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DEVELOPMENT OF NOVEL LIPID-BASED NANO-CARRIERS OF POLYHERBAL BLEND FOR ANTI-DIABETIC ACTIVITY

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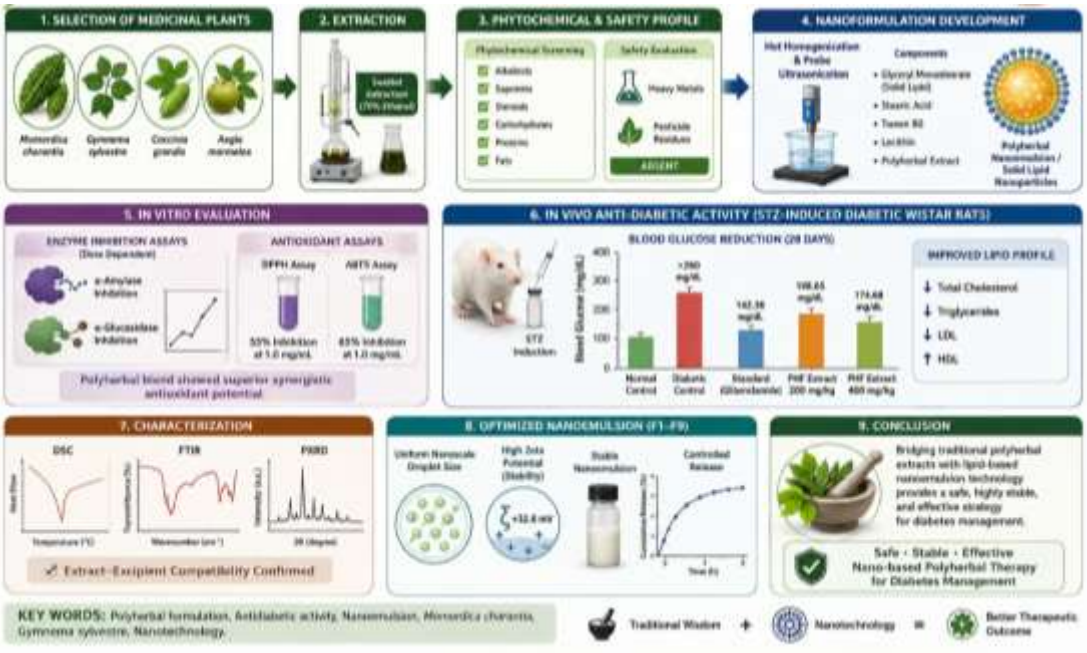
ABSTRACT
Polyherbal formulations offer synergistic therapeutic benefits. This study evaluated the authentication, extraction, phytochemical profile, and nanoemulsion formulation of four traditional Indian medicinal plants (Momordica charantia, Gymnema sylvestre, Coccinia grandis, and Aegle marmelos) to enhance antidiabetic efficacy. Plant materials were extracted using 70% ethanol via Soxhlet extraction. Phytochemical screening confirmed the presence of alkaloids, saponins, steroids, carbohydrates, proteins, and fats, with heavy metals and pesticide residues absent. In vitro assays demonstrated dose-dependent enzyme inhibition (alpha-amylase and alpha-glucosidase) and substantial antioxidant potential (DPPH and ABTS). The combined polyherbal extract demonstrated superior synergistic antioxidant potential (55% DPPH and 65% ABTS inhibition at 1.0 mg/mL). In vivo evaluation in streptozotocin-induced diabetic Wistar rats treated over 28 days showed significant blood glucose reduction at 200mg/kg 198.65 mg/dL and 400 mg/kg 174.68 mg/dL compared to baseline hyperglycemia >260 mg/dL, alongside improved lipid profiles. To optimize delivery, solid lipid nanoparticles and polyherbal nanoemulsions (F1–F9) were engineered via hot homogenization and probe ultrasonication using glyceryl monostearate, stearic acid, Tween 80, and lecithin. DSC, FTIR, and PXRD confirmed extract-excipient compatibility. The optimized nanoemulsion system displayed uniform nanoscale droplet size, high Zeta potential stability, and controlled release. These findings demonstrate that bridging traditional polyherbal extracts with nanoemulsion technology provides a safe, highly stable, and effective strategy for diabetes management.

KEYWORDS: Polyherbal formulation, Antidiabetic activity, Nanoemulsion, Momordica charantia, Gymnema sylvestre, Nanotechnology.

GRAPHICAL ABSTRACT:



1. INTRODUCTION
Diabetes mellitus remains one of the most critical metabolic disorders worldwide, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The continuous rise in global

diabetes prevalence has driven an urgent need for effective therapeutic strategies that not only regulate blood glucose levels but also mitigate secondary complications associated with oxidative stress [2]. Although conventional synthetic oral hypoglycemic agents are widely used, their long-term administration is frequently associated with adverse side effects, drug tolerance, and secondary failure rates [3]. Consequently, natural plant-based therapies have gained significant interest as safer, alternative or complementary therapeutic options [4].

Traditional Indian medicine, particularly Ayurveda, relies heavily on polyherbal formulations that exploit synergistic biological mechanisms to manage complex metabolic disorders [5]. Four plants with recognized antidiabetic and antioxidant properties include Momordica charantia (bitter gourd), Gymnema sylvestre (gurmar), Coccinia grandis (ivy gourd), and Aegle marmelos (bael). M. charantia contains active charantin and vicine, which possess potent insulin-mimetic activity and inhibit carbohydrate-hydrolyzing enzymes alpha-amylase and alpha-glucosidase) [6]. G. sylvestre contains gymnemic acids known to suppress intestinal glucose absorption and stimulate pancreatic beta-cell regeneration [7]. C. grandis and A. marmelos contribute rich polyphenolic and flavonoid profiles that reduce oxidative stress and enhance systemic glucose utilization [8,9]. Combined, these botanical extracts provide a multi-target therapeutic approach that addresses both glycemic control and oxidative cellular damage.

Despite their strong pharmacological potential, crude botanical extracts face significant biopharmaceutical limitations. Most active phytochemical constituents—such as lipophilic flavonoids, saponins, and complex glycosides—exhibit poor aqueous solubility, high intestinal degradation, and poor epithelial permeability, resulting in low oral bioavailability [10]. Pharmaceutical nanotechnology offers an effective strategy to overcome these physicochemical barriers [11]. Specifically, nanoemulsions and solid lipid nanoparticles (SLNs) serve as submicron lipid-based carrier systems that enhance the solubility, stability, and cellular uptake of poorly soluble phytoconstituents [12,13]. Droplet sizes within the nanoscale regime 20-200nm significantly increase surface area, prolong systemic circulation, and allow for controlled release kinetics [14].

While the individual antidiabetic properties of M. charantia, G. sylvestre, C. grandis, and A. marmelos have been documented, systematic studies evaluating their combined synergistic potential when encapsulated into a lipid-based nanoemulsion system remain limited. Therefore, this study aimed to prepare, formulate, and evaluate a polyherbal nanoemulsion combining 70% ethanolic extracts of these four medicinal plants. We evaluated the phytochemical composition, in vitro antioxidant and carbohydrate-hydrolyzing enzyme inhibition activities, in vivo hypoglycemic efficacy in streptozotocin (STZ)-induced diabetic Wistar rats, and the physicochemical characteristics of the engineered nanoemulsion formulations to establish a scientifically validated, high-bioavailability platform for diabetes management.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh plant materials of Momordica charantia (fruits), Gymnema sylvestre (leaves), Coccinia grandis (fruits), and Aegle marmelos (fruits) were collected from local areas near Buldana District, Maharashtra, India. Herbarium specimens were prepared, preserved, and authenticated at the Department of Botany, Shri Shivaji Science College, Chikhali, Buldana District, Maharashtra, India [15].

2.2 Preparation of Plant Extracts

Collected plant materials were washed thoroughly under running tap water to eliminate foreign matter, shade-dried at room temperature 25 pm 2^0C for 2–3 weeks, and pulverized into fine powder using an electric grinder. For extraction, 500g of dried powder from each plant species was packed into filter paper thimbles and subjected to Soxhlet extraction using 70% ethanol as the solvent [16]. Extraction was carried out over a minimum of 9 h (10 syphon cycles) until complete exhaustion of the plant material. The resulting liquid extracts were evaporated under reduced pressure using a rotary evaporator and dried under laminar airflow to yield dark green to reddish-brown crude extracts. The dried extracts were stored in airtight containers at room temperature for further analysis.

2.3 Physicochemical and Preliminary Phytochemical Screening

Physicochemical parameters of the crude drug powders, including loss on drying (LOD at 105^0C, total ash, acid-insoluble ash, water-soluble ash, sulfated ash, and alcohol/water-soluble extractive values, were determined according to standard Indian Pharmacopoeia [17] and WHO guidelines [18].

Extracts were qualitatively screened for major phytoconstituents according to standard procedures [19] **Assays included:**

- **Carbohydrates and Sugars:** Molisch, Fehling, Benedict, and Barfoed tests [20].
- **Proteins and Amino Acids:** Biuret, Millon, Xanthoproteic, and Ninhydrin tests [21].
- **Fats and Fixed Oils:** Saponification and solubility tests[22].
- **Steroids:** Salkowski, Liebermann-Burchard, and Liebermann reactions [23].
- **Glycosides:** Keller-Kiliani and Legal tests for cardiac glycosides; Borntrager and modified Borntrager tests for anthraquinones; foam test for saponins [24].
- **Flavonoids:** Shinoda, lead acetate, ferric chloride, and alkali tests [25].
- **Alkaloids:** Dragendorff, Mayer, Wagner, and Hager reagents [26].
- **Phenolic Compounds and Tannins:** Ferric chloride, lead acetate, gelatin, bromine water, and dilute iodine

tests [27].
Heavy metal contents (arsenic, cadmium, lead, mercury, zinc, copper, chromium, and manganese) were quantified using atomic absorption spectrometry (AAS) following microwave-assisted nitric acid HNO_3 digestion. Pesticide residues were quantified using gas chromatography equipped with a 63 Ni electron capture detector (GC-ECD) [28].

2.4 In Vitro Antioxidant and Antidiabetic Assays
Free radical scavenging activity was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays according to established methods [29,30] Absorbance was recorded spectrophotometrically at 517nm (DPPH) and 734 nm (ABTS).
In vitro antidiabetic activity was assessed via alpha-amylase and alpha-glucosidase inhibition assays [31,32]. Extracts at concentrations of 100, 200, and 400 g/mL were incubated with alpha-amylase enzyme and starch substrate; released maltose was measured at 540 nm using dinitrosalicylic acid (DNS) reagent [33]. Alpha-Glucosidase inhibition was evaluated using p-nitrophenyl-alpha-D-glucopyranoside as the substrate, measuring released p-nitrophenol at 405 nm [34]. Acarbose served as the reference standard. The half-maximal inhibitory concentration IC_{50} was calculated for individual and polyherbal extract combinations.

2.5 In Vivo Antidiabetic Study in STZ-Induced Diabetic Rats
2.5.1 Experimental Animals and Ethics
Healthy adult Wistar albino rats 150-200g of either sex were housed in polypropylene cages under standard environmental conditions 25 $\pm 2^0$C, 55-65 % relative humidity, 12-h light/dark cycle) with standard pellet diet and water ad libitum. Animals were acclimatized for 7 days prior to experimentation. All animal protocols received prior approval from the Institutional Animal Ethics Committee (IAEC Approval No. 31/IAEC-I/SLSRPL/2024, dated 20/11/2025) and complied with CPCSEA guidelines.
2.5.2 Acute Toxicity Study
Acute oral toxicity was conducted per OECD Guideline 425[35]. A single dose of 5000 mg/kg body weight of the polyherbal extract suspended in Tween-80 was administered orally to overnight-fasted rats (n = 5). Animals were observed continuously for the first 4 h, daily for 14 days, for mortality, behavioral profiles, neurological indicators, and autonomic responses.
2.5.3 Diabetes Induction and Treatment Protocol
Diabetes was induced via a single intraperitoneal injection of streptozotocin STZ, 45 mg/kg b.w.) dissolved in freshly prepared 0.1M citrate buffer pH 4.5 [36]. To prevent initial hypoglycemic shock, rats received a 5% glucose solution for 24 h post-injection. Hyperglycemia was confirmed 72 h post-STZ administration; animals with fasting blood glucose levels > 250mg/dL were included in the study.
Polyherbal extract combinations (G. sylvestre : M. charantia : C. grandis : A. marmelos) were prepared at two ratios: **1:1:1:1** and **4:3:2:1**. Diabetic rats received daily oral doses of 200 mg/kg or 400 mg/kg b.w. for 28 days. Fasting blood glucose levels were monitored on Days 0, 7, 14, 21, and 28 using a glucometer. At the study endpoint (Day 28), serum total cholesterol, triglycerides, HDL, LDL, and VLDL were quantified using enzymatic kits and Friedewald's formula [37]. Standard hematological parameters were measured to evaluate overall safety.

2.6 Preformulation Extract and Excipient Compatibility Studies
Physicochemical interactions between individual extracts (G. sylvestre, M. charantia, C. grandis, and A. marmelos) and solid lipid excipients (glyceryl monostearate [GMS] and stearic acid) were evaluated using:
1. **Differential Scanning Calorimetry (DSC):** Samples 5-10 mg were sealed in aluminum pans and heated from 25^0C to 300^0C at a rate of 10^0C/min under nitrogen purge [38].
2. **Fourier Transform Infrared Spectroscopy (FTIR):** Spectra were recorded on KBr pellets across 4000-400-cm^{-1} to observe potential shifts in major functional group peaks [39].
3. **Powder X-ray Diffraction (PXRD):** Diffraction patterns were obtained across a 2theta range of 5^0-80^0 to monitor shifts in material crystallinity.

2.7 Preparation of Polyherbal Solid Lipid Nanoparticles (SLNs)
Polyherbal extract-loaded solid lipid nanoparticles (SLNs, formulations F1–F9) were engineered using a hot high-speed homogenization technique followed by probe ultrasonication.
Briefly, the lipid phase consisting of stearic acid and glyceryl monostearate was melted at 5-10^0C above its melting temperature. The combined polyherbal extract was dissolved/dispersed uniformly into the molten lipid matrix under continuous stirring. Concurrently, an aqueous phase containing Tween 80 (or Poloxamer 188) and lecithin as co-surfactants was prepared in distilled water and heated to the same temperature. The hot aqueous phase was added dropwise to the lipid phase under high-speed homogenization 10000-15000rpm for 5-10min. to yield a primary emulsion.
The primary emulsion was immediately subjected to probe ultrasonication in pulse mode for 5-10min to achieve nanoscale droplet reduction. The resulting nanoemulsion was allowed to cool to room temperature under gentle stirring, facilitating lipid recrystallization to form SLNs. The dispersions were sealed in airtight glass containers for stability and characterization.

2.8 Formulation Design and Optimization via 3^2 Factorial Design

To systematically optimize the polyherbal solid lipid nanoparticles (SLNs) and nanoemulsions, a 3^2 full factorial design was implemented using Design-Expert software. Two independent variables—polyherbal extract concentration (X_1 , % w/v) and surfactant concentration (Tween 80, X_2, % w/v)—were evaluated at three levels to determine their main and interactive effects on formulation parameters (Tables 1 and 2).

Response data were evaluated using analysis of variance (ANOVA) based on the following second-order polynomial regression model:

$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2$

where Y represents the measured response variable, b_0 is the intercept, and b_1, b_2, b_{12} represent the linear and interaction regression coefficients.

Table 1. Composition of polyherbal extract–loaded solid lipid nanoparticles and nanoemulsion formulations (F1–F9).

Formulation Code	Polyherbal Extract (% w/v)	Oil Phase (% w/v)	Tween 80 (% w/v) Surfactant	Lecithin (% w/v) Co-surfactant	Distilled Water (q.s. to ml)
F1	0.5	2.0	1.0	0.5	100
F2	0.5	2.0	1.5	0.5	100
F3	0.5	2.0	2.0	0.5	100
F4	1.0	2.5	1.0	0.5	100
F5	1.0	2.5	1.5	0.5	100
F6	1.0	2.5	2.0	0.5	100
F7	1.5	3.0	1.0	0.5	100
F8	1.5	3.0	1.5	0.5	100
F9	1.5	3.0	2.0	0.5	100

Table 2. Factorial design matrix displaying independent variables.

Batch	X1: Extract (%)	X2: Tween 80 (%)
F-1	0.5	1.0
F-2	0.5	1.5
F-3	0.5	2.0
F-4	1.0	1.0
F-5	1.0	1.5
F-6	1.0	2.0
F-7	1.5	1.0
F-8	1.5	1.5
F-9	1.5	2.0

2.9 Preparation of Polyherbal Nanoemulsion

Polyherbal nanoemulsion formulations were prepared via high-energy emulsification coupled with probe ultrasonication. Briefly, polyherbal extract-loaded SLNs were dissolved in the chosen oil phase (Capryol 90, Capmul MCM, oleic acid, or isopropyl myristate). Tween 80 (surfactant) and lecithin (co-surfactant) were added to the oil phase under continuous agitation to obtain a homogeneous mixture. Distilled water was added dropwise under high-speed homogenization 10000-15000 rpm for 5-10min to yield a primary oil-in-water (o/w) emulsion. Droplet size was further reduced by probe ultrasonication in pulse mode (5-10 min) to produce stable nanoemulsions (F1–F9).

2.10 Characterization and Physicochemical Evaluation

2.10.1 Particle Size, Polydispersity Index (PDI), and Zeta Potential

Mean globule/particle size and PDI were determined using dynamic light scattering (DLS) / photon correlation spectroscopy. Samples were diluted with double-distilled water and analyzed at 25^0C with a scattering angle of 90^0. Zeta potential was derived from electrophoretic mobility measurements using disposable folded capillary cells to evaluate colloidal stability.

2.10.2 Entrapment Efficiency, Drug Content, and Production Yield

To quantify entrapment efficiency EE, the nanoformulation was centrifuged 12000rpm 4^0C for 30 min to separate unentrapped free extract in the supernatant. Total extract content was determined by dissolving the formulation in methanol and measuring absorbance spectrophotometrically. Equations applied were:

$$\% \text{ EE} = \frac{\text{weight of drug added} - \text{weight of drug in supernatant}}{\text{weight of drug added}} \times 100$$

$$\text{Production yield} = \frac{\text{Amount of freezed dried powder}}{\text{Amount of extract and Polymer in feed}} \times 100$$

2.11 Morphological and Structural Analysis

Surface morphology and droplet architecture were examined using Transmission Electron Microscopy (TEM). Samples were diluted to 1mg/mL, placed onto formvar-coated copper grids, negative-stained with phosphotungstic acid, air-dried, and viewed under TEM.

2.12 Physical Attributes, pH, Conductivity, and Dilution Tests

Formulations were visually inspected for clarity, color, homogeneity, and phase separation. System pH was recorded using a calibrated digital pH meter at 25^0C. Viscosity was measured using a Brookfield rotational viscometer at room temperature. Thermodynamic type (o/w vs. w/o) and phase integrity were confirmed using dilution tests with distilled water and electrical conductivity measurements.

2.13 In Vitro Drug Release

In vitro extract release was evaluated via dialysis membrane diffusion in phosphate-buffered saline PBS pH 6.8 at 37 $\pm 0.5^0$C under continuous stirring. Aliquots were withdrawn at specified time intervals, replaced immediately with fresh pre-warmed buffer, and quantified spectrophotometrically.

2.14 Stability Testing (ICH Guidelines)

Optimized formulations were subjected to stability studies in accordance with ICH Q1A guidelines under three conditions: refrigerated 4 $\pm 2^0$ C room temperature 25 $\pm$ 2^0C /60 $\pm$5% RH, and accelerated conditions 40 $\pm 2^0$C / 75 $\pm$5% RH. Physical appearance, phase separation, mean particle size, pH, and drug retention were recorded at 0, 1, 2, and 3 months.

3. RESULTS

3.1 Physicochemical Evaluation

3.1.1 Physicochemical Examination of Plant Powders

The physicochemical properties of powdered leaves of Momordica charantia, Gymnema sylvestre, Aegle marmelos (Bael), and Coccinia grandis (Ivy Gourd) were evaluated to establish foundational quality control and stability parameters (Table 3).

Table 3: Physicochemical Constituents of Powdered Herbs (% w/w)

Parameter	Momordica charantia	Gymnema sylvestre	Aegle marmelos	Coccinia grandis
Moisture content (% w/w)	8.50±0.90	6.70±0.90	7.00±0.80	6.50±0.80
Ash content (% w/w)	9.20±1.10	6.90±1.00	8.50±0.95	5.75±0.90
Acid insoluble content (% w/w)	7.80±1.00	5.80±0.70	7.00±0.75	4.80±0.70
Water soluble content (% w/w)	8.00±1.20	7.10±0.80	7.20±0.85	6.25±0.75
Water extractive value (% w/w)	15.50±1.20	16.50±1.20	15.80±1.10	12.75±1.00
Ethanol extractive (% w/w)	22.00±2.00	23.03±2.62	21.50±2.20	20.50±2.00

3.2 Organoleptic Analysis of Extracts

Ethanolic extracts of the four selected plants were evaluated for physical parameters. All extracts complied with organoleptic standards (Table 4).

Table 4: Organoleptic Characteristics of Ethanolic Herbal Extracts

Parameters	Gymnema Sylvestre	Momordica Charantia	Coccinia Grandis	Aegle Marmelos
Color	Reddish Brown (Light)	Greenish	Reddish Brown (Light)	Brown (Dark)
Odour	Agreeable	Agreeable	Agreeable	Agreeable
Taste	Slightly bitter	Bitter	Bitter	Bitter
Texture	Soft Semisolid	Semisolid	Resinous	Semisolid

3.2.1 Physicochemical Parameters of Herbal Extracts

The extracts were subjected to comprehensive chemical analysis, including pH determination, loss on drying (LOD), foreign matter detection, and contaminant screening (Table 5). Foreign organic content, heavy metals, and pesticide residues were absent across all samples.

Table 5: Physicochemical Parameters of Ethanolic Extracts

Parameters	Gymnema Sylvestre	Momordica Charantia	Coccinia Grandis	Aegle Marmelos

Ph				
1% Solution	7.1	7.5	6.8	7.0
10% Solution	6.2	7.0	6.5	6.1
Foreign Content	0%	0%	0%	0%
LOD	5.51%	4.81%	5.11%	6.12%
Ash values				
Total Ash Value	4.91%	6.82%	5.21%	6.5%
Acid insoluble ash value	0.80%	1.09%	1.17%	0.31%
Water soluble ash value	2.28%	4.24%	4.14%	3.11%
Sulphated ash value	4.23%	3.06%	2.08%	2.21%
Extractive values				
Alcohol-solubleb Extractive	12.87%	11.88%	12.38%	11.27%
Water-soluble Extractive	07.65%	09.29%	08.38%	08.78%
Heavy metals estimation (present)	---	---	---	---
Pesticide Residues	Absent	Absent	Absent	Absent

3.3 Preliminary Phytochemical Screening

Qualitative phytochemical screening revealed the universal presence of carbohydrates, proteins, fats/oils, steroids, alkaloids, and saponins (++ve) across all four individual plant extracts. Glycosides, flavonoids, tannins, free amino acids, and phenolic compounds were below detectable limits (Table 6).

Table 6: Phytochemical Profile of Herbal Extracts

Sr. No	Phyto- constituents	Momordica charantia	Gymnema sylvestre	Aegle marmelos (Bael)	Coccinia grandis (Ivy Gourd)
1	Carbohydrates	Positive	Positive	Positive	Positive
2	Proteins	Positive	Positive	Positive	Positive
3	Amino-acids	Negative	Negative	Negative	Negative
4	Fats & Oil	Positive	Positive	Positive	Positive
5	Steroids	Positive	Positive	Positive	Positive
6	Glycosides	Negative	Negative	Negative	Negative
7	Flavonoids	Negative	Negative	Negative	Negative
8	Alkaloids	Positive	Positive	Positive	Positive
9	Tannins	Negative	Negative	Negative	Negative
10	Phenol test	Negative	Negative	Negative	Negative
11	Saponins test	Positive (++)	Positive (++)	Positive (++)	Positive (++)

3.4 In-Vitro Antioxidant Activity

Antioxidant activity evaluated via DPPH, ABTS radical scavenging assays, and Total Antioxidant Capacity (TAC) demonstrated dose-dependent behavior across all extracts (0.1–1.0 mg/mL). The Polyherbal extract exhibited synergistic radical scavenging capacity compared to individual plant extracts, approaching the efficiency of Ascorbic Acid (Table 7).

Table 7: In Vitro Antioxidant Activity

Sample	Concentration (mg/mL)	DPPH Radical Scavenging (%)	ABTS Radical Scavenging (%)	Total antioxidant Capacity (mg AAE/g)
Momordica charantia	0.1	20 ± 1.50	30 ± 2.00	15 ± 1.00
	0.5	30 ± 2.00	40 ± 2.50	18 ± 1.20
	1.0	40 ± 2.50	50 ± 3.00	20 ± 1.50
Gymnema sylvestre	0.1	25 ± 2.00	35 ± 2.50	18 ± 1.20
	0.5	35 ± 2.50	45 ± 3.00	22 ± 1.50
	1.0	45 ± 3.00	55 ± 3.50	25 ± 2.00
Aegle marmelos (Bael)	0.1	22 ± 1.80	32 ± 2.20	17 ± 1.10
	0.5	32 ± 2.20	42 ± 2.80	20 ± 1.30

	1.0	42 ± 2.80	52 ± 3.30	24 ± 1.50
Coccinia grandis (Ivy Gourd)	0.1	18 ± 1.20	28 ± 1.80	14 ± 0.90
	0.5	28 ± 1.80	38 ± 2.30	18 ± 1.10
	1.0	38 ± 2.30	48 ± 2.80	22 ± 1.30
Polyherbal extract	0.1	35 ± 3.00	45 ± 3.50	25 ± 2.00
	0.5	45 ± 3.50	55 ± 4.00	30 ± 2.20
	1.0	55 ± 4.00	65 ± 4.50	35 ± 2.50
Ascorbic acid	0.1	42 ± 2.93	56 ± 3.14	32.5 ± 1.18
	0.5	63 ± 1.76	76 ± 1.02	52.6 ± 2.07
	1.0	76 ± 1.86	89 ± 2.83	64.4 ± 1.06

3.5 In Vitro Antidiabetic Efficacy

All extracts demonstrated dose-dependent inhibition of alpha-amylase and alpha-glucosidase enzymes (100–400g/mL). Among the single extracts, M. charantia displayed the highest potency (IC_{50} = 120.5µg/mL), followed by G. sylvestre (IC_{50}= 130.8 µg /mL), C. grandis (IC_{50}= 138.2 µg /mL), and A. marmelos (IC_{50}= 145.4 µg /mL) (Table 8).

Table 8: In Vitro Carbohydrate Enzyme Inhibitory Potential

Plant Extract	Concentration (µg/mL)	% α-Amylase Inhibition (Mean ± SD)	% α-Glucosidase Inhibition (Mean ± SD)	IC_{50} (µg/mL)	Comparison with Standard (Acarbose)
Momordica charantia	100	45.23 ± 1.12	50.17 ± 1.08	120.5	Moderate inhibition
	200	58.64 ± 1.35	63.42 ± 1.22		
	400	72.11 ± 1.56	77.28 ± 1.45		
Gymnema sylvestre	100	40.42 ± 1.18	47.63 ± 1.11	130.8	Moderate inhibition
	200	55.76 ± 1.42	60.34 ± 1.29		
	400	69.87 ± 1.53	75.66 ± 1.33		
Aegle marmelos (Bael)	100	35.38 ± 1.06	39.87 ± 1.00	145.4	Mild inhibition
	200	50.66 ± 1.25	55.78 ± 1.19		
	400	66.12 ± 1.39	70.25 ± 1.27		
Coccinia grandis (Ivy gourd)	100	38.19 ± 1.08	42.54 ± 1.03	138.2	Moderate inhibition
	200	53.24 ± 1.30	58.32 ± 1.22		
	400	68.75 ± 1.41	73.19 ± 1.34		
Acarbose (Standard)	100	62.54 ± 1.25	68.27 ± 1.20	98.6	Strong inhibition
	200	78.69 ± 1.38	82.47 ± 1.32		
	400	91.42 ± 1.51	94.63 ± 1.43		

3.6 In-Vivo Antidiabetic Efficacy in STZ-Induced Diabetic Rats

3.6.1 Blood Glucose Regulation (Day 0 to Day 28)

At Day 0 baseline, mean blood glucose across groups was non-significantly uniform (89.17 ± 3.71 to 92.25 ±2.16 mg/dL, $p > 0.05$). Induction of STZ resulted in marked hyperglycemia in positive control rats, reaching 313.67 ± 23.4mg/dL by Day 28 (###$p < 0.001$).

Treatment with polyherbal extract (200 and 400 mg/kg) significantly lowered blood glucose levels in a time-dependent manner (Table 6.7). By Day 28, the 400 mg/kg dose achieved profound glycemic reduction (174.68 ± 24.5mg/dL, ***$p < 0.001$), compared to 198.65 ± 25.6 mg/dL $p < 0.01$) for 200 mg/kg dose and 04.67 ± 17.8 mg/dL for standard treatment.

Table 9: Longitudinal Effect of Treatments on Blood Glucose Levels (mg/dL)

Group	Treatment	Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal Control	92.25±2.16	93.540±4.23	91.97±3.72	93.50±3.72	93.00±2.83
II	Positive Control (STZ)	92.09±3.12	261.09±20.44	276.20±18.6 ###	286.00±18.6 ###	313.67±23.4
III	Standard Group	89.17±3.71	110.17±34.8	209.50±33.43	185.50±33.43 **	104.67±17.8
IV	Polyherbal (200mg/kg)	90.51±4.46	269.98±16.3	236.30±22.5**	241.30±22.5	198.65±25.6
V	Polyherbal (400mg/kg)	90.31±4.23	267.96±29.5	220.32±23.38 ***	218.32±23.38 **	174.68±24.5

Data expressed as Mean ± SEM (n=6). Statistical comparison via One-Way ANOVA followed by Duncan's Multiple Range Test. ### $p < 0.001$ vs Normal Control; **$p < 0.01$,** $p < 0.001$ vs Positive Control.*

3.6.2 Effect on Serum Lipid Profile

Assessment of lipid parameters on Day 28 revealed significant improvements in serum triglycerides and VLDL, whereas total cholesterol, LDL, and HDL changes remained statistically non-significant ($p > 0.05$) relative to the diabetic positive control (Table 10).

Table 10: Effect of Treatments on Lipid Parameters on Day 28 (mg/dL)

Group	Treatment	Total Cholesterol	Triglycerides	VLDL	LDL	HDL
I	Normal control	90.5±7.72	94.3±4.55	19.8±3.31	48.5±3.94	25.9±2.79
II	Positive control	91.7±6.02	84.2±5.56	30.2±3.60	55.3±4.76	21.9±2.03
III	Standard Group	87.45.±10.2	75.8±6.01	21.0±2.45	49.2±5.67	23.5±1.51
IV	Poly-herbal extract 200 mg/kg	85.51±2.46	79.51±4.46	26.8±1.94	51.2 ± 3.67	21.2±1.51
V	Poly-herbal extract 400 mg/kg	86.57±2.23	75.07±4.23	24.8±1.03	50.5±3.42	22.1±1.41

3.6.3 Hematological Parameters

Polyherbal extract administration normalized diabetic-induced hematological alterations (Table 11). Elevated WBC counts in positive controls ($13.16 \pm 1.03 \times 10^9$/L) were significantly attenuated ($p < 0.05$) to $8.80 \pm 0.90 \times 10^9$/L at 400mg/kg. Furthermore, treatment restored RBC count ($7.92 \pm 0.26 \times 10^{12}$/L), hemoglobin (15.07 ± 0.46 g/dL), and differential leukocyte counts towards normoglycemic baseline values.

Table 11: Hematological Profile Across Treatment Groups

Group	Group-I (Normal Control)	Group- II (Positive control)	Group-III (Standard Group)	Group-IV (Poly-herbal extract 200 mg/kg)	Group – V (Poly-herbal extract 400 mg/kg)
White blood cells (WBC) ($\times 10^9$ /L)	7.76 ± 0.96	13.16 ± 1.03	8.63 ± 0.66	8.71 ± 0.1	8.80 ± 0.90
Red blood cells (RBC) ($\times 10^{12}$ /L)	8.29 ± 0.27	6.56 ± 0.20	8.09 ± 0.37	7.94 ± 0.37	7.92 ± 0.26
Haemoglobin (Hb) (g/dL)	15.43 ± 0.30	12.55 ± 0.35	15.36 ± 0.47	15.30 ± 0.42	15.07 ± 0.46
Platelets ($\times 10^9$/L)	926 ± 11.36	639 ± 12.85	765.1 ± 14.12	746.34 ± 15.8	746.02 ± 13.16
Neutrophils (%)	13.21 ± 0.27	4.53 ± 0.23	8.12 ± 0.42	7.83 ± 0.35	7.88 ± 0.31
Lymphocytes (%)	60.24 ± 1.94	67.69 ± 1.08	61.32 ± 1.44	62.33 ± 1.47	62.42 ± 1.25
Eosinophils(%)	3.04 ± 0.50	1.17 ± 0.17	2.67 ± 0.48	2.52 ± 0.18	2.48 ± 0.32

3.6.4 Acute Oral Toxicity (OECD Guideline 425)

Oral administration of polyherbal extract up to a limit dose of 5000mg/kg body weight produced no mortality or treatment-related signs of toxicity. Observations confirmed normal motor activity, respiration, ocular indicators, and absence of gastrointestinal anomalies.

3.6.5 Extract Compatibility Studies

DSC: Thermograms of the polyherbal extract showed endothermic peaks at 57.50 ^{0}C (desolvation), 109^{0}C (amorphous phase transition), and 233^{0}C (thermal degradation). Physical mixtures with lipid excipients (GMS and stearic acid) preserved key characteristic endotherms (58^{0}C, 66^{0}C, 105^{0}C), confirming absence of incompatibility up to 200^{0}C.

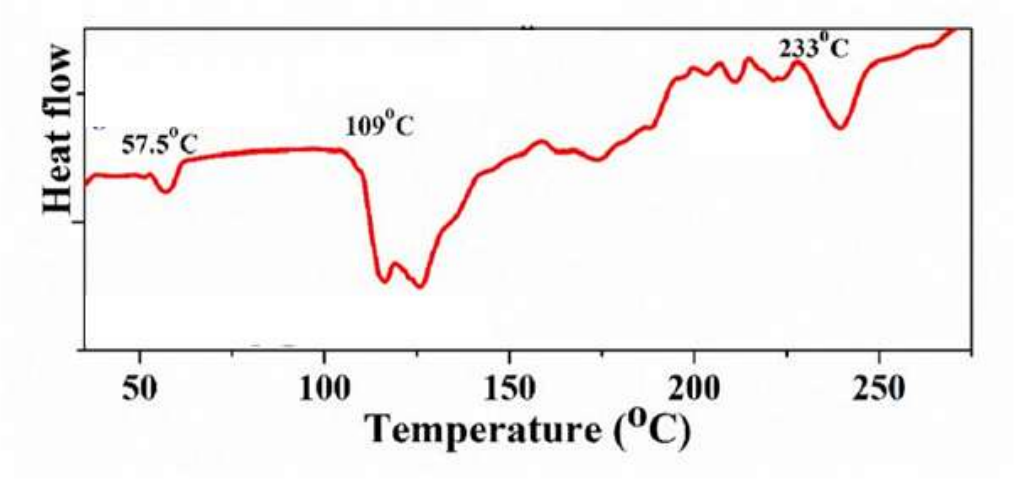


Figure 1: The DSC thermogram of polyherbal extract

FTIR: Polyherbal extracts demonstrated characteristic absorption bands at 3391cm^{-1} (O–H stretching), 1087cm^{-1} (C–O–C glycosidic linkage), 1638cm^{-1},and 1551cm^{-1} (aromatic/amide bonds). FTIR of physical mixtures retained functional group signatures (3332cm^{-1}, 1705 cm^{-1}, 1734cm^{-1}) without new peak formation.

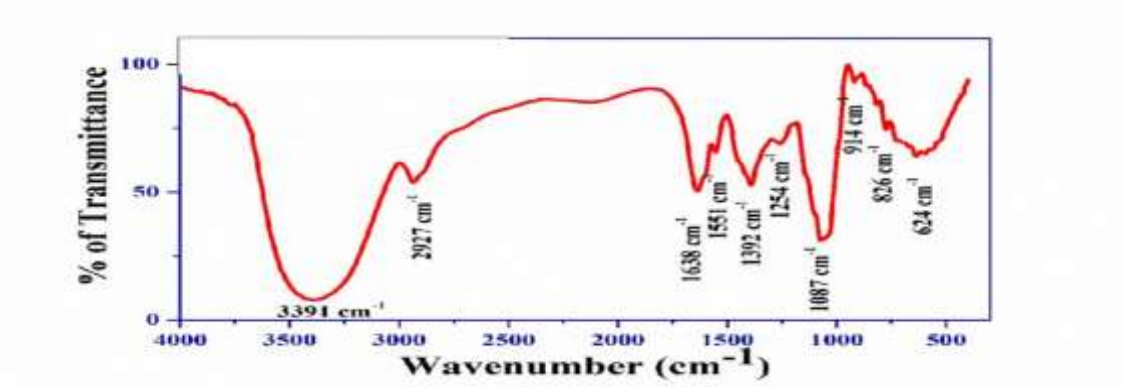


Figure 2: The FTIR spectra of Polyherbal extract

PXRD: Powder X-ray diffraction patterns of extracts exhibited a characteristic broad amorphous halo (2 Θ = 10–35^0 with minor crystalline reflections at 9.8^0, 13.6^0, 16.4^0, 18.9^0, 22.7^0, 25.3^0 and 28.1^0 indicating a semi-crystalline system. Physical mixtures displayed combined diffraction lines of lipids and extract without phase conversion.

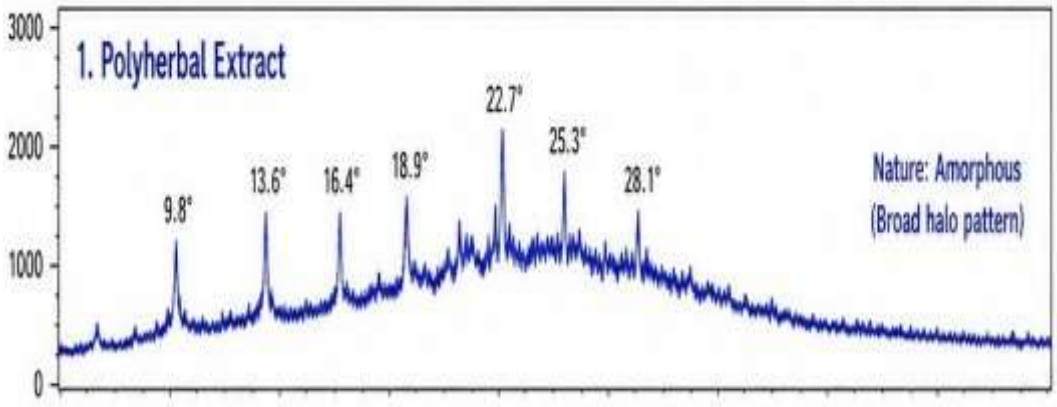


Figure 3: The PXRD pattern of combined extract

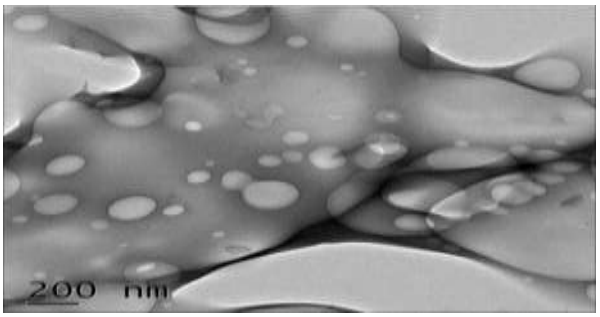
3.7 Characterization of Polyherbal Solid Lipid Nanoparticles (SLNs, F1–F9)

SLN batches F1–F9 were characterized for physicochemical properties (Table 12). Formulation **F6** achieved optimal particle metrics: particle size of 154.7 ± 3.2nm, low polydispersity index (0.18 ± 0.01), high zeta potential (-33.5 ±1.8 mV), highest entrapment efficiency (86.7 ± 1.4%), and drug content of 13.8 ±0.6%.

Table 12: Characterization Metrics for Polyherbal SLNs (F1–F9)

Formulation	Size of Particle (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)	Drug Entrapment Efficiency (%)	Content of Drug (%)
F1	245.7 ± 6.2	0.39 ± 0.02	−21.4 ± 1.2	62.3 ± 2.4	8.5 ± 0.6
F2	221.3 ± 5.4	0.34 ± 0.01	−23.1 ± 1.3	65.8 ± 2.1	9.1 ± 0.5
F3	198.7 ± 4.9	0.29 ± 0.02	−25.6 ± 1.5	68.4 ± 1.9	9.8 ± 0.4
F4	210.4 ± 5.7	0.32 ± 0.01	−24.2 ± 1.2	71.6 ± 2.3	10.6 ± 0.7
F5	185.9 ± 4.1	0.26 ± 0.01	−27.8 ± 1.4	75.9 ± 2.0	11.4 ± 0.5
F6	**154.7 ± 3.2**	**0.18 ± 0.01**	**−33.5 ± 1.8**	**86.7 ± 1.4**	**13.8 ± 0.6**
F7	190.6 ± 4.6	0.28 ± 0.02	−26.5 ± 1.3	76.8 ± 2.1	11.7 ± 0.4
F8	168.2 ± 3.5	0.21 ± 0.01	−31.2 ± 1.7	82.4 ± 1.6	12.9 ± 0.5
F9	172.3 ± 3.8	0.22 ± 0.01	−29.6 ± 1.6	79.2 ± 1.8	12.1 ± 0.6

Transmission Electron Microscopy (TEM) confirmed spherical, smooth-surfaced morphology ranging from 100 to 500 nm.



3.8 In-Vitro Release Kinetics

In-vitro dissolution in phosphate buffer (pH 6.8, 37^0C) over 24 hours demonstrated cumulative drug release ranging between 81% and 92% across batches (Table 13). Formulation **F6** achieved the highest drug release at 24 hours (92%).

Table 13: Cumulative In Vitro Percentage Drug Release Profiles (F1–F9)

Time (hr)	% Drug Release F1	% Drug Release F2	% Drug Release F3	% Drug Release F4	% Drug Release F5	% Drug Release F6	% Drug Release F7	% Drug Release F8	% Drug Release F9
1	6	5	6	4	5	3	6	4	5
2	13	12	14	10	12	8	13	10	12
4	22	20	23	18	21	15	22	18	20
6	32	30	33	27	31	24	32	27	30
8	43	40	43	37	42	34	42	37	40
10	53	50	52	47	52	45	51	46	50
12	62	59	61	56	61	57	60	55	59
16	73	71	72	74	74	75	72	68	71
20	80	78	80	82	82	82	79	76	78
24	88	83	82	85	86	92	86	81	83

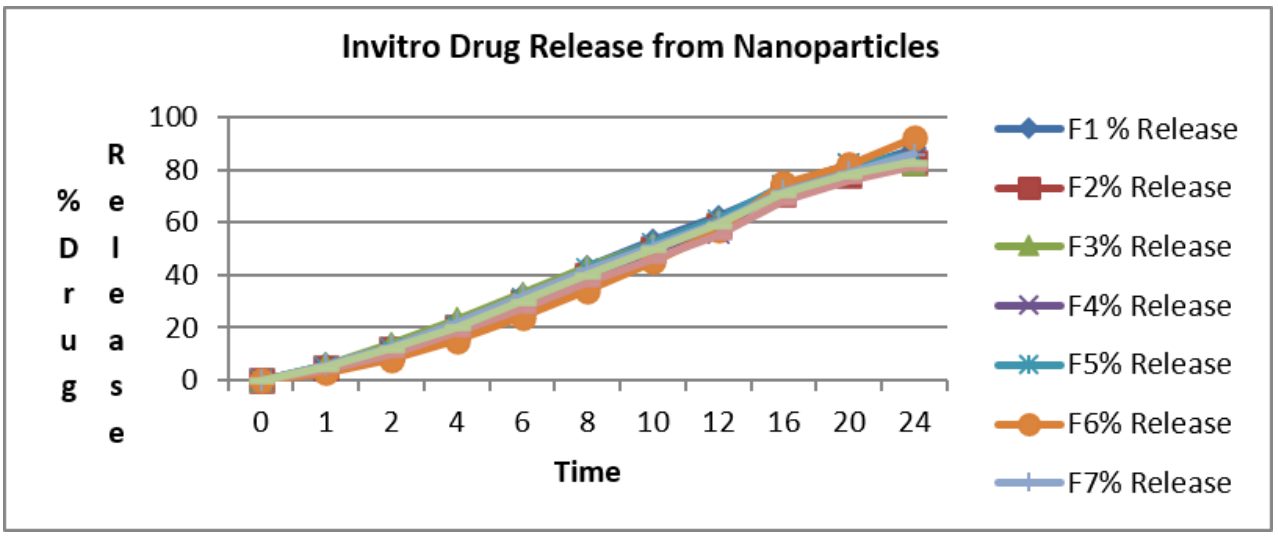


Figure 4: In-vitro drug release from nanoparticles

3.9 Characterization and Stability of Polyherbal Nanoemulsion

Conductivity and Dilution tests confirmed the prepared emulsion was O/W category.

Table 14: Physicochemical Characterization of Nanoemulsions (F1–F9)

Code of Formulation	Droplet Size (nm)	PDI	Zeta Potential (mV)	pH	Viscosity (CP)	Drug Content (%)
F1	162.4 ± 5.1	0.34 ± 0.02	−22.6 ± 1.3	6.1 ± 0.1	68 ± 3	94.2 ± 1.6
F2	148.7 ± 4.6	0.31 ± 0.01	−24.3 ± 1.4	6.1 ± 0.1	71 ± 4	95.6 ± 1.4
F3	132.9 ± 3.9	0.28 ± 0.01	−26.8 ± 1.5	6.2 ± 0.1	75 ± 3	96.8 ± 1.2
F4	140.6 ± 4.2	0.30 ± 0.02	−25.4 ± 1.2	6.2 ± 0.1	78 ± 4	97.1 ± 1.3
F5	121.8 ± 3.6	0.25 ± 0.01	−28.9 ± 1.6	6.3 ± 0.1	82 ± 5	98.3 ± 1.1
F6	109.4 ± 3.1	0.22 ± 0.01	−30.7 ± 1.7	6.3 ± 0.1	86 ± 4	99.1 ± 0.9
F7	118.6 ± 3.4	0.24 ± 0.02	−29.2 ± 1.4	6.4 ± 0.1	89 ± 5	98.6 ± 1.0
F8	101.3 ± 2.8	0.20 ± 0.01	−32.8 ± 1.8	6.4 ± 0.1	93 ± 4	99.4 ± 0.8
F9	92.7 ± 2.5	0.18 ± 0.01	−35.6 ± 1.9	6.5 ± 0.1	96 ± 5	99.8 ± 0.7

Stability Studies: Physical stability evaluation over 90 days under controlled storage conditions demonstrated absence of phase separation, phase inversion. **(Table 15 and Table 16).**

Table 15: Physical Stability Under Accelerated Storage

Storage Condition	Duration	Appearance	Phase Separation	Stability
25°C	0 day	Clear	Absent	Stable
25°C	30 days	Clear	Absent	Stable
40°C	60 days	Slight turbidity	Absent	Stable
40°C	90 days	Mild turbidity	Absent	Acceptable

Time (Days)	Globule Size (nm)	pH	Viscosity (cP)
0	78	6.5	112
30	81	6.4	115
60	84	6.3	118
90	88	6.2	121

4. CONCLUSION

This study confirms that combining M. charantia, G. sylvestre, A. marmelos, and C. grandis into a polyherbal system yields synergistic antioxidant and antidiabetic efficacy. The successful formulation of stable SLNs and nanoemulsions provides a promising nanomedicine delivery platform for the long-term management of metabolic disorders.

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