In-Vitro, In-Vivo Evaluation And Formulation Development Of Polyherbal Extract In Streptozotocin-Induced Diabetic Rat

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Polyherbal, metabolic disease, Glibenclamide, antidiabetic, tablet, antihyperglycemic, hyperglycemia, streptozotocin.

ABSTRACT
The current study focuses on developing an optimized tablet dosage form for a polyherbal blend consisting of ten herbs known for their potent antidiabetic effects. Polyherbal formulations, extensively utilized worldwide for long-term diabetes management, naturally contain bioactive components such as glycosides, flavonoids, alkaloids, and various other substances with targeted therapeutic activities. For this study, tablets were designed by combining polyherbal extracts from *Gymnema sylvestre, Syzygium cumini, Pterocarpus marsupium, Psidium guajava, Mangifera indica, Costus igneus, Aloe barbadensis, Abelmoschus esculentus, Camellia sinensis,* and *Tinospora cordifolia*, along with adequate excipients. Diabetes, a metabolic disorder characterized by persistent hyperglycemia, is a growing concern, and studies highlight that the synergistic antidiabetic actions offered by polyherbal mixtures often outperform those of single herbs. A total of nine formulations (F1–F9) were developed during the research. Of these, F3, F7, and F9, which were identified through promising in-vitro results, were designated PHF I, PHF II, and PHF III, respectively. These formulations were subsequently administered to animal models at a dosage of 200 mg/kg body weight. Thirty Male Wistar Albino rats were categorized into six groups: Group I (Normal Control), Group II (Positive Control treated with Standard Glibenclamide), Group III (Negative Control), Group IV (treated with PHF I), Group V (treated with PHF II), and Group VI (treated with PHF III). Diabetes was chemically induced in Groups II–VI by administering streptozotocin (STZ) to elevate blood sugar levels. Thereafter, PHF I, II, and III were tested for their antihyperglycemic effects over a 28-day period in the STZ-induced diabetic rats. Among the three tested formulations, PHF II (200 mg/kg) exhibited the most pronounced and consistent hypoglycemic activity, even outperforming the standard Glibenclamide treatment at 50 µg/kg. Results suggest PHF II demonstrates remarkable antihyperglycemic efficacy. Additionally, in-vitro findings consistently pointed to F7 as the most effective formulation. This study underscores the promising role of polyherbal formulations in managing diabetes. Enhanced formulations hold the potential for subsequent scale-up trials. Future research should focus on in vivo studies involving human participants and evaluations of in vitro-in vivo correlations (IVIVC). Overall, the results indicate that this polyherbal combination could provide a robust basis for developing alternative anti-diabetic treatments.

1. Introduction

Nature plays a fundamental role in the rise, development, and sustenance of life. By offering us resources like food, shelter, plants, minerals, animals, and healing agents, it fulfills our basic needs. When humans first began inhabiting the Earth, they gradually explored a variety of plant species, identifying those that were safe and useful for nutrition and medicine, as opposed to those that posed dangers. Additionally, natural sources became an accidental avenue for discovering treatments to combat illnesses. Through trial and error, people began gathering plants to address wounds, pain, bites, and other ailments. This knowledge was largely experiential, and the remedies they developed were handed down across generations. Since time immemorial, even prior to recorded history, humans have harnessed the medicinal properties of plants [1].

Traditional medicine, rooted in deeply ingrained cultural values and life experiences, encompasses the knowledge, practices, and skills passed down through generations. It is applied in diagnosing, preventing, managing, and treating both mental and physical ailments to promote overall health, as well as addressing conditions that modern medicine may not fully explain [2]. Throughout history, traditional medical systems have played a crucial role in meeting global healthcare needs, retaining their significance in modern clinical practices, and are anticipated to remain indispensable in the future [3-4].

India is home to some of the oldest and most diverse traditional medical practices in the world. The phrase "Indian Systems of Medicine" encompasses these practices, whether they originated within the country itself or were adopted from other regions and woven into the local culture. India formally recognizes six medical systems: Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy. Although Homoeopathy was introduced to India in the 18^{th} century, it has since become deeply integrated into Indian society, evolving alongside native traditions and gaining recognition as a legitimate medical discipline [5]. The use of medicinal plants in India dates back to the Rig Veda—one of the earliest sacred texts—which, beginning around 1500 BC, references plants with healing properties and describes 67 specific medicinal plants. Among the 2,50,000 known higher plant species, an estimated 80,000 possess medicinal value. Uniquely, India stands out as one of the world's twelve biodiversity hotspots [6].

India has long been one of the richest sources of medicinal herbs among ancient civilizations. Its forests act as major reserves for numerous plants used in drug development. Within the country, the AYUSH systems incorporate 8,000 herbal remedies. The Indian herbal industry makes use of 960 plant species, out of which 178 species demonstrate high usage levels, with an annual turnover exceeding 100 metric tons [7]. An overview of the distinctive features of various Traditional Medicine (TM) systems is outlined in Table 1.1 [8]. Diabetes, a chronic medical condition, occurs when the pancreas either produces insufficient insulin or when the body fails to properly utilize this hormone, which regulates blood sugar levels. Healthcare professionals recognize it as a pressing public health concern and a key area of focus [9]. The primary signs of elevated blood sugar include polyuria (frequent urination), polydipsia (excessive thirst), and polyphagia (heightened appetite). These symptoms, alongside elevated blood sugar levels and unexplained weight loss, are critical criteria for diagnosing diabetes [10, 11].

1.1 Mechanism of action of phytoconstituents for DM

Insulin is essential for regulating blood glucose levels, with several metabolic pathways contributing to carbohydrate metabolism, including Glycolysis, Glycogenesis, Glycogenolysis, the HMP shunt pathway, Gluconeogenesis, and the Kreb's cycle.

• Phytochemicals like polyphenols, particularly phenolic acids (such as gallic acid) and tannins, hinder the activities of α-glucosidase and α-amylase while also mitigating long-term diabetic complications.
• Alkaloids reduce glucose absorption across the intestinal lining by inhibiting α-glucosidase.
• Flavonoids enhance hepatic glucokinase activity, lowering triglycerides, cholesterol, and glucose levels. Additionally, dietary fibers limit glucose absorption and curb α-amylase activity.
• Saponins boost insulin secretion and effectively lower blood glucose levels [12, 13].

1.2. Polyherbal Formulation

Polyherbal formulations offer a more holistic and enduring therapeutic impact in comparison to using individual herbs in isolation. They improve pharmacological potency while simultaneously reducing the required doses of each herb. These preparations are composed of a synergistic blend of multiple herbal components [14-16]. In traditional Indian medicine, remedies derived from a combination of plant extracts have consistently been favored over single-herb preparations due to their enhanced therapeutic potential. These formulations have shown exceptional efficacy in treating a wide array of health conditions. The use of polyherbal combinations for disease management is well-documented in ancient medical literature [17-19].

1.3. Tablet Formulation

The pharmacological composition of a drug plays a crucial role in determining its bioavailability and therapeutic performance [20]. Developing a new medicinal product—whether sourced synthetically, naturally, or via biotechnological techniques—represents a highly complex and time-intensive undertaking, requiring multidisciplinary teamwork [21]. Powders constitute a key element in the creation of all solid dosage forms. They are made up of solid particles interspersed with air-filled voids, exhibiting properties of all three states of matter: solids, liquids, and gases [22]. The process of tablet manufacturing utilizes tablet presses, where powders are compacted between two punches positioned in a die to a preset depth. Following the removal of the compression force, the upper punch withdraws, while the lower punch rises to eject the formed tablet. Typically, tablets are produced using one of two techniques: direct compression or granulation [23]. Direct compression entails mixing the active pharmaceutical ingredient with excipients and directly compressing the mixture to form tablets, bypassing any prior material preparation. Granulation, on the other hand, involves increasing the particle size by forming agglomerates without compromising the original particles' structure

[24]. These agglomerates are prepared through two primary methods—wet granulation, where a liquid binding agent is utilized, and dry granulation, which avoids the use of liquid media [25].

2. Material and methods

2.1. Collection and Authentication:

The leaves of *Gymnema sylvestre* (Gurmar), seeds of *Syzygium cumini* (Jamun), bark of *Pterocarpus marsupium* (Vijaysar), leaves of *Psidium guajava* (Guava), leaves of *Mangifera indica* (Mango), leaves of *Costus igneus* (Insulin Plant), leaves of *Aloe barbadensis* (Ghrit kumari), leaves and flowers of *Abelmoschus bammia* (Lady Finger), leaves of *Camellia sinensis* (Green Tea), and the stem of *Tinospora cordifolia* (Giloy) were collected from local sources, shade-dried, and later identified and authenticated by Dr. Vijay Malik, the Head of the Botany Department at Chaudhary Charan Singh University, Meerut.

2.2. Preparation of Formulations:

Table 1 outlines the pH formulation prepared in the laboratory, integrating a specific quantity of each distinct herb. The herbs, after being cleaned, will be ground using a grinder and passed through a mesh with a size of 40. Accurate quantities of each herb will then be weighed individually. The herbs will be mixed geometrically using a double-cone blender. Finally, the prepared mixtures will be weighed and stored in labeled glass bottles.

Table 1. Constituents of polyherbal main formulation (PHMF)

S. N.	Botanical name	Quantity
1.	*Gymnema sylvestre*	7.93
2.	*Syzygium cumini*	32.83
3.	*Pterocarpus marsupium*	5.44
4.	*Psidium guajava*	8.84
5.	*Mangifera indica*	16.94
6.	*Costus igneus*	15.72
7.	*Aloe barbadensis*	1.27
8.	*Abelmoschus bammia*	1.46
9.	*Camellia sinensis*	8.62
10.	*Tinospora cordifolia*	129.63
Total		229 mg

2.3. Polyherbal Tablet Preparation:

Polyherbal tablets were formulated using a precise blend of herbal medications, polymers, and other essential ingredients. These tablets were produced using the direct compression technique. Accurate proportions of the herbal extract, D-mannitol, lactose anhydrous, starch, magnesium stearate, microcrystalline cellulose, sodium carboxymethyl cellulose (Na CMC), sodium starch glycolate, and talc were thoroughly mixed using a mortar and pestle. The resulting mixture was sieved through a 22 mm mesh to form granules, which were subsequently dried in a hot air oven. Talc and magnesium stearate were then added to the dried granules, as mentioned in reference. To facilitate smooth powder flow, the extract was absorbed onto lactose. Isopropanol served as a granulating agent during granule formation. A single-rotor tablet punching machine equipped with twelve stations was employed to compact the mixture into tablet form [26].

The composition of the tablets, as outlined in **Table 2**, features various quantities of disintegration agents, such as sodium carboxymethyl cellulose, microcrystalline cellulose, and sodium starch glycolate. Each 350 mg tablet utilized magnesium stearate as a lubricant, starch as a binder, and lactose anhydrous as a diluent for cohesive and consistent production.

Table 2. Composition of polyherbal tablet

Batch	Weight of granule (mg)	Na CMC (mg)	MCC (mg)	SSG (mg)	Magnesium stearate (mg)	Starch (mg)	Lactose Anhydrous (mg)	Total weight of Tablet (mg)
F1	229	25	-	-	15	50	q.s.	350
F2	229	35	-	-	15	50	q.s.	350
F3	229	45	-	-	15	50	q.s.	350
F4	229	-	25	-	15	50	q.s.	350
F5	229	-	35	-	15	50	q.s.	350
F6	229	-	45	-	15	50	q.s.	350
F7	229	-	-	25	15	50	q.s.	350
F8	229	-	-	35	15	50	q.s.	350
F9	229	-	-	45	15	50	q.s.	350

2.4. Standardization parameters of polyherbal tablet:

The organoleptic characteristics like color, odor and taste, physicochemical like moisture content, ash value ethanol-soluble and post compression parameter like hardness, thickness, friability, average weight, disintegration time and dissolution time was studied.

2.5. *α-Amylase inhibition activity:*

To evaluate the in vitro antidiabetic activity, absorbance was recorded at 540 nm with the help of a UV-Visible spectrophotometer. A sample blank reaction was similarly prepared, substituting the enzyme with the plant extract. Acarbose served as the positive control in the study [27-30].

2.6. *α-glucosidase inhibition activity*:

In order to estimate the *in vitro* antidiabetic activity by α-glucosidase assay method.

2.7 Experimental Model

2.7.1. Animal Selection:

Albino rats of the Wistar strain, aged 90 to 120 days and weighing between 150 and 200 g, were utilized in the study. The animals were housed in individual standard propylene cages under controlled laboratory conditions, which included a temperature of 25 ± 2°C, a relative humidity of 45–55%, and a 12-hour light/dark cycle, with food and water provided ad libitum. The IAEC of the School of Pharmacy, Bharat Institute of Pharmacy, Meerut, granted approval for the procurement of the experimental animals. All procedures for the care and handling of animals adhered to the guidelines outlined by CPCSEA and the "Guide for the Care and Use of Laboratory Animals." Furthermore, all experimental protocols complied with CPCSEA standards (Reg. No. 1147/ab/07/CPCSEA), and approval was documented under Ref. No. BIT/SOP/IAEC/23/47(14) on February 25, 2023.

2.7.2. Experimental Design (Animal Groups and Their Division):

Male Wistar albino rats, weighing between 150-200 g and bred in the institutional animal facility, were utilized for in vivo pharmacological evaluations. Before the experiments began, the rats were housed in sanitized polypropylene cages lined with sterile paddy husk bedding to facilitate acclimatization [31-33]. The experiment was conducted over a period of 28 days. Daily monitoring included measurements of water and food intake. Body weight was recorded across all groups on days 0, 7, 14, 21, 28, and on the day of sacrifice. Details regarding the grouping of the animals are outlined below.

Group I (Normal Control): Administered with the vehicle only.
Group II (Positive Control): Administered with the standard antidiabetic drug Glibenclamide at a dose of 500 mcg/kg body weight.
Group III (Negative Control): Induced hyperglycaemia through administration of Streptozotocin (55 mg/kg in 0.1 mol/L citrate buffer), without any treatment.
Group IV (Test Formulation-1): Induced hyperglycaemia with Streptozotocin + treated with PHF-1 at 200 mg/kg body weight (orally).
Group V (Test Formulation-2): Induced hyperglycaemia with Streptozotocin + treated with PHF-2 at 200 mg/kg body weight (orally).

Group VI (Test Formulation-3): Induced hyperglycaemia with Streptozotocin + treated with PHF-3 at 200 mg/kg body weight (orally).

3. Result

3.1. Standardization parameters of polyherbal tablet:

After organoleptic characteristics, physicochemical and post compression parameter the obtained results outlined in the **Table 3**.

Table 3. Standardization parameters of polyherbal tablet

Organoleptic Characters		Physicochemical Parameters		Post Compression Parameter	
color	Brown	Moisture content	4.03%	Hardness (kg/cm2	5.79±0.061
odor	Characteristic	Total ash	4.67%	Thickness (mm)	4.65±0.012
Taste	Bitter	Acid-insoluble ash	1.15%	Friability (%)	0.44±0.006
		Water-soluble ash	2.89%	Average weight (mg)	350.15±2.23
		Water-soluble extractive value	16.37%	Disintegration time (min)	14.27±0.23
		Ethanol-soluble extractive value	10.19%	Dissolution time (min)	97.54±0.47

3.2. *In-vitro* antidiabetic activity:

Table 4. *In-Vitro* antidiabetic activity of polyherbal extract

Concentration (µg/ml)	α- amylase		α- glucosidase	
	Acarbose	**PHE**	**Acarbose**	**PHE**
10	26.31±0.23	23.26±0.17	18.21±1.7	17.39±2.1
20	36.63±0.12	35.51±0.24	27.17±2.2	20.27±2.6
50	47.34±0.27	46.33±0.36	48.21±2.4	40.21±4.3
75	66.24±0.19	61.37±0.33	69.09±5.2	58.33±4.6
100	77.23±0.71	74.34±0.27	75.26±7.6	73.32±3.8
IC_{50}	49.64	54.42	54.65	63.60

In-vitro experiments were carried out to evaluate the effects of polyherbal methanolic extracts on the enzymatic activities of α-amylase and α-glucosidase. The IC50 values for acarbose and the polyherbal extract in inhibiting these enzymes are shown in **Table 4**. Specifically, the polyherbal extract exhibited IC50 values of 54.42 for α-amylase inhibition and 63.60 for *α-glucosidase* inhibition.

3.3. Study of Fasting Blood Glucose:

Table 5 and **Fig. 1** present the average fasting blood glucose levels at various time intervals following treatment with PHF I, II, III, and standards. Measurements were taken on days 0, 1, 7, 14, 21, and 28. Prior to administering PHFs or the standard medications, there were no significant variations observed in the fasting glucose levels across or within the different groups of animals.

However, distinct differences in blood glucose levels were noticed between the PHF-treated groups and those treated with the standard medications after 28 days of treatment.

For diabetic rats, the oral administration of PHF I, II, and III at a dose of 200 mg/kg body weight over 28 days significantly ($p<0.05$) reduced the fasting glucose levels to 96.20±1.4832, 93.20±0.8367, and 101.4±2.0736, respectively, compared to pre-treatment levels. Similarly, Glibenclamide at a dose of 500 mcg/kg also resulted in a significant post-treatment reduction ($p<0.05$) in blood glucose levels, recorded at 104.6±1.1402. In contrast, the diabetic control group that did not receive any treatment showed consistently elevated blood glucose levels throughout the 28-day period.

Among the tested treatments, PHF II at 200 mg/kg b.w. demonstrated the highest antidiabetic efficacy by the 28th day, achieving a 68.55% reduction in fasting blood glucose from day 1 levels. Meanwhile, reductions of 64.99% and 67.74% were noted for PHF I and PHF III (200 mg/kg b.w.), respectively, between days 1 and 28, indicating that the treated groups effectively restored their hyperglycemic states closer to normal levels.

Additionally, treatment with standard Glibenclamide led to a 62.61% reduction in fasting glucose levels by the 28th day.

Table 5. Effect of PHFs on fasting blood glucose levels in STZ induced diabetic rats

Group	Fasting blood glucose (mg/dl)					
	Day 0	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Normal Control	91.60±1.6733	92.4±1.1402	93.2±1.3038	95.6±1.1402	96.6±1.1402	95.2±1.3038
Diabetic Control	92.80±1.9235	284.8±5.0695	274.6±5.6833	288.4±4.9295	305.6±3.0496	329.2±5.9330
Diabetic + Glibenclamide 500 mcg/kg bw	94.20±0.8367	279.8±3.5637	215.8±3.5637	148.2±3.5637	108.6±1.9235	104.6±1.1402
Diabetic + PHF I 200 mg/kg bw	95.20±1.4832	274.8±3.1937	208.2±1.6432	182.4±2.4083	103.6±2.0736	96.2±1.4832
Diabetic + PHF II 200 mg/kg bw	93.00±2.0000	296.4±3.8471	192.8±2.1679	176.2±3.1145	97.6±2.1909	93.2±0.8367
Diabetic + PHF III 200mg/kg bw	92.40±1.5166	314.4±4.0373	213.6±2.9665	184.6±3.3615	110.2±2.9496	101.4±2.0736

n=5/group and $p<0.05$ use for significant difference between pre-treatment and post-treatment

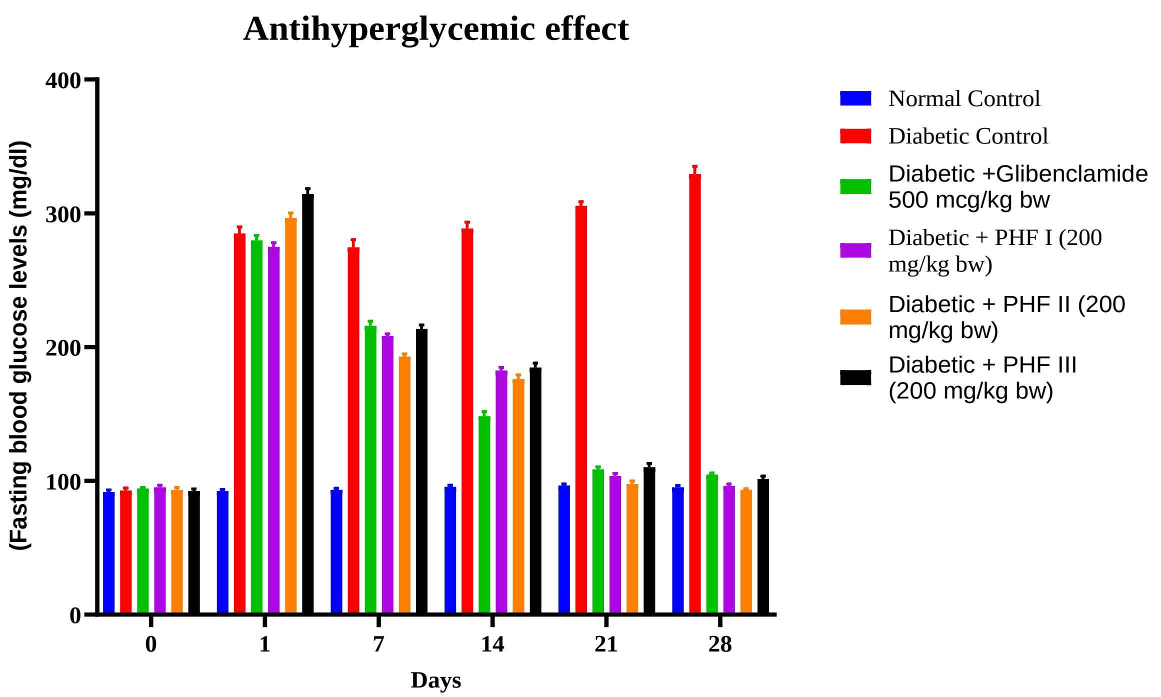

Fig. 1 Effect of PHF on fasting blood glucose levels in STZ induced diabetic rats

4. Conclusion

Herbal medicine stands as the oldest form of healthcare practiced across the globe. Many traditional practices involving these natural remedies have been seamlessly incorporated into modern approaches for diagnosing, treating, and preventing diseases. Following an extensive literature review, ten natural herbs were selected to develop the polyherbal tablet formulation. Each of these herbs underwent thorough inspection to confirm their identity, quality, and purity, ensuring compliance with the standards prescribed by the WHO and the Indian Ayurvedic Pharmacopoeia. The polyherbal ingredients were dried and pulverized using a mixer grinder, and the resultant powders underwent extraction prior to their incorporation into the final formulation. The dried herbal powders were further optimized to meet quality benchmarks and ensure uniformity across batches, which were tested during the production of three independent trial batches (Trial I, II, and III). Outcomes demonstrated that all trial batches achieved exceptional quality, with evaluated parameters adhering to established regulatory standards. In the study, the impact of various treatments on fasting blood glucose levels in diabetic rats was examined over a 28-day period, with findings presented in Table 5 and Figure 1. At the start of the experiment (Day 0), prior to diabetes induction, there were no significant differences in blood glucose levels across all groups. The normal control group maintained stable fasting blood glucose levels throughout the study, with negligible variation from its baseline value of 91.60±1.6733 mg/dl. Meanwhile, the diabetic control group exhibited a sharp increase in fasting blood glucose, soaring from 92.8±1.9235 mg/dl on Day 0 to 329.2±5.9330 mg/dl on Day 28. In contrast, the positive control group treated with Glibenclamide showed a marked reduction in blood glucose levels, decreasing from 279.8±3.5637 mg/dl on Day 1 to 104.6±1.1402 mg/dl on Day 28. This represented a significant 62.61% drop, demonstrating robust antihyperglycemic efficacy. Oral administration of PHF I, II, and III at a dosage of 200 mg/kg body weight for 28 days led to significant ($p<0.05$) reductions in blood glucose levels, measuring 96.2±1.4832, 93.2±0.8367, and 101.4±2.0736, respectively. All treatment groups—including Glibenclamide, PHF I, PHF II, and PHF III—demonstrated marked decreases in fasting blood glucose levels when compared to the diabetic control group. Among these, PHF II displayed the strongest antihyperglycemic activity, closely followed by PHF III and Glibenclamide. These findings indicate that the PHF formulations possess powerful antihyperglycemic effects, comparable to the standard treatment with Glibenclamide.

5. Consent for publication

Not applicable.

6. Availability of data and materials

Yes.

7. Funding

None.

8. Conflict of Interest

The authors declare no conflict of interest.

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