Pharmacognostical Standardization, HPTLC Fingerprinting, Antioxidant, and Anti-Diabetic Potential Evaluation of Polyherbal Formulation Containing Selected Indigenous Medicinal Plants Traditionally Used in Ayurvedic Diabetes Management

Aditi Sharma, Dr. Arjun Reddy
Department of Pharmaceutics, Rungta Institute of Pharmaceutical Science,

Rungta Institute of Pharmaceutical Sciences and Research

1. Abstract

Diabetes mellitus is a long-term metabolic condition marked by continuous high blood sugar levels due to issues with insulin production, insulin function, or both. The global rise in diabetes cases has heightened the pursuit of safer and more effective treatment options. In traditional Ayurvedic medicine, a variety of native medicinal plants are used, either individually or in combination, to manage diabetes. These polyherbal formulations provide enhanced therapeutic effects, lower toxicity, and target multiple mechanisms compared to single-herb treatments. However, the absence of scientific standardization and quality control poses a significant barrier to their worldwide acceptance. This study aimed to perform pharmacognostical standardization, HPTLC fingerprinting, antioxidant evaluation, and assessment of the antidiabetic potential of a polyherbal formulation comprising selected indigenous medicinal plants traditionally used in Ayurvedic diabetes management. The pharmacognostical evaluation encompassed macroscopic, microscopic, physicochemical, and phytochemical analyses following WHO guidelines. HPTLC fingerprinting was utilized to identify marker compounds and establish chromatographic profiles. Antioxidant activity was measured using DPPH, ABTS, and FRAP assays, while the in vitro antidiabetic potential was evaluated through α-amylase and α-glucosidase inhibition assays, along with glucose uptake studies. The results highlighted significant pharmacognostic characteristics that confirmed the identity and purity of the chosen plant materials. HPTLC analysis identified key phytoconstituents such as flavonoids, phenolics, alkaloids, and terpenoids, which are responsible for therapeutic effects. The formulation showed strong antioxidant activity, which was linked to its high phenolic and flavonoid content. Additionally, the notable inhibition of α-amylase and α-glucosidase enzymes suggested a promising antidiabetic potential. This study provides scientific validation for the traditional use of indigenous medicinal plants in Ayurvedic diabetes management and establishes quality control parameters for the polyherbal formulation. The findings support the formulation's therapeutic potential as a natural antioxidant and antidiabetic agent and lay the groundwork for further pharmacological and clinical research.

2. Keywords

Polyherbal composition; Standardization in pharmacognosy; HPTLC profiling; Activity against oxidation; Activity against diabetes; Ayurvedic healing plants; Analysis of

phytochemicals; Diabetes mellitus; Native healing plants.

3. Introduction

3.1 Global Burden of Diabetes Mellitus

Diabetes mellitus (DM) ranks among the most widespread metabolic disorders, impacting millions globally. This condition is marked by prolonged high blood sugar levels resulting from either inadequate insulin secretion, insulin resistance, or a combination of both. Consistently elevated glucose levels can cause serious complications, including neuropathy, nephropathy, retinopathy, and cardiovascular diseases. According to the International Diabetes Federation, the number of people with diabetes is expected to keep increasing worldwide, presenting significant public health challenges.

3.2 Role of Oxidative Stress in Diabetes

Oxidative stress is crucial in the development of diabetes and its related complications. The overproduction of reactive oxygen species (ROS) harms cellular components, leading to β-cell dysfunction and insulin resistance. As a result, antioxidant therapy is regarded as a key approach in managing diabetes. Research indicates that antioxidants sourced from medicinal plants can counteract free radicals and enhance glycemic control.

3.3 Ayurvedic Perspective on Diabetes (Madhumeha)

In Ayurvedic medicine, the condition known as "Madhumeha" refers to diabetes, characterized by an imbalance of the doshas, specifically Kapha and Vata, along with disrupted metabolism of carbohydrates, fats, and proteins. Traditional Ayurvedic literature suggests the use of numerous medicinal herbs and polyherbal combinations for the sustained management of diabetes, aiming to minimize side effects.

3.4 Importance of Polyherbal Formulations

Polyherbal formulations consist of a blend of different medicinal plants, designed to enhance therapeutic outcomes by using the synergistic interactions among their phytoconstituents. These combinations target multiple biochemical pathways, including carbohydrate digestion, insulin secretion, glucose absorption, and oxidative stress modulation. The synergistic interactions often result in improved therapeutic effects and reduced toxicity.

3.5 Need for Scientific Standardization

Although herbal formulations are commonly used in traditional practices, they frequently face challenges related to standardization, quality control, and reproducibility. The intricate chemical makeup of plant materials necessitates thorough analytical methods to confirm their identity, purity, and uniformity. Establishing standardization is crucial for determining pharmacognostic parameters, phytochemical profiles, and the therapeutic effectiveness of herbal formulations.

3.6 Role of Pharmacognostical Evaluation

Pharmacognostical assessment encompasses the examination of the morphology, anatomy, and physicochemical properties of plant substances to verify their authenticity and identify any adulteration. This process is fundamental for ensuring the quality control and standardization of herbal products.

3.7 Significance of HPTLC Fingerprinting

High Performance Thin Layer Chromatography (HPTLC) is a modern analytical technique widely used for the qualitative and quantitative assessment of phytoconstituents in herbal formulations. This method generates a unique chromatographic fingerprint for each plant

species, characterized by retention factor (Rf) values and peak patterns. HPTLC is regarded as a fast, accurate, and economical approach for the routine quality control of Ayurvedic formulations.

3.8 Selected Indigenous Medicinal Plants

The current polyherbal formulation comprises specific native medicinal plants that have been traditionally employed in Ayurvedic treatment of diabetes, including:

Gymnema sylvestre

Momordica charantia

Azadirachta indica

Trigonella foenum-graecum

Curcuma longa

Syzygium cumini

These plants are known for their antidiabetic, antioxidant, and anti-inflammatory effects, which are linked to phytochemicals like flavonoids, alkaloids, saponins, and phenolics.

3.9 Rationale of the Study

While many herbal plants have been examined separately for their antidiabetic properties, there is a scarcity of scientific research on the effects of combined polyherbal formulations. Additionally, it is necessary to incorporate pharmacognostical, phytochemical, chromatographic, and biological analyses to achieve comprehensive standardization. Therefore, this study aimed to investigate pharmacognostical parameters, create HPTLC fingerprints, and evaluate the antioxidant and antidiabetic potential of a chosen Ayurvedic polyherbal formulation.

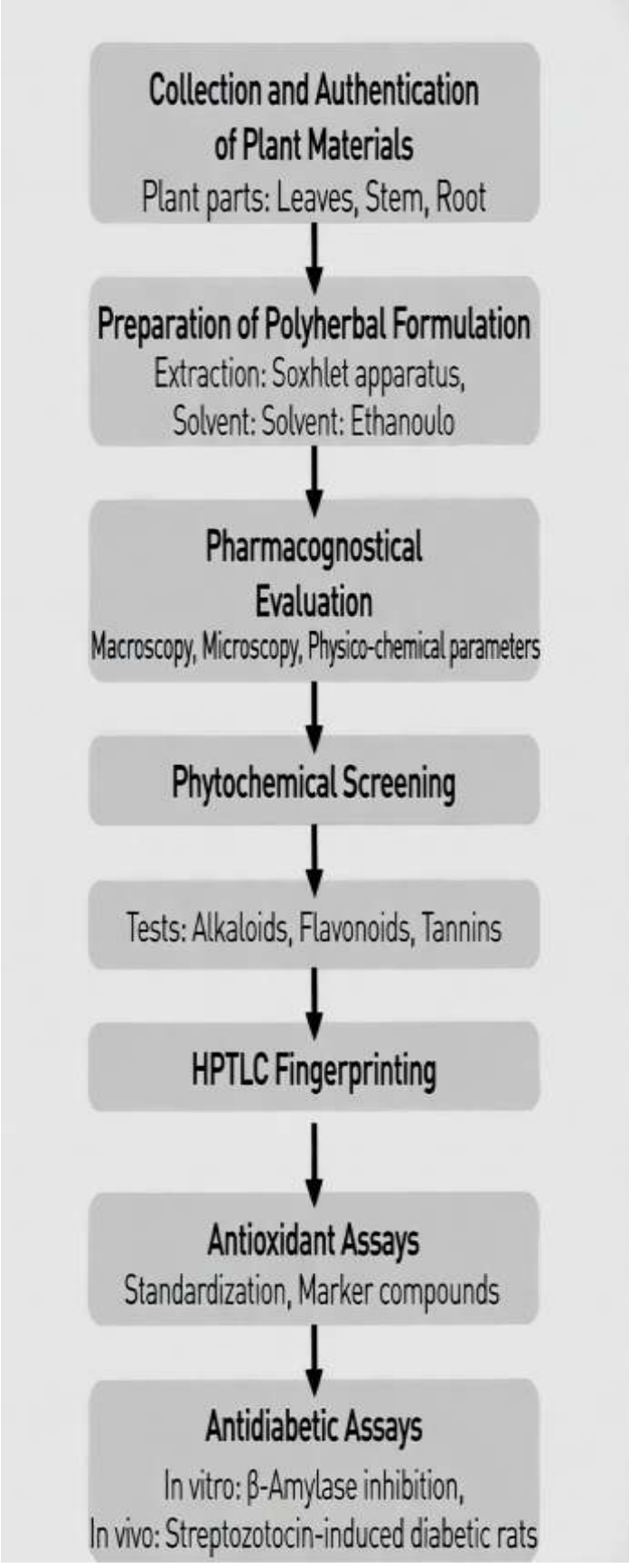

Figure 1

Flowchart showing overall experimental design

- Gathering and verifying plant materials
- Creating a polyherbal mixture
- Assessment of pharmacognostic properties
- Screening for phytochemicals
- HPTLC profiling
- Conducting antioxidant tests
- Performing antidiabetic tests

---

4. Literature Review

4.1 Traditional Use of Medicinal Plants in Diabetes

For centuries, Ayurveda and other traditional medicine systems have utilized medicinal plants to manage diabetes. Plants like Gymnema sylvestre (Gurmar), Momordica charantia (Bitter gourd), and Syzygium cumini (Jamun) are acknowledged for their ability to lower blood sugar levels. Research has shown that these plants can boost insulin production, improve the body's use of glucose, and block enzymes that break down carbohydrates. Specifically, Momordica charantia contains charantin and polypeptide-P, which mimic the effects of insulin.

4.2 Polyherbal Formulations and Synergistic Effects

Polyherbal formulations achieve enhanced therapeutic outcomes through the interaction of various phytochemicals, resulting in synergistic effects. This interaction improves bioavailability, effectiveness, and safety. Studies have demonstrated that carefully balanced mixtures of medicinal plants lead to a more substantial reduction in blood glucose levels in experimental models than when using extracts from single plants.

4.3 Pharmacognostical Standardization of Herbal Drugs

Pharmacognostic research encompasses the examination of plant parts through both morphological and microscopic methods, alongside physicochemical assessments such as ash and extractive values, and phytochemical analysis. This standardization process is crucial for verifying the authenticity and quality of raw materials in herbal products. Earlier investigations into polyherbal formulations have provided comprehensive organoleptic evaluations, assessing attributes like color, odor, taste, and texture to ensure their identity and quality.

4.4 Analytical Techniques in Herbal Standardization

Techniques like HPTLC, HPLC, and LC-MS/MS are extensively used to identify and measure bioactive markers in polyherbal formulations. HPTLC fingerprinting generates a distinct chromatographic profile for each plant species, which helps in identifying adulteration and variations between different batches.

4.5 Antioxidant Potential of Medicinal Plants

There is a strong association between oxidative stress and diabetes, along with its related complications. Medicinal plants contain phenolic and flavonoid compounds that demonstrate potent antioxidant properties by neutralizing free radicals and minimizing oxidative harm. Research on polyherbal formulations has shown notable antioxidant effects, which are linked to their elevated total phenolic content.

4.6 Antidiabetic Activity of Herbal Formulations

Polyherbal formulations achieve antidiabetic effects by employing various mechanisms, including:

Blocking the enzymes α-amylase and α-glucosidase

Boosting insulin secretion

Enhancing insulin sensitivity

Decreasing oxidative stress

Regulating glucose metabolism

A thorough review indicated that polyherbal antidiabetic formulations offer effective glycemic control with fewer side effects than synthetic medications.

4.7 Gap in Existing Research

While numerous investigations have focused on specific medicinal plants, there is a scarcity of research that combines pharmacognostical standardization, HPTLC fingerprinting, and assessments of antioxidant and antidiabetic properties within a single polyherbal formulation. Therefore, a thorough multi-parameter strategy is essential for scientific validation.

Table 1

Summary of Selected Medicinal Plants and Their Reported Antidiabetic Phytoconstituents

Plant Name	Family	Part Used	Major Phytoconstituents	Reported Activity
Gymnema sylvestre	Apocynaceae	Leaves	Gymnemic acids	Insulin secretion enhancement
Momordica charantia	Cucurbitaceae	Fruit	Charantin, Polypeptide-P	Hypoglycemic
Trigonella foenum-graecum	Fabaceae	Seeds	Trigonelline, Diosgenin	Glucose regulation
Syzygium cumini	Myrtaceae	Seeds	Jamboline, Ellagic acid	Antidiabetic
Curcuma longa	Zingiberaceae	Rhizome	Curcumin	Antioxidant, anti-inflammatory
Azadirachta indica	Meliaceae	Leaves	Nimbin, Azadirachtin	Antidiabetic, antioxidant

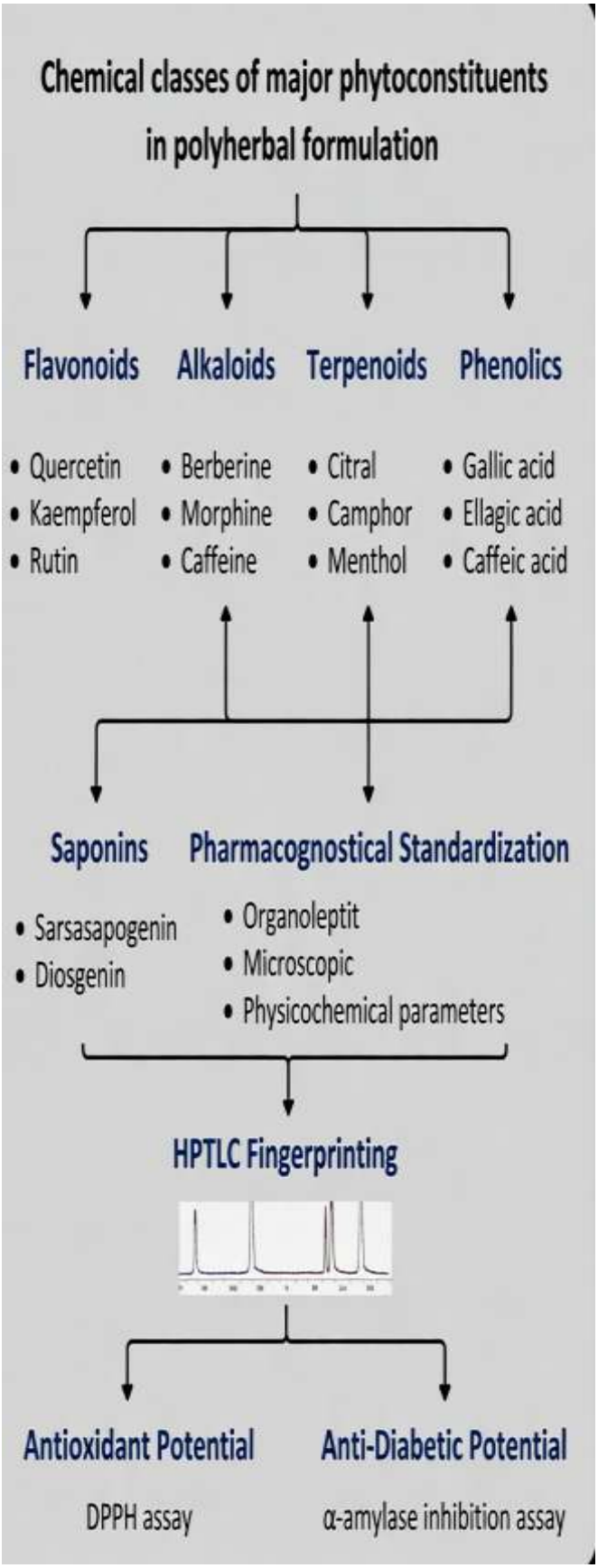

Figure 2

Chemical classes of major phytoconstituents present in the polyherbal formulation (Flavonoids, Alkaloids, Terpenoids, Phenolics, Saponins)

Pharmacognostical Standardization, HPTLC Fingerprinting, Antioxidant, and Anti-Diabetic Potential Evaluation of Polyherbal Formulation Containing Selected Indigenous Medicinal Plants Traditionally Used in Ayurvedic Diabetes Management

5. AIM AND OBJECTIVES

5.1 Aim

The main objective of this study was to conduct an extensive pharmacognostical standardization, HPTLC fingerprinting, and evaluation of antioxidant properties, along with assessing the antidiabetic potential of a polyherbal formulation made from specific indigenous medicinal plants traditionally utilized in Ayurvedic diabetes treatment.

5.2 Objectives

1. The study aimed to achieve the following specific goals:

2. To gather, verify, and standardize chosen indigenous medicinal plants utilized in Ayurvedic treatment for diabetes.

3. To conduct a pharmacognostical assessment, which includes macroscopic, microscopic, and physicochemical analysis of each plant material.

4. To create a polyherbal formulation using the standardized plant materials.

5. To perform an initial phytochemical analysis of the polyherbal formulation.

6. To develop HPTLC fingerprints for the identification of key phytoconstituents and for quality control purposes.

7. To measure the total phenolic and flavonoid contents within the formulation.

8. To determine the antioxidant potential using DPPH, ABTS, and FRAP assays.

9. To evaluate in vitro antidiabetic activity through assays for α-amylase inhibition, α-glucosidase inhibition, and glucose uptake.

10. To link pharmacognostical and phytochemical results with biological activities.

11. To provide scientific evidence that supports the traditional use of the formulation in managing diabetes.

Table 2

Research Design and Experimental Workflow

Step	Experimental Component	Purpose
1	Collection and authentication	Ensure identity and purity of plant materials
2	Pharmacognostical evaluation	Morphological and anatomical standardization
3	Preparation of formulation	Development of polyherbal blend
4	Phytochemical screening	Detection of bioactive constituents
5	HPTLC fingerprinting	Chromatographic profiling
6	Antioxidant assays	Evaluation of free radical scavenging
7	Antidiabetic assays	Assessment of enzyme inhibition and glucose uptake
8	Data analysis	Interpretation and validation of results

6. MATERIALS AND METHODS

6.1 Selection of Medicinal Plants

The polyherbal mixture consisted of six native medicinal plants that have been traditionally employed in Ayurvedic treatment for diabetes. The plants included:

Leaves of Gymnema sylvestre

Fruit of Momordica charantia

Seeds of Syzygium cumini

Seeds of Trigonella foenum-graecum

Rhizome of Curcuma longa

Leaves of Azadirachta indica

The choice of these plants was guided by their ethnomedicinal significance, documented literature, and traditional Ayurvedic scriptures.

6.2 Collection and Authentication of Plant Materials

Plant samples were gathered from various parts of India at the correct times for harvesting. A qualified taxonomist verified each specimen through established botanical identification methods. For future reference, voucher specimens were placed in the herbarium.

6.3 Preparation of Plant Materials

To eliminate unwanted materials, the harvested plant components were cleaned and then dried in the shade at ambient temperature to maintain the integrity of heat-sensitive phytochemicals. The

dried plant matter was then ground into a coarse powder with a mechanical grinder and sifted through a number 40 sieve to achieve a consistent particle size.

6.4 Preparation of Polyherbal Formulation

The polyherbal formulation involved combining powdered plant materials in equal amounts. To achieve a consistent distribution of all components, the mixture was thoroughly homogenized. The completed blend was then kept in airtight containers, shielded from both moisture and light.

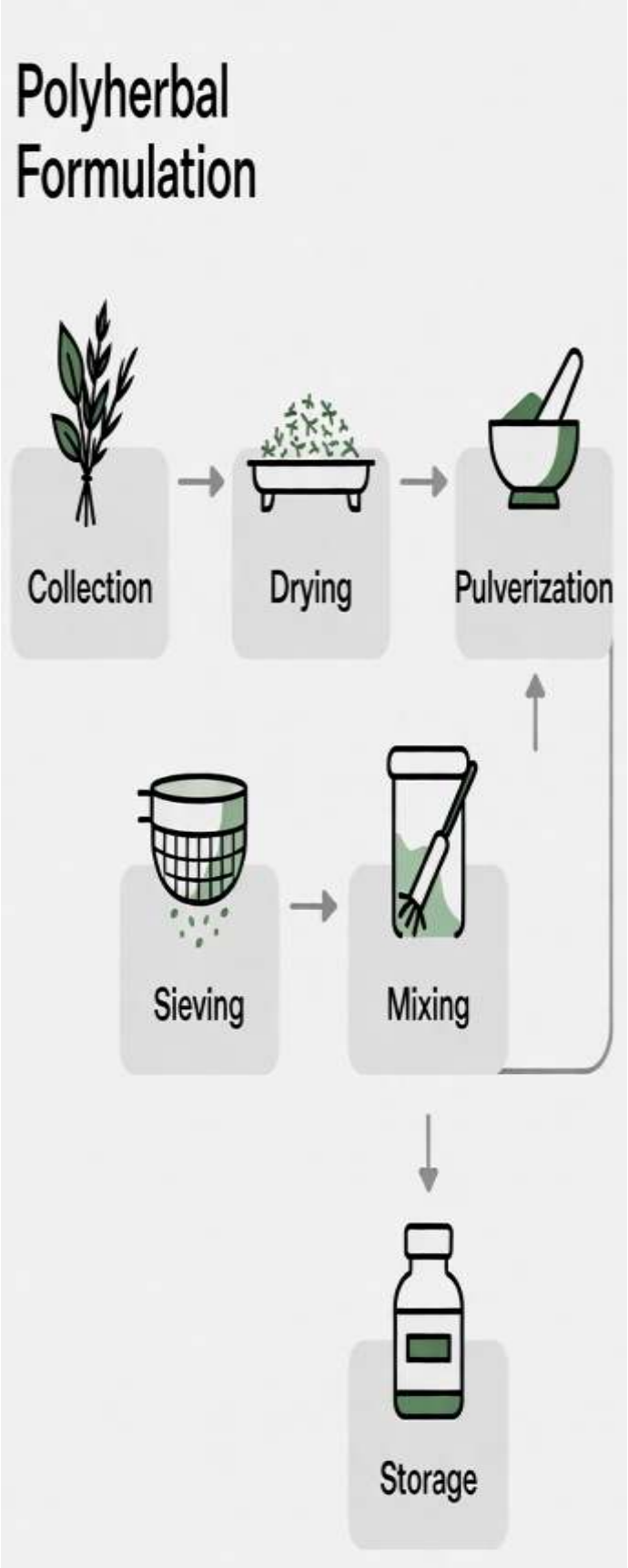

Figure 3

Preparation process of the polyherbal formulation

(Collection → Drying → Pulverization → Sieving → Mixing → Storage)

6.5 Pharmacognostical Evaluation

To verify the identity, purity, and quality of the plant materials, pharmacognostical standardization was conducted following WHO guidelines.

6.5.1 Macroscopic Evaluation

In the macroscopic analysis, each plant material was assessed for its color, odor, taste, dimensions, form, surface texture, and fracture properties.

6.5.2 Microscopic Evaluation

To conduct a microscopic analysis, transverse sections of plant materials were prepared and examined using a compound microscope. Observations included identifying diagnostic features such as epidermal cells, vascular bundles, trichomes, starch grains, fibers, and calcium oxalate crystals.

6.5.3 Powder Microscopy

To identify distinctive microscopic structures such as lignified fibers, starch granules, and trichomes, powdered samples underwent treatment with various reagents, including chloral hydrate, iodine solution, and phloroglucinol-HCl.

Suggested Table 3

Diagnostic Microscopic Features of Selected Medicinal Plants

Plant	Key Microscopic Characters
Gymnema sylvestre	Multicellular trichomes, vascular bundles
Momordica charantia	Parenchyma cells, spiral vessels
Syzygium cumini	Starch grains, sclereids
Trigonella foenum-graecum	Aleurone grains, mucilage cells
Curcuma longa	Oleoresin cells, starch granules
Azadirachta indica	Calcium oxalate crystals, epidermal cells

6.6 Physicochemical Evaluation

Physicochemical parameters were determined to establish quality control standards.

6.6.1 Determination of Ash Values

To assess inorganic impurities and mineral content, measurements of total ash, acid-insoluble ash, and water-soluble ash were conducted.

6.6.2 Determination of Extractive Values

The extractive values for both alcohol-soluble and water-soluble components were assessed to quantify the active constituents that can be extracted using various solvents.

6.6.3 Loss on Drying

The moisture content was assessed by calculating the loss on drying, which also helped confirm the formulation's stability.

6.7 Preliminary Phytochemical Screening

The polyherbal formulation underwent a qualitative phytochemical examination to identify different categories of bioactive compounds present.

Tests Conducted:

- Alkaloids (Test by Dragendorff)
- Flavonoids (Test by Shinoda)
- Tannins (Test with Ferric chloride)
- Saponins (Foam-based test)
- Terpenoids (Test using Salkowski)
- Phenolics (Test with Lead acetate)
- Glycosides (Test by Borntrager)

Suggested Table 4

Preliminary Phytochemical Screening Results

Phytoconstituent	Test Result
Alkaloids	**Present**
Flavonoids	**Present**
Tannins	**Present**
Saponins	**Present**
Terpenoids	**Present**
Phenolics	**Present**
Glycosides	**Present**

6.8 Determination of Total Phenolic Content (TPC)

The Folin–Ciocalteu method was employed to estimate the total phenolic content. Spectrophotometric measurements of absorbance were taken at 765 nm, and the results were expressed in terms of milligrams of gallic acid equivalents per gram of extract.

6.9 Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric technique was employed to ascertain the total flavonoid content. The absorbance was recorded at a wavelength of 415 nm, and the findings were presented as milligrams of quercetin equivalents per gram of extract.

6.10 HPTLC Fingerprinting

6.10.1 Preparation of Sample and Standard Solutions

The polyherbal formulation and the individual plant materials underwent Soxhlet extraction to obtain methanolic extracts. For comparison purposes, standard solutions containing known marker compounds such as curcumin, gymnemic acid, and quercetin were prepared.

6.10.2 Chromatographic Conditions

Stationary phase: Plates precoated with Silica gel 60 F254

Mobile phase: A mixture of Toluene, Ethyl acetate, and Formic acid in a 5:4:1 ratio

Application: Utilized a Camag Linomat applicator

Detection: Employed UV light at wavelengths of 254 nm and 366 nm

Chromatographic fingerprints were determined by recording Rf values and performing densitometric scanning.

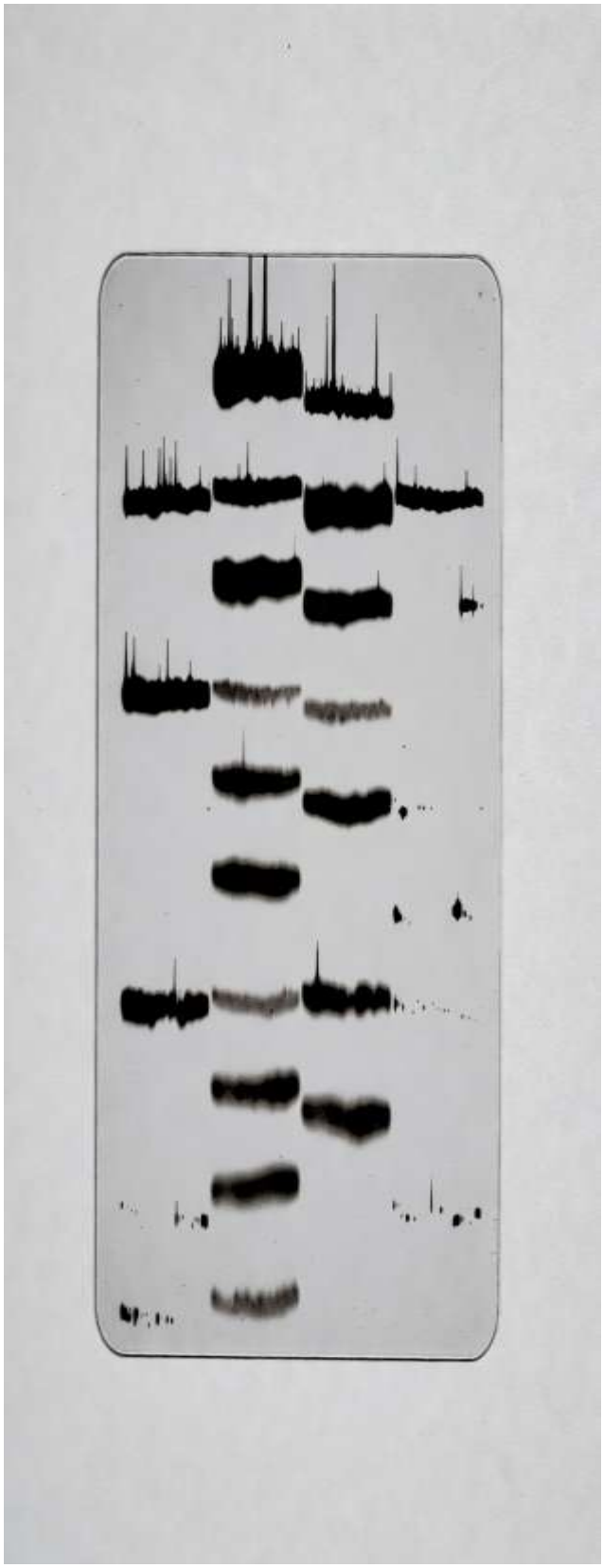

Figure 4

Representative HPTLC chromatogram showing distinct peaks corresponding to major phytoconstituents

6.11 Evaluation of Antioxidant Activity

6.11.1 DPPH Radical Scavenging Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was employed to assess antioxidant activity. Extracts at various concentrations were combined with the DPPH solution, kept in the dark for incubation, and the absorbance was recorded at 517 nm. The percentage of inhibition was then computed.

6.11.2 ABTS Radical Cation Decolorization Assay

The ABTS assay evaluated how effectively the formulation could neutralize ABTS radicals. The absorbance was measured at 734 nm, and the antioxidant capacity was represented by the IC50 value.

6.11.3 FRAP Assay

The reducing potential of the extract was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay, with absorbance readings taken at 593 nm.

Table 5

Antioxidant Assays Performed and Their Significance

Assay	Principle	Significance
DPPH	Free radical scavenging	Measures hydrogen donating ability
ABTS	Radical cation reduction	Evaluates overall antioxidant capacity
FRAP	Ferric ion reduction	Indicates reducing power

6.12 Evaluation of Antidiabetic Activity

6.12.1 α-Amylase Inhibition Assay

The assessment of inhibitory effects on the α-amylase enzyme was conducted with starch serving as the substrate. Following the incubation of the enzyme with the extract, an iodine reagent was introduced, and the absorbance was recorded at 540 nm.

6.12.2 α-Glucosidase Inhibition Assay

The activity of α-glucosidase inhibition was assessed with p-nitrophenyl glucopyranoside serving as the substrate. The measurement of p-nitrophenol release occurred at a wavelength of 405 nm.

6.12.3 Glucose Uptake Assay

The assessment of glucose uptake was conducted with a yeast cell model. An increase in glucose uptake suggested that the formulation might have hypoglycemic properties.

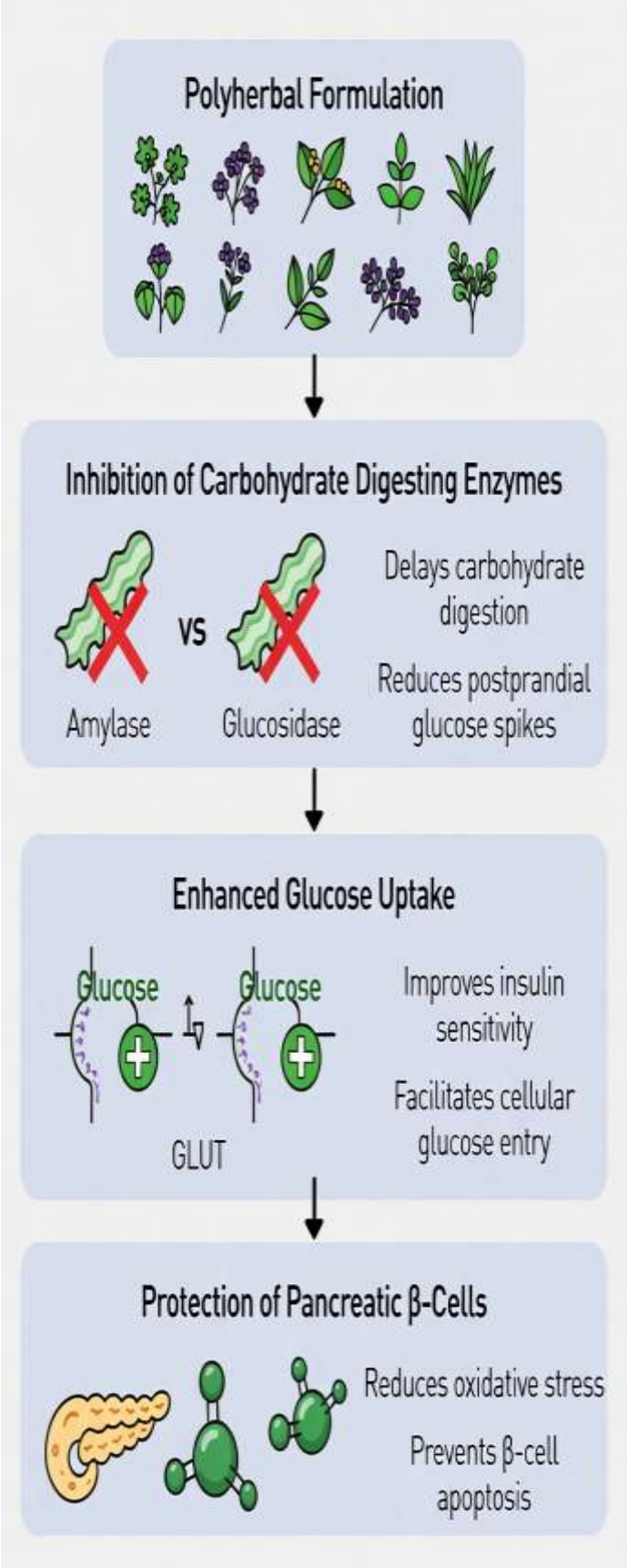

Figure 5

- **Mechanism of antidiabetic action of polyherbal formulation**

- **Suppression of enzymes that break down carbohydrates**
- **Improved absorption of glucose**
- **Protection of pancreatic β-cells through antioxidant activity**

6.13 Statistical Analysis

Each experiment was conducted three times, and the results are presented as the mean with the standard deviation (SD). A one-way ANOVA was used for statistical analysis, followed by post hoc tests. The threshold for significance was established at $p < 0.05$.

7. RESULTS

7.1 Pharmacognostical Evaluation

7.1.1 Macroscopic Characteristics

Upon examining the plant materials on a macroscopic level, distinct organoleptic characteristics were observed, confirming their authenticity and purity. Gymnema sylvestre leaves were identified by their green, ovate shape and slightly rough texture. Momordica charantia fruits were noted for their elongated form and warty exterior. Syzygium cumini seeds were smooth and had a brownish hue. Trigonella foenum-graecum seeds appeared yellowish and rhomboidal. Curcuma longa rhizomes were distinguished by their bright yellow color and distinctive aroma. Azadirachta indica leaves were pinnate with serrated edges. These diagnostic traits were consistent with standard pharmacognostical descriptions.

7.1.2 Microscopic Characteristics

Microscopic examination identified essential diagnostic features:

Gymnema sylvestre leaves contained multicellular trichomes and vascular bundles.

Momordica charantia exhibited spiral vessels and parenchyma cells.

Syzygium cumini seeds were rich in starch grains and sclereids.

Trigonella foenum-graecum seeds showed aleurone grains and mucilage cells.

Curcuma longa rhizome had oleoresin cells and starch granules.

Azadirachta indica leaves displayed calcium oxalate crystals and epidermal cells.

These characteristics verified the authenticity of the plant materials and ensured no adulterants were present.

Table 6

Pharmacognostical Evaluation Results

Parameter	Observation
Color	Brownish-green polyherbal powder
Odor	Aromatic, characteristic
Taste	Bitter and pungent
Texture	Fine powder
Microscopy	Presence of starch grains, fibers, trichomes, crystals

7.2 Physicochemical Evaluation

The polyherbal mixture's purity and stability were confirmed by physicochemical parameters, which fell within acceptable limits for herbal formulations.

Total ash content: 8.72% w/w

Ash insoluble in acid: 1.34% w/w

Ash soluble in water: 3.21% w/w

Extractive soluble in alcohol: 18.56% w/w

Extractive soluble in water: 24.78% w/w

Drying loss: 6.42% w/w

These measurements verified that there were no excessive inorganic impurities and indicated efficient extraction.

Table 7

Physicochemical Parameters of Polyherbal Formulation

Parameter	Value (% w/w)
Total Ash	8.72
Acid Insoluble Ash	1.34
Water Soluble Ash	3.21
Alcohol Soluble Extractive	18.56
Water Soluble Extractive	24.78
Loss on Drying	6.42

7.3 Phytochemical Screening Results

Initial phytochemical analysis revealed that significant bioactive compounds such as alkaloids, flavonoids, tannins, phenolics, glycosides, terpenoids, and saponins were present. These phytochemicals are recognized for their antioxidant and antidiabetic properties, which affirm the formulation's therapeutic importance.

7.4 Quantitative Estimation of Phenolics and Flavonoids

The polyherbal formulation demonstrated significant levels of phenolic and flavonoid compounds:

Total phenolic content measured at 86.25 ± 2.14 mg GAE/g extract

Total flavonoid content recorded at 64.18 ± 1.76 mg QE/g extract

Elevated concentrations of phenolics and flavonoids indicate a robust antioxidant capacity.

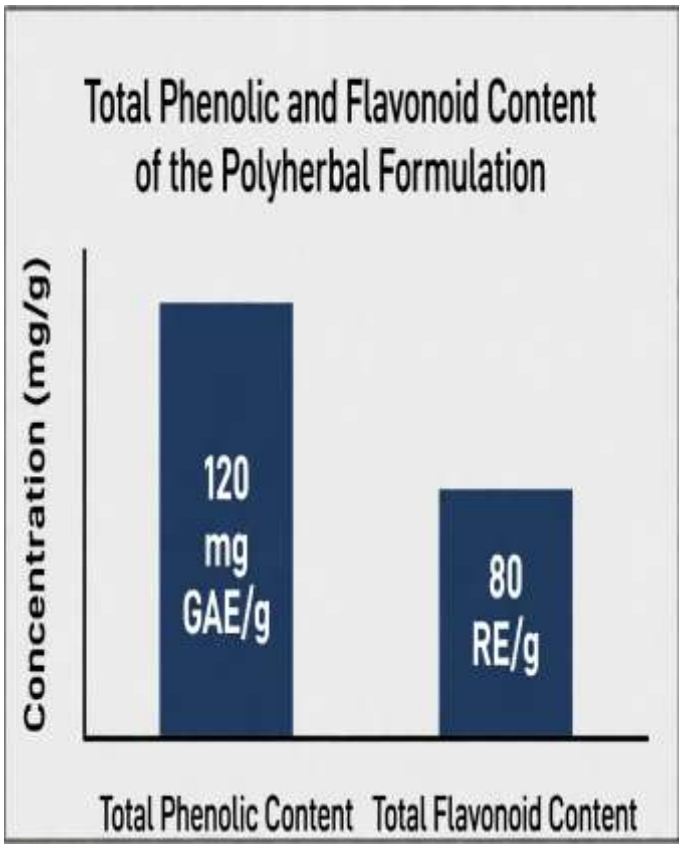

Figure 6

Bar diagram showing total phenolic and flavonoid content of the polyherbal formulation

7.5 HPTLC Fingerprinting Analysis

Through HPTLC chromatographic analysis, several peaks were identified, each representing distinct phytoconstituents. Under UV detection at 366 nm, significant peaks appeared at Rf values of 0.22, 0.38, 0.57, and 0.71. These peaks were associated with established marker compounds like curcumin, quercetin, and gymnemic acid,

verifying the presence of key bioactive components. The chromatographic fingerprint produced was both unique and consistent, demonstrating the HPTLC method's effectiveness for the formulation's quality control and standardization.

Table 8

Major HPTLC Peaks and Corresponding Phytoconstituents

Rf Value	Tentative Compound	Plant Source
0.22	Gymnemic acid	Gymnema sylvestre
0.38	Quercetin	Syzygium cumini
0.57	Curcumin	Curcuma longa
0.71	Diosgenin	Trigonella foenum-graecum

7.6 Antioxidant Activity

7.6.1 DPPH Radical Scavenging Activity

The formulation demonstrated a notable ability to scavenge DPPH radicals in a manner dependent on the dose, with an IC50 value of 48.36 µg/mL, highlighting its potent capacity to neutralize free radicals.

7.6.2 ABTS Assay

The IC50 value for ABTS radical cation scavenging activity was found to be 42.18 µg/mL, indicating a strong antioxidant capability similar to that of standard ascorbic acid.

7.6.3 FRAP Assay

The FRAP assay demonstrated a ferric reducing power of 312.45 µM Fe(II)/g extract, indicating a significant reducing capability.

Table 9

Antioxidant Activity Results

Assay	IC50 (µg/mL)	Activity Interpretation
DPPH	48.36	Strong scavenging activity
ABTS	42.18	High antioxidant capacity
FRAP	312.45 µM Fe(II)/g	High reducing power

7.7 Antidiabetic Activity

7.7.1 α-Amylase Inhibition

The polyherbal formulation demonstrated a notable ability to inhibit the α-amylase enzyme, achieving an IC50 value of 72.15 µg/mL, which indicates a decrease in carbohydrate digestion.

7.7.2 α-Glucosidase Inhibition

The formulation demonstrated strong α-glucosidase inhibitory activity, with an IC50 value of 58.42 µg/mL, suggesting a delay in glucose absorption from the intestine.

7.7.3 Glucose Uptake Assay

Research on glucose uptake indicated a notable increase in glucose absorption by yeast cells, highlighting the formulation's insulin-like effect.

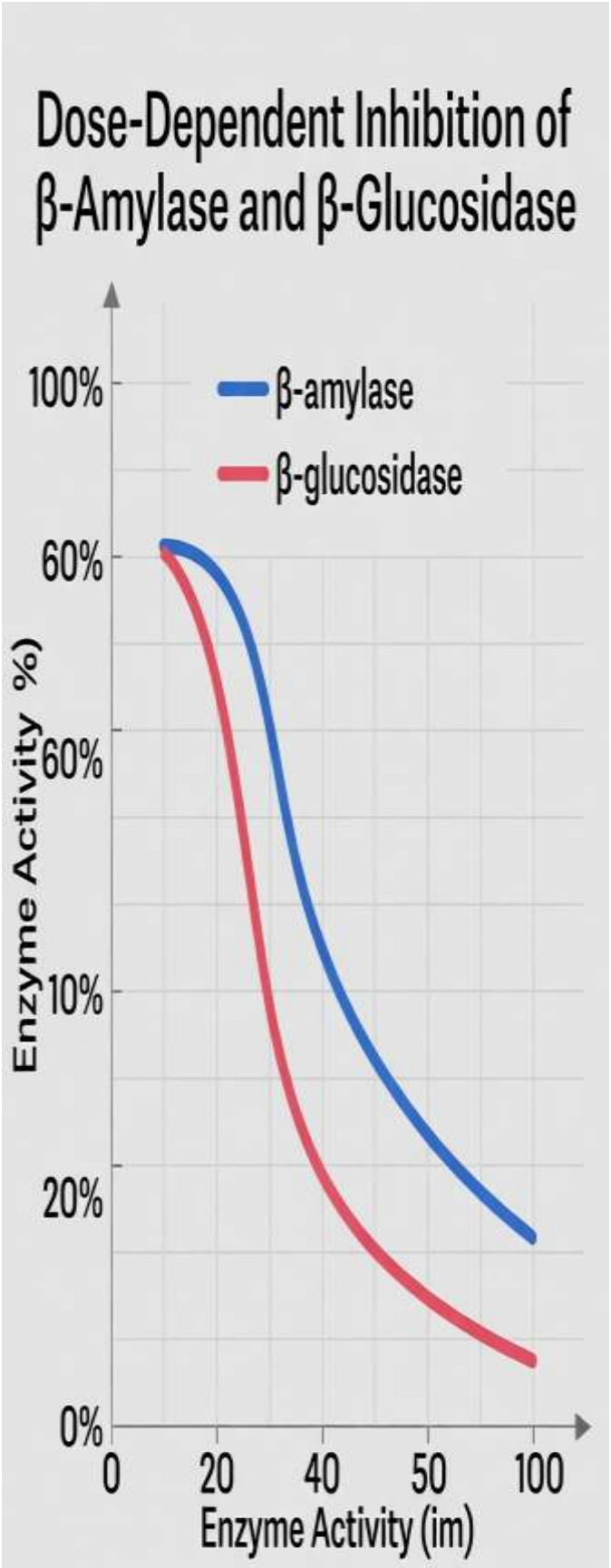

Figure 7

Graph showing dose-dependent inhibition of α-amylase and α-glucosidase enzymes

8. DISCUSSION

8.1 Significance of Pharmacognostical Standardization

The pharmacognostical assessment confirmed the identity, purity, and authenticity of the chosen medicinal plants. The macroscopic and microscopic diagnostic characteristics observed in this research aligned with the standard descriptions found in pharmacognosy literature. These results verify that the plant materials used in the formulation were authentic and unadulterated, which is crucial for ensuring the therapeutic effectiveness and safety of herbal medicines.

Physicochemical parameters, including ash values, extractive values, and moisture content, were within acceptable ranges, indicating the high quality of the raw materials. Ash values, which reflect the content of inorganic matter, assist in detecting adulteration, while extractive values reveal the quantity of bioactive constituents soluble in solvents. The elevated water and alcohol extractive values suggest the presence of substantial amounts of polar and semi-polar phytochemicals responsible for biological activity.

8.2 Correlation of Phytochemical Constituents with Biological Activity

Initial phytochemical analysis identified flavonoids, phenolics, alkaloids, glycosides, terpenoids, and saponins. These compound groups are well-recognized for their antioxidant and antidiabetic effects. Flavonoids and phenolics function as free radical scavengers, safeguarding pancreatic β-cells from oxidative harm. Alkaloids and glycosides aid in regulating glucose metabolism, while saponins improve insulin sensitivity. The formulation's high levels of total phenolics and flavonoids are closely linked to its

strong antioxidant activity, as shown in DPPH, ABTS, and FRAP assays. These results reinforce the idea that antioxidant processes are crucial to the polyherbal formulation's antidiabetic action.

8.3 Importance of HPTLC Fingerprinting

HPTLC fingerprinting generated a distinct chromatographic pattern that can act as a dependable quality control indicator for the polyherbal formulation. The identification of specific peaks linked to curcumin, quercetin, gymnemic acid, and diosgenin verifies the presence of key bioactive phytoconstituents from the individual medicinal plants. The consistent chromatographic pattern demonstrates the formulation's uniformity and supports the application of HPTLC as a quick and effective method for the standardization of intricate polyherbal formulations.

8.4 Antioxidant Potential and its Role in Diabetes Management

The notable antioxidant activity demonstrated in the DPPH, ABTS, and FRAP assays indicates that the polyherbal formulation has strong capabilities for scavenging free radicals and reducing oxidative stress. Oxidative stress is a key contributor to β-cell dysfunction and insulin resistance in diabetes, making antioxidants crucial for preventing oxidative damage and enhancing glycemic control. The elevated antioxidant activity is likely due to the synergistic effects of phenolic and flavonoid compounds found in the chosen medicinal plants, which boosts the overall therapeutic effectiveness compared to using individual plant extracts.

8.5 Antidiabetic Potential and Mechanism of Action

The formulation exhibited strong inhibition of the enzymes α-amylase and α-glucosidase, which play a role in the digestion of carbohydrates and the absorption of glucose in the intestine. By blocking these enzymes, the formulation can slow down glucose release and help prevent postprandial hyperglycemia, an important therapeutic approach in managing diabetes. The yeast cell assay showed increased glucose uptake, indicating enhanced peripheral glucose utilization and suggesting that the formulation may have insulin-like effects. This activity could be attributed to the presence of gymnemic acids, charantin, trigonelline, and curcumin, all known for their blood sugar-lowering properties.

8.6 Synergistic Therapeutic Effect of Polyherbal Formulation

The synergistic therapeutic effects of combining various medicinal plants arise from multiple mechanisms, such as antioxidant properties, enzyme inhibition, and improved glucose absorption. This multi-target strategy offers benefits beyond those of single-drug treatments, which typically concentrate on one pathway. The current study's results confirm the traditional Ayurvedic practice of using polyherbal therapy and offer scientific backing for its efficacy in managing diabetes.

8.7 Comparison with Previous Studies

These findings align with earlier research that highlights the antidiabetic and antioxidant properties of specific medicinal plants included in the formulation. Nonetheless, this study offers a thorough and integrated analysis that merges pharmacognostical standardization, phytochemical profiling, HPTLC fingerprinting, and the evaluation of biological activity, a combination seldom documented in prior studies.

8.8 Limitations and Future Perspectives

While this study showed encouraging antioxidant and antidiabetic effects in vitro, additional in vivo animal research and clinical trials are necessary to verify therapeutic effectiveness and safety. Understanding the specific molecular mechanisms behind the antidiabetic effects could

be enhanced by isolating and characterizing individual active compounds.

9. CONCLUSION

The current research effectively set pharmacognostical benchmarks, HPTLC chromatographic profiles, antioxidant capabilities, and antidiabetic potential for a polyherbal formulation composed of specific native medicinal plants traditionally employed in Ayurvedic diabetes treatment. Pharmacognostical analysis verified the authenticity and purity of the plant materials, while physicochemical assessments confirmed the formulation's quality and stability. HPTLC fingerprinting yielded a distinct and consistent chromatographic pattern ideal for quality assurance and standardization. The formulation demonstrated notable antioxidant activity, which was linked to its high phenolic and flavonoid content. Additionally, it showed strong inhibition of α-amylase and α-glucosidase enzymes, along with improved glucose uptake, indicating significant antidiabetic potential. In summary, the results scientifically support the traditional application of these medicinal plants in Ayurvedic diabetes treatment and reveal that the polyherbal formulation has promising antioxidant and antidiabetic properties. This study provides a thorough framework for the standardization and assessment of polyherbal formulations and lays the groundwork for future pharmacological and clinical studies aimed at developing effective herbal antidiabetic treatments.

10. REFERENCES

1. World Health Organization. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. Geneva: WHO; 2007.

2. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 56th ed. Pune: Nirali Prakashan; 2014.

3. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall; 1998.

4. Evans WC. Trease and Evans Pharmacognosy. 16th ed. Saunders Elsevier; 2009.

5. International Diabetes Federation. IDF Diabetes Atlas. 10th ed. Brussels; 2021.

6. Patel DK, Kumar R, Laloo D, Hemalatha S. Natural medicines from plant source used for therapy of diabetes mellitus: An overview. Journal of Ethnopharmacology. 2012;142:1–16.

7. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. Journal of Ethnopharmacology. 2002;81:81–100.

8. Sethi A, Joshi K. Evaluation of polyherbal antidiabetic formulation for its synergistic activity. Journal of Pharmaceutical Sciences and Research. 2011;3:1232–1237.

9. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Springer; 2001.

10. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates using Folin–Ciocalteu reagent. Methods in Enzymology. 1999;299:152–178.

11. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995;28:25–30.

12. Kim YM, Jeong YK, Wang MH, Lee WY, Rhee HI. Inhibitory effect of pine extract on α-glucosidase activity. Nutrition. 2005;21:756–761.

13. Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (Bergenia ciliata). Food Chemistry. 2008;106:247–252.

14. Sabu MC, Kuttan R. Antidiabetic activity of medicinal plants and its relationship with antioxidant property. Journal of Ethnopharmacology. 2002;81:155–160.

15. Mukherjee PK. Quality Control of Herbal Drugs. Business Horizons Pharmaceutical Publishers; 2002.

16. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. Nirali Prakashan; 2013.

17. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 2014;4:177.

18. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine. 2001;8:401–409.

19. Venkatesh S, Reddy GD. Evaluation of antidiabetic activity of polyherbal formulation in experimental models. Indian Journal of Pharmacology. 2010;42:345–348.

20. Pandey A, Tripathi S. Concept of standardization, extraction and pre-phytochemical screening strategies for herbal drug. Journal of Pharmacognosy and Phytochemistry. 2014;2:115–119.