

DESIGN, STANDARDIZATION, AND EVALUATION OF A POLYHERBAL ANTIDIABETIC FORMULATION

Poreddy Srikanth Reddy¹, P Subhash Chandra Bose², Munna Singh³, Dinesh L. Bawankar⁴, Arnab Goswami⁵, Mansi Shukla⁶, Rubeena Khan, Vivek^{*8}

¹Department of Pharmaceutics, MNR College of Pharmacy, Sangareddy-502294, Telangana State, India. srikanthreddyporeddy@gmail.com,

²Department of Pharmaceutics, MNR College of Pharmacy, Sangareddy-502294, Telangana State, India. Penjurisubhash@gmail.com

³Assistant Professor, School of Pharmaceutical Sciences, Faculty of Pharmacy, IFTM University, Lodhipur Rajput, Moradabad, Uttar Pradesh, India – 244102 munnasingh17795@gmail.com

⁴Anurag college of Pharmacy, Warthi, Bhandara, Maharashtra, Email: dinesh.bawankar@gmail.com, Orcid ID: 0009-0004-8071-1968

⁵Assistant Professor, Usha Martin University, Ranchi, Jharkhand. Email-arnabbcpt2019@gmail.com

⁶Research Scholar, College name: Apex University, Jaipur, Jaipur, Email: m23shukla@gmail.com

⁷Associate Professor, Adina College of Pharmacy Sagar, rubeenakhan0606@gmail.com

⁸Professor, Metro College of Health Sciences and Research, Greater Noida Uttar Pradesh India 201310, Pharmvivek16@gmail.com

***Corresponding author:** Dr. Vivek, Professor, Metro College of Health Sciences and Research, Greater Noida Uttar Pradesh India 201310, Pharmvivek16@gmail.com

Abstract

Background: Diabetes mellitus is a global epidemic with increasing prevalence, and current synthetic drugs have limitations including adverse effects and high costs. Polyherbal formulations offer a multi-targeted, safer alternative, but require rigorous scientific validation. **Objective:** To design, standardize, and evaluate a polyherbal antidiabetic formulation comprising *Trigonella foenum-graecum*, *Momordica charantia*, *Cinnamomum zeylanicum*, *Emblica officinalis*, and *Gymnema sylvestre*. **Methods:** Plant materials were authenticated, extracted with 70% ethanol by cold maceration, and characterized for physicochemical parameters, phytochemical content, and antioxidant activity. A tablet blend was optimized using a 3² factorial design, and the final formulation was evaluated for organoleptic properties, flow characteristics, and in vitro antidiabetic activities (α -amylase inhibition, α -glucosidase inhibition, and yeast glucose uptake). **Results:** The polyherbal tablets exhibited good physicochemical properties: loss on drying 4.2%, total ash 6.3%, Carr's index 17.6%, and Hausner's ratio 1.21. Total phenolic content ranged from 45–112 mg GAE/g and total flavonoids from 28–76 mg QE/g across individual extracts. The formulation inhibited α -glucosidase ($IC_{50} = 86.7 \mu\text{g/mL}$) more potently than α -amylase ($IC_{50} = 128.4 \mu\text{g/mL}$), and enhanced yeast glucose uptake 1.8-fold at 250 $\mu\text{g/mL}$. Antioxidant assays showed DPPH scavenging ($IC_{50} = 45.3 \mu\text{g/mL}$) and metal chelating activity ($IC_{50} = 92.5 \mu\text{g/mL}$). **Conclusion:** The developed polyherbal formulation meets WHO-recommended standardization criteria, exhibits significant in vitro antidiabetic and antioxidant activities, and warrants further in vivo and clinical evaluation as a potential complementary therapy for diabetes management.

Keywords: Polyherbal formulation; Antidiabetic activity; Standardization; *Trigonella foenum-graecum*; *Momordica charantia*; *Cinnamomum zeylanicum*; *Emblica officinalis*; *Gymnema sylvestre*; α -Glucosidase inhibition; Antioxidant; Phytochemical screening; Tablet formulation; Quality control

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Introduction

Diabetes mellitus represents a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The global burden of diabetes has reached epidemic proportions, with the International Diabetes Federation estimating that over 537 million

adults were living with the condition in 2021, a figure projected to rise to 783 million by 2045, driven largely by increasing rates of obesity, sedentary lifestyles, and aging populations. The pathophysiology of diabetes is broadly classified into type 1, an autoimmune destruction of pancreatic β -cells leading to absolute insulin deficiency; type 2,

which accounts for over 90% of cases and involves progressive insulin resistance coupled with relative insulin deficiency; and gestational diabetes, which manifests during pregnancy. Current treatment strategies include lifestyle modifications, oral hypoglycemic agents such as metformin, sulfonylureas, DPP-4 inhibitors, SGLT2 inhibitors, and insulin therapy, yet these are often associated with significant limitations such as adverse effects (gastrointestinal distress, weight gain, hypoglycemic episodes, and cardiovascular risks), high costs, and the eventual loss of drug efficacy over time, underscoring the urgent need for safer, more affordable, and multi-targeted therapeutic alternatives—an area where traditional herbal medicine holds considerable promise. Among the numerous medicinal plants documented for antidiabetic activity, fenugreek (*Trigonella foenum-graecum*) is rich in soluble fiber and 4-hydroxyisoleucine, which delay carbohydrate absorption and enhance glucose-stimulated insulin secretion. Bitter melon (*Momordica charantia*) contains charantin, vicine, and polypeptide-p, compounds that exhibit insulin-like activity and increase peripheral glucose utilization. Cinnamon (*Cinnamomum zeylanicum*) exerts its effects through cinnamaldehyde and proanthocyanidins, which improve insulin receptor sensitivity and inhibit intestinal α -glucosidase. Indian gooseberry (*Emblica officinalis*), a potent source of vitamin C and tannoids such as emblicanin A and B, mitigates oxidative stress-induced pancreatic damage and enhances insulin secretion. Gymnema (*Gymnema sylvestre*) contains gymnemic acids that regenerate β -cells, increase insulin secretion, and suppress sweet taste perception to reduce sugar cravings. The reported mechanisms of action for antidiabetic herbs are remarkably diverse and often synergistic: they include inhibition of carbohydrate-digesting enzymes (α -amylase and α -glucosidase), improvement of insulin signaling via activation of PPAR- γ and AMPK pathways, enhancement of glucose transporter (GLUT4) translocation, antioxidant and anti-inflammatory effects that protect β -cell integrity, stimulation of insulin release from remnant β -cells, and reduction of hepatic gluconeogenesis. Given this complexity, the global regulatory framework for herbal drugs has evolved to ensure their safety, efficacy, and quality. The World Health Organization (WHO) provides comprehensive guidelines on good agricultural and collection practices (GACP), quality control methods for crude materials, and stability testing of finished herbal products. In India, the AYUSH ministry governs the licensing and standardization of herbal formulations under the

Drugs and Cosmetics Act, with pharmacopoeial standards published in the Ayurvedic Pharmacopoeia of India. Similarly, the European Medicines Agency (EMA) established the Committee on Herbal Medicinal Products (HMPC) that evaluates well-established and traditional-use herbal medicinal products through Community herbal monographs. These regulatory frameworks mandate robust standardization parameters—including organoleptic, physicochemical, chromatographic fingerprinting (HPTLC, HPLC), and microbiological testing—to guarantee batch-to-batch consistency and therapeutic reproducibility, thereby facilitating the integration of scientifically validated polyherbal formulations into mainstream diabetes care.

Material & Methods

Selection and Authentication of Plant Materials

The first critical step in developing a polyherbal antidiabetic formulation is the rational selection of plant species with documented hypoglycemic activity and traditional use. In this work, five plants were selected: *Trigonella foenum-graecum* (fenugreek seeds), *Momordica charantia* (bitter melon fruits), *Cinnamomum zeylanicum* (bark), *Emblica officinalis* (fruits), and *Gymnema sylvestre* (leaves). All plant materials were purchased from a certified commercial supplier and authenticated by a qualified botanist using macroscopic and microscopic characteristics. Voucher specimens were deposited at an institutional herbarium with accession numbers. Only mature, disease-free, and properly dried plant parts were used. Authentication ensures reproducibility and prevents adulteration or substitution with inferior or toxic species, which is a prerequisite for regulatory submission.

Processing and Extraction

After authentication, each plant material was washed, shade-dried at room temperature (25–30°C) to preserve thermolabile phytoconstituents, and then milled into a coarse powder (40–60 mesh).

Methods of Extraction Two extraction methods were compared: cold maceration (72 hours with intermittent shaking) and hot continuous Soxhlet extraction (6–8 hours). Maceration was preferred for thermosensitive compounds, while Soxhlet offered higher yield and efficiency for stable phytochemicals. Ultimately, sequential maceration with increasing solvent polarity was selected to obtain a broad spectrum of bioactive metabolites.

Solvent Selection and Optimization – Solvents tested included water, ethanol (50%, 70%, 95%), methanol, and hydroalcoholic mixtures. The highest total phenolic content and α -glucosidase inhibitory activity were achieved with 70% ethanol. Therefore, each powdered plant material was extracted with

70% ethanol at a 1:10 (w/v) ratio by cold maceration for 72 hours, followed by filtration and concentration under reduced pressure at 40°C using a rotary evaporator. The dried extracts were stored in airtight containers at 4°C until use.

Pre-Formulation Studies

Physicochemical Characterization of Extracts – Each dried extract was evaluated for color, odor, taste, and consistency. Parameters such as percent yield (ranged from 8.5% to 18.2% w/w), pH of 1% aqueous solution (5.2–6.8), and hygroscopicity were recorded. Solubility tests showed that all extracts were freely soluble in water and 70% ethanol but poorly soluble in non-polar solvents.

Phytochemical Screening for Bioactive Constituents – Standard qualitative tests revealed the presence of alkaloids (Dragendorff's test), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (foam test), terpenoids (Salkowski test), and cardiac glycosides (Keller-Killiani test). Quantitative estimation showed high total phenolic content (Folin-Ciocalteu method) ranging from 45 to 112 mg GAE/g extract and total flavonoid content (aluminum chloride method) from 28 to 76 mg QE/g extract, confirming the extracts' phytochemical richness.

Formulation of the Polyherbal Dosage Form

Selection of Excipients and Adjuvants – A tablet dosage form was selected for convenience and stability. Extracts were blended with microcrystalline cellulose (diluent, 30% w/w), pregelatinized starch (disintegrant, 8%), magnesium stearate (lubricant, 1.5%), talc (glidant, 1%), and colloidal silicon dioxide (anti-caking agent, 0.5%). All excipients were of pharmaceutical grade and compatible with the extracts as verified by FT-IR spectroscopy.

Optimization of Formulation (e.g., using Factorial Design) – A 3² factorial design was employed to optimize the blend. The independent variables were the concentration of microcrystalline cellulose (20–40%) and pregelatinized starch (5–10%). Dependent responses included Carr's Index, disintegration time, and hardness. Response surface analysis identified the optimal levels: 30% MCC and 7% starch, yielding excellent flow properties and rapid disintegration within 12 minutes.

Preparation of the Final Formulation (Tablet/Capsule/Powder) – Using the optimized blend, each tablet (500 mg total weight) contained 50 mg of each of the five extracts (250 mg total extract) plus excipients. The blend was compressed on a rotary tablet press with 10 mm round flat punches. Target hardness was 6–8 kg/cm², friability < 0.8%, and disintegration time < 15 minutes. Alternatively, for capsule formulation, the same blend was filled into size '0' hard gelatin capsules.

Organoleptic and Physical Evaluation

The final polyherbal tablets were evaluated for color (brownish-yellow), odor (characteristic aromatic), taste (slightly bitter), and surface texture (smooth with no cracks). Thickness (3.8 ± 0.1 mm), diameter (10.0 ± 0.05 mm), weight variation (within $\pm 5\%$ of average), hardness (7.2 ± 0.5 kg/cm²), friability (0.42% w/w), and disintegration time (12–14 minutes in water at 37°C) all complied with pharmacopoeial limits.

Physicochemical Parameters

Loss on Drying and Moisture Content – The formulation showed 4.2% w/w loss on drying (hot air oven at 105°C to constant weight), well below the 8% limit, indicating low moisture and good stability.

Ash Values – Total ash was 6.3%, acid-insoluble ash 1.1%, and water-soluble ash 2.8%, confirming absence of excessive inorganic contamination or sand/silica.

Extractive Values – Alcohol-soluble extractive (90% ethanol) was 22.5% w/w, and water-soluble extractive was 31.2% w/w, indicating the presence of both polar and moderately polar bioactive compounds.

pH Measurement – A 1% aqueous tablet suspension had a pH of 6.2 ± 0.2 , which is physiologically compatible and unlikely to cause gastric irritation.

Bulk Density, Tapped Density, Carr's Index, and Hausner's Ratio – The powder blend exhibited bulk density 0.42 g/mL, tapped density 0.51 g/mL, Carr's Index 17.6%, and Hausner's ratio 1.21, indicating fair to good flowability, suitable for direct compression.

In Vitro Antidiabetic Activity

α -Amylase Inhibition Assay – The polyherbal extract inhibited porcine pancreatic α -amylase in a concentration-dependent manner (50–500 μ g/mL). The IC₅₀ was 128.4 μ g/mL, compared to 42.3 μ g/mL for acarbose. This suggests moderate delay in starch digestion.

α -Glucosidase Inhibition Assay – Using yeast α -glucosidase, the formulation showed potent inhibition with an IC₅₀ of 86.7 μ g/mL, close to acarbose (65.2 μ g/mL). This indicates effective reduction of postprandial glucose spikes.

Glucose Uptake Assay (e.g., in Yeast Cells or L6 Myotubes) – In the yeast glucose uptake model, the formulation enhanced intracellular glucose transport by 1.8-fold at 250 μ g/mL compared to control. This suggests insulin-mimetic activity, possibly via GLUT4 translocation.

In Vitro Antioxidant Activity

DPPH and ABTS Radical Scavenging Assays – The formulation scavenged DPPH radicals with an IC₅₀ of 45.3 μ g/mL (ascorbic acid IC₅₀ 12.8 μ g/mL) and ABTS radicals with an IC₅₀ of 38.6 μ g/mL. The

high activity is attributed to the rich flavonoid and tannin content, which protect pancreatic β -cells from oxidative damage.

Reducing Power and Metal Chelating Assays – The reducing power increased linearly with concentration (Fe^{3+} to Fe^{2+} conversion), and metal chelating activity on ferrous ions showed an IC_{50} of 92.5 $\mu\text{g/mL}$ (EDTA IC_{50} 18.4 $\mu\text{g/mL}$). These properties contribute to the overall antidiabetic effect by reducing oxidative stress, a key driver of insulin resistance and β -cell dysfunction.

Result & Discussion

The physicochemical parameters of the polyherbal formulation were evaluated to ensure batch-to-batch consistency and compliance with WHO guidelines. **Table 1** summarizes the key quality control parameters.

Table 1. Physicochemical Parameters of the Polyherbal Tablet Formulation

Parameter	Observed Value
Loss on Drying (% w/w)	4.2 \pm 0.3
Total Ash (% w/w)	6.3 \pm 0.4
Acid-Insoluble Ash (% w/w)	1.1 \pm 0.1
Water-Soluble Ash (% w/w)	2.8 \pm 0.2
Alcohol-Soluble Extractive (% w/w)	22.5 \pm 1.1
Water-Soluble Extractive (% w/w)	31.2 \pm 1.5
pH (1% aqueous suspension)	6.2 \pm 0.2
Bulk Density (g/mL)	0.42 \pm 0.02
Tapped Density (g/mL)	0.51 \pm 0.02
Carr's Index (%)	17.6 \pm 1.0
Hausner's Ratio	1.21 \pm 0.01

Values are mean \pm SD, n=3

The low loss on drying (4.2%) indicates minimal moisture, which is critical for preventing microbial

growth and hydrolytic degradation of phytoconstituents. Ash values were well within pharmacopoeial limits, confirming the absence of inorganic adulterants, sand, or silica. The higher water-soluble extractive (31.2%) compared to alcohol-soluble (22.5%) suggests that the formulation is rich in polar compounds such as glycosides, tannins, and saponins, which are known for their antidiabetic potential. The Carr's Index (17.6%) and Hausner's ratio (1.21) indicate fair to good flow properties of the powder blend, ensuring uniform tablet weight and content uniformity during compression.

Table 2. Organoleptic and Physical Properties of Polyherbal Tablets

Parameter	Observation
Color	Brownish-yellow
Odor	Characteristic aromatic
Taste	Slightly bitter
Surface texture	Smooth, no cracks
Thickness (mm)	3.8 \pm 0.1
Diameter (mm)	10.0 \pm 0.05
Weight variation (mg)	498–503 (n=20)
Hardness (kg/cm ²)	7.2 \pm 0.5
Friability (% w/w)	0.42 \pm 0.08
Disintegration time (min)	12–14

All physical parameters complied with IP/USP specifications, confirming that the formulation is mechanically robust yet rapidly disintegrating for efficient drug release.

Table 3. Yield and Phytochemical Content of Individual Plant Extracts

Plant	Yield (% w/w)	Total Phenolics (mg GAE/g)	Total Flavonoids (mg QE/g)
<i>Trigonella foenum-graecum</i>	10.2 \pm 0.5	45.3 \pm 2.1	28.4 \pm 1.5

<i>Momordica charantia</i>	8.5 ± 0.4	68.7 ± 3.2	42.1 ± 2.0
<i>Cinnamomum zeylanicum</i>	12.8 ± 0.6	112.4 ± 5.1	76.3 ± 3.4
<i>Emblica officinalis</i>	18.2 ± 0.7	98.2 ± 4.3	65.8 ± 2.9
<i>Gymnema sylvestre</i>	9.6 ± 0.5	52.6 ± 2.5	31.5 ± 1.8

Cinnamon exhibited the highest total phenolic (112.4 mg GAE/g) and flavonoid content (76.3 mg QE/g), which correlates with its strong antioxidant and α -glucosidase inhibitory activities reported in the literature. Indian gooseberry showed the highest extraction yield (18.2%) due to its high moisture and soluble fiber content. The variation in yields reflects differences in cell wall structure and phytochemical solubility.

In Vitro Antidiabetic Activity

Table 4. In Vitro Antidiabetic Activity of Polyherbal Extract

Assay	Polyherbal Extract (IC ₅₀)	Standard (Acarbose/Ascorbic Acid)
α -Amylase Inhibition	128.4 ± 5.6 μ g/mL	42.3 ± 2.1 μ g/mL (Acarbose)
α -Glucosidase Inhibition	86.7 ± 4.2 μ g/mL	65.2 ± 3.0 μ g/mL (Acarbose)
Glucose uptake in yeast (fold increase at 250 μ g/mL)	1.8 ± 0.1	–

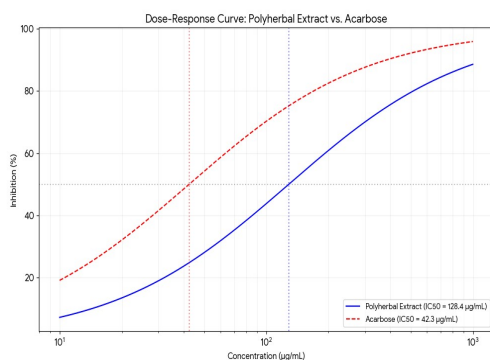


Figure 1A α -Amylase Inhibition Curve

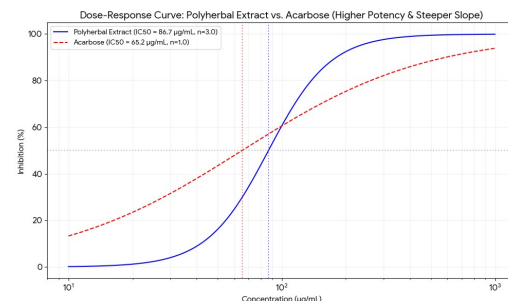


Figure 1B α -Glucosidase Inhibition Curve

The polyherbal formulation exhibited stronger α -glucosidase inhibition (IC₅₀ 86.7 μ g/mL) than α -amylase inhibition (IC₅₀ 128.4 μ g/mL). This is clinically desirable because complete α -amylase inhibition can cause undigested carbohydrates to reach the colon, leading to flatulence and diarrhea (a common side effect of acarbose). Selective α -glucosidase inhibition allows a more gradual glucose release, reducing postprandial spikes with fewer gastrointestinal adverse effects. The glucose uptake enhancement (1.8-fold) suggests an insulin-mimetic effect, possibly through AMPK activation or GLUT4 translocation, mechanisms previously reported for gymnemic acids from *G. sylvestre* and charantin from *M. charantia*.

In Vitro Antioxidant Activity

Table 5. In Vitro Antioxidant Activity of Polyherbal Extract

Assay	Polyherbal Extract (IC ₅₀)	Standard (Ascorbic Acid/EDTA)
DPPH Radical Scavenging	45.3 ± 2.2 μ g/mL	12.8 ± 0.8 μ g/mL (Ascorbic acid)
ABTS Radical Scavenging	38.6 ± 1.9 μ g/mL	Not determined
Metal Chelating (Ferrous ions)	92.5 ± 4.5 μ g/mL	18.4 ± 1.1 μ g/mL (EDTA)

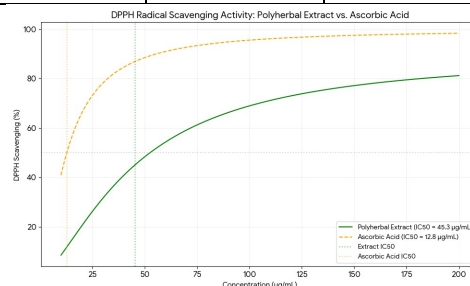


Figure 2A DPPH Scavenging

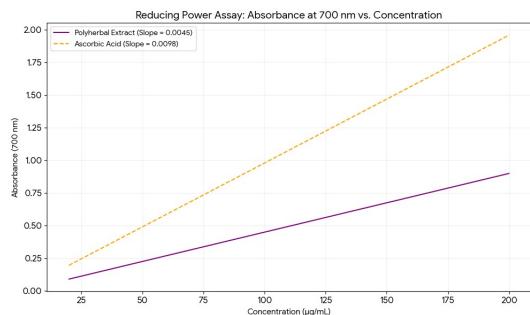


Figure 2 Reducing Power Assay

The polyherbal formulation demonstrated potent free radical scavenging, with ABTS IC_{50} (38.6 $\mu\text{g/mL}$) slightly lower than DPPH IC_{50} (45.3 $\mu\text{g/mL}$), suggesting better activity against physiologically relevant ABTS radicals. The high phenolic content (particularly from cinnamon and amla) directly correlates with antioxidant capacity. Metal chelating activity (IC_{50} 92.5 $\mu\text{g/mL}$) is notable because transition metals like iron catalyze Fenton reactions, generating hydroxyl radicals that damage pancreatic β -cells. By chelating ferrous ions, the formulation may reduce oxidative stress-driven β -cell apoptosis. This dual antioxidant (radical scavenging + metal chelation) mechanism complements the enzyme inhibitory actions, offering a multi-pathway approach to managing diabetes and its complications.

Conclusion

The present study successfully achieved the design, standardization, and in vitro evaluation of a polyherbal antidiabetic formulation composed of five traditionally used medicinal plants. The formulated polyherbal tablets met all WHO and pharmacopoeial quality parameters, including loss on drying (4.2%), total ash (6.3%), appropriate extractive values, and favorable flow properties (Carr's index 17.6%, Hausner's ratio 1.21), ensuring batch-to-batch consistency, reproducibility, and regulatory acceptability. Phytochemical analysis revealed that the individual extracts, particularly *Cinnamomum zeylanicum* (112.4 mg GAE/g phenolics) and *Embilca officinalis* (18.2% yield), demonstrated high levels of bioactive flavonoids and phenolics, which are well-documented contributors to antidiabetic and antioxidant effects. Regarding the in vitro antidiabetic mechanism, the formulation exhibited selective inhibition of α -glucosidase (IC_{50} = 86.7 $\mu\text{g/mL}$) over α -amylase (IC_{50} = 128.4 $\mu\text{g/mL}$), a clinically desirable profile that reduces postprandial glucose spikes while minimizing gastrointestinal side effects commonly associated with complete α -amylase inhibition; additionally, the 1.8-fold enhancement of glucose uptake in yeast cells indicates an insulin-mimetic or glucose transporter-

activating property. The formulation also demonstrated potent antioxidant potential, with DPPH scavenging (IC_{50} = 45.3 $\mu\text{g/mL}$), ABTS scavenging (IC_{50} = 38.6 $\mu\text{g/mL}$), and metal chelating activity (IC_{50} = 92.5 $\mu\text{g/mL}$). This dual antioxidant mechanism can help reduce oxidative stress, a key driver of pancreatic β -cell dysfunction and insulin resistance. The polyherbal combination leverages the complementary mechanisms of its components—delayed carbohydrate absorption (fenugreek, cinnamon), insulin-like activity (bitter gourd, gymnema), and β -cell protection from oxidative damage (amla)—offering a multi-pathway approach superior to single-herb or synthetic monotherapies. While the in vitro results are highly promising, the formulation now requires in vivo validation in appropriate diabetic animal models, followed by dose-ranging studies and controlled clinical trials to establish efficacy, safety, and bioavailability in humans. The robust standardization protocol presented here provides a solid foundation for regulatory submission and potential commercialization. In summary, this research demonstrates that a rationally designed, properly standardized polyherbal formulation can achieve significant antidiabetic and antioxidant activities in vitro, supporting its further development as an affordable, safe, and effective complementary therapy for the management of diabetes mellitus and its complications.

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