

Development and Optimization of Phytosome for Enhancement of Therapeutic Potential of Epiyangambin in *Tinospora cordifolia* Extract Identified by GC–MS and Docking Analysis

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Abstract

Background: To enhance the therapeutic potential of *Tinospora cordifolia* phytosomes, a chloroform extract was prepared.

Objectives: The goal of this study was to create and test the therapeutic potential of a phytosome containing *T. cordifolia* extract.

Materials and Methods: The extraction of nonpolar compounds was carried out using chloroform as solvent. The active constituent of the extract was subjected to phytochemical and Gas Chromatography–Mass Spectrometry (GC–MS) analyses. Docking studies against multiple targeted proteins confirmed the anti-diabetic, anti-inflammatory, and analgesic properties of *T. cordifolia* extract. The phytosomes of *T. cordifolia* extract were prepared by solvent evaporation technique. The phytosomes were characterized for vesicle size, entrapment efficiency, surface morphology, FTIR, *in vitro* drug release and *in vivo* anti-diabetic, anti-inflammatory, and analgesic activities.

Results: The highest concentration was found to be 16.58% for epiyangambin. The reported compound inhibits GLUT1 and COX2 with 9.25 and 8.34 kcal/mol binding scores. The optimized TCP4 exhibited 454.2 ± 8.1 nm of vesicle size, –43.1 ± 7.5 mV of zeta potential, and 0.33 of polydispersity index (PDI). The phytosomes exhibited a spherical shape confirmed by TEM analysis. The formulation TCP4 showed a significantly higher release (94.7% ± 1.7%) than pure extract. TCP4 exhibited potent therapeutic potential for anti-diabetic, anti-inflammatory, and analgesic activity.

Conclusion: Chloroform extract loaded phytosomes with cholesterol as lipids exhibited significant therapeutic potential due to the presence of epiyangambin.

Keywords

T. cordifolia, Epiyangambin, Phytosome, Anti-diabetic, Anti-inflammatory, Analgesic.

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Introduction

Herbal formulations consist of individual or combined herbal extracts used to treat various diseases.¹ Due to the rising use of allopathic drugs, herbal remedies have lost their significance.² Before introducing the allopathic system, traditional medicine has operated for over two thousand years.³ The effectiveness of plant-based traditional medicine is gaining attraction due to its cheap cost and minimum adverse effects.⁴ India and China reported consuming approximately 1000 tons of *Tinospora cordifolia* (Willd.) per year.^{5,6} The primary active constituents are alkaloids, furano diterpenoids, norditerpenoids, sesquiterpenoids, phenolics, lignin, and sterols. It also contained aliphatic compounds, polysaccharides, essential oils, and fatty acids.^{7,8} *T. cordifolia*

(TC) is used as bitter tonics in jaundice, arthritis, diabetes, and other skin ailments.^{9–11} The whole plant extract is responsible for hepatoprotective, anti-ulcer, and antioxidant properties. The extract of the stem exhibits the hepatoprotective effect. In addition, stems also showed hepatoprotective,

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anti-pyretic, cytotoxic, anti-diabetic, and immunomodulatory properties.^{12–17} The roots have emetic, anti-stress, anti-oxidant, anti-ulcer, anti-diabetic, and visceral obstructions.¹⁸ Hyperbilirubinemia and rheumatic disorders are treated with dried fruits, whereas diabetes is treated with the leaves.¹⁹

The structure-based drug design is widely accepted for determining the binding affinity with specific proteins. Strong protein–ligand interaction indicates a high binding relationship between specific proteins and ligands. The molecular docking approach is applied to simulate a biomolecular interaction, and it provides data on the affinity of each ligand.²⁰

A lipid-compatible molecular complex between lipids and phytoconstituents is termed phytosomes.²¹ It enhanced permeation and intestinal permeability compared to pure phytochemical extracts. It also has a preventive impact on herbal extracts by shielding them from digestive fluids and destroying gut flora. They may readily pass through the bilayer membrane and bloodstream.²² The lipids and nonionic surfactants generate unilamellar or bi-lamellar structures of phytosomes. Spans and tweens have better entrapment and release characteristics among the nonionic surfactants.²³

Type 2 diabetes is a complex disease associated with genetic defects. Type 2 diabetes is 20 times more common compared to type 1. Patients with prolonged exposure lead to overweight and obesity due to poor lifestyles and uncontrolled diets. The chances of type 2 diabetes increase with an increase in age. Because of their high incidence and common risk factors, type 2 diabetes and arthritis are linked illnesses. Approximately 48% of patients who have type 2 diabetes are associated with the complication of arthritis. Aging is also a critical risk factor for diabetes and arthritis. Different treatment options are available to manage arthritis and diabetes.²⁴

The present research work involves extracting the phytoconstituents from *T. cordifolia* using chloroform and identifying the phytoconstituents by Gas Chromatography–Mass Spectrometry (GC–MS). The molecular docking analysis was applied to determine the anti-diabetic, anti-inflammatory, and analgesic effects of the active ingredient. Phytosomes of *T. cordifolia* chloroform extract was developed by the reverse phase solvent evaporation method. Phytosomes were evaluated for various parameters, that is, physico-chemical characterization, morphological examinations, and *in vitro* release study. *In vivo* anti-diabetic, anti-inflammatory, and analgesic effects of phytosomes were also evaluated.

Materials and Methods

Chemicals and Reagents

T. cordifolia hydroalcoholic extract was procured from Herbal Creations (Nainital, India). Span 60, cholesterol, and soylacethin were purchased from Sigma Aldrich (Bangalore, India). Aspirin, glibenclamide, and indomethacin were

procured from Dr. Reddy's Laboratory (Hyderabad, India). All solvents and other chemical reagents of analytical grade were procured from Fischer Scientific Laboratory (Mumbai, India).

T. cordifolia Extract Preparation

An amount of 500 ml chloroform was added into 200 g of defatted hydroalcoholic extract and macerated for 24 h with continuous shaking. The chloroform layer was collected and evaporated to dryness to get the semisolid mass. The extract was air-dried and stored in an amber color container for further study.²⁵

Phytochemicals Analysis

A detailed phytochemical investigation was carried out for chloroform extracts. The chemical tests were performed for alkaloids, glycosides, terpenoids, phenolics, lignans, steroids, aliphatic compounds, polysaccharides, essential oils, and fatty acids.²⁶

GC–MS Analysis

Agilent 7890A GC System (Model: AccuTOFGCv/JMS-T100GCv, Tokyo, Japan) was used for GC–MS analysis as per previous published work with slight modification.²⁵ Electron Impact Ionization (EI) positive ionization source mass spectrometry was used to identify the separated chemicals. The structure of the compounds was determined by mass spectral data from the National Institute of Standard and Technology (NIST) library database.²⁵

Preparation Structures for Docking Analysis

Chemdraw ultra 12.0.2 and Chem 3D Pro 12 were used to create the structure of the identified molecule in mol2 format.

Analysis of Docking

The docking analysis was performed with the Swiss Dock analysis tool (<http://www.swissdock.ch/docking>) and the UCSF Chimera 1.5.3. The anti-diabetic and anti-inflammatory studies were conducted against specific proteins reported by Kumar et al.²⁵ The targeted proteins 5ycp (Human PPAR-gamma), 4pyp (human glucose transporter GLUT1), 1eqg (COX-1), 3ln1 (COX-2) was collected from RSCB protein data bank in pdb format.

Formulation of *T. cordifolia* Loaded Phytosomes

The phytosomes containing *T. cordifolia* chloroform dry extract were prepared using the reverse phase solvent evaporation method.²⁷ Table 1 shows the compositions of several phytosome preparations. The varying quantities of cholesterol and soya-lecithin as per given in the Table 1 were

Table 1. Composition for the *T. cordifolia* Chloroform Extract Phytosome. Formulation.

Ingredients (g)	TCP1	TCP2	TCP3	TCP4	TCP5
Chloroform extract of <i>T. cordifolia</i>	1	1	1	1	1
Cholesterol	0.25	0.50	0.75	1	1.25
Span 60	1	1	1	1	1
Soya lecithin	0.30	0.30	0.30	0.30	0.30

transferred in the conical flask and dissolved with ether (15 ml). The required quantity of span 60 (1 g) was dissolved in methanol (5 ml) and mixed with lipid mixture. One gram of extract was added to the lipid surfactant mixture and stirred magnetically at 500 rpm (Remi magnetic stirrer 2MLH, Mumbai, India). Precisely 10 ml of phosphate buffer (pH 7.4) was transferred in a round bottom flask at 1 ml/min. The resultant mixtures were refluxed at 60°C–65°C for 1 h with continuous stirring. A rotary flash evaporator was used to recover the organic solvent at 65°C and 50 rpm (Cyberlab Corporation, CR2001, Delhi, India). The aqueous phase was homogenized at 8000 rpm for 2 h at 4°C (Remi homogenizer, RQT-1274, Mumbai, India). Finally, the phytosomes were sonicated at 8°C using a probe sonicator (Citizen Digital ultrasonicator, CD4820) for 5 min at 30 s intervals. The formulation was stored in an amber glass bottle at 2°C–8°C for further study.

Entrapment Efficiency (% EE)

The ultracentrifugation technique was used to measure the entrapment efficiency. Two milliliters of freshly prepared phytosomes were centrifuged for 30 min at 10,000 rpm (Remi cooling centrifuge, Remi, India). Carefully pipette out 1 ml supernatant liquid and added 9 ml of buffer and made the final volume 10 ml. The absorbance was analyzed by UV–Visible spectrophotometer (Lab India-UV 3000) at 216 nm. The % EE was calculated by following formula.²⁸ Each study was carried out in triplicate and data was represented in the form of mean $\pm$ SDs.

$$\% \text{ EE} = \frac{C1 - C2}{C1} \times 100$$

where C1 is the drug concentration in the liquid supernatant and C2 is the total amount of extract in the formulation.

The Size Distribution, Zeta Potential and Polydispersity Index (PDI)

The Malvern Zetasizer (Malvern Instruments, Southborough, UK) was used to study the vesicle size, PDI and zeta potential of phytosomes.²⁹ The diluted sample was filled in the cuvette and analysis was carried out at 25°C $\pm$ 2°C and 90° scattering angle. The measurement was done at a 1.330 refractive index

and 0.050 absorptions. The zeta potential of the sample was analyzed using an electrode cuvette. Each study was carried out in triplicate and data was represented in the form of mean $\pm$ SDs.

Transmission Electron Microscopy (TEM)

TEM instrument (JEM 2100F, Tokyo, Japan) was used for morphological examination of phytosomes. On a 200-mesh carbon-coated copper grid one drop of a sample dropped. Remove excess solvent by blotting with filter paper. The samples were air-dried and placed into TEM and the image was captured using software.

FTIR Analysis

Bruker instrument (3000 Hyperion Microscope with Vertex 80 FTIR System, Germany) equipped with Micro ATR, Grazing angle was used for FTIR analysis of samples. Approximately 2 mg of the samples were applied to the sample plate and scanned at 4000–500 cm⁻¹ with a spectral resolution of FTIR 1.0 cm⁻¹.

In vitro Release and Release Kinetic Studies

The USP dissolution apparatus (Dissolution tester, USP, TDT-08L, Elctrolab, Mumbai, India) was used for the release study using a dialysis bag.³⁰ The 900 ml of freshly prepared phosphate buffer (pH 6.8) was filled into basket as release media and maintained at 37°C $\pm$ 0.5°C in a pretreated dialysis bag (Dialysis Membrane-60, Av. flat width 25.27 mm, Av. diameter 15.9 mm, approximate capacity 1.99 ml/cm, Mumbai, India) 5 ml of the sample (250 mg equivalent extract) was filled and fitted with pad of dissolution apparatus and rotated at 50 rpm. The 5 ml sample was collected at predefined intervals and replaced with 5 ml of fresh medium to maintain a constant volume. The concentration of the drug was analyzed by UV-spectrophotometric at 216 nm. The absorbance data were used to calculate the percentage of cumulative drug release (% CDR). The release profile fitted to zero order, first order, Higuchi diffusion, and Korsmeyer–Peppas models. The correlation coefficient (R^2) was calculated to determine the best-fitted releases model and mechanism of drug release. Each study was carried out in triplicate and data was represented in the form of mean $\pm$ SDs.

In vivo Study

Animals

Wister rats (180 and 200 g) of either sex were used for anti-diabetic, anti-inflammatory, and analgesic activity. Wister mice of 20–25 g of either sex were selected for analgesic activity. The animals were housed in standard climatic conditions for 24 h before the experiment. The Institutional Animal Ethical Committee (IAEC) approved all of the experimental protocols of Translamin Institute of Pharmaceutical Education and Research, Meerut (Reg. Number 1207/PO/RE/S/08CPCSEA, Ref. no. TIPER/CPCSEA/21/247).

Anti-diabetic Properties

Induction of Diabetic Condition

Insulin-dependent diabetes is induced by intraperitoneal administration of a single dose of streptozocin (25 mg/kg) and continuous supply of sugar solution for drinking.²⁸ The animal was standing for 72 h for stabilization of blood glucose level. Blood glucose level was determined by taking blood samples from the dorsal vein. Fasting blood glucose levels of 270–310 mg/100 dl is considered a diabetic condition.

Dose Schedule and Experimental Procedure

The rats were divided into four groups and each group contained six rats. Group 1 is a normal control (10 ml/kg normal saline), group 2 is disease control, group 3 is treated with glibenclamide (50 mg/kg), group 4 is treated with pure *T. cordifolia* extract (250 mg/kg), and group 5 is treated with phytosomes (TCP4, 250 mg/kg). Treatment was done for 21 days and at different time intervals (1, 7, 14, and 21 days) and the blood glucose level was measured using glucose oxidase-peroxidase (GOD-POD) kit (Accurex, India).

Biochemical Parameters

All biochemical parameters like total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), triglycerides, creatinine, urea, and uric acid were measured using published methods.³¹ Commercially available kits were used to carry out the estimations by Erba diagnostic kit Mannheim GmbH, Mallaustr, Germany. The concentrations of different biochemical parameters were determined using the auto-analyzer Erba Chem-7.³²

Histological Examination

A semi-automated microtome (RM2155 Leica Microsystems) was employed to slice the paraffin-embedded pancreas.³⁴

The tissue pieces were placed on a glass, slide and deparaffinized with xylene and 70% ethanol. Hematoxylin and eosin were used to stain the sections. All slides were viewed at a magnification of X200 using light microscopy (Yamto 100, Japan).

Anti-inflammatory Activity

Carrageenan-induced hind paw edema technique was used to evaluate the anti-inflammatory effect.³⁴ Rats were divided into four different groups and each group having six rats. Group 1 was treated with normal saline (10 ml/kg), group 2 treated with indomethacin (50 mg/kg), group 3 treated with pure *T. cordifolia* extract (250 mg/kg), and group 4 treated with TCP4 (250 mg/kg). After one hour of therapy, 1% carrageenan was injected into the left hind leg to induce edema. The rats were given water at a 2 ml/100 g body weight to limit variance in edema development. Paw volume measured up to 5 h at 1 h of interval. A decrease in paw volume is considered an anti-inflammatory action.

% Inhibition =

$$\frac{\text{Paw volume of control} - \text{Paw volume of test}}{\text{Paw volume of control}} \times 100$$

Acetic Acid-Induced Writhing in Mice

This study was conducted on mice. The mice were divided into four groups and each group contain six mice. Group 1 received normal saline 10 ml/kg, group 2 received aspirin 100 mg/kg, group 3 treated with pure *T. cordifolia* extract (250 mg/kg), group 4 received TCP4 (250 mg/kg). Glacial acetic acid solution (IP injection (0.6% v/v, 1 ml/kg glacial acetic acid solution) was injected 30 min before receiving the treatments. The number of writhes was counted for 3 h with 1 h of interval. The % inhibition was calculated by the given formula. A decrease in the number of writhes indicates the presence of analgesic properties.³⁵

% inhibition =

$$\frac{\text{Number of writhes for control} - \text{Number of writhes for test}}{\text{Number of writhes for control}} \times 100$$

Statistical Analysis

The data were analyzed using Graph Pad Prism 5 (GraphPad Prism 5.00.288). The data were presented as mean and standard error mean (SEM), with a one-way analysis of variance (ANOVA). Dunnett's test was used to compare the control and test substances ($p \leq .05$ considered significant).

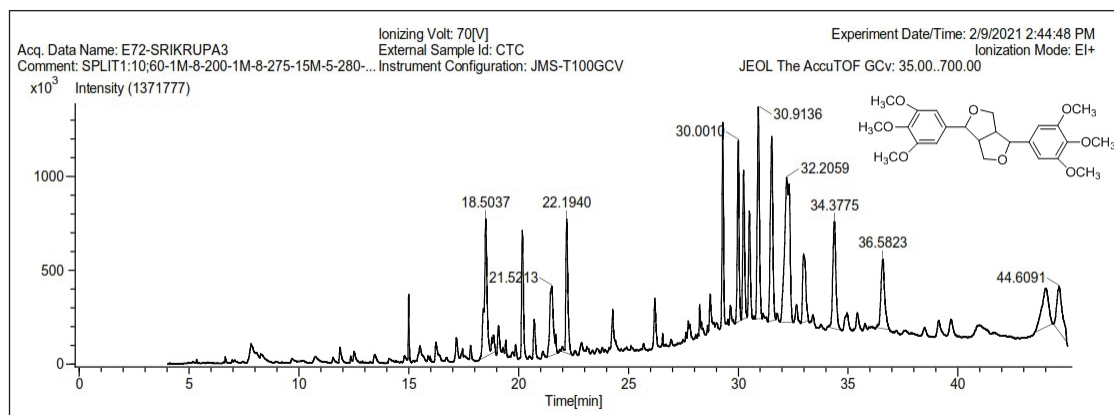


Figure 1. Chromatogram Diagram for Chloroform Extract of *T.cordifolia* by GC–MS.

Source: Kumar et al. (2022).²⁵

Abbreviation: GC–MS, Gas Chromatography–Mass Spectrometry.

Results and Discussion

Percentage Yield and Phytochemical Analysis

Extraction was carried out by the cold maceration method using chloroform as solvent. The percentage yield was found to be 29.8%. Phytochemical screening showed the presence of alkaloids, flavonoids, steroids, phenolic compounds, lignin, terpenoids, and aliphatic chemicals in the extract.

GC–MS analysis

The GC–MS chromatogram of the *T. cordifolia* extract is shown in Figure 1. Epiyangambin exhibited in highest concentration as compared to another compound (16.58% at 32.50 min).²⁵ Chemically it is a furofuran lignan with PAF antagonist activity.³⁶

Docking Analysis

The ligand–protein interactions with strong binding affinity are represented in Figure 2. The epiyangambin exhibited good binding affinity against peroxisome proliferator-activated receptor gamma ligand (PPAR- γ), glucose transporter 1 (GLUT-1), cyclo-oxygenase-1 (COX1) and cyclo-oxygenase-2 (COX-2) inhibition, and compared to standards (rosiglitazone, glibenclamide, aspirin, and celecoxib). The binding energies (ΔG) were found to be -8.84 , -9.25 , -8.24 , and -8.34 kcal/mol, respectively. The molecule epiyangambin has more GLUT-1 and COX-2 inhibitory action.²⁵ High blood sugar levels promote the formation of reactive oxygen species (ROS), which are chemicals that stimulated the release of pro-inflammatory cytokines (substances secreted by specific immune cells). It also causes the formation of chemicals known as advanced glycation end products (AGEs), which accumulate in and destroy joints.³⁷ Lignins have limited medicinal applications

because of the lack of solubility in aqueous and organic phases.³⁸ Phytosomes are considered an ideal approach to improving the therapeutic efficacy of water-insoluble phytoconstituents.²¹ The solubility profile of lignin epiyangambin and its GLUT-1, and COX-2 inhibitory action suggests further formulation of phytosomes of the extract to increase oral bioavailability and therapeutic efficacy.

Formulation Development

The extract-loaded phytosomes were successfully prepared by the solvent evaporation method. The composition of all phytosomes was given in Table 1.

Vesicle Size, PDI, and Zeta Potential

Table 2 represented the result of vesicle size, PDI, and zeta potential of all phytosomes formulation. The vesicle size, PDI, and zeta potential of all phytosomes were found in the range of 265.4–814.5 nm, 0.23–0.57, and 40.8–58.1 mV (negative), respectively. It was observed that cholesterol concentration increased the vesicle size because it interferes with the closed packing of the lipid bilayer and increases the fluidity of the membrane.³⁹ In addition, the PDI of phytosomes increases on increasing the cholesterol concentration because of vesicle size increases.⁴⁰ Zeta potential of phytosomes increases by increasing the cholesterol concentration. A high value of zeta potential indicated the high stability of formulation (more repulsion between vesicles) and that vesicles did not coagulate with each other.

Entrapment Efficiency

The entrapment efficiency is an important parameter because of its direct effect on the concentration of the drug for pharmacological activity. The results of entrapment efficiency

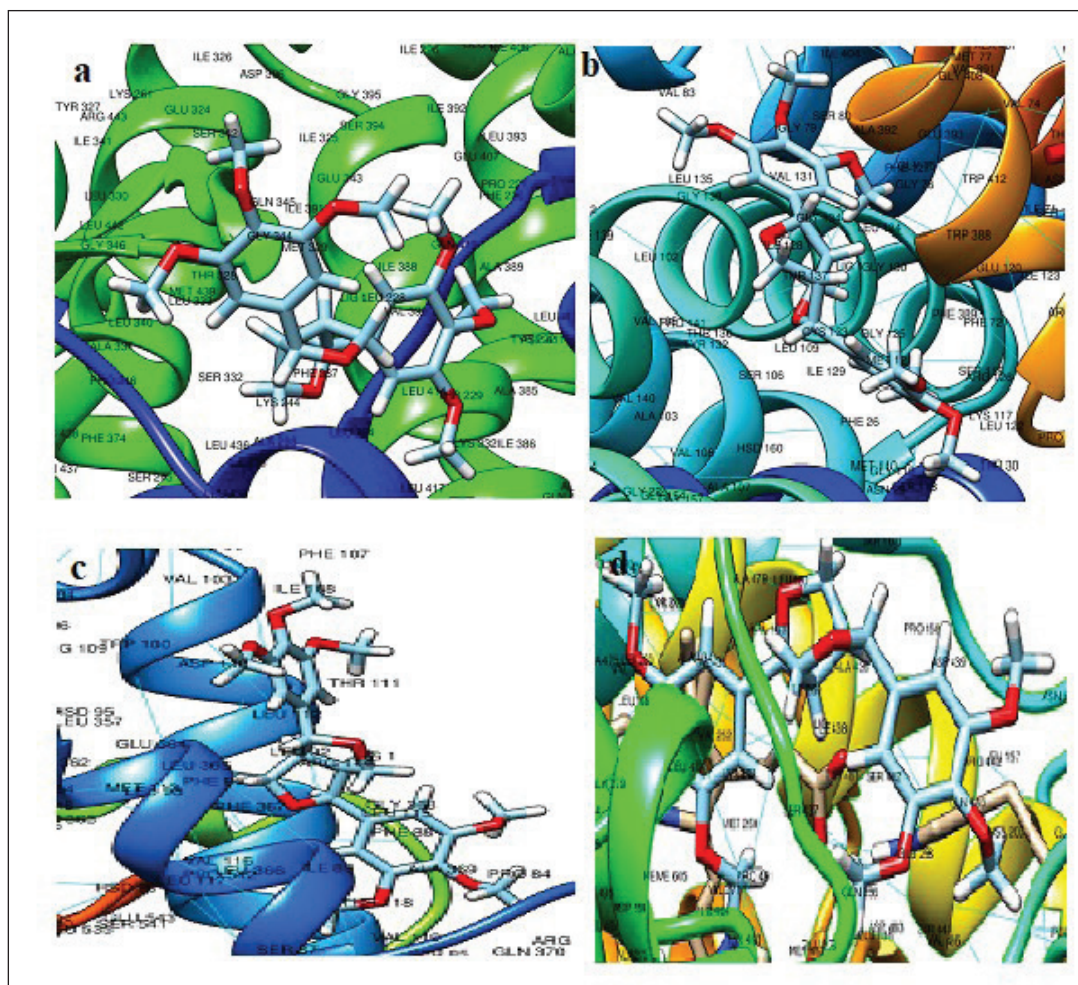


Figure 2. Ligand Protein Interaction of CEC7 with 5cyp (a), 4pyr (b), 1eqg (c), and 3lnl (d).

were given in Table 2. All the formulations exhibited % EE in the range of 55.8%–92.1%. The entrapment efficiency of the extract in phytosomes increases by increasing cholesterol concentration because cholesterol increases the rigidity and permeability of lipid bilayer and prevents the leakage of the drug, hence increasing the entrapment efficiency.⁴¹

On the basis of optimum vesicle size, PDI, zeta potential and entrapment efficiency, the formulation TPC4 was selected as the best formulation. The optimized phytosomes TPC4 have a vesicle size of 454.2 ± 8.1 nm (Figure 3a), PDI of 0.33, zeta potential of -43.1 ± 7.5 mv (Figure 3b) and entrapment efficiency of $82.7\% \pm 1.6\%$, respectively. TPC4 is used for further evaluation.

TEM Analysis

The morphology of the optimized TCP4 formulation was analyzed by TEM instruments and the image was shown in Figure 4 and found to be a spherical shape. The concentric

bilayer vesicle was formed due to hydrophobic and hydrophilic interaction between lipid and drugs. The nonionic surfactants reduce the interfacial tension between both surfaces.⁴² The shape of the phytosomes depends on the nature of the lipid and the temperature of the hydration medium. The hydrogen bonding interaction of lipid with surfactant is essential for encapsulation efficiency and release rate.⁴³ The stronger interaction reduces vesicle size and release rate. The asymmetric vesicle morphologies are stable; promote cellular polarity and consequently biological effectiveness.⁴⁴

FTIR Analysis

The FTIR spectra of pure *T. cordifolia* extract, cholesterol, span 60 and optimized formulation (TCP4) were recorded and spectra were depicted in Figure 5. FTIR spectra of pure TC extract exhibited characteristic peaks at 3391 (–OH, str), 1620 (C=O, str), 1384 (–C–O–, str), and 1153 (–C–N, str).

Table 2. Vesicle Size, Zeta Potential and PDI of Optimized Phytosomes.

Formulation	Z Average (d nm) $\pm$ SD	Zeta Potential (mV) $\pm$ SD	PDI	Entrapment Efficiency $\pm$ SD
TCP1	265.4 $\pm$ 4.5	-58.1 $\pm$ 3.2	0.23	55.8 $\pm$ 3.1
TCP2	310.5 $\pm$ 6.4	-48.2 $\pm$ 3.4	0.29	63.6 $\pm$ 2.5
TCP3	390.1 $\pm$ 5.2	-45.2 $\pm$ 4.1	0.31	73.5 $\pm$ 2.1
TCP4	454.2 $\pm$ 8.1	-43.1 $\pm$ 7.5	0.33	82.6 $\pm$ 1.6
TCP5	814.5 $\pm$ 7.3	-40.8 $\pm$ 7.1	0.57	92.1 $\pm$ 1.1

Note: All the studies were carried out in triplicate and data were represented in mean $\pm$ SD.

Abbreviations: SD, standard deviation; PDI, polydispersity index.

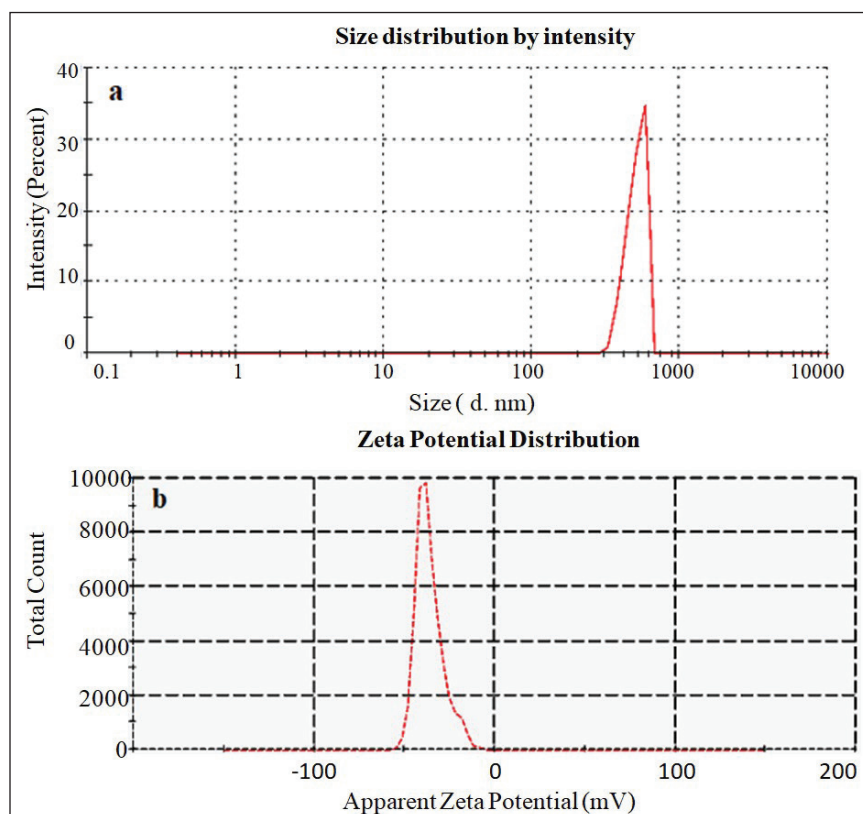


Figure 3. (a) Vesicle Size Distribution Graph of TCP4 and (b) Zeta Potential Analysis TCP4.

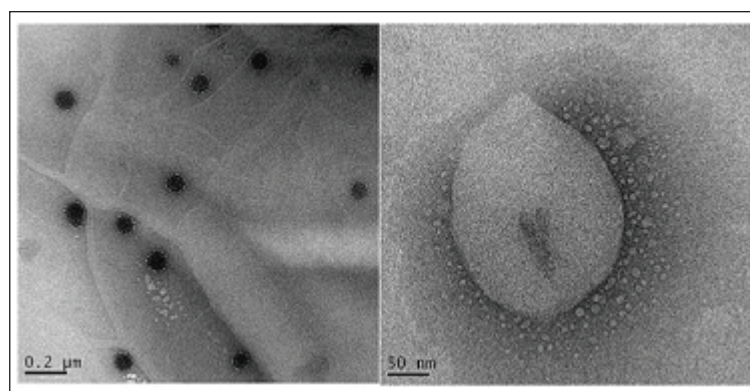


Figure 4. HR-TEM Image of TCP4.

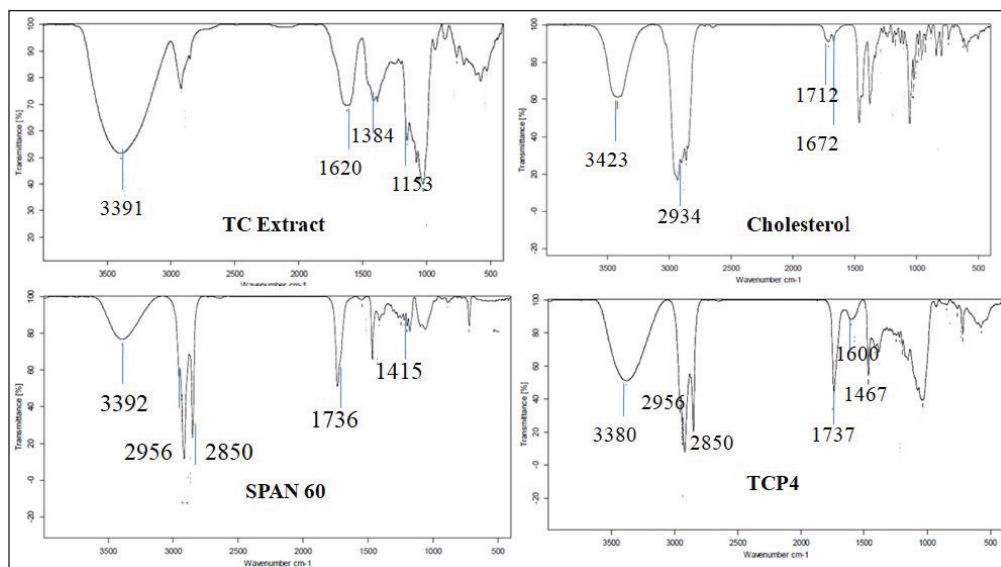


Figure 5. *In vitro* Incompatibility Study by FTIR Analysis for Optimized Phytosomes.

The cholesterol exhibited characteristic peaks at 3423 (O–H, str), 2934 (CH₃, str), 1712 (C–H, str), and 1672 (C=C, str). The span 60 exhibited characteristic peaks at 3392 (O–H, str), 2956 (–CH₃, str), 2850 (–CH₂–, str), 1736 (C=O, str), and 1415 (–C–O–, str). However, the TCP4 exhibited characteristic peaks at 3380 (O–H, str), 2956 (CH₃, str), 2850 (–CH₂–, str), 1600 (C=C, str), 1737 (C=O, str), and 1467 (–C–O–, str). In TCP4 all the characteristic peaks of pure *T. cordifolia* extract present without significant shift revealed that there is no interaction between drug and excipients. The change in intensity of the characteristic IR peaks also confirmed the formation of van der Waals forces, repulsive forces, entropic repulsive forces and short-acting repulsive forces, and so on, among surfactant lipid and extract molecules. The van der Waals forces provide stability in the phytosomes. The repulsive forces played an important role in drug release characteristics.⁴⁵

In vitro Release Study

In vitro release study of pure *T. cordifolia* extract and optimized formulation TCP4 dispersion was analyzed by dialysis bag and data were represented in Figure 6. The *in vitro* release study was done to determine the release behavior of drugs from the formulation in body circulation. The optimized formulation TCP4 showed initial fast-release (49.43% in 1 h) and later sustained-release (94.7% $\pm$ 1.7% in 12 h). The higher release of extract from phytosomes is due to the surface deposition of a drug as well as increased solubility due to the presence of lipid and surfactant in the formulation. The surfactant and lipid reduced the interfacial tension between the aqueous and lipid phase. However, the pure extract exhibited only

26.2% $\pm$ 1.8% in 12 h, which is due to the low aqueous solubility of the extract.

Drug Release Kinetics

Drug release kinetics of optimized formulation (TCP4) was assessed by plotting zero-order, first-order, Higuchi, and Korsmeyer–Peppas model through Microsoft Excel as shown in Figure 7. The mean correlation coefficient (R^2) is 0.814 for zero-order, 0.952 for first order, 0.970 for Higuchi and 0.504 for Korsmeyer–Peppas model. The maximum R^2 was found to be more maximum for the Higuchi model ($R^2 = 0.97$) than in other models. So, Higuchi is the best fit kinetic release model. The release exponent is 0.532 indicates correlation coefficient value of drug release.

Anti-diabetic Activity

The anti-diabetic activity for 21 days of the study were given in Table 3. The body weight of toxic control rats was 136.6 $\pm$ 1.9 g at 21 days. However, the body weight of the glibenclamide and TCP4 treated groups increases significantly i.e., 183.8 $\pm$ 2.9 g for the glibenclamide group, 160.5 $\pm$ 2.3 g for *T. cardifolia* extract and 163.5 $\pm$ 2.4 g for TCP4 group on 21 days treatment. The blood glucose levels of disease control groups was 427 $\pm$ 15.10 mg/dl at 21 days. The glibenclamide group and TCP4 treated group showed significant decreases ($p < .05$) in blood glucose levels up to 21 days of study which is 112.8 $\pm$ 10.31 mg/dl (glibenclamide), 216.7 $\pm$ 12.2 mg/dl (TC) and 206.7 $\pm$ 13.9 mg/dl (TCP4) respectively. *T. cordifolia* exhibits stimulatory glucose absorption. The TCP4 demonstrated anti-hyperglycemic action owing to epiyangambin inhibition of GLUT1. GLUT1 and GLUT3 do

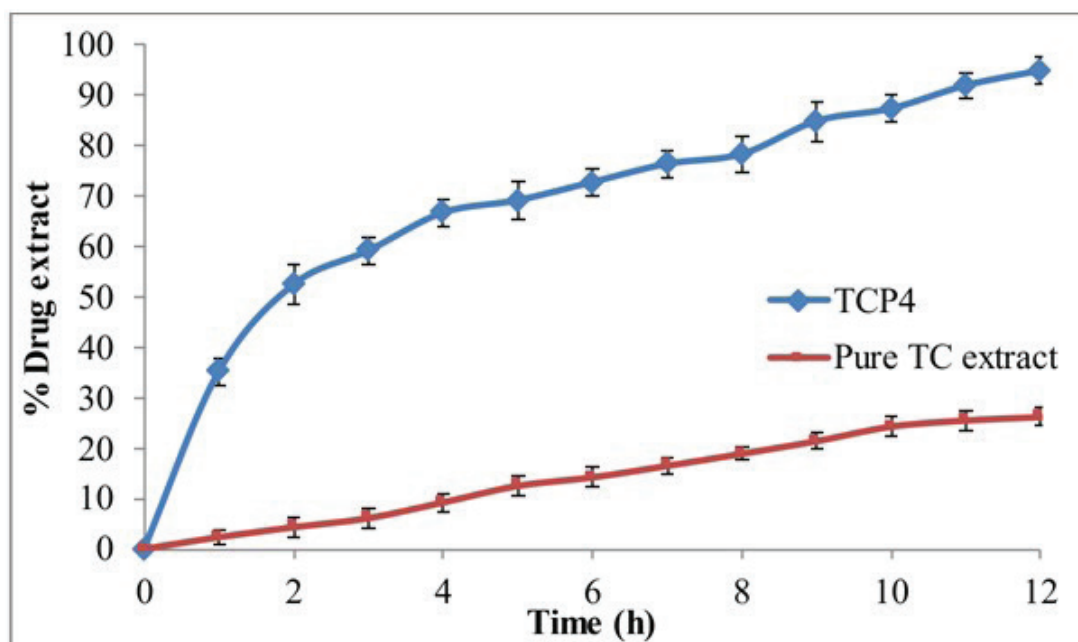


Figure 6. In vitro Drug Release Study of Phytosomes and Pure *T. cordifolia* Extract.

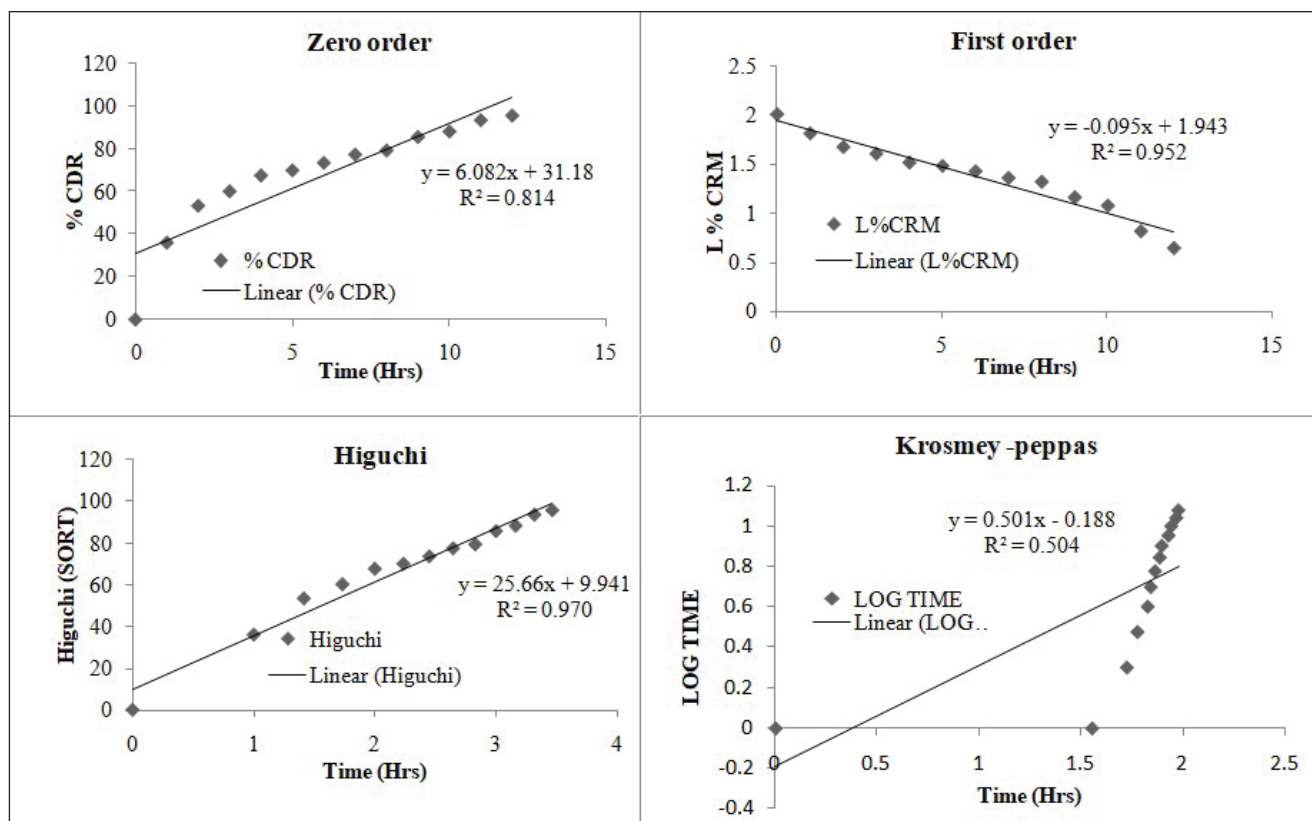


Figure 7. In vitro Drug Release Kinetics Diagrams of Optimized Formulation (TCP4).

Table 3. Effect of Phytosomes on Body Weight and Blood Glucose Level.

Treatment Group	Day 1	Day 7	Day 14	Day 21
Body weight (gm, mean $\pm$ SEM)				
Normal control	166.6 $\pm$ 11.5	173.5 $\pm$ 12.5	182.7 $\pm$ 3.1	193.7 $\pm$ 1.5
Disease control	159.2 $\pm$ 12.3	156.2 $\pm$ 13.6	148.3 $\pm$ 2.9	136.6 $\pm$ 1.9
Standard (glibenclamide)	161.3 $\pm$ 12.7	166.2 $\pm$ 13.2	173.5 $\pm$ 3.1**	183.8 $\pm$ 2.9**
TC	157.0 $\pm$ 10.2	159.1 $\pm$ 13.1	158.5 $\pm$ 5.9*	160.5 $\pm$ 2.3*
TCP4	159.0 $\pm$ 11.9	160.3 $\pm$ 12.2	162.5 $\pm$ 4.9*	163.5 $\pm$ 2.4*
Change in blood glucose level (mg/dl, mean $\pm$ SEM)				
Normal control	98.1 $\pm$ 10.2	99.1 $\pm$ 9.8	98.1 $\pm$ 8.6	98.1 $\pm$ 7.6
Disease control	283.3 $\pm$ 12.6	367.5 $\pm$ 26.3	395.3 $\pm$ 18.3	427.1 $\pm$ 15.1
Standard (glibenclamide)	308.5 $\pm$ 11.8	169.1 $\pm$ 10.5	131.5 $\pm$ 10.4**	112.8 $\pm$ 10.3**
<i>T. cordifolia</i> extract	303.3 $\pm$ 12.2	275.3 $\pm$ 12.1	248.8 $\pm$ 19.1*	216.7 $\pm$ 12.2*
TCP4	302.3 $\pm$ 11.7	270.3 $\pm$ 11.1	238.8 $\pm$ 29.5*	206.7 $\pm$ 13.9**

Note: Values are expressed as Mean $\pm$ SEM, N = 6. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Comparison with toxic control.

Abbreviation: SEM, standard error mean.

Table 4. Effect of Phytosomes on Lipid Profile and Kidney Functions on 21 Days of Study.

Biochemical Parameters	Control	Toxic Control	Standard	<i>T. cordifolia</i> Extract	TCP4
LDL	39.3 $\pm$ 3.5	138.3 $\pm$ 4.7	43.2 $\pm$ 3.57***	101.7 $\pm$ 5.8**	81.8 $\pm$ 4.7**
Total cholesterol	86.6 $\pm$ 2.2	201.7 $\pm$ 15.7	94.2 $\pm$ 12.7*	138.9 $\pm$ 7.6*	118.8 $\pm$ 7.5*
Triglycerides	72.2 $\pm$ 3.2	182.1 $\pm$ 3.9	73.2 $\pm$ 3.3**	95.8 $\pm$ 3.4**	90.8 $\pm$ 3.4**
HDL	31.8 $\pm$ 2.3	18.4 $\pm$ 2.1	32.7 $\pm$ 2.4**	27.4 $\pm$ 2.1*	23.5 $\pm$ 2.2*
VLDL	14.5 $\pm$ 0.9	37.6 $\pm$ 1.2	19.8 $\pm$ 1.3**	32.2 $\pm$ 1.7**	29.2 $\pm$ 1.6**
Urea	25.3 $\pm$ 1.1	72.3 $\pm$ 1.2	26.3 $\pm$ 1.4**	40.3 $\pm$ 1.3	36.3 $\pm$ 1.4
Creatinine	0.8 $\pm$ 0.1	3.8 $\pm$ 0.1	0.9 $\pm$ 0.1**	1.9 $\pm$ 0.07***	1.6 $\pm$ 0.1**
Uric acid	3.3 $\pm$ 0.3	6.7 $\pm$ 0.3	3.7 $\pm$ 0.3**	4.9 $\pm$ 0.4*	4.4 $\pm$ 0.3*

Note: Values are expressed as Mean $\pm$ SEM, N = 6. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Comparison with toxic control.

Abbreviations: LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein.

not require insulin to translocate glucose, and these are abundant in human tissues. These are the most prevalent glucose transporter isoforms expressed in the adult brain and are responsible for baseline glucose absorption.⁴⁶

Biochemical Parameters

The biochemical evaluation was done on the 21st day of treatment and data was expressed in Table 4. The result showed that glibenclamide, pure *T. cordifolia* extract and TCP4 caused a significant ($p < 0.05$) decrease in lipid levels, for instance, cholesterol, LDL, VLDL, and triglycerides, except for HDL, as compared to disease control. Similarly, kidney function tests, for instance, creatinine, urea, and uric acid also exhibited a significant decrease ($p < 0.05$) in the levels as compared to disease control. Destruction of pancreatic β cells is responsible for the decrease in

glucose-stimulated insulin release. Due to the reduction of plasma insulin, a change in lipid profile occurred. The movement of fatty acids from peripheral tissues is also responsible for the increase in lipid levels. This condition is caused by insulin-sensitive lipase inhibition.⁴⁷ Similarly, urea, creatinine, and uric acid are important renal parameters, especially in the case of type 2 diabetic patients.⁴⁸ Different types of complications like hyperlipidemia, edema, obesity, and diabetic nephropathy are evoked due to increased lipid levels and alteration in kidney functions, which are crucial factors in developing arthritis, gout inflammation, and pain.

Histopathological Study

The histopathological examination of rat pancreatic cells is depicted in Figure 8. The normal control group of rats showed normal cellular morphology in islet cells

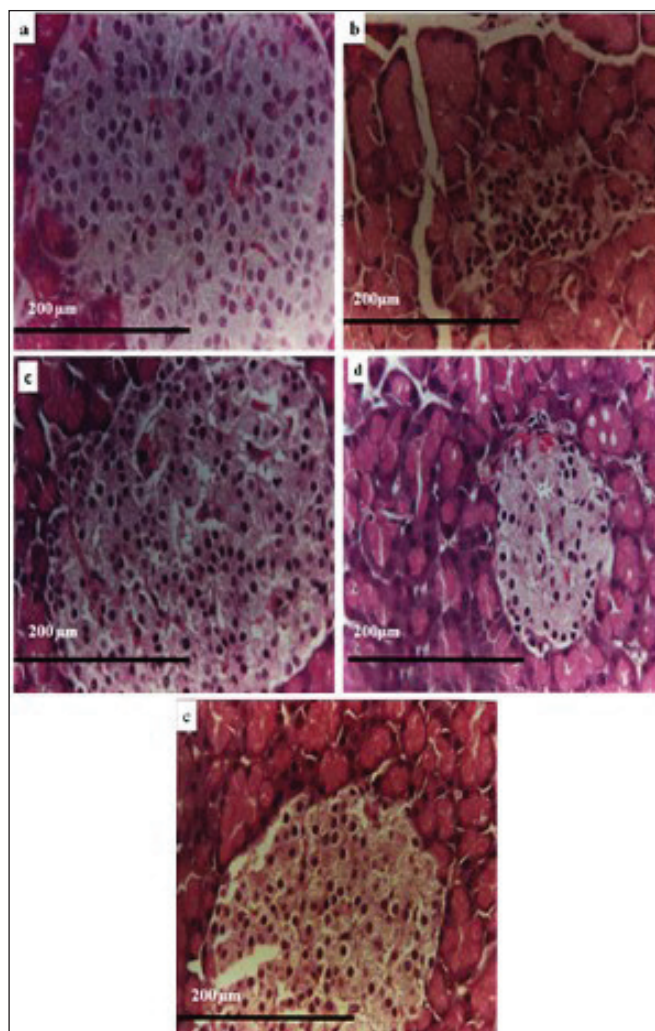


Figure 8. Histopathological Image of (a) Normal Control, (b) Disease Control, (c) Treated with Glibenclamide, (d) Treated with *T. cordifolia* Extract, and (e) Treated with TCP4 of Pancreas.

Table 5. Effect of Phytosomes on Paw Volume and Percentage Inhibition by Carcinogen-induced Paw Edema.

	1 h	2 h	3 h	4 h	5 h	
	Paw Volume (Mean $\pm$ SEM)					Percentage Inhibition at 5 h
Disease control	1.82 $\pm$ 0.03	1.84 $\pm$ 0.11	1.87 $\pm$ 0.13	1.91 $\pm$ 0.14	1.93 $\pm$ 0.17	—
Standard	1.56 $\pm$ 0.01	1.53 $\pm$ 0.07	1.45 $\pm$ 0.15	1.41 $\pm$ 0.14	1.37 $\pm$ 0.11**	29.17
<i>T. cordifolia</i> extract	1.66 $\pm$ 0.02	1.64 $\pm$ 0.13	1.64 $\pm$ 0.15	1.62 $\pm$ 0.19	1.66 $\pm$ 0.18**	13.99
TCP4	1.74 $\pm$ 0.03	1.70 $\pm$ 0.10	1.70 $\pm$ 0.13	1.64 $\pm$ 0.15	1.61 $\pm$ 0.11**	16.74

Note: Values are expressed as Mean $\pm$ SEM, N = 6. * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.001$. Comparison with toxic control.

Abbreviation: SEM, standard error mean.

(Figure 8a). There was necrosis and shrinkage of islet cells with dilated acini of the pancreas observed in diabetic rats (Figure 8b). The treatment group with glibenclamide showed regular pancreatic architecture, increasing islet

size, along with the proliferation of β -cells (Figure 8c). However, pure *T. cordifolia* extract and TCP4 also exhibited increases in the islet cells with dilated acini than extract and disease control (Figure 8d and e). It revealed

that *T. cordifolia* extract-loaded phytosomes have good anti-diabetic activity due to an increase in the solubility of lipid and surfactant.

Anti-inflammatory Activity

Table 5 shows the results of the anti-inflammatory activity of indomethacin and TCP4. It was observed that indomethacin, *T. cordifolia* extract and TCP4 exhibited significant inhibition in paw edema (29.17%, 13.99%, and 16.74%) as compared to disease control. The significant anti-inflammatory activity of TCP4 is due to the increased solubility of extract as well as the high release of extract.

Analgesic Activity

The result of analgesic activity was depicted in Table 6. TCP4 exhibited good analgesic effects (43.19%) than disease control. However standard (aspirin) exhibited analgesic activity (78.26%) than *T. cordifolia* extract (37.55%) and TCP4 (43.19%). The analgesic and anti-inflammatory activity of *T. cordifolia*-loaded phytosomes (TCP4) are due to the inhibition of COX-2.⁴⁹ The significant activity of *T. cordifolia* in the formulation may be due to high solubility in the lipid system.

Conclusion

The phytochemical analysis reported the presence of lignin as one of the major phytoconstituents. GC–MS chromatogram reported the highest concentrations of lignin compound epiyangambin. The reported compound also exhibited good anti-diabetic, anti-inflammatory, and analgesic potential by GLUT-1 and COX-2 inhibition. Phytosomes were prepared by a reverse-phase solvent evaporation method using span 60, soyalecithin, and cholesterol as formulation ingredients. Formulations exhibited high entrapment efficiency and nanoscale of vesicle size. The morphological behavior of vesicles with bilayer membranes validates their spherical form. The FTIR peaks show no interaction between the extract and excipients. The physico-chemical and morphological studies revealed that formulation at the equal ratio of drug lipid and surfactant demonstrated sustained-release behavior with the Fickian diffusion mechanism. Phytosomes loaded with *T. cordifolia* extract showed significant anti-diabetic, anti-inflammatory, and analgesic activity compared to extract alone. This might be due to the presence of epiyangambin in the formulation and enhanced solubility in the lipid and surfactant of the formulation.

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Declaration of Conflicting Interests

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Statement of Informed Consent and Ethical Approval

Necessary ethical clearances and informed consent was received and obtained respectively before initiating the study from all participants.

References

1. Olabiyi AS, Nkemhule FE, Odukoya OA, et al. Inhibition of glycosylation as an index of activity in plants with antidiabetic potentials. *Biochem Pharmacol* 2013; 2: 181–85.
2. Turmeric: Singletary K. An overview of potential health benefits. *Nutr Today* 2010; 45(5): 216–25. DOI: 10.1097/NT.0b013e3181f1d72c.
3. Wilken R, Veena MS, Wang MB, et al. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer* 2011; 10: 12. DOI: 10.1186/1476-4598-10-12, PMID 21299897.
4. Patel P, Patel D, Patel N. Experimental investigation of anti-rheumatoid activity of *Pleurotus sajorcaju* in adjuvant-induced arthritic rats. *Chin J Nat Med* 2012; 10(4): 269–74. DOI: 10.1016/S1875-5364(12)60054-5.
5. Chadha YR. *The Wealth of India, Publication and Information Directorate*. New Delhi: Council of Scientific and Industrial Research, 1948, p. 33.
6. Sharma A, Shanker C, Tyagi LK, et al. Herbal medicine for market potential in India: An overview. *Acad J Plant Sci* 2008; 1: 26–36.
7. Upadhyay AK, Kumar K, Kumar A, et al. *Tinospora cordifolia* (Willd.) Hook. f. and Thoms. (Guduchi)—validation of the Ayurvedic pharmacology through experimental and clinical studies. *Int J Ayurveda Res* 2010; 1(2): 112–21. DOI: 10.4103/0974-7788.64405, PMID 20814526.
8. Ghosal S and Vishwakarma RA. Tinocordiside, a new rearranged cadinane sesquiterpene glycoside from *Tinospora cordifolia*. *J Nat Prod* 1997; 60(8): 839–41. DOI: 10.1021/np970169z.
9. Nadkarni AK. *Indian Materia Medica*. 3rd ed., Bombay: Popular Prakashan, 1954. p. 1221.
10. Chadha YR. *The Wealth of India, Publication and Information Directorate*. New Delhi: Council of Scientific and Industrial Research, 1976, p. 251.

11. Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytother Res* 1999; 13(4): 275–91. DOI: 10.1002/(SICI)1099-1573(199906)13:4<275::AID-PTR510>3.0.CO;2-S, PMID 10404532.
12. Bafna PA and Balaraman R. Anti-ulcer and anti-oxidant activity of pepticare, a herbomineral formulation. *Phytomedicine* 2005; 12(4): 264–70. DOI: 10.1016/j.phymed.2003.12.009, PMID 15898703.
13. Atal CK, Sharma ML, Kaul A, et al. Immunomodulating agents of plant origin. I: Preliminary screening. *J Ethnopharmacol* 1986; 18(2): 133–41. DOI: 10.1016/0378-8741(86)90025-5, PMID 3560991.
14. Vedavathy S and Rao KN. Antipyretic activity of six indigenous medicinal plants of Tirumala Hills, Andhra Pradesh, India. *J Ethnopharmacol* 1991; 33(1–2): 193–96. DOI: 10.1016/0378-8741(91)90178-G.
15. Jagetia GC and Rao SK. Evaluation of cytotoxic effects of dichloromethane extract of Guduchi (*Tinospora cordifolia* Miers ex Hook F and THOMS) on cultured HeLa cells. *Evid Based Complement Alternat Med* 2006; 3(2): 267–72. DOI: 10.1093/ecam/nel011, PMID 16786058.
16. Jagetia GC and Rao SK. Evaluation of the antineoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascites carcinoma bearing mice. *Biol Pharm Bull* 2006; 29(3): 460–66. DOI: 10.1248/bpb.29.460, PMID 16508146.
17. Gupta SS, Verma SCL, Garg VP, et al. Anti-diabetic effects of *Tinospora cordifolia*. I. Effect on fasting blood sugar level, glucose tolerance and adrenaline induced hyperglycaemia. *Indian J Med Res* 1967; 55(7): 733–45. PMID 6056285.
18. Prince PSM and Menon VP. Antioxidant activity of *Tinospora cordifolia* roots in experimental diabetes. *J Ethnopharmacol* 1999; 65(3): 277–81. DOI: 10.1016/S0378-8741(98)00164-0, PMID 10404427.
19. Wadood N, Wadood A, Shah SA. Effect of *Tinospora cordifolia* on blood glucose and total lipid levels of normal and alloxan-diabetic rabbits. *Planta Med* 1992; 58(2): 131–36. DOI: 10.1055/s-2006-961414, PMID 1529024.
20. Leach AR, Shoichet BK, Peishoff CE. Prediction of protein-ligand interactions. Docking and scoring: Successes and gaps. *J Med Chem* 2006; 49(20): 5851–55. DOI: 10.1021/jm060999m, PMID 17004700.
21. Bhise JJ, Bhusnure OG, Jagtap SR, et al. Phytosomes: A novel drug delivery for herbal extracts. *J Drug Deliv Ther* 2019; 9: 924–30.
22. Poorani V, Selvakumar K, Venkat Kumar G. Improving bio-availability of phytochemicals through niosomes. *J Drug Deliv Ther* 2020; 10(6-s): 119–21. DOI: 10.22270/jddt.v10i6-s.4459.
23. Xu YQ, Chen WR, Tsosie JK, et al. Niosome encapsulation of curcumin: Characterization and cytotoxic effect on ovarian cancer cells. *J Nanomater* 2016; 2016: 1–9. DOI: 10.1155/2016/6365295.
24. Veronese N, Cooper C, Reginster JY, et al. Type 2 diabetes mellitus and osteoarthritis. *Semin Arthritis Rheum* 2019; 49(1): 9–19. DOI: 10.1016/j.semarthrit.2019.01.005, PMID 30712918.
25. Kumar DS, Karthikeyan D, Roy B. *In silico*, ADMET and docking analysis for the compounds of chloroform extract of *Tinospora cordifolia* (Willd.) identified by GC-MS and spectral analysis for antidiabetic and anti-inflammatory activity. *Asian J Chem* 2022; 34(2): 342–54. DOI: 10.14233/ajchem.2022.23521.
26. Chaudhary N, Siddiqui MB, Khatoon S. Pharmacognostic evaluation of *Tinospora cordifolia* (Willd.) Mier and identification of biomarker. *Indian J Trad Knowl* 2014; 13: 543–50.
27. Barani M, Sangiovanni E, Angarano M, et al. Phytosomes as innovative delivery systems for phytochemicals: A comprehensive review of literature. *Int J Nanomed* 2021; 16: 6983–7022. DOI: 10.2147/IJN.S318416, PMID 34703224.
28. Anwar E and Farhana N. Formulation and evaluation of phytosome-loaded maltodextrin-gum Arabic microsphere system for delivery of *Camellia sinensis* extract. *J Young Pharm* 2018; 10(2s): S56–S62. DOI: 10.5530/jyp.2018.2s.11.
29. Maryana W, Rahma A, Mudhakir D, et al. Phytosome containing silymarin for oral administration: Formulation and physical evaluation. *JBBBE*. 2015; 25: 54–65. DOI: 10.4028/www.scientific.net/JBBBE.25.54.
30. Nnamani PO, Kenekchukwu FC, Asogwa FO, et al. Novel anti-ulcer phytosomal formulation of ethanol extract of *Pentaclethra macrophylla* Stem-Bark. *Trop J Nat Prod Res* 2020; 4: 385–91. DOI: 10.26538/tjnpr/v4i8.11.
31. Sen S, Bhaumik P, Roy B, et al. Antimicrobial and antinociceptive profile of some novel pyrazole derivatives. *Indian J Chem B* 2016; 55: 507–16.
32. Sen S, De B, Easwari TS. Synthesized 2-substituted-3-phenylthiazolidine-4-ones as potent antioxidants and antidiabetic agents. *Trop J Pharm Res* 2014; 13(9): 1445–54. DOI: 10.4314/tjpr.v13i9.10.
33. Nurdiana S, Goh YM, Ahmad HN, et al. Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with *Ficus deltoidea* and vitexin. *BMC Complement Altern Med* 2017; 17(1): 290. DOI: 10.1186/s12906-017-1762-8, PMID 28576138.
34. Philip S, Tom G, Vasumathi AV. Evaluation of the anti-inflammatory activity of *Tinospora cordifolia* (Willd.) Miers chloroform extract—a preclinical study. *J Pharm Pharmacol* 2018; 70(8): 1113–25. DOI: 10.1111/jphp.12932, PMID 29770441.
35. Adeyemi OO, Adeneye AA, Alabi TE. Analgesic activity of the aqueous seed extract of *Hunteria umbellata* (K. Schum.) Hallier f. in rodents. *Indian J Exp Biol* 2011; 49(9): 698–703. PMID 21941944.
36. Castro-Faria-Neto HC, Martins MA, Silva PM, et al. Pharmacological profile of epiyangambin: A furofuran lignan with PAF antagonist activity. *J Lipid Mediat* 1993; 7(1): 1–9. PMID 8358020.
37. Singh VP, Bali A, Singh N, et al. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol* 2014; 18(1): 1–14. DOI: 10.4196/kjpp.2014.18.1.1, PMID 24634591.
38. Evstigneev EI. Factors affecting lignin solubility. *Russ J Appl Chem* 2011; 84(6): 1040–45. DOI: 10.1134/S1070427211060243.
39. Shaker S, Gardouh AR, Ghorab MM. Factors affecting liposomes particle size prepared by ethanol injection method.

- Res Pharm Sci* 2017; 12(5): 346–52. DOI: 10.4103/1735-5362.213979, PMID 28974972.
40. Refai H, Hassan D, Abdelmonem R. Development and characterization of polymer-coated liposomes for vaginal delivery of sildenafil citrate. *Drug Deliv* 2017; 24(1): 278–88. DOI: 10.1080/10717544.2016.1247925, PMID 28165805.
41. Haeri A, Alinaghian B, Daeihamed M, et al. Preparation and characterization of stable nanoliposomal formulation of fluoxetine as a potential adjuvant therapy for drug-resistant tumors. *Iran J Pharm Res* 2014; 13(Suppl): 3–14. PMID 24711824.
42. Ge X, Wei M, He S, et al. Advances of non-ionic surfactant vesicles (niosomes) and their application in drug delivery. *Pharmaceutics* 2019; 11(2): 55. DOI: 10.3390/pharmaceutics11020055, PMID 30700021.
43. Kumar GP and Rajeshwarrao P. Nonionic surfactant vesicular systems for effective drug delivery—an overview. *Acta Pharm Sin B* 2011; 1(4): 208–19. doi: 10.1016/j.apsb.2011.09.002.
44. Svetina S and Zeks B. Shape behavior of lipid vesicles as the basis of some cellular processes. *Anat Rec* 2002; 268(3): 215–25. DOI: 10.1002/ar.10156, PMID 12382320.
45. Kazi KM, Mandal AS, Biswas N, et al. Niosome: A future of targeted drug delivery systems. *J Adv Pharm Technol Res* 2010; 1(4): 374–80. DOI: 10.4103/0110-5558.76435, PMID 22247876.
46. Bharti SK, Krishnan S, Kumar A, et al. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. *Ther Adv Endocrinol Metab* 2018; 9(3): 81–100. DOI: 10.1177/2042018818755019, PMID 29492244.
47. Das S, Khan I, Dhasmana S, et al. Formulation and evaluation of the antidiabetic, hypolipidemic and antioxidant potential of the tablets of arbortristoside-A from the Seeds of *Nyctanthes arbortristis* Linn. *Indian J Pharm Educ Res* 2021; 55(4): 1074–83. DOI: 10.5530/ijper.55.4.208.
48. Idonije BO, Festus O, Oluba OM. Plasma glucose, creatinine and urea levels in Type 2 diabetic patients attending a Nigerian teaching hospital. *Res J Med Sci* 2011; 5(1): 1–3. DOI: 10.3923/rjmsci.2011.1.3.
49. Ghatpande NS, Misar AV, Waghole RJ, et al. *Tinospora cordifolia* protects against inflammation associated anemia by modulating inflammatory cytokines and hepcidin expression in male Wistar rats. *Sci Rep* 2019; 9(1): 10969. DOI: 10.1038/s41598-019-47458-0, PMID 31358831.