceration over 48-72 hours with occasional stirring. After maceration, the solvent was evaporated on a water bath to dryness, and the extract was weighed to calculate the percentage yield. The color and consistency of the extract were recorded (19).

Preformulation studies

Organoleptic evaluation

Research examined the organoleptic features of the plant extracts Ixora coccinea, Tinospora cordifolia, and Chrysanthemum morifolium through evaluations of their color appearance and scent detection and solution behavior. The researchers collected dried plant materials which received cleaning treatment and subsequent shade-drying then powdering and extraction occurred with three solvent solutions of water ethanol methanol. The scientists conducted filtration then concentration of the extracts to create a semi-solid or dry compound. Testing the extracts under natural outdoor lighting and artificial indoor conditions allowed researchers to observe and document their standard color descriptors. Multiple evaluators used sensory evaluation to determine the direct inhalation odours of the materials which they described as aromatic, pungent, floral or earthy or bitter in nature. The testing procedure for extract solubility involved evaluating small amounts of each extract in water as a polar solvent and two non-polar solvents composed of ethanol and chloroform/petroleum ether. The extraction solutions were stirred in the respective solvents and scientists observed their solubility status as either freely soluble or partially soluble or insoluble until the time expired (20). The recorded data delivered crucial information about extract chemical properties to support assessment of pharmaceutical and treatment potential. The organoleptic characteristics were deputed in Table 1.

Table 1: Organoleptic Characteristics of *Tinospora cordifolia*

S. No.	Name of the Plant	Colour	Odour	Solubility
1	*Tinospora cordifolia*	Green	Characteristic odour	Freely soluble

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
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Melting point

Tinospora cordifolia extract melting point determination operated through the capillary method with help from a melting point apparatus. The personnel obtained a small portion of the extract while filling a capillary tube containing fine powder before placing it inside the apparatus (21). Results were recorded when the extract started to become liquid and completely melted during the temperature increase. The thermal properties alongside purity underwent assessment through this analysis to become an essential tool for quality evaluation and pharmacological assessment of the extract.

FTIR Spectroscopy

The drug spectra analysis happened through a Shimadzu-8400S FT-IR Spectrophotometer from Tokyo, Japan. The semi-solid paste analysis required the combination of processed *Tinospora cordifolia* and cholesterol. FT-IR spectroscopy analyzed characteristic functional group identification of the sample by scanning it from 3500 to 1000 cm^{-1} using a Fourier Transform Infrared (FT-IR) spectrometer. The UV PROB version 14 software processed data to interpret spectra for precise evaluation of molecular chemistry and interactions.

Determination of solubility

The evaluation of *Tinospora cordifolia* extract solubility took place through testing six different organic solvents composed of ethyl acetate, ethanol, dichloromethane, dimethyl sulfoxide (DMSO) and distilled water. The evaluation purpose sought to determine which solvent demonstrated maximum effectiveness for extracting active components from Tinospora cordifolia. The different solvents revealed dissimilar solubility behaviors of the extract which created essential knowledge about its structural makeup and possible medical applications. These solubility research findings will play an essential role in guiding future studies about the extraction and purification as well as utilization of bioactive compounds contained within *Tinospora cordifolia* (22).

UV Spectroscopy study (determination of λmax)

The researchers created a pure standard stock solution at 50 µg/mL concentration of Ixora coccinea, Chrysanthemum morifolium, and Tinospora cordifolia. Tests with UV spectrophotometry were conducted between 200–800 nm wavelengths to examine extract absorbance by using both water and ethanol as analytical solvents. The evaluation of the plant extract optical features through this analysis induced vital information about bioactive elements and pharmaceutical applications (23).

Formulation and development

Tinospora cordifolia extract became the selection for phytosome preparation because it contains abundant natural carotenoid phyto-constituents which demonstrate therapeutic properties. A complete bioavailability study measured the extract-cholesterol relationship to produce an optimized formulation (24, 25, 26, 27). The evaluation process proved essential in establishing a stable and efficient phytosomal formulation because it enabled better absorption along with enhanced bioactivity of the bioactive compounds.

Preparation Methods

Research investigated three different approaches for creating phytosomes through anti-solvent precipitation and cholesterol complexation and rotary evaporation methods. The anti-solvent precipitation method proved best among multiple possibilities because of its simplified process and effective outcomes. The system combined *Tinospora cordifolia* extract and cholesterol while refluxing them in 30 mL of methanol below a temperature set point at 60°C for a period of two hours. The solution required concentration to 5 mL and then mixers added 20 mL n-hexane to cause phytosome formation while maintaining

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

constant stirring. Dryness of the obtained precipitate was achieved through storage in vacuum desiccators during an overnight period (28).

Selected plant extract received cholesterol at equal molar proportions prior to being dissolved within dichloromethane solvent solution. The reflux continued until the mixture reduced to 3–5 mL and then n-hexane addition provided the conditions to produce the complex through precipitation. The phytosomal formulation continued its development through the vacuum-dried precipitation process of the filtered material.

Determination of % Yield and Average Particle Size

The yield of the phytosomal formulation was calculated using the equation:

Yield (%) =

(Practical yield)/(Theoretical yield)*100

We analyzed the phytosomal formulation particle size under optical microscopy for exact characterization purposes. The research aimed to verify that *Tinospora cordifolia* phytosomes possessed appropriate particle dimensions which guarantee successful drug delivery and intensified therapeutic outcomes. The correct measurement of particle size remains essential because it enhances bioavailability and stability alongside pharmaceutical activities of their new formulation.

Drug Entrapment Efficiency of Phytosomes for *Tinospora cordifolia* (29-30)

Testing the Drug Entrapment Efficiency (DEE) helped assess how well *Tinospora cordifolia* phytosomal formulation maintained its active components. One hundred milligrams of the *Tinospora cordifolia* phytosomal product received accurate weighing before placement into a 100 mL volumetric flask with phosphate buffer holding a pH level of 6.8. The flask rested for a duration of 24 hours to permit complete drug release from the encapsulation process. A two-hour mixing process at 35°C with continuous stirring maintained uniform distribution of all contents while the solution rested in the phosphate buffer at pH 6.8.

Percentage Drug Entrapment Efficiency=
Actual Drug Content X 100

Theortical Drug Content

After filtering the solution all undissolved impurities were eliminated. Analysis of the clear filtrate required a 1 mL portion to get diluted to 10 mL for analysis. UV spectrophotometry analysis at 410 nm wavelength of diluted samples determined the drug content through measurements of absorbance values that correspond to Tinospora cordifolia. This formulation determined drug entrapment efficiency percentage through the following calculation:
al bioavailability and therapeutic performance

***Preparation of Final Batches of Phytosomes for* Tinospora cordifolia**

Tinospora cordifolia phytosomes through cholesterol complexation after analyzing initial formulation batches. The researchers altered the molar proportion between *Tinospora cordifolia* extract and cholesterol during optimization to obtain appropriate physicochemical properties in the phytosomes. The Table 2 provides a complete

Table 2: Multiple composition of Phytosomes Formulation

S. No.	Plant Extract Phytosomes	Extract	Cholesterol
1	F1	1	1
2	F2	1	2
3	F3	2	1
4	F4	2	2
5	F5	1	1
6	F6	3	1
7	F7	1	3
8	F8	3	2
9	F9	2	3

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

comparison of the multiple compositions by presenting the final phytosome formulations together with their molar ratios.

Evaluation of Final Batches of Phytosomes for Tinospora cordifolia

The functional properties of *Tinospora cordifolia* phytosomes were examined through FTIR spectroscopy after completing the calibration studies to reveal structural details. The research group devoted attention to examine every batch through systematic evaluation of essential metrics which included percentage yield combined with drug entrapment efficiency and particle size and *in vitro* drug release. These series of extensive evaluations establish the pharmacokinetic conditions along with stability and effectiveness potentials of the phytosomal formulations to evaluate their suitability as therapeutical candidates (31, 32).

***Optimization of Final Batch of Phytosomes for* Tinospora cordifolia**

The optimized *Tinospora cordifolia* formulations by using *in vitro* dissolution tests to enhance drug release characteristics which maintained stability and therapeutic effectiveness for phytosomes. The evaluation findings will guide future development and clinical utilization for the most promising formulation choice. Such optimization methods are crucial to enhance *Tinospora cordifolia* effectiveness in phytosomal formulations while boosting its bioavailability and pharmacological properties.

Results and Discussion

Organoleptic Evaluation

An organoleptic evaluation of *Tinospora cordifolia* generates vital descriptive information about physical properties to assist in both quality determination and identification. The plant shows distinct features of green coloration and produces odor that serves as essential elements for identifying the species. The high solubility properties of *Tinospora cordifolia* suggest that it could serve well in extraction methods which lead to pharmaceutical formulation creation. The organoleptic properties stand as essential identifiers which distinguish *Tinospora cordifolia* from related plants because they demonstrate its distinct therapeutic capabilities. The λ_{max} for the stem extract of *Tinospora cordifolia* was found to be at 410nm.

Standard Calibration Curve of Tinospora cordifolia in Ethanol at 410 nm

A standard calibration curve for *Tinospora cordifolia* solutions in ethanol was prepared through UV spectrophotometric determination of sample absorbance from 0 to 50 µg/mL at 410 nm wavelength. The continual rise of absorbance values as the concentration increases shows ethanol functions properly as a spectrophotometric solvent for measuring Tinospora cordifolia. The Beer-Lambert law applies because the absorbance measurements show a direct correlation with the concentrations measured within the chosen detection range. The linear trend proves this method provides a quantitative measurement approach for *Tinospora cordifolia* identification in various formulations.

The calibrated curve deputed in Table 3 and Figure 1 provides a standard measurement tool for pharmaceutical preparation testing laboratories to detect *Tinospora cordifolia* concentration accurately. The research confirms that the UV spectrophotometric method functions effectively as an easy, sensitive and

Table 3: Standard Calibration Curve Data of *Tinospora cordifolia* in Ethanol at 410 nm

S. No.	Concentration (µg/ml)	Absorbance
1.	0	0
2.	10	0.064
3.	20	0.091
4.	30	0.118
5.	40	0.173
6.	50	0.220

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

repeatable analytical method to determine *Tinospora cordifolia* levels in ethanol-based solutions.

Determination of Solubility Studies

Solubility of Tinospora cordifolia

A test of soluble *Tinospora cordifolia* extract was conducted through liquid solution assessments with four different solvents for pharmaceutical suitability testing as well as method optimization. The extract partially dissolved in water because its active component bioavailability becomes restricted in aqueous formulations so water-based extractions require the implementation of solubility-enhancing methodologies. The extract remained partially soluble when submerged in ethanol indicating the solvent is not the optimum choice for extracting all bioactive components in their entirety. The complete solubility of the extract in ethyl acetate showcases its strong affinity with this solvent thus establishing ethyl acetate as an efficient method for phytochemical isolation of specific bioactive compounds. DMSO (Dimethyl Sulfoxide) displays complete solubilization properties with the extract which indicates its value for pharmaceutical development. The solvent property of DMSO permits it to dissolve compounds of both polar and non-polar nature which makes it an adaptable solvent for drug delivery enhancement and bioavailability improvement. The identified findings provide crucial information for developing optimal extraction methods and increasing phytoconstituent availability and selecting suitable solvents for herbal drugs. The basic solubility behavior of *Tinospora cordifolia* demonstrates its therapeutic value as a herbal therapy which aids pharmaceutical development of improved effectual medicines.

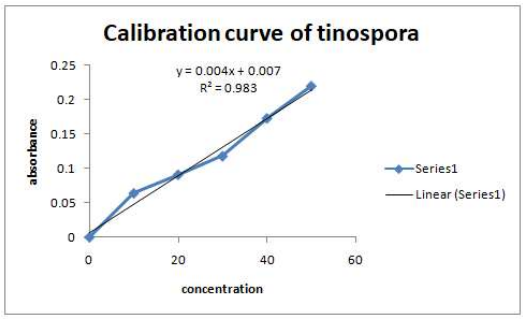

Figure 1: Standard Calibration curve of *Tinospora* in ethanol at 410 nm

FTIR Studies

FTIR Spectral analysis of Tinospora cordifolia Extract

An FTIR spectral analysis of *Tinospora cordifolia* extract detected essential functional groups that signify its bioactive elements shown in Figure 2. The O-

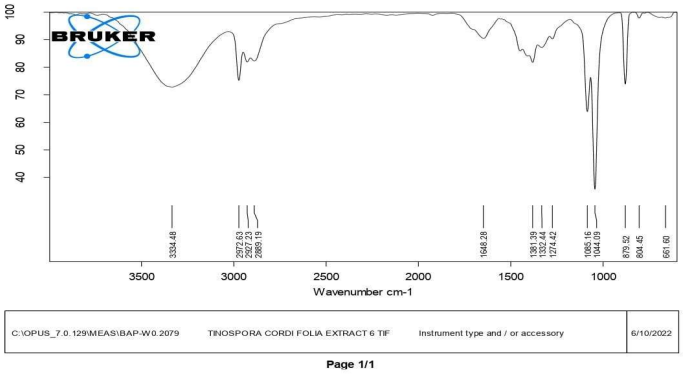

Figure 2: Fourier –Transform infrared spectroscopy absorption of *Tinospora cordifolia*

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

H stretching at 3334.48 cm^{-1} indicates hydroxyl groups that are characteristic of phenols and flavonoids. FTIR analysis shows the presence of aliphatic hydrocarbons through C-H stretching peaks which appear between 2972.63 cm^{-1} – 2882.19 cm^{-1}.

A robust IR spectrum band at 1648.28 cm^{-1} signifies the stretch of C=O functional group that exists within phenolic flavonoids together with alkaloids. The presence of C-O and C-N stretching absorption bands at 1381.39 cm^{-1}, 1233.44 cm^{-1} and 1217.42 cm^{-1} proves that esters, ethers and amines exist in the extract. Multiple C-O stretching vibrations appear in intense peaks at 1085.16 cm^{-1} and 1044.80 cm^{-1} which might indicate the presence of polysaccharides and glycosides. Aromatic ring deformations along with possible alkaloid structures are indicated through absorption bands at 879.45 cm^{-1}, 844.45 cm^{-1} and 661.80 cm^{-1}. The analysis confirms that *Tinospora cordifolia* contains various functional groups showing its complex phytochemical characteristics with possible range of pharmacological effects.

FTIR Analysis of Tinospora cordifolia & cholesterol

A stable complex occurred between *Tinospora cordifolia* extract and cholesterol based on the FTIR spectrum of the interaction shown in Figure 3. The wide peaks at 3658.59 cm^{-1} and 3739.91 cm^{-1} identify O-H stretching that suggests both hydroxyl groups from cholesterol and bioactive compounds in *Tinospora cordifolia* are present. C-H stretching bands at 2953.92 and 2856.68 cm^{-1} verify the existence of aliphatic hydrocarbon structures in the substance. The 1657.84 cm^{-1} strong peak indicates C=O stretching which suggests the extract contains carbonyl groups that might originate from flavonoids and other phytoconstituents.

The infrared spectrum shows the C-O bond stretching frequencies at 1212.51 cm−1 and 1161.37 cm−1 which proves the complex contains esters and ethers. The IR spectroscopic results show C-N stretching combined with C-O bending vibrations at either 1084.32 cm^{-1} or 1039.32 cm^{-1} or 927.28 cm^{-1} that may stem from polysaccharides and glycosides and alkaloids. Both peaks at 872.99 cm^{-1} and 720.00 cm^{-1} reveal aromatic ring deformations as well as potential sterol structures in the compound. Observing different functional groups vibrations between the phytosome mixture and its individual components supports the hypothesis that complex formation occurred generating a stable structure with better bioavailability potential.

Homeland Security uses FTIR spectra to disclose the chemical structure of *Tinospora cordifolia* extract and its cholesterol complex. The FTIR spectrum of pure *Tinospora cordifolia* extract displayed O-H stretching at 3334.48 cm^{-1} and C-H stretching at 2927.63 cm^{-1} and 2882.19 cm^{-1}. Absorption bands in the 1600–

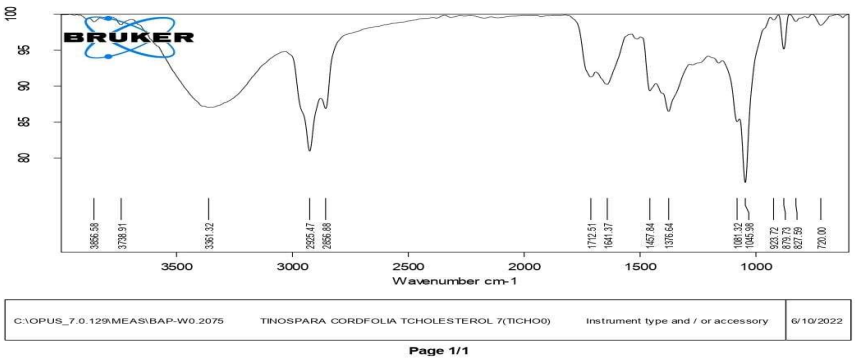

Figure 3: Fourier –Transform infrared spectroscopy absorption of *Tinospora cordifolia* & cholesterol

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

1000 cm^{-1} range demonstrated the presence of C=O, C-O and C-N functional groups that indicate the presence of bioactive alkaloids and flavonoids and polysaccharides.

The successful phytosome complex formation was confirmed by the peak position changes that appeared when *Tinospora cordifolia* extract bound with cholesterol molecules. The O-H stretching of Tinospora cordifolia-cholesterol complex moved to 3658.59 cm^{-1} and 3739.91 cm^{-1} through hydrogen bonding between the compounds. An analysis of the peaks at 2953.92 cm^{-1} and 2856.68 cm^{-1} validated aliphatic hydrocarbon presence in the sample while C=O stretching at 1657.84 cm^{-1} confirmed cholesterol's interaction with bioactive compounds. The phytosome complex formation was confirmed through two peaks at 1212.51 cm^{-1} and 1161.37 cm^{-1} which indicated C-O stretching vibrations. The formation of new peaks coupled with changes in functional group vibrations indicates improved stability together with potential enhanced bioavailability when *Tinospora cordifolia* is developed as a cholesterol-based phytosome.

Determination of percentage yield

The different *Tinospora cordifolia* phytosomal formulations yielded varying percentage results because the preparation methods had unique efficiencies. The production efficiency measures from F1 to F2 reached 80% to 70% while showing promising phytosome formation process parameters. The evaluation of F4 and F6 and F5 showed 50% and 53% and 46% moderate yield levels because precipitation and filtration procedures likely caused some extraction losses. Yields reached 33% in F8 and 40% in F7 F9 and F3 (Table 4).

These results potentially stem from complexation being imperfect or evaporation of the solvent or a lack of satisfactory extract and cholesterol binding. Various yield levels require optimization of parameters such as solvent selection combined with temperature management alongside proper extract-to-cholesterol ratio design for better manufacturing outcomes of phytosomes.The following equation was used to calculate the assurance of percentage yield of formulations:

Table 4: Production efficiency for the Formulations F1 to F9

S. No.	Formulation	Percentage yield (%)
1.	F1	80%
2.	F2	70%
3.	F3	40%
4.	F4	50%
5.	F5	46%
6.	F6	53%
7.	F7	40%
8.	F8	33%
9.	F9	40%

$$\%yield = (practical\ yield/theoretical\ yield) \times 100$$

Determination of particle size

To determine *Tinospora cordifolia* phytosomal formulations had an average 76 µm particle size. The documented particle size measurement stands critical because it determines drug delivery performance through its impact on pharmaceutical attributes including solubility and release rate and bioavailability and total therapeutic strength. The 76 µm measurement shows that phytosomal formulations possess dimensions suitable for pharmaceutical applications which helps them maintain uniform dispersion while being stable.

The drug absorption and bioavailability benefits from smaller particles because they create greater surface area which proves advantageous for active constituents that need release in herbal medicines. Additional assessment through dynamic light scattering (DLS) together with scanning electron microscopy (SEM) would supply advanced knowledge about phytosome dimensions and shapes. The method for preparing phytosomes requires optimized stirring techniques and sonication procedures together with solvent evaporation protocols to achieve optimal particle sizes for effective drug delivery systems.

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

Drug Entrapment Efficiency

The *Tinospora cordifolia* phytosomal formulations achieved different entrapment efficiencies between 72.34% (F8) and 95.91% (F2) shown in Table 5. The drug release profile together with formulation stability and bioavailability depends heavily on the entrapped efficiency measurement of *Tinospora cordifolia* phytosomal formulations. Stable phytosomal complexes were achieved in F2 since its EE measurement reached 95.91%, confirming excellent extract and cholesterol binding with better drug retention and reduced premature leakage. High values of entrapment efficiency were detected in F1 (90.90%) and F4 (92.31%) as well as F6 (90.01%) and F7 (92.91%) indicating effective bioactive constituent encapsulation for sustained drug release.

Table 5: Drug Entrapment Efficiency of Formulations

S. No.	Formulation	% Entrapment efficiency
1.	F1	90.90
2.	F2	95.91
3.	F3	83.31
4.	F4	92.31
5.	F5	83.64
6.	F6	90.01
7.	F7	92.91
8.	F8	72.34
9.	F9	76.79

The entrapment efficiency of F8 (72.34%) and F9 (76.79%) came out lower than other formulations because the encapsulation or complex formation between extract and cholesterol components was likely inefficient. Efficient phytosome formulation optimization requires attention to parameters because it directly impacts both stability and therapeutic effectiveness of *Tinospora cordifolia* phytosomes (Figure 4).

***In vitro* Drug Release Studies**

In vitro drug release tests of *Tinospora cordifolia* phytosomal formulations showed varying drug dissolution rates throughout an 8-hour experiment for different phytosomal varieties (Table 6). F1 along with F3 and F6 attained maximum drug release values of 98.7%, 96.5% and 92.5% respectively due to their swift dissolution characteristics thus offering the best choice for quick therapeutic application. The sustained drug delivery capabilities of phytosomes F2, F7, F8, and F9 can be seen in their drug release pattern of 92.3%, 80.2%, 82.5%, and 81.3% respectively. These results demonstrate their potential to provide drug release over longer periods. The drug release from F4 and F5 was slow while providing potential benefits for sustained drug delivery technology with stable plasma drug

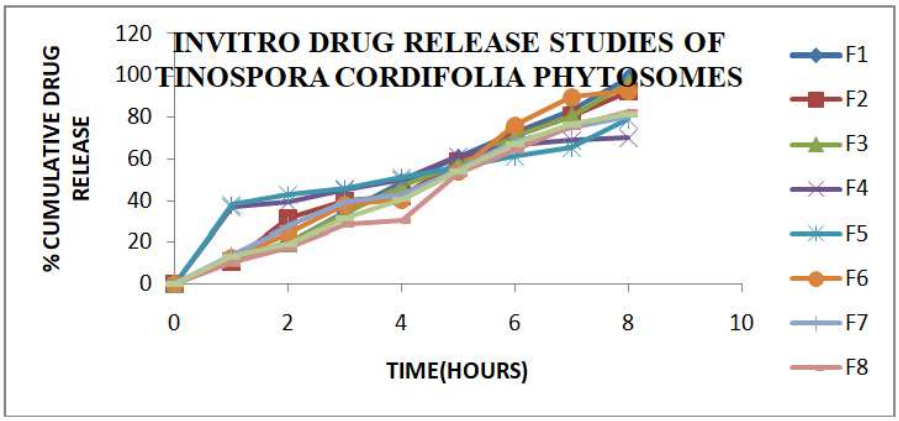

Figure 4: Dissolution curves of *Tinospora cordifolia* phytosomes

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

levels. The inital drug release patterns of F4 and F5 create a burst effect reaching 36.9% and 38.1% respectively within the first few hours but the release patterns became sustained afterward. The most suitable formulations that combine high drug release efficiency with controlled release profiles are F1, F3 and F6 according to their overall release kinetics measurements. The research findings will support the optimization of phytosomal drugs because they help improve *Tinospora cordifolia* bioavailability and therapeutic effects.

Formulation F4 is recommended as the best formulation due to its excellent balance of burst and controlled release properties, making it ideal for practical applications in drug delivery systems. Further studies can focus on optimizing this formulation to enhance its performance even further, ensuring that it meets the desired pharmacokinetic profiles for therapeutic efficacy.

An 8-hour *in vitro* drug release study of *Tinospora cordifolia* phytosomal formulations demonstrates different rates of cumulative drug release that appear in the provided graph. The drug dissolution charts indicate that the drug delivery increased steadily through time yet specific formulations reached almost 100% drug release by the study duration. Among the test formulations F1 F3 F6 show the fastest drug release rates thus indicating they have the best potential for pharmaceutical applications. The drug release kinetics of F4 and F5 is steady which indicates a sustained therapeutic effect duration.

The drug release through F4 and F5 shows a brief initial stage of accelerated release during the first 2 hours that should benefit patients by providing rapid therapeutic effects. Most of the evaluated formulations release drugs through a controlled process which provides both stability and continuous drug availability throughout the period. The formation capabilities combined with formulation composition and particle dimensions determine the differences in released drug amounts.

The rate analysis shows F1 F3 and F6 deliver maximum drug release efficiency thus making them superior candidates for therapeutic effectiveness. Further pharmacokinetic studies along with clinical applications will benefit from these findings by selecting the best formulation which ensures *Tinospora cordifolia* phytosomes become effective components for both herbal medicine and pharmaceutical products.

Drug Release kinetics of optimized formulation

The drug release kinetics data enables researchers to gain essential information about drug release percentages along with remaining drug levels and different mathematical transformations of the release

Table 6: *In vitro* Drug Release Studies

Time (hour)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	11.5	10.6	12.6	36.9	38.1	12.3	13.5	10.3	13.3
2	19.9	31.5	19.7	39.4	42.5	24.6	27.7	17.1	18.9
3	34.7	39.6	33.8	45.3	45.6	38.2	39.1	28.4	31.4
4	48.9	42.3	46.8	49.7	51.1	40.2	42.9	30.5	40.6
5	60.4	58.4	57.2	61.2	55.6	53.9	54.5	52.7	53.8
6	72.5	71.5	70.8	66.4	61.3	75.9	68.3	64.2	67.2
7	83.2	80.5	80.6	69.1	65.4	89.5	74.6	75.2	76.5
8	98.7	92.3	96.5	70.1	78.9	92.5	80.2	82.5	81.3

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

pattern. The sustained drug release over an 8-hour period led to the cumulative drug release growing from 0% to 92.3% and a parallel decrease from 100% to 7.7% of drug remaining deputed in Table 7.

A first-order release model becomes apparent through the drug remaining profile because it shows a gradual decline which depends on available drug concentration levels. Drug diffusion proves significant in the release process because the drug release mechanism follows both the square root of time transformation and the Higuchi release model. The controlled release pattern emerges through increased log cumulative drug release values together with decreasing cube root of drug remaining (Wt) values that demonstrate diffusion-control as a likely mechanism which follows the Hixson-Crowell cube root law for dissolving objects with changing surface areas. The drug release data shows that the continuous drug depletion from the formulation causes the Wo - Wt value to progressively increase. The drug release mechanism appears to be governed by first-order kinetics and Higuchi kinetics and it follows a controlled diffusion pattern. The rate-controlled drug delivery demonstrates constant drug release which indicates improved therapeutic outcomes and drug bioavailability deputed in Figure 5-9.

Kinetics of Phytosome Release Together with Optimization Process

The data of drug dissolution was evaluated using various kinetic models to identify the release mechanism. Optimized phytosome formulation exhibits zero-order kinetic behavior along with R^2 = 0.974 value which indicates time-consistent drug release. The drug release parameters match the Hixon-Crowell model (R^2 = 0.965) which indicates the dissolution aspects related to cholesterol amphiphilic nature drive the incremental matrix erosion of phytosomes.

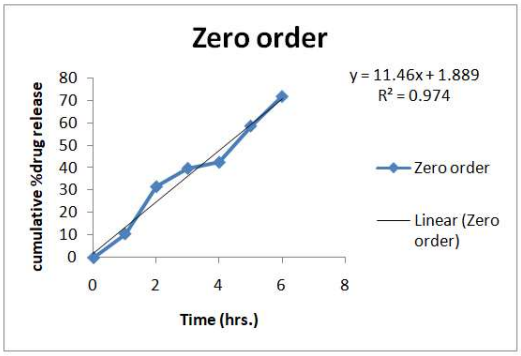

Figure 5: Zero order release kinetics of optimized *Tinospora cordifolia* phytosome formulation

Table 7: Drug Release kinetics of optimized formulation

Time (Hr)	cumulative % drug released	% drug remaining	Square root time	log Cumu % drug remainining	log time	log Cumu % drug released	% Drug released	Cube Root of % drug Remaining (Wt)	Wo-Wt
0	0	100	0.000	2.000	0.000	0.000	100	4.642	0.000
1	10.6	89.4	1.000	1.951	0.000	1.025	10.6	4.471	0.171
2	31.5	68.5	1.414	1.836	0.301	1.498	20.9	4.092	0.550
3	39.6	60.4	1.732	1.781	0.477	1.598	8.1	3.924	0.718
4	42.3	57.7	2.000	1.761	0.602	1.626	2.7	3.864	0.778
5	58.4	41.6	2.236	1.619	0.699	1.766	16.1	3.465	1.177
6	71.5	28.5	2.449	1.455	0.778	1.854	13.1	3.055	1.587
7	80.5	19.5	2.646	1.290	0.845	1.906	9	2.692	1.950
8	92.3	7.7	2.828	0.886	0.903	1.965	11.8	1.975	2.667

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

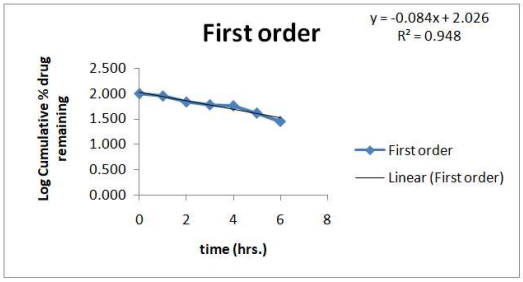

Figure 6: First order release kinetics of optimized *Tinospora cordifolia* phytosome formulation

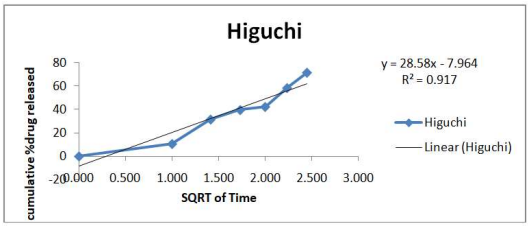

Figure 7: Higuchi release kinetics of optimized *Tinospora cordifolia* phytosome formulation

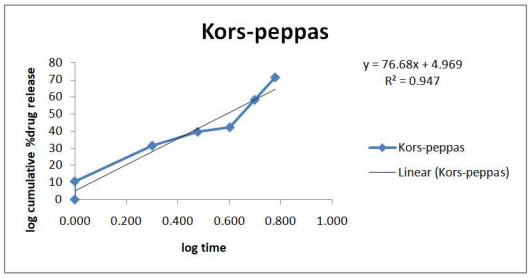

Figure 8: korsmeyer peppas release kinetics of optimized *Tinospora cordifolia* phytosome formulation

Optimization of Phytosome Formulation

The optimized formulation F2 outperformed all other variants regarding percentage yield and entrapment efficiency and drug release and particle size measurements. The research findings led investigators to choose F2 phytosome formulation as their optimum selection because it exhibited improved drug accessibility through sustained

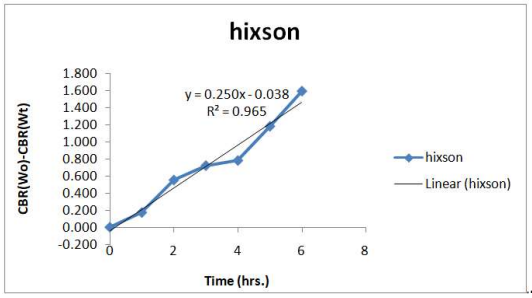

Figure 9: Hixson release kinetics of optimized *Tinospora cordifolia* phytosome formulation

release kinetics for potential pharmaceutical implementation.

Conclusion

This research project developed and characterized *Tinospora cordifolia* phytosomes for improving the biological absorption and therapeutic effect of its active pharmaceutical ingredients. The experimental samples underwent multiple tests which provided data regarding percentage yield together with entrapment efficiency and particle size measurements and drug release characteristics *in vitro* tests. The research team selected F2 as the best formulation which demonstrated superior performance throughout various examinations. The drug release pattern observed in the optimized formulation exhibited zero-order kinetics (R^2 = 0.974) which led to a consistent drug delivery during the time period. The drug release pattern followed the Hixson-Crowell model (R^2 = 0.965) because the dissolution behavior affected by cholesterol's amphiphilic properties controlled the drug release mechanism. Phytosomes gain effective membrane permeation because of their amphiphilic design which enhances drug uptake and bioavailability.

The findings establish that phytosome-based drug delivery approaches demonstrate potential as effective mechanisms for optimizing therapeutic release of herbal bioactives. The phytosomal formulation of *Tinospora cordifolia* demonstrates successful potential in pharmaceutical and nutraceutical

Vol. 19 (Supplementry Issue 1A), June 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.2s.11

applications because it improves plant-derived compound pharmacokinetic properties. The therapeutic potential of the drug needs additional research through in vivo pharmacokinetic assessments along with clinical studies to establish its effectiveness.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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