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RESEARCH ARTICLE

Development and Standardization of a Polyherbal Formulation for the Management of Diabetes

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Abstract: Diabetes mellitus is a chronic metabolic syndrome characterized by persistent hyperglycemia, which frequently leads to debilitating systemic complications. While conventional oral hypoglycemic agents are effective, their long-term utilization is often constrained by adverse metabolic effects and the high cost of treatment. This research presents the systematic formulation, standardization, and pharmacological evaluation of a polyherbal powder designed for synergistic glycemic management. The formulation incorporates a specific ratio of *Gymnema sylvestre* (30g), *Momordica charantia* (25g), *Trigonella foenum-graecum* (20g), *Ocimum sanctum* (15g), and *Azadirachta indica* (10g). Systematic processing through controlled drying and comminution ensured a uniform distribution of bioactive phytochemicals. Physicochemical assessment demonstrated a moisture content of 5.8% and a total ash value of 6.2%, indicating high purity. The *in vivo* antidiabetic potential was evaluated in Streptozotocin (STZ)-induced diabetic Wistar rats. Oral administration of the polyherbal formulation at doses of 200 mg/kg and 400 mg/kg significantly ($p < 0.01$) reduced fasting blood glucose levels from 285.4 ± 10.2 mg/dL to 115.6 ± 6.8 mg/dL by the 21st day of treatment, comparable to the standard drug Glibenclamide (105.2 ± 4.5 mg/dL). Micromeritic profiling further confirmed excellent flow characteristics (Carr's index: 12.7%) suitable for pharmaceutical scale-up. These results provide scientific validation for the formulation's safety and potent antihyperglycemic efficacy, supporting its use as a standardized phytotherapeutic agent.

Keywords: Diabetes Mellitus; Polyherbalism; *Gymnema sylvestre*; Physicochemical Characterization; Phytotherapeutic Standardization.

1. Introduction

Diabetes mellitus refers to a group of metabolic disorders characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin resistance, or a combination of both [1]. The global prevalence of the disease has reached alarming levels, driven by sedentary lifestyles, dietary shifts, and aging populations [2]. Prolonged elevation of blood glucose levels initiates oxidative stress and non-enzymatic glycation of proteins, eventually leading to severe microvascular and macrovascular complications, including nephropathy, neuropathy, and cardiovascular disease [3]. Conventional management strategies predominantly involve the use of synthetic drugs such as metformin, sulfonylureas, and thiazolidinediones. While effective in the short term, these agents are often associated with gastrointestinal discomfort, weight gain, and secondary failure, necessitating the exploration of safer alternatives [4].

Natural products have historically served as a cornerstone for diabetes management, particularly in traditional systems like Ayurveda. Modern pharmacology increasingly recognizes the benefits of polyherbalism, where the combination of multiple botanical entities produces a therapeutic effect that exceeds the efficacy of single-herb treatments [5]. This synergistic approach allows for the simultaneous modulation of diverse pathological pathways, such as the inhibition of alpha-glucosidase in the intestine, the stimulation of pancreatic insulin release, and the enhancement of glucose utilization in peripheral tissues [6]. By utilizing herbs with complementary mechanisms, a polyherbal formulation can achieve superior glycemic control with reduced dosages, thereby minimizing potential toxicity [7].

The current formulation targets five medicinal plants with established antidiabetic profiles. *Gymnema sylvestre* contains gymnemic acids that block intestinal glucose receptors and promote the regeneration of pancreatic beta-islet cells [8]. *Momordica charantia* provides insulin-mimetic compounds like charantin and polypeptide-P, which facilitate glucose uptake in muscle and adipose tissue [9]. *Trigonella foenum-graecum* contributes high levels of soluble fiber and 4-hydroxyisoleucine to delay carbohydrate absorption and stimulate insulin secretion [10]. *Ocimum sanctum* and *Azadirachta indica* offer potent antioxidant and anti-inflammatory properties,

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protecting the pancreas from oxidative damage and improving systemic insulin sensitivity [11]. The standardization of such complex mixtures require rigorous physicochemical and phytochemical evaluation to ensure therapeutic consistency and safety for clinical applications [12].

2. Materials and Methods

2.1. Collection and Authentication of Plant Materials

The botanical ingredients utilized in this study were procured from reputable herbal suppliers and authenticated by the Pharmacognosy Department at K.G.R.L College of Pharmacy (Specimen No. KGRL/25/037). The selected plant parts included the leaves of *Gymnema sylvestre*, the fruits of *Momordica charantia*, the seeds of *Trigonella foenum-graecum*, and the leaves of *Ocimum sanctum* and *Azadirachta indica*. Each sample was meticulously inspected for the absence of foreign organic matter, fungal growth, or physical damage prior to processing, adhering to the World Health Organization (WHO) guidelines for assessing the quality of herbal materials [13].

2.2. Processing and Comminution

The plant materials underwent a thorough cleaning process under running water to eliminate environmental dust and debris. Drying was conducted under controlled conditions to preserve the integrity of heat-sensitive secondary metabolites [14]. Leaves and fruits were shade-dried for a period of 7 to 10 days in a well-ventilated environment, while the seeds were oven-dried at a constant temperature of 40°C to 45°C for 12 hours. Upon reaching a brittle consistency, each herb was pulverized individually using a mechanical grinder. The resulting powders were passed through a #60 mesh sieve to achieve a uniform particle size distribution, ensuring homogeneity during the subsequent blending phase as per standard pharmaceutical powder processing protocols [15].

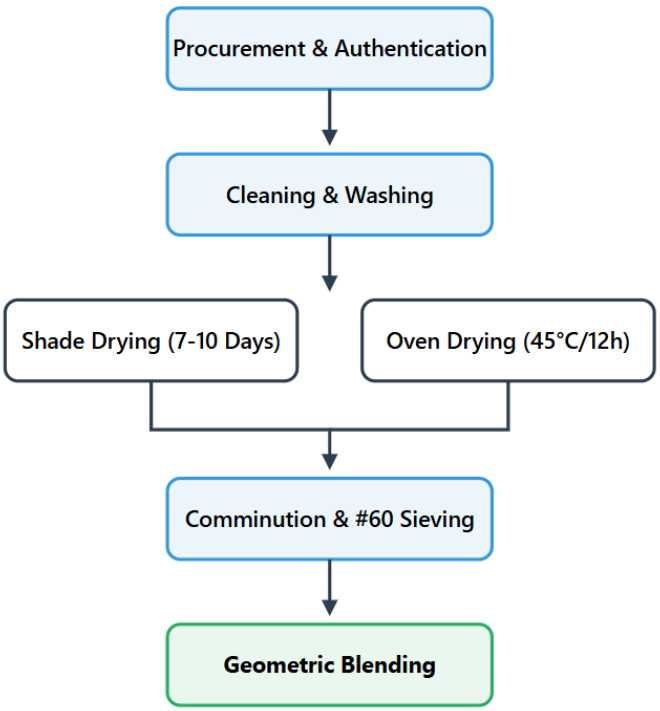

Figure 1. Formulation Process of Polyherbal Powder

2.3. Compounding and Blending

The polyherbal powder was prepared by blending the individual herb powders in precise ratios as detailed in Table 1. The formulation consisted of *Gymnema sylvestre* (30g), *Momordica charantia* (25g), *Trigonella foenum-graecum* (20g), *Ocimum sanctum* (15g), and *Azadirachta indica* (10g). The mixture was homogenized using the geometric dilution method in a mortar and pestle for 20 minutes, followed by tumbling in a closed container to ensure the uniform distribution of each component [16]. The final formulation was stored in an airtight, light-resistant glass container to maintain stability, following standard storage for polyherbal churnas [17].

2.4. Physicochemical Standardization

2.4.1. Determination of Moisture Content

The moisture content, or loss on drying (LOD), was determined by heating a 2g sample of the polyherbal powder in a hot air oven at 105°C until a constant weight was achieved, following the procedure described in the Indian Pharmacopoeia [18]. This parameter is critical for assessing the susceptibility of the formulation to microbial degradation and chemical hydrolysis during storage.

2.4.2. Ash Value

Total ash, acid-insoluble ash, and water-soluble ash values were determined to evaluate the inorganic content and purity of the formulation. The procedures were conducted in accordance with the Ayurvedic Pharmacopoeia of India [19]. Total ash reflects the overall inorganic residue, while acid-insoluble ash specifically indicates contamination with siliceous matter like earth or sand. Water-soluble ash provides an estimate of the amount of soluble inorganic salts present in the herbal matrix.

2.4.3. Extractive Value

The extractive values were calculated using water and alcohol as solvents as per the maceration method [20]. A known weight of the powder was macerated with the respective solvent for 24 hours. The resulting filtrate was evaporated to dryness, and the weight of the residue was used to calculate the percentage of soluble constituents. This analysis helps in estimating the yield of bioactive compounds that would be available upon oral administration [21].

2.5. Micromeritic and Flow Properties

The flow properties of the powder were evaluated through several parameters based on the methods described by Aulton [22]. Bulk density and tapped density were measured using a graduated cylinder and a tapping apparatus. These values were subsequently used to calculate Carr's compressibility index and the Hausner's ratio, which characterize the packing geometry and inter-particulate friction of the powder. The angle of repose was determined using the fixed funnel method to assess the gravity flow characteristics, which is essential for ensuring uniform filling during industrial manufacturing [23].

2.6. Qualitative Phytochemical Screening

A series of chemical tests were performed on the aqueous and alcoholic extracts of the polyherbal powder to identify the major classes of secondary metabolites [24]. The presence of alkaloids was tested using Mayer's and Dragendorff's reagents, while flavonoids were identified using the Shinoda test. Saponins were detected via the foam formation test, and tannins were confirmed using the ferric chloride reaction. These tests provide a qualitative baseline for the therapeutic potential of the formulation [25].

2.7. Anti-diabetic activity

Healthy adult Wistar albino rats (180–220 g) of either sex were utilized for the pharmacological study. The animals were housed in polyacrylic cages under standard laboratory conditions (temperature 25 ± 2ºC, 12-hour light/dark cycle) and provided with a standard pellet diet and water *ad libitum*. All experimental procedures were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) and received approval (IAEC/KGRL/2025/86) from the Institutional Animal Ethics Committee (IAEC) [26].

2.7.1. Induction of Experimental Diabetes

Diabetes was induced in overnight-fasted rats by a single intraperitoneal (i.p.) injection of Streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH 4.5) at a dose of 55 mg/kg body weight [27]. To prevent initial drug-induced fatal hypoglycemia, animals were provided with a 5% glucose solution for 24 hours. Blood glucose levels were measured 72 hours post-induction; rats with fasting blood glucose levels exceeding 250 mg/dL were considered diabetic and included in the study [28].

2.7.2. Experimental Design

The animals were divided into five groups (n=6):

- Group I (Normal Control): Received distilled water (5 mL/kg).
- Group II (Diabetic Control): STZ-induced diabetic rats receiving distilled water.

- Group III (Standard): Diabetic rats receiving Glibenclamide (5 mg/kg, p.o.).
- Group IV (Test Low Dose): Diabetic rats receiving Polyherbal Powder (200 mg/kg, p.o.).
- Group V (Test High Dose): Diabetic rats receiving Polyherbal Powder (400 mg/kg, p.o.).

The treatments were administered orally once daily for 21 days. Fasting blood glucose (FBG) levels were monitored on days 0, 7, 14, and 21 using a digital glucometer via the tail-vein pricking method [29].

3. Results and Discussion

3.1. Formulation Composition and Organoleptic Profile

The quantitative composition of the developed polyherbal formulation is shown in Table 1. The prepared powder was evaluated for its sensory and macroscopic properties. The formulation presented as a light brown, fine, and uniform powder with a characteristic herbal odor and a slightly bitter taste. These organoleptic features (summarized in Table 2) are primarily attributed to the presence of triterpenoids in *Gymnema sylvestre* and bitter glycosides in *Momordica charantia* [26]. The absence of lumps and foreign matter indicates a successful homogenization process, which is essential for ensuring dose uniformity in traditional churna formulations [27].

Table 1. Composition of the Polyherbal Antidiabetic Formulation

Ingredient	Plant Part Used	Quantity (g)	Percentage (%)
Gymnema sylvestre	Leaves	30	30%
Momordica charantia	Fruit	25	25%
Trigonella foenum-graecum	Seeds	20	20%
Ocimum sanctum	Leaves	15	15%
Azadirachta indica	Leaves	10	10%
Total	-	100	100%

3.2. Physicochemical Evaluation

The results of the physicochemical evaluation are summarized in Table 2. The loss on drying was found to be 5.8 ± 0.3%. Moisture content is a critical quality attribute; values below 10% are generally considered optimal for herbal powders to prevent microbial proliferation and the enzymatic degradation of active constituents [28].

The total ash value was 6.2 ± 0.4%, while the acid-insoluble ash was low at 1.1 ± 0.2%. High ash values often indicate contamination with inorganic salts or earthy matter. The low acid-insoluble ash specifically confirms the purity of the raw materials and suggests that the cleaning and processing stages were effective in removing siliceous contaminants [29]. Water-soluble and alcohol-soluble extractive values were 15.6 ± 0.6% and 12.4 ± 0.5%, respectively. The higher water-soluble extractive value suggests that a significant portion of the formulation's antihyperglycemic constituents, such as mucilage and certain saponins, are polar in nature [30].

Table 2. Organoleptic and Physicochemical Characteristics

Parameter	Observation / Result (% w/w)
Organoleptic Properties	
Color	Light brown
Odor	Characteristic herbal
Taste	Slightly bitter
Texture	Fine, uniform powder
Physicochemical Markers	**Mean ± SD (n=3)**
Loss on Drying (at 105°C)	5.8 ± 0.3
Total Ash	6.2 ± 0.4
Acid-Insoluble Ash	1.1 ± 0.2
Water-Soluble Ash	2.8 ± 0.3
Alcohol-Soluble Extractive	12.4 ± 0.5
Water-Soluble Extractive	15.6 ± 0.6

3.3. Micromeritic Properties and Flowability

The flow characteristics of the powder are vital for packaging and industrial handling. The results for micromeritic properties are detailed in Table 3. The bulk density was 0.48 ± 0.02 g/ml and the tapped density was 0.55 ± 0.03 g/ml. From these values, the Carr's Index was calculated as 12.7% and the Hausner's Ratio as 1.14. According to USP standards, a Carr's Index below 15% and a Hausner's Ratio below 1.25 signify "good" flow properties [31]. The angle of repose was found to be 28.4°, which further corroborates the excellent flowability of the formulation. These results indicate that the powder can be easily processed into secondary dosage forms like capsules or sachets without significant friction-related issues [32].

Table 3. Micromeritic and Flow Properties of the Formulation

Parameter	Observed Value (Mean ± SD)
Bulk Density (g/ml)	0.48 ± 0.02
Tapped Density (g/ml)	0.55 ± 0.03
Carr's Compressibility Index (%)	12.7%
Hausner's Ratio	1.14
Angle of Repose (°)	28.4 ± 1.2
pH (1% aqueous solution)	6.4 ± 0.2

3.4. Qualitative Phytochemical Profile

Preliminary screening (Table 4) confirmed the presence of diverse secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and glycosides. These compounds are known to exert multi-targeted antidiabetic effects. Flavonoids and tannins are potent antioxidants that mitigate the oxidative stress associated with chronic hyperglycemia, thereby protecting pancreatic beta-islet cells from apoptosis [33]. Saponins, particularly those from *Gymnema*, are credited with inhibiting intestinal glucose transporters, while alkaloids like trigonelline contribute to improved insulin sensitivity [34].

Table 4. Preliminary Phytochemical Screening Results

Phytoconstituent	Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Tannins	Present (+)
Saponins	Present (+)
Glycosides	Present (+)
Terpenoids	Present (+)

3.5. Safety

Heavy metal screening (Table 5) showed that lead (0.8 ppm), arsenic (0.5 ppm), and mercury (0.2 ppm) were all well within the permissible limits defined by the WHO [13]. Microbial limit tests showed that the total aerobic count was within acceptable thresholds, confirming the hygienic processing and low moisture-related risk. The near-neutral pH (6.4 ± 0.2) of the 1% aqueous solution suggests that the formulation is likely to be well-tolerated by the gastrointestinal mucosa upon oral administration [35].

Table 5. Heavy Metal Analysis Compared to WHO Limits

Heavy Metal	Observed Value (ppm)	Permissible Limit (ppm)
Lead (Pb)	0.8	≤10
Arsenic (As)	0.5	≤ 3
Mercury (Hg)	0.2	≤ 1

3.6. Antidiabetic Activity in Wistar Rats

The effect of the polyherbal powder on fasting blood glucose (FBG) levels in STZ-induced diabetic rats is summarized in Table 6. Following STZ induction, FBG levels increased significantly compared to the normal control group. Administration of the polyherbal formulation at doses of 200 mg/kg and 400 mg/kg resulted in a progressive and significant ($p < 0.01$) reduction in blood glucose. By day 21, the 400 mg/kg dose reduced glucose levels to 115.6 ± 6.8 mg/dL, showing high efficacy comparable to the standard Glibenclamide (105.2 ± 4.5 mg/dL).

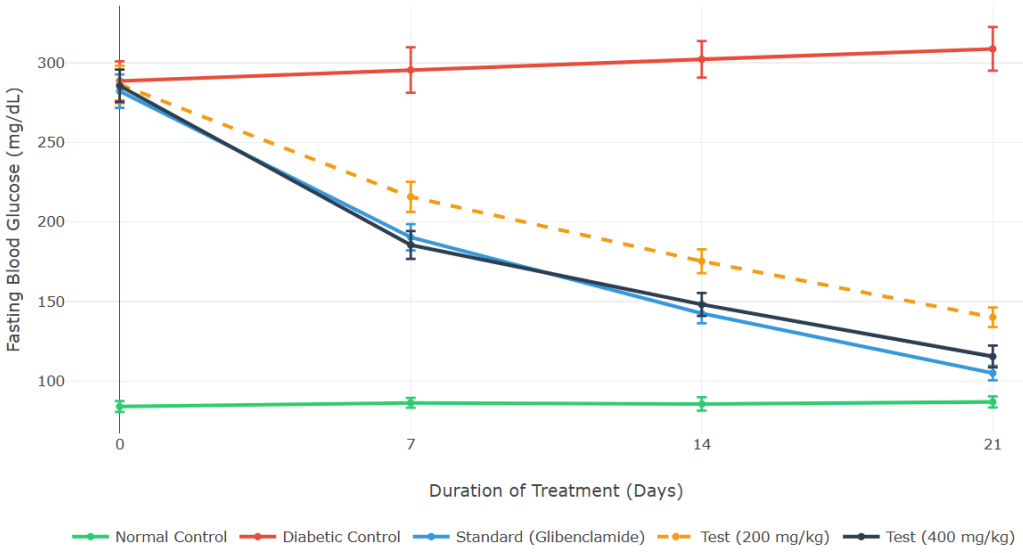

Figure 2. Fasting Blood Glucose levels over a 21-day treatment period in Wistar rats. Both 200 mg/kg and 400 mg/kg doses show significant ($p < 0.01$) reductions in hyperglycemia compared to the diabetic control.

Table 6. Effect of Polyherbal Powder on Fasting Blood Glucose (mg/dL) in Wistar Rats

Experimental Group	Day 0 (Baseline)	Day 7	Day 14	Day 21
Normal Control	84.2 ± 3.4	86.5 ± 3.1	85.8 ± 4.2	87.1 ± 3.5
Diabetic Control (STZ)	288.5 ± 12.4#	295.4 ± 14.2#	302.1 ± 11.5#	308.6 ± 13.7#
Standard (Glibenclamide)	282.1 ± 10.5	190.4 ± 8.2**	142.6 ± 6.1**	105.2 ± 4.5**
Polyherbal Powder (200mg/kg)	286.3 ± 11.8	215.8 ± 9.4**	175.4 ± 7.5**	140.2 ± 6.1**
Polyherbal Powder (400mg/kg)	285.4 ± 10.2	185.6 ± 8.7**	148.2 ± 7.2**	115.6 ± 6.8**

**Values are Mean ± SEM (n=6). #$p < 0.01$ compared to Normal Control; *$p < 0.01$ compared to Diabetic Control.*

The pharmacological evaluation confirms that the formulated polyherbal powder possesses potent antihyperglycemic activity in a chronic diabetic model. STZ selectively destroys pancreatic beta-islet cells, leading to insulin deficiency and elevated glucose levels [29]. The significant reduction in FBG by the polyherbal formulation likely stems from the synergistic action of its constituents. *Gymnema sylvestre* and *Trigonella* have been reported to stimulate insulin release and regenerate beta-cells, while *Momordica charantia* enhances peripheral glucose utilization through insulin-mimetic peptides [8, 9]. The antioxidant properties provided by *Ocimum sanctum* and *Azadirachta indica* likely contributed to the protection of surviving beta-cells from STZ-induced oxidative damage [11]. The dose-dependent response observed between 200 mg/kg and 400 mg/kg indicates a scientifically consistent therapeutic window for the formulation.

4. Conclusion

The present study successfully standardized a synergistic polyherbal powder incorporating five potent medicinal plants. The formulation demonstrates a robust physicochemical profile with low moisture content and high extractive yields, ensuring both stability and the availability of bioactive constituents. The excellent micromeritic properties suggest that the powder is suitable for standardized pharmaceutical manufacturing. Qualitative analysis confirmed a rich matrix of phytochemicals capable of targeting multiple diabetic pathways. These results provide a scientific validation for the traditional use of these herbs in combination and establish a standardized quality control template for further pharmacological and clinical investigations.

Compliance with ethical standards

Acknowledgements

The authors express their sincere gratitude to the management and laboratory staff of K.G.R.L College of Pharmacy for providing the necessary facilities, botanical authentication, and technical support throughout the formulation development and pharmacological evaluation. Special thanks are extended to the animal house technicians for their assistance in the maintenance and handling of the experimental models during the *in vivo* study.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest or competing interests regarding the publication of this manuscript. There are no financial or personal relationships with institutions, pharmaceutical companies, or products that could inappropriately influence the outcomes or integrity of the research presented in this study.

Statement of Ethical Approval

Ethical approval (IAEC/KGRL/2025/86)) for this experimental research was obtained from the Institutional Animal Ethics Committee (IAEC). All procedures involving Wistar albino rats were performed in strict accordance with the ethical standards of the institutional research committee and the guidelines prescribed by the Committee for Control and Supervision of Experiments on Animals (CCSEA). The study also adhered to the principles outlined in the Declaration of Helsinki regarding the humane treatment of experimental models.

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