

Formulation and Evaluation of Nanoformulation Containing Phytoconstituent of *Cardiospermum halicacabum* Extract

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ABSTRACT

The study aimed to formulate and evaluate polymeric nanoparticles containing phytoconstituents of *Cardiospermum halicacabum* L. Methods: Ethanolic extract of *Cardiospermum halicacabum* L. was incorporated into polymeric nanoparticles using the solvent evaporation technique. Poly (lactic-co-glycolic acid) (PLGA) was used as the biodegradable polymer, and polyvinyl alcohol (PVA) served as a stabilizer. Five different formulations (F1–F5) were prepared by varying polymer and stabilizer concentrations. Evaluation parameters included particle size, polydispersity index (PDI), zeta potential, encapsulation efficiency, in vitro drug release, and antioxidant activity using the DPPH assay. The stability of the optimized batch (F3) was assessed under accelerated storage conditions. Results: Formulation F3 was optimized, showing the smallest particle size (165 ± 4 nm), low PDI (0.280), and high zeta potential (-28.3 mV), indicating good physical stability. It also showed superior entrapment efficiency (75.6%) and maximum drug release (85.2% at 24 h). Antioxidant studies confirmed free radical scavenging potential of the extract. Stability studies revealed minimal changes in physicochemical parameters over time. Conclusion: The nanoformulation of *Cardiospermum halicacabum* L. significantly enhances its stability and drug delivery potential. The developed system holds promise for further in vivo studies and future use in the effective management of diabetes.

Keywords- *Lantana camara*, Silver Nanoparticles, Nanogel, Topical Delivery, Antibacterial Activity, Antioxidant, Phytoconstituents, Green Synthesis, Drug Release.

I. INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder characterized by persistent hyperglycemia, has emerged as a global public health concern due to its increasing prevalence and associated complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases. The limitations of current antidiabetic therapies, including adverse side effects, limited bioavailability, [1] and the inability to halt disease progression, have driven the search for alternative treatment strategies. In this context, herbal medicine has gained considerable attention due to its multi-targeted approach, safety profile, and cost-

effectiveness. Among various medicinal plants, *Cardiospermum halicacabum* L., commonly known as balloon vine, holds promising potential due to its rich reservoir of bioactive phytoconstituents with reported anti-inflammatory, antioxidant, and antidiabetic properties.[2]

Cardiospermum halicacabum, traditionally used in Ayurvedic and folk medicine, contains a wide range of phytochemicals such as flavonoids, alkaloids, saponins, and sterols. These constituents are known to exhibit potent antioxidant activity, which plays a vital role in mitigating oxidative stress—a key factor implicated in the pathogenesis of diabetes and its complications. Despite its therapeutic potential, the

clinical utility of *C. halicacabum* extract is hampered by poor aqueous solubility, limited bioavailability, and rapid degradation in the gastrointestinal tract. To overcome these pharmacokinetic challenges, the

incorporation of phytoconstituents into advanced drug delivery systems, particularly nanoformulations, offers a promising solution.[3,4]



Fig. 1: *Cardiospermum halicacabum*

Nanotechnology-based drug delivery systems such as polymeric nanoparticles provide a strategic platform for the targeted and controlled release of phytoconstituents. Poly (lactic-co-glycolic acid) (PLGA), a biodegradable and biocompatible polymer approved by the FDA, has emerged as a preferred carrier due to its ability to encapsulate hydrophobic plant extracts, protect them from enzymatic degradation, and enhance their bioavailability. The use of PLGA-based nanoparticles can significantly improve the therapeutic efficacy of *C. halicacabum* extract by facilitating its sustained release and effective transport across biological membranes.[5,6]

The present study is focused on the formulation and evaluation of a nanoformulation containing the ethanolic extract of *Cardiospermum halicacabum* using the solvent evaporation method. Various preformulation and formulation parameters were optimized to obtain stable and effective nanoparticles. The prepared nanoparticles were subjected to comprehensive physicochemical and biological evaluations, including particle size analysis, zeta potential, entrapment efficiency, in vitro drug release, and antioxidant activity using DPPH assay. Additionally, the optimized formulation was evaluated for its stability under different storage conditions. This research aims to provide a scientific basis for the development of a novel, plant-based nanocarrier system for potential antidiabetic therapy, highlighting the integration of traditional medicine with modern pharmaceutical technology.

II. MATERIALS AND METHODS

Materials:

The materials used in this study included authenticated *Cardiospermum halicacabum* leaves obtained from a herbal garden and extracted using

analytical grade ethanol (Merck, India). PLGA (Resomer® RG 503H, 50:50) sourced from Evonik Industries, Germany, was used as the polymer, while PVA (MW ~30,000–70,000, 88% hydrolyzed) from Loba Chemie served as a stabilizer. Dichloromethane (DCM, HPLC grade) from Rankem, and laboratory-prepared distilled water were used during formulation. For antioxidant evaluation, analytical grade DPPH (Sigma-Aldrich, USA) and methanol (Fisher Scientific, India) were utilized. Phosphate buffer saline (pH 7.4) was prepared in-house for in vitro release studies. All materials were of high analytical or pharmaceutical grade.

Collection and authentication of plant materials:

The leaves of *Cardiospermum halicacabum* were carefully collected from a biodiverse region, ensuring sustainable harvesting at the optimal time for phytochemical content. Species authentication was carried out by the Pharmacognosy Department of SVP College of Pharmacy, Hatta Hingoli. This step confirmed the botanical identity and ensured scientific accuracy, enhancing the reliability and credibility of the study's findings.[7]

Morphological Study

The morphological examination of the leaves of *Cardiospermum halicacabum* was performed to confirm the identity and authenticity of the plant material prior to formulation development. Standard pharmacognostic procedures were employed to assess the macroscopic and organoleptic characteristics, including leaf color, shape, size, texture, and odor. These visual and sensory parameters are vital for the primary screening and verification of raw herbal drugs, helping to distinguish genuine samples from adulterated or substituted ones. Consistent documentation of such morphological traits ensures the reproducibility and reliability of future research and herbal product development. This step is essential not only for standardization but also for

maintaining the quality and efficacy of phytopharmaceuticals derived from the plant.[8]

Physicochemical Parameters of *Cardiospermum halicacabum*

In addition to morphological identification, a comprehensive physicochemical evaluation of *Cardiospermum halicacabum* leaves was undertaken to determine the purity, stability, and suitability of the material for therapeutic application. Analytical methods recommended by recognized pharmacopoeias were followed to assess key parameters. The moisture content was measured to evaluate the potential for microbial contamination and degradation during storage. Ash values, including total ash, acid-insoluble ash, and water-soluble ash, were analyzed to detect the presence of inorganic impurities and determine the level of physiological and non-physiological matter. Furthermore, extractive values in solvents like water and alcohol were determined to quantify the amount of active constituents soluble in different media. The pH of the aqueous extract was also recorded as an indicator of chemical stability and compatibility with formulation excipients.[9]

Preparation of *Cardiospermum halicacabum* Leaf Extracts

To obtain the phytoconstituents, the dried leaves of *Cardiospermum halicacabum* were ground into a fine powder using a mechanical grinder, sieved for uniformity, and stored in airtight containers away from moisture and light. Extraction was carried out using the Soxhlet method with solvents of increasing polarity—starting from petroleum ether, followed by chloroform, ethyl acetate, methanol, and finally water. Each solvent was used for 6–8 hours until a clear siphon indicated complete extraction. The extracts were filtered, concentrated using a rotary evaporator under reduced pressure, and dried in a desiccator. These were then stored in amber-colored bottles at 4°C to maintain the integrity and potency of light- and heat-sensitive bioactive compounds for future use.[10]

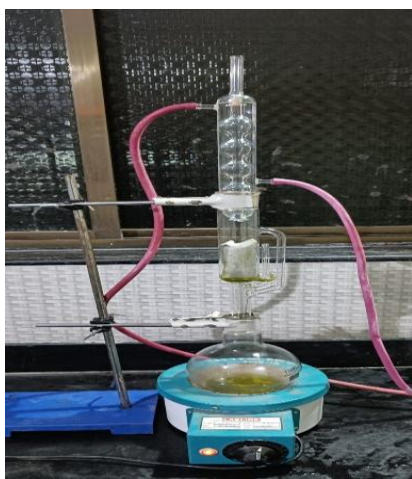


Fig. 2: Extraction *Cardiospermum halicacabum* Leaves powder

Phytochemical Investigation Methods for *Cardiospermum halicacabum* Extract

The phytochemical screening of *Cardiospermum halicacabum* involved qualitative and quantitative methods to identify and estimate key bioactive constituents. Standard phytochemical tests were used to detect major groups such as alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. Each test was conducted using specific reagents and protocols to confirm the presence and estimate the concentration of these compounds, forming a comprehensive phytochemical profile of the plant extract suitable for further pharmacological and formulation research.[11]

Quantitative Evaluation Methods for *Cardiospermum halicacabum* Extract

Quantitative estimation of phytochemicals in *Cardiospermum halicacabum* extract is essential to validate its pharmacological efficacy. Key constituents such as phenolics and flavonoids were analyzed using established colorimetric methods to correlate their presence with therapeutic actions.[12]

Total Phenolic Content (TPC):

The TPC was determined by the Folin–Ciocalteu method, where phenolic compounds react with the Folin reagent and sodium carbonate, forming a blue-green complex. Absorbance was measured at 666 nm, and values were calculated using a gallic acid standard curve ($R^2 = 0.9919$). The results were expressed in mg of gallic acid equivalents (GAE) per gram of dry extract, indicating the antioxidant potential of the extract.[13]

Total Flavonoid Content (TFC):

The TFC was assessed using the aluminum chloride colorimetric assay. Flavonoids form a colored complex with aluminum chloride, and absorbance was measured at 510 nm. Quercetin was used as the standard ($R^2 = 0.9911$), and the results were reported as mg of quercetin equivalents (QE) per gram of dry extract, reflecting the contribution of flavonoids to the extract's biological activity. These quantitative evaluations provide crucial insights into the bioactive profile of the extract, supporting its potential use in therapeutic formulations.[14]

Qualitative Analysis of Inorganic Elements

The ash of *Cardiospermum halicacabum* extract leaves was digested using nitric acid and hydrochloric acid in a 3:1 ratio and filtered. The resulting filtrate was tested for inorganic elements. Calcium was identified by the formation of a white calcium oxalate precipitate. Magnesium was detected by a white crystalline precipitate upon reaction with sodium phosphate. Sodium was confirmed by a yellow crystalline precipitate with uranyl magnesium acetate. Potassium showed a yellow precipitate with sodium cobalt nitrite. Iron presence was confirmed by dark blue coloration with potassium ferrocyanide. Sulphate formed a white precipitate with lead acetate, soluble in sodium hydroxide. Phosphate produced a yellow crystalline

precipitate with ammonium molybdate upon heating. Chloride was indicated by a white precipitate with lead acetate, soluble in hot water. Nitrate was identified by a brown ring formed at the junction of layers after adding ferrous sulphate and sulfuric acid. [15]

In-vitro Antioxidant Studies

Antioxidants play a vital role in neutralizing free radicals generated through oxidative processes in the body, thereby preventing cellular damage and reducing the risk of chronic diseases such as diabetes and inflammation. To assess the antioxidant potential of *Cardiospermum halicacabum* leaf extract, two widely accepted in-vitro methods were employed: the DPPH radical scavenging assay and the Ferric Reducing Antioxidant Power (FRAP) assay. [16-18]

DPPH Radical Scavenging Assay:

The DPPH method measures the extract's ability to donate hydrogen atoms or electrons to neutralize free radicals. The extract and standard (quercetin) were dissolved in methanol and tested at different concentrations (20–100 µg/mL) against a 0.1 mM DPPH methanolic solution. A decrease in absorbance at 513 nm indicated effective radical scavenging. The percentage inhibition and EC₅₀ values were calculated, with lower EC₅₀ values reflecting stronger antioxidant activity.

FRAP Assay:

The FRAP assay evaluates the reducing capacity of antioxidants in the extract by their ability to convert ferric (Fe³⁺) to ferrous (Fe²⁺) ions. The reaction forms a blue ferrous–TPTZ complex, with absorbance measured at 593 nm. The ethanolic extract was tested at various concentrations (10–500 µg/mL), and results were compared against a standard ferrous sulfate calibration curve. The antioxidant power was expressed as µmol Fe²⁺ equivalents per gram of extract.

Preparation of Polymeric Nanoparticles

50 mg of PLGA along with 10 mg of dried up ethanolic extract of CH were co dissolved in 10 ml of organic solvent acetone/ acetonitrile. This organic phase was then added to 25 ml of aqueous phase containing 0.1% PVA using a 50-watt homogenizer. The solution thus obtained was magnetically stirred for at least 4 h to completely evaporate the organic solvent acetonitrile from it. After stirring the solution was centrifuged (REMI, INDIA) for 25 minutes at 15,000 rpm to settle down the suspended nanoparticles which were further washed using deionised water and then stored at 40 C. [19-21]

Evaluation Parameters of Polymeric Nanoparticles

To comprehensively assess the quality and functionality of the prepared nanoformulations containing phytoconstituents of *Cardiospermum halicacabum*, several physicochemical and performance-related parameters were systematically evaluated. These evaluations ensured that the developed nanoparticles possessed optimal characteristics for enhanced drug delivery, stability, and therapeutic efficacy. [23]

Particle Size and Polydispersity Index (PDI):

Dynamic Light Scattering (DLS) was employed to determine the average particle size and size distribution (PDI) of the polymeric nanoparticles. Particle size is a key determinant in nanoparticle-based drug delivery as it affects absorption, bioavailability, and cellular uptake. A PDI value less than 0.3 indicated a uniform size distribution, which is essential for consistent drug delivery. The optimized formulation aimed to maintain particle sizes below 200 nm to ensure effective tissue penetration and targeted delivery. [24-25]

Zeta Potential:

The surface charge of the nanoparticles, represented by zeta potential, was measured using a zeta sizer. Zeta potential values greater than ±20 mV are indicative of good colloidal stability, reducing the risk of particle aggregation due to electrostatic repulsion. The optimized formulation demonstrated a negative zeta potential attributed to the carboxyl groups of PLGA, enhancing suspension stability during storage.

Entrapment Efficiency (%EE):

Entrapment efficiency was calculated to determine the proportion of active phytoconstituents successfully encapsulated within the polymer matrix. This was done by centrifuging the formulation and analyzing the untrapped drug in the supernatant using a UV-Visible spectrophotometer (Shimadzu UV-1800). A standard calibration curve for the methanolic extract was prepared to quantify drug entrapment.

In-vitro Drug Release Studies:

Drug release profiles were studied using a dialysis method, where drug-loaded nanoparticles were sealed in dialysis bags and immersed in phosphate buffer saline (PBS, pH 6.8). The assembly was stirred continuously, and aliquots were withdrawn at specific intervals (3 to 24 hours) and analyzed using UV-spectrophotometry. Fresh buffer was replenished after each sampling. Comparative drug release profiles were obtained for both the nanoparticle formulation and the plain extract, with all values expressed as mean ± standard deviation from triplicate experiments.

Morphological Analysis:

The surface morphology and particle shape were examined using Scanning Electron Microscopy (SEM) or Transmission Electron Microscopy (TEM). Ideal nanoparticles appeared spherical with a smooth surface, which favors better cellular interactions and sustained drug release.

Stability Studies:

Stability testing was conducted by storing the nanoformulations under varied environmental conditions (room temperature and refrigeration) for up to three months. Particle size, zeta potential, and drug content were periodically assessed to monitor any physical or chemical changes, ensuring that the formulation remained stable over time.

III. RESULTS AND DISCUSSION

Morphological and Organoleptic Evaluation of *Cardiospermum halicacabum* Leaves

The morphological and organoleptic assessment of *Cardiospermum halicacabum* leaves was conducted as a preliminary step for authentication and quality control. Fresh leaves were observed to be vibrant green, turning light green upon drying—indicating proper drying without degradation of active components. The leaves emitted a mild, earthy herbal odor with a slightly bitter taste, suggestive of bioactive constituents like alkaloids and flavonoids. The texture of dried leaves was coarse and brittle, confirming adequate moisture

removal. Morphologically, the leaves were ovate with serrated margins and measured 4–6 cm in length. These distinct features provide essential parameters for crude drug identification.

Table 1: Morphological and organoleptic Evaluation of *Cardiospermum halicacabum* leaves

Parameter	Observation
Colour	Green (dried: light green)
Odour	Characteristic herbal odour
Taste	Slightly bitter
Texture	Coarse, brittle (dried)
Shape	Ovate with serrated margins
Size	4–6 cm in length

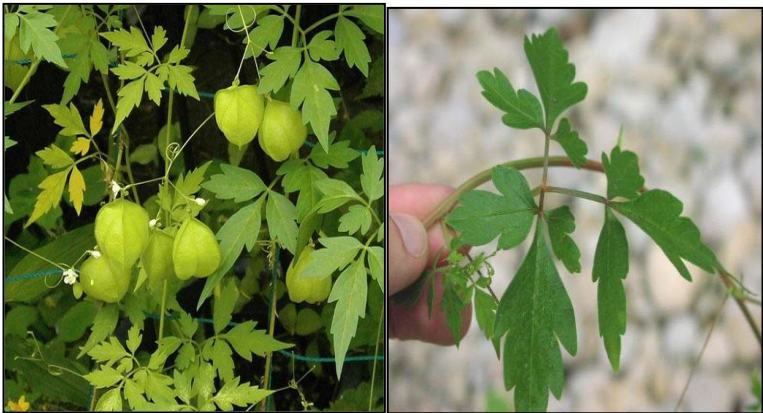


Fig. 3: *Cardiospermum halicacabum* Plant Leaves

Physicochemical Parameters of *Cardiospermum halicacabum* Leaves

Physicochemical analysis was performed to evaluate the quality and purity of *C. halicacabum* leaves. The moisture content (7.85% w/w) was within acceptable limits, ensuring minimal microbial growth. Total ash (10.4%) and acid-insoluble ash (2.6%) values indicated low contamination with inorganic material. Water- and alcohol-soluble extractives were 18.9% and 12.3%, respectively, reflecting a high concentration of polar and semi-polar phytoconstituents. The foreign organic matter was minimal (0.5%), and the pH of a 1% solution was 6.1, indicating compatibility for various pharmaceutical formulations. These parameters collectively support the standardization of the plant for herbal applications.

Table 2: Physicochemical Parameters of *Cardiospermum halicacabum* Leaves

Parameter	Value
Moisture content (% w/w)	7.85
Total ash (% w/w)	10.4
Acid-insoluble ash (% w/w)	2.6
Water-soluble ash (% w/w)	5.2
Alcohol-soluble extractive (% w/w)	12.3
Water-soluble extractive (% w/w)	18.9
Foreign organic matter (%)	0.5

pH (1% solution)	6.1
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Phytochemical Screening of *Cardiospermum halicacabum* Leaf Extracts

Preliminary phytochemical screening of *C. halicacabum* leaves, using solvents such as ethanol, methanol, acetone, chloroform, and water, revealed the presence of several key phytoconstituents. Ethanol and methanol extracts showed the widest range of compounds, including alkaloids, flavonoids, tannins, terpenoids, glycosides, steroids, and phenolics, indicating their effectiveness in extracting polar bioactives. Acetone and chloroform extracts showed moderate phytochemical profiles, whereas the aqueous extract, though rich in some constituents like alkaloids and terpenoids, lacked several non-polar components. Ethanol emerged as the most suitable solvent for broad-spectrum phytochemical extraction, underscoring its relevance for future pharmacological exploration of the plant.

Table 3: Phytochemical Investigation of *Cardiospermum halicacabum* leaves

Phytochemical Test	Ethanol	Methanol	Acetone	Chloroform	Water
Alkaloids (Wagner's Test)	+	+	+	+	+

Flavonoids (Alkaline Reagent Test)	+	+	-	+	+
Tannins (Ferric Chloride Test)	+	+	+	+	+
Saponins (Foam Test)	+	+	-	+	-
Terpenoids (Salkowski Test)	+	+	+	-	+
Phenolic Compounds (Ferric Chloride)	+	+	+	+	-
Glycosides (Borntrager's Test)	+	+	-	-	-
Steroids (Liebermann-Burchard Test)	+	+	+	+	-
Coumarins	+	+	-	-	+
Quinones	+	+	+	-	+

Note: "+" indicates presence; "-" indicates absence of the phytochemical.

IV. QUANTITATIVE ANALYSIS

Total Phenolic Content (TPC) of Ethanol Extract of *Cardiospermum halicacabum*

To determine the phenolic content in the ethanol extract of the selected plant material, a standard calibration curve was generated using Gallic acid. The absorbance values were recorded at 666 nm and plotted against various concentrations (0–10 µg/ml). The linear relationship obtained from the calibration curve showed good correlation, confirming the suitability of the method for estimating total phenolic content.

Table 4: Total Phenolic Content of Ethanol Extract

Sr. No.	Extract	Concentration (µg/ml)	Absorbance	TPC (mg GAE/g DW)
1	Ethanol Extract of <i>Cardiospermum halicacabum</i>	50	0.202 ± 0.004	108

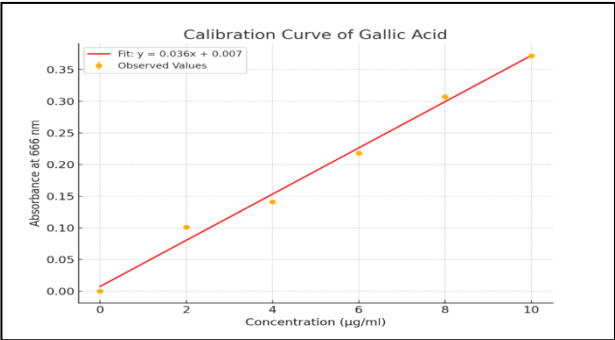


Fig. 4: Calibration Curve of Gallic Acid

The above results reveal that the ethanol extract of the plant material exhibits a high total phenolic content of 108 mg GAE/g DW. This indicates that

ethanol is an effective solvent for extracting phenolic compounds, which are known for their antioxidant, anti-inflammatory, and potential therapeutic properties. The high phenolic content of the ethanol extract underscores its potential utility in pharmaceutical and nutraceutical applications.

Total Flavonoid Content (TFC) of Ethanol Extract of *Cardiospermum halicacabum*

To determine the total flavonoid content in the ethanol extract of the plant material, a standard calibration curve was constructed using quercetin as the reference standard. The absorbance was measured at 510 nm for various concentrations of quercetin.

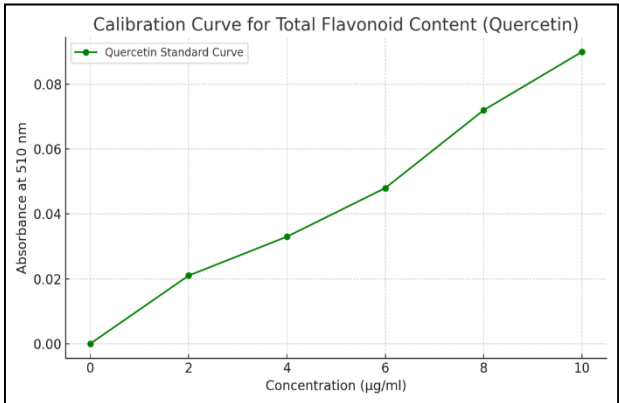


Fig. 5: Calibration Curve for total flavonoid content (Quercetin)

Table 5: Total Flavonoid Content (TFC) of Ethanol Extract

Sr. No.	Extract	Concentration (µg/ml)	Absorbance	TFC (mg QE/g DW)
1	Ethanol Extract of <i>Cardiospermum halicacabum</i>	50	0.180 ± 0.003	160.2

The ethanol extract of the plant material exhibited a high total flavonoid content of 160.2 mg QE/g DW, indicating that it is rich in flavonoid compounds. This is a significant finding, as flavonoids are known for their potent antioxidant and pharmacological activities. The strong absorbance observed at 510 nm in the ethanol extract confirms the presence of a considerable amount of flavonoid compounds.

Qualitative analysis of inorganic elements in *Cardiospermum halicacabum* leaves

The inorganic elements were investigated in *Cardiospermum halicacabum* leaves and represented in following table. *Cardiospermum halicacabum* leaves showed the presence of calcium, magnesium, potassium, phosphate, chloride, sodium, iron, sulphate and nitrate. These elements are known important for the growth and development of organisms.

Table 6: Qualitative inorganic elemental analysis of *Cardiospermum halicacabum*

S. No	Inorganic elements	Results
1	Calcium	+
2	Magnesium	+
3	Sodium	+
4	Potassium	+
5	Iron	+
6	Sulphate	+
7	Phosphate	+
8	Chloride	+
9	Nitrate	+

(+) indicates presence

In-vitro Antioxidant Evaluation
DPPH Radical Scavenging Activity

The antioxidant potential of the ethanolic extract of *Cardiospermum halicacabum* leaves was evaluated using the DPPH assay, which measures the ability to scavenge free radicals by observing a decrease in absorbance at 517 nm. Standard antioxidants—Gallic acid, Rutin, and Quercetin—served as references. Among them, Quercetin showed the highest inhibition (88.92%) at 100 µg/ml. The ethanolic extract demonstrated significant dose-dependent antioxidant activity, with 57.36% inhibition at 20 µg/ml and a peak of 86.39% at 100 µg/ml, nearly matching Quercetin. The extract exhibited an IC₅₀ of approximately 15 µg/ml, indicating stronger radical scavenging ability than Quercetin (IC₅₀ ≈ 38 µg/ml). These results suggest the extract’s potent antioxidant nature, likely due to its rich content of phenolics and flavonoids.

Table 7: % Inhibition of Standard Antioxidants in DPPH Assay

Sr. No.	Concentration (µg/ml)	% Inhibition (Gallic acid)	% Inhibition (Rutin)	% Inhibition (Quercetin)
1	20	48.30 ± 0.36	69.26 ± 0.28	44.40 ± 0.10
2	40	53.59 ± 0.21	73.33 ± 0.17	54.20 ± 0.18
3	60	61.37 ± 0.28	76.43 ± 0.18	65.56 ± 0.18
4	80	64.95 ± 0.18	80.09 ± 0.25	76.22 ± 0.17
5	100	76.14 ± 0.24	81.68 ± 0.13	88.92 ± 0.10

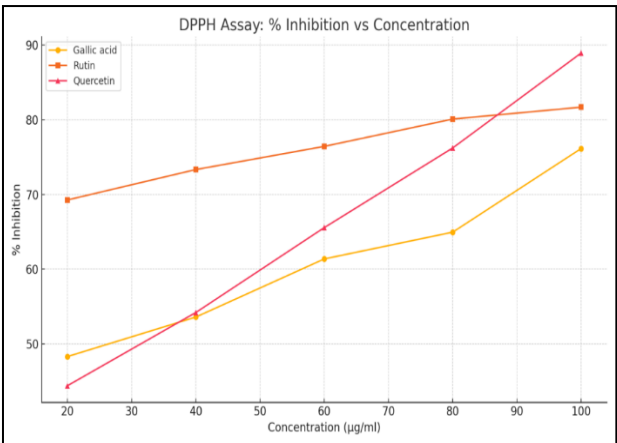


Fig. 6: % inhibition of Gallic acid, Rutin, and Quercetin at various concentrations based on the DPPH assay

FRAP Assay (Ferric Reducing Antioxidant Power)

The FRAP assay further assessed the antioxidant capacity of the ethanolic extract, focusing on its ferric ion (Fe³⁺) reducing potential. A concentration-dependent increase in absorbance at 593 nm was observed, with values rising from 0.142 at 20 µg/ml to 0.484 at 100 µg/ml. While ascorbic acid showed 100% inhibition at 100 µg/ml (IC₅₀ = 36.24 µg/ml), the extract achieved 76.12% inhibition at the same concentration with an IC₅₀ of 62.85 µg/ml. Though less potent than the standard, the extract displayed notable reducing power, reinforcing its antioxidant capability. This activity is attributed to phytoconstituents like flavonoids and polyphenols, which effectively donate electrons to neutralize oxidative species.

Table 8: % Antioxidant Activity (% Inhibition) and IC₅₀ of Ethanolic Extract vs. Standard

Sr. No	Concentration (µg/ml)	% Inhibition (Ascorbic Acid)	% Inhibition (Ethanolic Extract of CH)
1	20	32.39 ± 0.45	22.67 ± 0.48
2	40	53.33 ± 0.38	35.45 ± 0.56
3	60	70.58 ± 0.34	50.34 ± 0.42
4	80	85.74 ± 0.26	63.85 ± 0.39
5	100	100.00 ± 0.00	76.12 ± 0.40

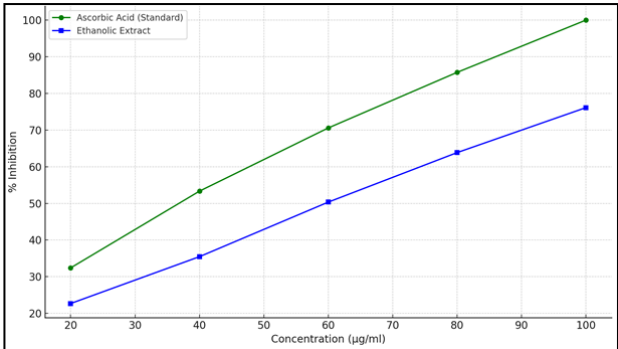


Fig. 7: FRAP Assay for ethanolic extract of CH leaves for *in-vitro* antioxidant

Formulation Development of *Cardiospermum halicacabum* leaves ethanolicextract Polymeric nanoparticles

Polymeric nanoparticles were successfully prepared using the solvent evaporation method, utilizing PLGA (poly(lactic-co-glycolic acid)) as the core polymer due to its biocompatibility and biodegradability. Polyvinyl alcohol (PVA) served as a surfactant and stabilizer to support the formation of a stable emulsion system. The ethanolic extract was incorporated into the nanoparticles at different drug-to-polymer ratios, and five formulations (F1 to F5) were developed by varying PLGA and PVA concentrations, as shown in following table.

Table 9: Composition of Polymeric Nanoparticles of *Cardiospermum halicacabum* Extract

Batch Code	Extract (mg)	PLGA (mg)	PVA (%)	Organic Solvent (mL)	Stirring Speed (rpm)	Stirring Time (min)
F1	10	100	0.5	10	1000	30
F2	10	150	0.5	10	1000	30
F3	10	200	0.5	10	1000	30
F4	10	100	1	10	1000	30
F5	10	150	1	10	1000	30

Each batch was subjected to extensive evaluation to assess its physical properties, encapsulation capability, and *in-vitro* drug release profile.

Evaluation of Nanoformulations

Particle Size and Polydispersity Index (PDI)

Particle size is a crucial parameter in nanoparticle design, significantly affecting drug absorption, biodistribution, and therapeutic efficacy. In this study, the particle sizes of all formulations ranged from 165 nm to 210 nm. Among them, Formulation F3 demonstrated the smallest average particle size of 165 ± 4 nm, which falls within the optimal range for enhanced bioavailability. Polydispersity Index (PDI) values, which reflect particle size distribution uniformity, remained below 0.35 for all formulations. F3 showed the lowest PDI (0.280), indicating a highly uniform and stable nanoparticulate system.

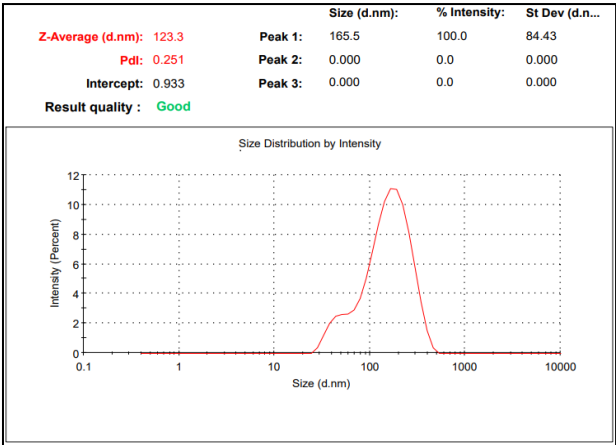


Fig. 8: Particle Size of Nano formulation F3

Zeta Potential

Zeta potential assesses nanoparticle surface charge and predicts dispersion stability. All formulations showed negative zeta potential values due to the anionic nature of PLGA. F3 exhibited the highest zeta potential of -28.3 mV, suggesting strong electrostatic repulsion and excellent colloidal stability, thereby minimizing aggregation risks during storage.

Table 10: Evaluation Parameters of Nanoformulations

Batch Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)	% Drug Release (24 h)
F1	210 ± 5	0.312	-22.4	58.2 ± 2.1	72.5 ± 1.8
F2	185 ± 6	0.295	-25.1	68.5 ± 1.6	78.4 ± 2.2
F3	165 ± 4	0.28	-28.3	75.6 ± 2.4	85.2 ± 1.9
F4	205 ± 7	0.31	-20.2	60.3 ± 1.9	70.6 ± 2.5
F5	180 ± 5	0.29	-24	70.2 ± 2.0	80.3 ± 2.1

Entrapment Efficiency (%EE)

Entrapment efficiency is essential for evaluating how effectively a formulation encapsulates the active constituents. Across batches, EE ranged from 58.2% to 75.6%. F3 achieved the highest entrapment efficiency of 75.6 ± 2.4%, suggesting that its polymer-drug interaction was most favorable, ensuring maximum loading and potential therapeutic impact.

Transmission Electron Microscopy (TEM)

TEM analysis revealed that the PLGA nanoparticles loaded with *Cardiospermum halicacabum* extract were spherical, uniformly dispersed, and under 100 nm in size. The use of acetonitrile as a solvent, along with sonication during the solvent evaporation process, led to the formation of nanosized droplets with a monodispersed profile, confirming the formation of consistent and stable nanoparticles.

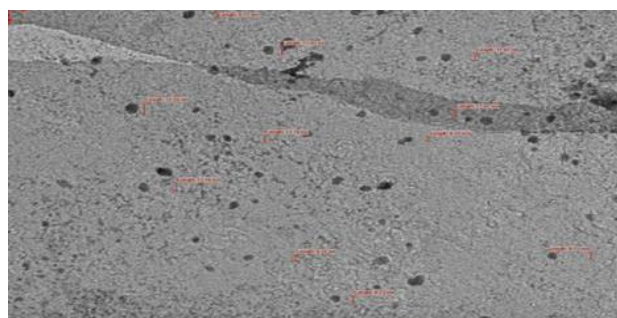


Fig. 9: TEM images of *Cardiospermum halicacabum* loaded PLGA nanoparticles

In-vitro Drug Release

The drug release study over 24 hours showed a biphasic pattern—an initial burst release followed by sustained diffusion. F3 demonstrated the most controlled release profile, with $85.2 \pm 1.9\%$ of drug release at 24 hours. This extended-release behavior supports its suitability for chronic conditions, offering prolonged therapeutic levels with minimal dosing frequency.

Comparative Drug Release Profile

When compared across all batches, F3 stood out with the most sustained release profile. Formulations like F1 and F4 showed lower cumulative release (72.5% and 70.6%, respectively), indicating a less controlled release possibly due to suboptimal polymer ratios. The superior performance of F3 is attributed to its optimized PLGA content and homogeneity.

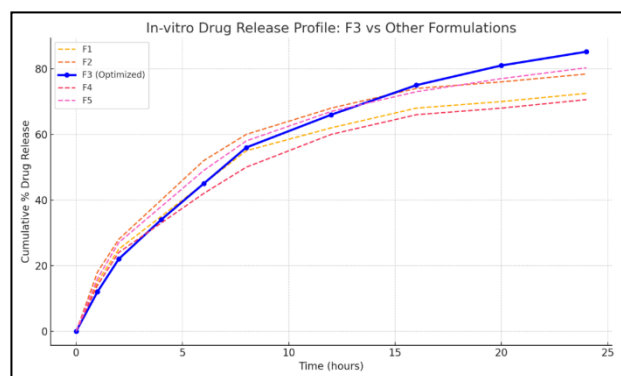


Fig. 10: In-vitro drug release profile of F3 and other formulations

Optimized Batch Selection

Considering all evaluation parameters, F3 was selected as the optimized formulation. It showed the best combination of particle size (165 nm), PDI (0.280), zeta potential (-28.3 mV), entrapment efficiency (75.6%), and drug release (85.2%). These properties confirm its potential as an effective nano-delivery system for *Cardiospermum halicacabum* phytoconstituents.

Stability Study of Optimized Nanoformulation (F3)

The stability of the optimized batch F3 was monitored over 90 days under two conditions—room temperature (25°C) and accelerated storage (40°C). Minor changes in appearance, such as slight turbidity

and opalescence, were noted after 60 days, more so at elevated temperatures. Particle size increased moderately (from 165 nm to 173 nm at 25°C and 182 nm at 40°C), remaining within acceptable limits. The PDI values rose marginally, but stayed below 0.3, indicating maintained uniformity. Zeta potential decreased slightly, though values remained above -20 mV, ensuring continued electrostatic stability. Entrapment efficiency showed a gradual reduction, particularly under accelerated conditions, yet over 90% of initial encapsulation was retained. Drug release slightly decreased but remained above 75% after 90 days, demonstrating that the formulation retained functional integrity over time.

V. CONCLUSION

The current research successfully demonstrates the formulation and in vitro evaluation of a nanoformulated delivery system for *Cardiospermum halicacabum* L., a traditionally known antidiabetic plant. The study confirmed that encapsulating its ethanolic extract in PLGA-based polymeric nanoparticles significantly enhanced its drug release, stability, and antioxidant efficacy. Among the prepared batches, formulation F3 exhibited superior physicochemical and release properties, making it the ideal candidate for further research. The promising antioxidant potential revealed by DPPH assay further reinforces the plant's therapeutic relevance in combating oxidative stress, commonly associated with diabetic complications. Stability studies confirmed that the formulation retained its integrity and efficacy under accelerated conditions, suggesting shelf-stability. This research contributes a novel, biocompatible, and effective nano-drug delivery strategy for herbal antidiabetic therapy and encourages future in vivo and clinical studies to validate its translational potential in modern diabetes treatment regimens.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors have contributed equally

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