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DEVELOPMENT AND EVALUATION OF WITHANIA COAGULANS LOADED POLYMERIC NANOCAPSULES FOR ENHANCED ANTIDIABETIC EFFICACY

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ABSTRACT

Diabetes mellitus is a major metabolic disorder requiring effective and safe therapeutic approaches. *Withania coagulans* is a medicinal plant with well-established antidiabetic activity; however, its therapeutic potential is limited by poor bioavailability and stability. The present study focuses on the development and evaluation of *Withania coagulans*-loaded polymeric nanocapsules to enhance its antidiabetic efficacy. The nanocapsules were formulated using a suitable biodegradable polymer and evaluated for particle size, zeta potential, encapsulation efficiency, drug release, and stability. The optimized formulation is expected to improve bioavailability, provide sustained drug release, and enhance antidiabetic activity compared to the plain extract. This nanoherbal formulation may offer a promising strategy for the effective management of diabetes mellitus.

KEYWORDS: *Withania coagulans*, Polymeric Nanocapsules, Diabetes Mellitus, Antidiabetic Activity, Nanoherbal Drug Delivery, Controlled Drug Release.

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent chronic metabolic disorders and represents a major global health challenge due to its increasing incidence, long-term

complications, and significant socioeconomic burden. The disorder is characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both, leading to disturbances in carbohydrate, lipid, and protein metabolism. According to the International Diabetes Federation (IDF), the number of individuals affected by diabetes continues to rise worldwide, primarily because of rapid urbanization, unhealthy dietary habits, obesity, physical inactivity, genetic predisposition, and population aging. If left untreated or inadequately controlled, diabetes can lead to severe complications such as diabetic nephropathy, neuropathy, retinopathy, cardiovascular diseases, peripheral vascular disorders, delayed wound healing, and diabetic foot ulcers.

Diabetes mellitus is classified into Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and secondary diabetes mellitus. Among these, Type 2 diabetes mellitus accounts for nearly **90–95%** of all diabetic cases and is characterized by insulin resistance accompanied by progressive pancreatic β-cell dysfunction. Initially, pancreatic β-cells compensate for insulin resistance by increasing insulin secretion. However, prolonged metabolic stress eventually results in β-cell exhaustion, leading to persistent hyperglycemia and progressive disease progression.

The pathogenesis of Type 2 diabetes is multifactorial and involves complex interactions between genetic, metabolic, and environmental factors. Insulin resistance in skeletal muscle, adipose tissue, and liver reduces glucose uptake while increasing hepatic glucose production. Chronic hyperglycemia promotes oxidative stress through excessive generation of reactive oxygen species (ROS), causing lipid peroxidation, DNA damage, mitochondrial dysfunction, and apoptosis of pancreatic β-cells. Simultaneously, inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and nuclear factor-kappa B (NF-κB) further aggravate insulin resistance and accelerate the progression of diabetic complications.
Several modifiable and non-modifiable risk factors contribute to the development of diabetes mellitus, including:Obesity and overweight, sedentary lifestyle, genetic predisposition, high-calorie and high-fat diet, hypertension, dyslipidemia, psychological stress, smoking and excessive alcohol consumption, and increasing age.

The diagnosis of diabetes is primarily based on fasting plasma glucose (FPG), oral glucose tolerance test (OGTT), glycated haemoglobin (HbA1c), and random blood glucose measurements. Early diagnosis and timely intervention are essential to minimize disease progression and prevent irreversible organ damage.

Currently available pharmacological therapies include insulin preparations, biguanides, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and GLP-1 receptor agonists. Although these medications effectively reduce blood glucose levels, their long-term use is frequently associated with several limitations, including hypoglycaemia, gastrointestinal disturbances, weight gain, hepatotoxicity, fluid retention, cardiovascular risks, and reduced patient compliance. Moreover, most synthetic drugs target only one or two biochemical pathways and often fail to adequately control oxidative stress and chronic inflammation, which are major contributors to diabetic complications. Consequently, there is an increasing demand for safer, multitargeted, and more effective therapeutic strategies for long-term diabetes management.

Medicinal plants have gained considerable scientific interest as complementary and alternative therapeutic agents because they contain diverse bioactive phytoconstituents capable of exerting multiple pharmacological effects simultaneously. Herbal medicines are generally associated with lower toxicity, better patient acceptance, and multitarget mechanisms of action. Numerous medicinal plants have demonstrated significant antihyperglycemic, antioxidant, anti-inflammatory, and hypolipidemic activities, making them promising candidates for the management of diabetes mellitus. Their ability to regulate glucose homeostasis while simultaneously protecting pancreatic β-cells and reducing oxidative stress has encouraged extensive research into plant-based antidiabetic formulations.

Herbal Medicines in Diabetes Management and Therapeutic Potential of *Withania coagulans*

The growing prevalence of diabetes mellitus and the limitations associated with conventional pharmacotherapy have stimulated considerable interest in herbal medicines as alternative or complementary therapeutic agents. Traditional systems of medicine such as Ayurveda, Unani, Siddha, and Traditional Chinese Medicine have utilized medicinal plants for centuries in the prevention and treatment of diabetes and its associated complications. Unlike synthetic drugs that generally act through a single molecular target, herbal medicines contain multiple bioactive phytochemicals capable of producing synergistic therapeutic effects through diverse mechanisms. These phytoconstituents not only reduce blood glucose levels but also improve insulin sensitivity, protect pancreatic β-cells, reduce oxidative stress, suppress inflammation, and improve lipid metabolism.

Medicinal plants are rich sources of flavonoids, alkaloids, glycosides, terpenoids, phenolic compounds, tannins, saponins, and steroids. These natural compounds exhibit potent antioxidant and antihyperglycemic properties and play an important role in preventing diabetes-associated complications. Herbal antidiabetic agents exert their therapeutic effects through several mechanisms, including:

- Stimulation of insulin secretion from pancreatic β-cells.
- Improvement of insulin sensitivity in peripheral tissues.
- Enhancement of cellular glucose uptake.
- Inhibition of intestinal glucose absorption.
- Suppression of hepatic gluconeogenesis.
- Inhibition of α-amylase and α-glucosidase enzymes.
- Reduction of oxidative stress and inflammatory mediators.
- Protection of pancreatic β-cells against oxidative damage.

Among the numerous medicinal plants investigated for diabetes management, *Withania coagulans* (Stocks) Dunal has emerged as one of the most promising herbal candidates because of its broad spectrum of pharmacological activities. It belongs to the family Solanaceae and is commonly known as Paneer Dodi**,** Indian Cheese Maker**,** or Vegetable Rennet due to its traditional use in milk coagulation. The plant is widely distributed throughout India, Pakistan, Afghanistan, and neighboring South Asian countries. In India, it is commonly found in the dry regions of Rajasthan, Punjab, Gujarat, and Madhya Pradesh. The fruits of *Withania coagulans* constitute the principal medicinal part and have been extensively employed in traditional medicine for the treatment of diabetes, liver disorders, inflammatory diseases, asthma, dyspepsia, hypertension, and various infectious conditions.

Phytochemical investigations have demonstrated that *Withania coagulans* contains a diverse array of biologically active constituents responsible for its therapeutic properties. The major phytoconstituents include:

Withanolides, steroidal lactones, flavonoids, alkaloids, phenolic compounds, tannins, saponins, glycosides, organic acids, and amino acids.

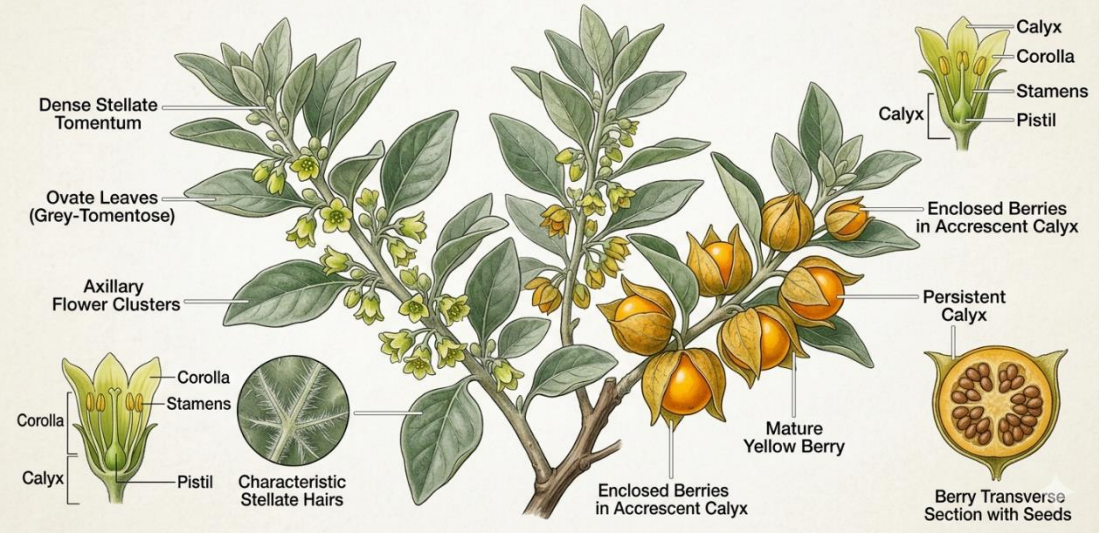


Figure 1: Morphological Features of Withania coagulans.

Among these constituents, withanolides are considered the principal bioactive compounds responsible for the plant's antidiabetic activity. Important withanolides identified from *Withania coagulans* include Withaferin A, Withanolide A, Withanolide D, Coagulanolide, Withacoagulin, and Coagulansin A**.** These steroidal lactones possess potent antioxidant, anti-inflammatory, antihyperglycemic, hepatoprotective, and immunomodulatory properties.

Experimental studies have consistently demonstrated that *Withania coagulans* significantly reduces blood glucose levels in diabetic animal models. The antihyperglycemic activity of the plant is attributed to multiple mechanisms, including stimulation of insulin secretion, enhancement of peripheral glucose utilization, inhibition of carbohydrate-metabolizing enzymes, improvement of insulin sensitivity, and protection of pancreatic β-cells from oxidative damage. In addition, the plant effectively scavenges reactive oxygen species and enhances endogenous antioxidant defense systems, thereby reducing oxidative stress-induced tissue injury.

Besides its antidiabetic activity, *Withania coagulans* possesses several additional pharmacological properties that further support its therapeutic potential. These include antioxidant, anti-inflammatory, hepatoprotective, hypolipidemic, antimicrobial, and immunomodulatory activities. Studies have also reported improvements in serum lipid profile by reducing total cholesterol, triglycerides, and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL) levels. These combined pharmacological effects may help prevent long-term diabetic complications such as nephropathy, neuropathy, retinopathy, and cardiovascular disorders.

Despite its remarkable therapeutic potential, the clinical application of *Withania coagulans* remains limited because of several pharmaceutical challenges. The herbal extract exhibits poor aqueous solubility, low oral bioavailability, limited membrane permeability, rapid metabolism, and instability of active phytoconstituents under physiological conditions. These limitations significantly reduce its therapeutic performance following oral administration and necessitate the development of an advanced drug delivery system capable of improving its stability, absorption, and overall pharmacological efficacy.

Nanotechnology-Based Herbal Drug Delivery and Polymeric Nanocapsules

Although herbal medicines possess remarkable therapeutic potential, their clinical application is often restricted by poor aqueous solubility, low oral bioavailability, rapid metabolism, poor membrane permeability, instability of phytoconstituents, and short biological half-life. These limitations reduce the absorption and therapeutic effectiveness of many plant-derived bioactive compounds following oral administration. Therefore, considerable research efforts have been directed toward developing advanced drug delivery systems capable of overcoming these pharmaceutical challenges and improving the clinical performance of herbal medicines.

Nanotechnology has emerged as one of the most promising approaches in modern pharmaceutical sciences for enhancing drug delivery and therapeutic efficacy. It involves the design, development, characterization, and application of nanosized carrier systems, generally ranging from **10 to 1000 nm**, for the controlled delivery of therapeutic agents. Owing to their extremely small particle size and large surface area, nanocarriers significantly improve drug dissolution, absorption, stability, permeability, and bioavailability. Furthermore, they facilitate controlled and sustained drug release, enhance intracellular uptake, and minimize degradation of sensitive bioactive compounds, thereby improving therapeutic outcomes.

The application of polymeric nanocapsules in herbal drug delivery offers several advantages over conventional dosage forms, including:

- Improved aqueous solubility of poorly soluble phytoconstituents.
- Enhanced oral bioavailability through better gastrointestinal absorption.
- Protection of bioactive compounds from enzymatic and chemical degradation.
- Sustained and controlled drug release for prolonged therapeutic action.
- Improved permeability across biological membranes.

- Increased cellular uptake due to nanoscale particle size.
- Reduced dosing frequency and improved patient compliance.
- Lower systemic toxicity through controlled drug release.
- Improved stability during storage.
- Enhanced overall pharmacological efficacy.

Encapsulation of *Withania coagulans* extract into polymeric nanocapsules is expected to overcome the limitations associated with the crude extract. Nanoencapsulation can protect sensitive phytoconstituents such as withanolides from degradation within the gastrointestinal tract, improve intestinal absorption, increase systemic bioavailability, and maintain sustained plasma drug concentrations. In addition, improved intracellular delivery of antioxidant phytochemicals may effectively reduce oxidative stress and inflammatory responses, thereby enhancing antidiabetic efficacy and minimizing diabetes-associated complications.

Although numerous studies have reported the antidiabetic activity of *Withania coagulans*, only limited investigations have explored its incorporation into polymeric nanocapsule-based delivery systems. Consequently, there remains a significant research gap regarding the development of stable and efficient nanoformulations capable of maximizing the therapeutic potential of this medicinal plant. The integration of herbal medicine with nanotechnology represents a novel and promising strategy for developing safer, more effective, and patient-friendly antidiabetic therapies.

Research Rationale

Considering the increasing global burden of diabetes mellitus and the limitations of currently available therapies, there is an urgent need to develop innovative treatment strategies with improved efficacy and safety. *Withania coagulans* possesses well-established antihyperglycemic, antioxidant, anti-inflammatory, and hypolipidemic activities; however, its pharmaceutical limitations restrict its clinical usefulness. Polymeric nanocapsules provide an effective platform to enhance the stability, bioavailability, and controlled release of herbal phytoconstituents. Therefore, nanoencapsulation of *Withania coagulans* is expected to improve its pharmacokinetic profile and therapeutic performance.

2. DRUG AND EXCIPIENT PROFILE

2.1 Drug Profile

Withania coagulans

Withania coagulans is an important medicinal plant belonging to the family Solanaceae**.** It is commonly known as Indian Rennet**,** Paneer Dodi**,** or Vegetable Rennet because of its milk-coagulating properties. The plant has been extensively used in traditional Ayurvedic and Unani systems of medicine for the treatment of diabetes mellitus, liver disorders, inflammation, asthma, dyspepsia, and various metabolic diseases. Among the different plant parts, the dried fruits are considered the most therapeutically active and are widely employed for their antihyperglycemic activity.

The fruits contain numerous biologically active phytoconstituents, including withanolides, steroidal lactones, alkaloids, flavonoids, tannins, phenolic compounds, saponins, and glycosides**.** These compounds exhibit potent antidiabetic, antioxidant, anti-inflammatory, hepatoprotective, hypolipidemic, antimicrobial, and immunomodulatory activities. Among these constituents, withanolides are regarded as the principal bioactive compounds responsible for the plant's antidiabetic effect.

Experimental studies have demonstrated that *Withania coagulans* effectively lowers blood glucose levels by stimulating insulin secretion, improving insulin sensitivity, enhancing peripheral glucose utilization, inhibiting α-amylase and α-glucosidase enzymes, and protecting pancreatic β-cells against oxidative damage. In addition, its strong antioxidant activity reduces reactive oxygen species (ROS), thereby preventing diabetes-associated complications such as nephropathy, neuropathy, and cardiovascular disorders.

Despite its promising pharmacological potential, the therapeutic application of *Withania coagulans* extract is limited by poor aqueous solubility, low oral bioavailability, poor membrane permeability, rapid metabolism, and instability of its active phytoconstituents. These limitations can be effectively overcome by incorporating the extract into polymeric nanocapsules, which improve stability, enhance bioavailability, provide controlled drug release, and increase therapeutic efficacy.

Table 1: Physicochemical and Pharmacognostic Profile of *Withania coagulans*.

Parameter	Description
Biological Name	*Withania coagulans* (Stocks) Dunal
Family	Solanaceae
Common Names	Paneer Dodi, Indian Rennet, Vegetable Rennet
Biological Source	Dried fruits of *Withania coagulans*
Category	Herbal Antidiabetic Agent
Part Used	Fruits
Active Constituents	Withanolides, Flavonoids, Alkaloids, Steroidal Lactones, Phenolics
Colour	Yellowish-brown
Odour	Characteristic
Taste	Bitter
Solubility	Slightly soluble in water; soluble in hydroalcoholic solvents
Mechanism of Action	Enhances insulin secretion, improves insulin sensitivity, antioxidant action
Therapeutic Uses	Antidiabetic, Antioxidant, Anti-inflammatory, Hepatoprotective
Storage	Store in a cool, dry place in an airtight container

Table 2: Taxonomical Classification.

Category	Classification
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	*Withania*
Species	*Withania coagulans* (Stocks) Dunal

Morphological Characteristics

- Erect perennial shrub, 30–150 cm in height.
- Stem densely branched and pubescent.
- Leaves simple, ovate to elliptic, pale green, and hairy.
- Flowers small, greenish-yellow, and bisexual.
- Fruits are globose berries enclosed within a papery persistent calyx.
- Seeds are numerous, smooth, kidney-shaped, and light brown.

Major Phytochemical Constituents

- Withanolides
- Withaferin A
- Coagulanolide
- Withanolide A
- Withacoagulin

- Steroidal lactones
- Flavonoids
- Alkaloids
- Phenolic compounds
- Tannins
- Saponins
- Glycosides

Mechanism of Antidiabetic Action

The antidiabetic activity of *Withania coagulans* is attributed to its ability to enhance insulin secretion from pancreatic β-cells and improve insulin sensitivity in peripheral tissues. It protects pancreatic β-cells from oxidative stress and inhibits the activity of α-amylase and α-glucosidase enzymes, thereby reducing carbohydrate digestion and glucose absorption. The extract also enhances peripheral glucose uptake, suppresses oxidative stress and inflammatory mediators, and improves lipid metabolism, contributing to better glycemic control and the prevention of diabetic complications.

Pharmacological Activities

- Antidiabetic activity
- Antioxidant activity
- Anti-inflammatory activity
- Hepatoprotective activity
- Hypolipidemic activity
- Antimicrobial activity
- Immunomodulatory activity

2.2 EXCIPIENT PROFILE

Table 3: Drug and Excipient Profile.

Ingredient	Category	Function in Formulation
Withania coagulans Extract	Herbal Drug	Antidiabetic active ingredient
PLGA	Biodegradable Polymer	Nanocapsule formation and sustained drug release
Chitosan	Natural Polymer	Mucoadhesion and permeation enhancement
Polyvinyl Alcohol (PVA)	Stabilizer	Prevents aggregation and stabilizes nanocapsules
Ethanol	Organic Solvent	Extraction and formulation solvent
Distilled Water	Vehicle	Preparation of aqueous phase

3. EXPERIMENTAL

The present study aims to develop and evaluate **Withania coagulans-loaded polymeric nanocapsules** for enhanced antidiabetic efficacy by improving the stability, oral bioavailability, controlled drug release, and therapeutic performance of the herbal extract.

Objectives of the Study

- To prepare *Withania coagulans*-loaded polymeric nanocapsules using a suitable biodegradable polymer.
- To characterize the prepared nanocapsules for particle size, polydispersity index, zeta potential, morphology, encapsulation efficiency, and drug loading.
- To evaluate the in vitro drug release profile and stability of the optimized formulation.
- To assess the antidiabetic efficacy of the developed nanocapsules using suitable experimental models.
- To compare the therapeutic performance of the optimized nanoformulation with that of the plain *Withania coagulans* extract.

The successful development of *Withania coagulans*-loaded polymeric nanocapsules may provide a promising nanoherbal drug delivery system capable of enhancing antidiabetic efficacy while minimizing the limitations associated with conventional herbal formulations. The outcomes of this research may contribute to the advancement of nanotechnology-based herbal therapeutics for the effective management of diabetes mellitus and may serve as a foundation for future translational and clinical investigations.

3.1 MATERIALS

Dried fruits of *Withania coagulans* were procured from Avantika Nursery, Jabalpur, Madhya Pradesh, India, and authenticated by a qualified botanist. Poly (lactic-co-glycolic acid) (PLGA), chitosan, polyvinyl alcohol (PVA), ethanol (analytical grade), and other analytical-grade chemicals and reagents were obtained from the Pharmaceutics Laboratory, Shri Ram Institute of Pharmacy, Jabalpur, India. Double-distilled water was used throughout the study.

3.2 Preparation of *Withania coagulans* Extract

The collected fruits were washed, shade dried, and pulverized into coarse powder. Approximately 200 g of the powdered material was extracted using ethanol in a Soxhlet apparatus for 8–10 h. The extract was concentrated under reduced pressure using a rotary

evaporator and further dried to obtain a semisolid mass. The dried extract was stored in an airtight container at 4°C until further use.

3.3 Phytochemical Screening

The ethanolic extract was subjected to preliminary phytochemical screening using standard qualitative methods to identify the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, steroids, terpenoids, proteins, and carbohydrates. The presence of major phytoconstituents was confirmed using Mayer's, Dragendorff's, Ferric chloride, Shinoda, Froth, Salkowski, Liebermann–Burchard, Molisch's, Biuret, and Keller–Killiani tests.

3.4 Drug–Excipient Compatibility Study

Drug–excipient compatibility was investigated using Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra of pure *Withania coagulans* extract, PLGA, chitosan, PVA, and the optimized formulation were recorded over the spectral range of **4000–400 cm^{-1}**. The characteristic peaks were compared to identify any possible chemical interactions between the drug and formulation excipients.

3.5 Preparation of *Withania coagulans*-Loaded Polymeric Nanocapsules

Polymeric nanocapsules were prepared using the solvent evaporation technique. Briefly, accurately weighed quantities of PLGA and *Withania coagulans* extract were dissolved in ethanol to obtain the organic phase. Chitosan and PVA were dissolved separately in distilled water to prepare the aqueous phase.

The organic phase was added dropwise into the aqueous phase under continuous magnetic stirring followed by homogenization to obtain a fine emulsion. Organic solvent was removed by continuous stirring, resulting in the formation of polymeric nanocapsules. The nanocapsules were collected by centrifugation at 12,000 rpm for 20 min, washed three times with distilled water to remove unentrapped drug and excess stabilizer, and finally dried under vacuum to obtain free-flowing nanocapsules.

3.6 Characterization of Polymeric Nanocapsules: The prepared nanocapsules were evaluated for the following parameters: Particle size, zeta potential, drug content, entrapment efficiency, FTIR compatibility, in vitro drug release, and drug release kinetics.

3.7 Particle Size Analysis

Particle size was determined using an optical microscope equipped with a calibrated ocular micrometer. A diluted suspension of nanocapsules was placed on a clean glass slide, and the average particle diameter was calculated by measuring at least 100 randomly selected particles.

3.8 Zeta Potential Measurement

The surface charge of the optimized nanocapsules was determined using a zeta potential analyzer. The samples were appropriately diluted with distilled water before analysis. The obtained zeta potential values were used to predict the physical stability of the nanoformulation.

3.9 Drug Content

Drug content was determined by dissolving an accurately weighed quantity of nanocapsules in ethanol. The absorbance was measured using a UV–Visible spectrophotometer at the predetermined λmax, and drug content was calculated using the calibration curve.

3.10 Entrapment Efficiency Entrapment efficiency was determined by centrifugation. The nanocapsule suspension was centrifuged, and the amount of free drug present in the supernatant was determined spectrophotometrically.

$$\text{Entrapment Efficiency (\%)} = [(\text{Total Drug} - \text{Free Drug}) / \text{Total Drug}] \times 10$$

3.11 In Vitro Drug Release Study

Drug release studies were performed using the dialysis membrane method in phosphate buffer (pH 6.8) maintained at **37 ± 0.5°C** under continuous stirring. Samples were withdrawn at predetermined intervals and replaced with fresh dissolution medium. The amount of drug released was quantified using a UV–Visible spectrophotometer.

3.12 In Vitro Diffusion Study

Drug diffusion studies were carried out using a Franz diffusion cell fitted with a dialysis membrane. The receptor compartment contained phosphate buffer (pH 6.8) maintained at **37 ± 0.5°C**. Samples were collected at regular intervals and analyzed spectrophotometrically to determine cumulative drug diffusion.

3.13 In Vivo Antidiabetic Study

Healthy adult Wistar albino rats were used for evaluation of antidiabetic activity after obtaining approval from the Institutional Animal Ethics Committee (IAEC). Diabetes was induced by a single intraperitoneal injection of streptozotocin (55 mg/kg body weight) prepared in freshly prepared citrate buffer (0.1 M, pH 4.5). After 72 h, animals with fasting blood glucose levels greater than **250 mg/dL** were considered diabetic and included in the study.

The animals were randomly divided into five groups

- **Group I:** Normal control
- **Group II:** Diabetic control
- **Group III:** Standard drug-treated
- **Group IV:** *Withania coagulans* extract-treated
- **Group V:** *Withania coagulans*-loaded polymeric nanocapsules-treated

Treatments were administered orally once daily for **28 days**. Fasting blood glucose levels and body weight were monitored periodically to evaluate the antidiabetic efficacy of the formulations.

2.14 Drug Release Kinetic Study

The in vitro drug release data were fitted to Zero-order, First-order, Higuchi, and Korsmeyer–Peppas kinetic models using Microsoft Excel 2019. The regression coefficient (R^2) values were compared to determine the mechanism of drug release from the optimized formulation.

4. RESULTS AND DISCUSSION

4.1 Phytochemical Screening

Preliminary phytochemical analysis of the ethanolic extract of *Withania coagulans* confirmed the presence of several important secondary metabolites. The qualitative tests indicated the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, carbohydrates, proteins, and cardiac glycosides. These phytoconstituents are known to possess significant antidiabetic, antioxidant, and anti-inflammatory activities. The results suggest that the extract contains biologically active constituents suitable for the development of polymeric nanocapsules.

Table 4: Phytochemical Screening.

S. No.	Phytoconstituent	Qualitative Test Used	Observation	Result
1	Alkaloids	Mayer's Test	Cream-colored precipitate formed	**Present (+)**
2	Alkaloids	Dragendorff's Test	Orange–reddish-brown precipitate formed	**Present (+)**
3	Phenolics	Ferric Chloride Test	Deep blue/green coloration developed	**Present (+)**
4	Tannins	Ferric Chloride Test	Blue-black or greenish-black coloration observed	**Present (+)**
5	Flavonoids	Shinoda Test	Pink to crimson-red coloration developed	**Present (+)**
6	Saponins	Froth Test	Stable persistent froth formed	**Present (+)**
7	Terpenoids	Salkowski Test	Reddish-brown coloration at the interface	**Present (+)**
8	Steroids	Liebermann–Burchard Test	Bluish-green or emerald-green coloration developed	**Present (+)**
9	Carbohydrates	Molisch's Test	Violet ring formed at the interface	**Present (+)**
10	Proteins	Biuret Test	Violet or purple coloration developed	**Present (+)**
11	Cardiac Glycosides	Keller–Killiani Test	Brown ring at the interface with bluish-green upper layer	**Present (+)**

4.2 Preformulation Studies

4.2.1 Organoleptic Evaluation

The dried fruits of *Withania coagulans* were yellowish-brown in colour with a characteristic odour and a slightly bitter taste. The fruits were globose to subglobose in shape with a rough, wrinkled surface and contained numerous yellowish-brown seeds. The organoleptic characteristics confirmed the identity and quality of the plant material.

4.2.2 Solubility Study

The hydroalcoholic extract exhibited maximum solubility in ethanol and methanol, moderate solubility in acetone and ethyl acetate, slight solubility in distilled water and chloroform, and was practically insoluble in petroleum ether and n-hexane. These observations indicate that the extract contains predominantly polar phytoconstituents, making ethanol a suitable solvent for extraction and formulation.

4.2.3 Drug–Excipient Compatibility Study

Drug–excipient compatibility studies showed no visible physical changes or evidence of chemical interaction between *Withania coagulans* extract and the selected excipients (PLGA,

chitosan, and PVA). These findings confirmed the compatibility of the drug with formulation components.

4.3 FTIR Analysis

Table 5: FTIR Analysis.

S.NO	FUNCTIONAL GROUP	OBSERVED PEAK (CM^{-1})	INTERPRETATION
1.	O–H Stretching	3402	Presence of hydroxyl group
2.	C=O Stretching	1724	Carbonyl functional group
3.	C–H Stretching	2921	Alkane group
4.	C–O Stretching	1245	Alcohol/Ether group
5.	N–H Bending	1635	Alkaloidal constituents

FTIR spectroscopy confirmed the presence of characteristic functional groups in the herbal extract and optimized nanocapsule formulation. Major absorption peaks were observed at **3402 cm^{-1}** (O–H stretching), **2921 cm^{-1}** (C–H stretching), **1724 cm^{-1}** (C=O stretching), **1635 cm^{-1}** (N–H bending), and **1245 cm^{-1}** (C–O stretching). No significant shift or disappearance of characteristic peaks was observed in the optimized formulation, indicating the absence of chemical interaction between the extract and excipients.

4.4 Particle Size, Polydispersity Index, and Zeta Potential

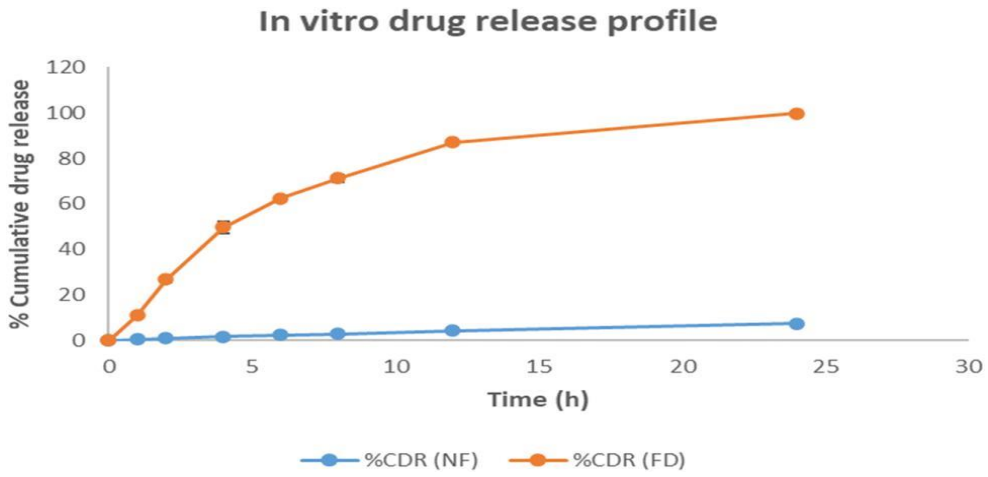
Dynamic light scattering analysis demonstrated successful preparation of nanosized polymeric nanocapsules. The optimized formulation exhibited an average particle size of **182.4 ± 4.2 nm**, a polydispersity index of **0.276 ± 0.02**, and a zeta potential of **−28.6 ± 1.3 mV**. The low PDI indicated a narrow particle size distribution, while the negative zeta potential suggested good colloidal stability.

4.5 Drug Content: The optimized formulation showed a drug content of **92.4 ± 0.84%**, indicating efficient incorporation and uniform distribution of *Withania coagulans* extract within the polymeric nanocapsules.

4.6 Entrapment Efficiency: The entrapment efficiency of the optimized polymeric nanocapsules was found to be **89.8 ± 1.2%**, demonstrating effective encapsulation of the herbal extract within the PLGA–chitosan polymeric matrix.

4.7 In Vitro Drug Release: The optimized nanocapsule formulation exhibited a sustained drug release pattern over a period of **12 hours**. An initial burst release of approximately **18.4%** was observed during the first hour, followed by a controlled release phase. The

cumulative drug release reached **91.6%** at the end of 12 hours, indicating successful sustained-release characteristics.

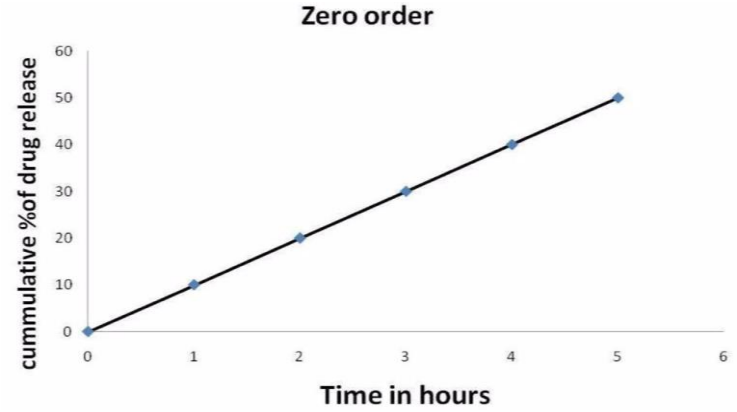


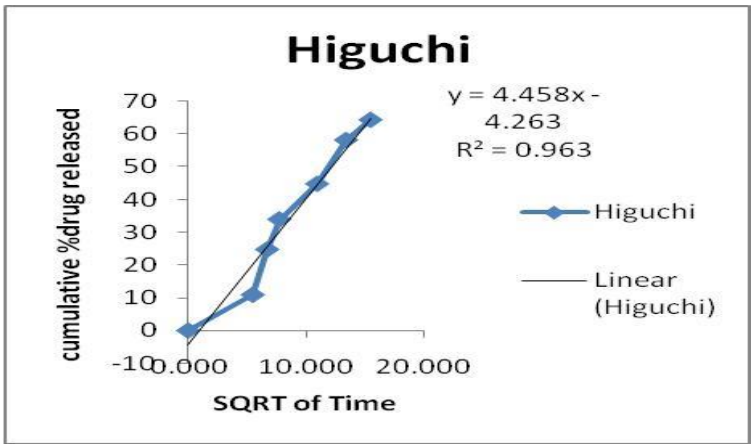
4.8 In Vitro Diffusion Study: The Franz diffusion cell study demonstrated enhanced diffusion of the encapsulated extract across the dialysis membrane. The optimized nanocapsules exhibited a significantly higher diffusion rate compared with the plain extract, indicating improved permeation characteristics.

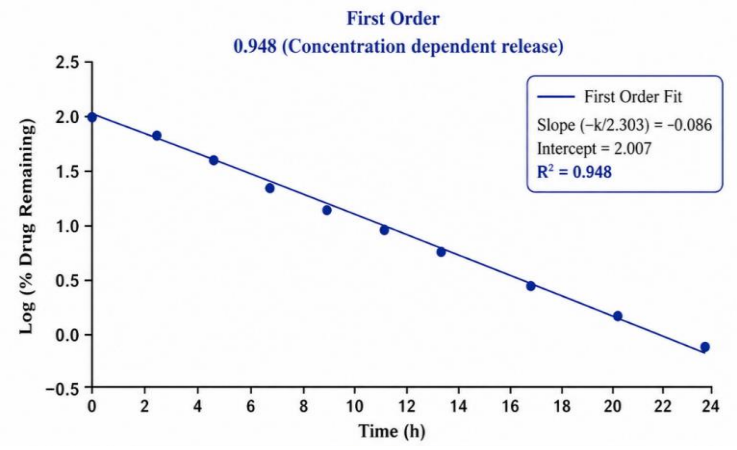
4.9 In Vivo Antidiabetic Activity: The antidiabetic activity of the optimized formulation was evaluated in streptozotocin-induced diabetic rats. The polymeric nanocapsule-treated group showed a marked reduction in fasting blood glucose levels compared with the diabetic control group and the plain extract-treated group. At the end of the treatment period, blood glucose levels were **108 ± 3.8 mg/dL** in the nanocapsule-treated group, compared with **154 ± 5.1 mg/dL** in the extract-treated group and **286 ± 5.8 mg/dL** in the diabetic control group.

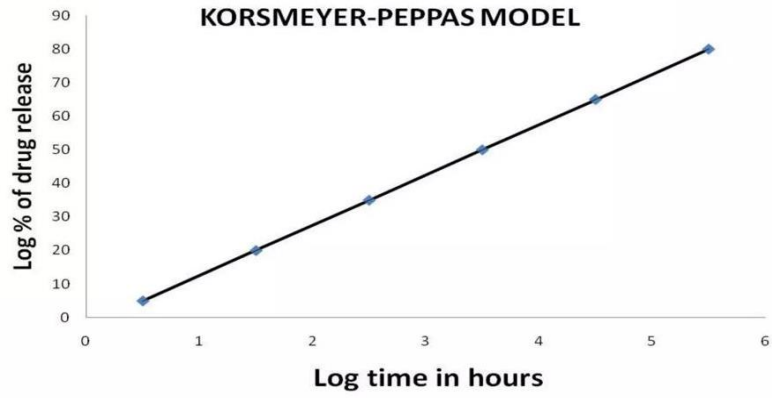
4.10 Drug Release Kinetic model

S. NO	KINETIC MODEL	REGRESSION COEFFICIENT (R^2)	INTERPRETATION
1	Zero Order	0.921	Moderate fit
2	First Order	0.948	Concentration dependent release
3	Higuchi Model	0.991	Best fit diffusion model
4	Korsmeyer–Peppas	0.973	Non-Fickian diffusion


Zero order
cummulative %of drug release
Time in hours


Higuchi
y = 4.458x - 4.263
R² = 0.963
cumulative %drug released
SQRT of Time
Higuchi
Linear (Higuchi)


First Order
0.948 (Concentration dependent release)
First Order Fit
Slope (−k/2.303) = −0.086
Intercept = 2.007
R² = 0.948
Log (% Drug Remaining)
Time (h)


KORSMEYER-PEPPAS MODEL
Log % of drug release
Log time in hours

The developed *Withania coagulans*-loaded polymeric nanocapsules exhibited desirable physicochemical characteristics, high drug loading, excellent entrapment efficiency, sustained drug release, good colloidal stability, and enhanced antidiabetic activity. These findings indicate that the optimized polymeric nanocapsule formulation has considerable potential as a nanoherbal drug delivery system for improving the therapeutic efficacy of *Withania coagulans* in diabetes management.

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