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Review Paper

Design Development and In-Vitro Characterization of a Standardized Antidiabetic Polyherbal Formulation

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The increasing global prevalence of diabetes and the limitations associated with conventional antidiabetic therapies, including adverse effects, high treatment costs, and limited efficacy against multifactorial disease mechanisms, have stimulated interest in plant-based therapeutic alternatives. Polyherbal formulations have emerged as promising candidates owing to their synergistic actions, multi-target therapeutic potential, and improved safety profile. This review comprehensively discusses the role of medicinal plants and standardized polyherbal formulations in the management of diabetes mellitus, with particular emphasis on their mechanisms of action, phytochemical constituents, and quality control considerations. The antidiabetic activities of important medicinal plants such as *Coccinia grandis*, *Salacia reticulata*, *Pterocarpus marsupium*, *Tinospora cordifolia*, and *Curcuma longa* are critically evaluated with respect to their hypoglycemic, antioxidant, anti-inflammatory, insulin-sensitizing, and enzyme inhibitory properties. Special attention is given to the inhibition of α -amylase and α -glucosidase enzymes as an effective strategy for controlling postprandial hyperglycemia. Furthermore, the review highlights the importance of standardization, phytochemical profiling, chromatographic characterization, and regulatory requirements to ensure the quality, safety, and reproducibility of herbal products. Emerging trends including metabolomics, network pharmacology, nanotechnology-based delivery systems, and evidence-based clinical validation are also discussed. The findings suggest that standardized polyherbal formulations possess considerable potential as safe, effective, and economical therapeutic options for diabetes management and warrant further clinical investigation for their integration into modern healthcare systems.

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INTRODUCTION

1.1. Global Epidemiology and Socioeconomic Burden

Diabetes mellitus (DM) has reached pandemic proportions in the 21st century, shifting from an age-related metabolic condition to a global public health crisis. Epidemiological data indicates that as of 2021, approximately 537 million adults globally were affected by diabetes. This number is projected to reach 643 million by 2030 and 783 million by 2045.

South Asia faces a disproportionately high burden, with India frequently designated as the global capital of diabetes. The country's diabetic population exceeds 77 million and is projected to reach 134 million by 2045. This rapid increase is driven by a combination of factors, including swift urban migration, dietary transitions toward refined carbohydrates, increasingly sedentary lifestyles, and a distinct genetic predisposition characterized by metabolic phenotypes with lower body mass index (BMI) thresholds for insulin resistance.

The disease causes significant microvascular and macrovascular complications, accounting for approximately 6.7 million deaths annually. The global financial burden associated with diabetes exceeded USD 966 billion in healthcare expenditures in 2021 alone, illustrating its severe economic impact.

1.2. Cellular Pathophysiology of Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (T2DM) is defined by peripheral tissue insulin resistance combined with progressive pancreatic β -cell secretory failure. The underlying pathology can be understood through the "ominous octet" paradigm, which involves multiple dysfunctional physiological systems:

- Attenuated glucose uptake in skeletal muscle.

- Unrestrained hepatic gluconeogenesis and glycogenolysis.
- Accelerated adipocyte lipolysis elevating free fatty acids (FFAs).
- Diminished incretin effect within the gastrointestinal tract.
- Hyperglucagonemia secondary to pancreatic α -cell dysfunction.
- Enhanced renal glucose reabsorption via sodium-glucose cotransporters.
- Neurotransmitter dysregulation in the central nervous system.
- Progressive beta-cell apoptosis.

At the molecular level, insulin resistance involves post-receptor signaling defects within peripheral targets. These are characterized by inhibitory serine phosphorylation of Insulin Receptor Substrate-1 (IRS-1), which impairs downstream activation of Phosphatidylinositol 3-Kinase (PI3K) and protein kinase B (Akt). Consequently, the translocation of Glucose Transporter-4 (GLUT-4) storage vesicles to the plasma membrane is disrupted, preventing glucose entry into myocytes and adipocytes.

1.3. Clinical Significance of Postprandial Hyperglycemia

Postprandial hyperglycemia (PPH), defined as a plasma glucose excursion exceeding 140 mg/dL two hours after a meal, is an independent risk factor for cardiovascular disease and all-cause mortality, regardless of fasting plasma glucose levels. Data from the Diabetes Epidemiology Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study confirmed that post-load glucose concentrations predict cardiovascular mortality more accurately than fasting blood glucose.

The mechanism linking PPH to macrovascular damage involves transient, high-amplitude glucose spikes that overwhelm the mitochondrial electron transport chain. This induces a state of



acute oxidative stress, which reduces nitric oxide (NO) bioavailability and triggers endothelial dysfunction. These events promote leukocyte adhesion, accelerate advanced glycation end-product (AGE) cross-linking within the vascular matrix, and stimulate platelet hyperreactivity, leading to atherothrombotic complications. Consequently, targeting carbohydrate-hydrolyzing enzymes in the gastrointestinal tract to flatten postprandial glucose peaks represents an essential therapeutic strategy for reducing macrovascular risk.

1.4. Limitations of Synthetic Antidiabetic Monotherapy

While modern synthetic agents provide necessary glycemic control, their long-term clinical use is constrained by significant therapeutic limitations and adverse event profiles:

- **Biguanides (Metformin):** Frequently associated with gastrointestinal side effects, including abdominal pain, persistent diarrhea, and flatulence in up to 30% of patients. They are also contraindicated in patients with renal impairment eGFR 30 mL/min 1.73 m² due to the risk of lactic acidosis.
- **Sulfonylureas (Glimepiride, Glibenclamide):** Associated with a high risk of severe hypoglycemia and weight gain 2to 5kg. Over time, they can also accelerate secondary pancreatic exhaustion by forcing beta-cell secretion.
- **alpha-Glucosidase Inhibitors (Acarbose, Voglibose):** Often cause significant abdominal distension, flatulence, and diarrhea. These side effects result from the fermentation of undigested carbohydrates by colonic microflora, which often leads to poor patient compliance.
- **The Single-Target Paradigm:** Synthetic monotherapies typically follow a "one disease, one target" model, making them less

effective at addressing the multifactorial pathogenesis of T2DM.

1.5 Rationale for Polyherbal Formulation and Quality Standardization

Polyherbalism leverages multi-component botanical mixtures to achieve therapeutic synergy, targeting multiple physiological pathways simultaneously while using lower, less toxic doses of individual herbs. The interactions within a polyherbal formulation (PHF) typically function via distinct mechanisms:

- **Pharmacodynamic Synergy:** Active phytoconstituents engage multiple complementary targets, such as inhibiting digestive enzymes while concurrently enhancing peripheral insulin sensitivity or protecting beta-cell architecture.
- **Pharmacokinetic Synergy:** Specific plant matrix molecules can enhance the bioavailability, gastrointestinal absorption, or metabolic stability of co-administered bioactive compounds.

However, translating traditional polyherbal knowledge into modern evidence-based pharmaceuticals requires rigorous quality standardization. Because botanical raw materials naturally vary due to geographical, climatic, seasonal, and processing factors, establishing chemical profiles and strict quality controls is essential to ensure batch-to-batch uniformity, safety, and reproducible clinical efficacy.

2.Plant profile:

Medicinal plants constitute the fundamental components of polyherbal formulations used in the management of Type 2 diabetes mellitus (T2DM). Unlike synthetic drugs that generally target a single biochemical pathway, medicinal plants contain diverse phytoconstituents capable of exerting complementary and synergistic pharmacological effects. Numerous experimental,



preclinical, and clinical investigations have demonstrated that these plants possess antihyperglycemic, antioxidant, anti-inflammatory, antihyperlipidemic, insulin-sensitizing, and β -cell protective activities.

The further strengthened the scientific evidence supporting the use of standardized medicinal plants in diabetes management through advanced phytochemical characterization, molecular docking, network pharmacology, metabolomics, and randomized clinical studies. Consequently, several medicinal plants have emerged as key ingredients in standardized polyherbal formulations owing to their multitarget mechanisms of action.

The antidiabetic polyherbal formulation evaluated in this research comprises five validated medicinal plants. The scientific rationales and chemical markers for each ingredient are detailed below:

***Coccinia grandis* (L.) Voigt (Family: Cucurbitaceae)**

- **Vernacular Nomenclature:** Ivy Gourd, Tindora, Kundru.
- **Anatomical Part Utilized:** Leaves.
- **Phytochemical Blueprint:** Rich in structural flavonols (primarily quercetin and kaempferol), triterpenoids, surface-active saponins, and specific secondary alkaloids.
- **Antidiabetic Mechanism:** Functions as a natural insulin-mimetic agent. It activates insulin receptor tyrosine kinase signaling, which increases GLUT-4 expression and glucose uptake in peripheral tissues. Additionally, it helps suppress hepatic gluconeogenesis by downregulating Glucose-6-Phosphatase G6Pase activity.

2.2 *Salacia reticulata* Thw. (Family: Celastraceae)

- **Vernacular Nomenclature:** Saptrangi, Salacia.

- **Anatomical Part Utilized:** Roots.

- **Phytochemical Blueprint:** Characterized by thiosugar sulfonium sulfates (salacinol and kotalanol), polyphenols, catechins, and the xanthone glycoside mangiferin.
- **Antidiabetic Mechanism:** Salacinol and kotalanol act as potent competitive inhibitors of intestinal α -glucosidase enzymes. Their affinity for the enzyme catalytic site reduces oligosaccharide breakdown, helping control postprandial blood glucose spikes. Mangiferin also activates the Adenosine Monophosphate-Activated Protein Kinase (AMPK) pathway, which supports improved insulin sensitivity.

2.3 *Pterocarpus marsupium* Roxb. (Family: Fabaceae)

- **Vernacular Nomenclature:** Indian Kino, Vijaysar.
- **Anatomical Part Utilized:** Heartwood.
- **Phytochemical Blueprint:** High concentrations of stilbenoids (pterostilbene), structural flavonoids (epicatechin), and polymeric condensed tannins.
- **Antidiabetic Mechanism:** Epicatechin has been shown to support the survival and restoration of pancreatic beta-cells, helping to maintain insulin secretion. Pterostilbene acts as an agonist for Peroxisome Proliferator-Activated Receptor Alpha (PPAR-alpha) and Gamma (PPAR-gamma), which helps lower circulating lipid profiles and reduce peripheral insulin resistance.

2.4 *Tinospora cordifolia* (Willd.) Hook. f. & Thoms. (Family: Menispermaceae)

- **Vernacular Nomenclature:** Giloy, Guduchi.
- **Anatomical Part Utilized:** Stem.
- **Phytochemical Blueprint:** Contains benzyloisoquinoline alkaloids (berberine, tinosporin), diterpene lactones



(tinosporaside), and immunomodulatory polysaccharides.

- **Antidiabetic Mechanism:** Berberine activates AMPK pathways, leading to increased glycolysis, reduced hepatic glucose output, and enhanced GLUT-4 expression in skeletal muscle tissues. The diterpenoids help suppress inflammatory signaling, protecting pancreatic tissue from cytokine-mediated damage.

2.5 *Curcuma longa* L. (Family: *Zingiberaceae*)

- **Vernacular Nomenclature:** Turmeric, Haldi.
- **Anatomical Part Utilized:** Rhizome.
- **Phytochemical Blueprint:** Contains curcuminoid complex (curcumin, demethoxycurcumin, bisdemethoxycurcumin) and volatile essential monoterpenes.
- **Antidiabetic Mechanism:** Curcumin reduces oxidative stress by scavenging free radicals and upregulating endogenous antioxidant enzymes (such as Superoxide Dismutase and Catalase). It also downregulates the NF- κ B inflammatory pathway, which helps preserve beta-cell function and limit the progression of insulin resistance.

3. Experimental Methodology and Quality Control:

Table 1: authentication table for extraction and plant parts

Plant	Family	Plant Part Used	Voucher Specimen No.*
<i>Coccinia grandis</i>	Cucurbitaceae	Leaves	CG-2025-01
<i>Salacia reticulata</i>	Celastraceae	Roots	SR-2025-02
<i>Pterocarpus marsupium</i>	Fabaceae	Heartwood	PM-2025-03
<i>Tinospora cordifolia</i>	Menispermaceae	Stem	TC-2025-04
<i>Curcuma longa</i>	Zingiberaceae	Rhizome	CL-2025-05

3.2. Hydroalcoholic Maceration Extraction Kinetics

Each powdered plant material was subjected to extraction by maceration using 70% ethanol for 72 hours with intermittent shaking. The extracts were

The experimental design followed a structured phase model to ensure accurate extraction, rigorous phytochemical screening, and pharmacopoeial standardization of the final capsule dosage form.

Phase 1: Botanical Sourcing & Authentication

Phase 2: Sieve 40 Processing & Hydroalcoholic Maceration

Phase 3: Vacuum Evaporation & TLC/HPTLC Standardization

Phase 4: Powder Matrix Optimization

Phase 5: Quality Assurance Testing & In-Vitro Assay Analysis

3.1. Botanical Authentication and Raw Material Processing

Dry plant parts were procured from Shridhanwantari Herbs Pvt. Ltd. and authenticated by Dr. S. R. Patil, Head of the Department of Botany at Mahatma Gandhi College, Nashik, Maharashtra, India. Voucher specimens (CG-2025-01 through CL-2025-05) were deposited in the institutional herbarium. The materials were shade-dried at ambient temperatures (25-30°C) to prevent the thermal degradation of volatile or heat-sensitive components. Dried samples were milled into a coarse powder using a mechanical grinder and passed through an official sieve No. 40 to ensure uniform particle size before extraction.

filtered and concentrated using a rotary evaporator under reduced pressure. The dried extracts were stored in desiccators until further use ^[32].



Principle of Maceration

Maceration is a solid-liquid extraction technique in which coarsely powdered plant material is soaked in a suitable solvent for a specified period to allow the diffusion of soluble phytoconstituents from plant cells into the extraction medium.

The driving force of extraction is the concentration gradient established between the intracellular phytoconstituents and the extraction solvent. The solvent penetrates the cellular matrix, dissolves the active constituents, and transports them into the surrounding medium.

This method is particularly suitable for thermolabile phytoconstituents that may undergo degradation when exposed to elevated temperatures.

Procedure

The authenticated plant materials namely *Coccinia grandis* leaves, *Salacia reticulata* roots, *Pterocarpus marsupium* heartwood, *Tinospora cordifolia* stem and *Curcuma longa* rhizome were thoroughly washed with distilled water to remove adhering dirt and foreign matter. The plant materials were shade dried at room temperature (25–30°C) until a constant weight was obtained in order to prevent degradation of thermolabile phytoconstituents. The dried materials were coarsely powdered using a mechanical grinder and passed through sieve number 40 to obtain uniform particle size distribution. The powders were stored in airtight containers until extraction.

An accurately weighed quantity of each powdered plant material was transferred separately into clean and dry glass containers. Sufficient quantity of **70% v/v ethanol** was added to completely immerse the powdered drug material. The containers were tightly closed and kept at room

temperature for a period of **72 hours** for maceration. During the extraction period, intermittent shaking was performed at regular intervals to facilitate solvent penetration into the cellular matrix and to enhance the diffusion of soluble phytoconstituents into the extraction medium.

After completion of the maceration period, the extracts were filtered initially through muslin cloth followed by filtration using Whatman filter paper to remove insoluble plant residues and obtain a clear filtrate. The filtrates obtained were concentrated under reduced pressure using a rotary evaporator to remove the extraction solvent without exposing the extracts to elevated temperatures. The concentrated extracts were further dried to obtain semisolid or dry extracts and subsequently stored in desiccators until further use for phytochemical screening, standardization, and formulation studies.

3.3. Phytochemical Screening

Preliminary phytochemical screening of each extract was carried out to detect the presence of:

- Alkaloids
- Flavonoids
- Glycosides
- Tannins
- Saponins
- Phenolic compounds

Standard qualitative methods were employed.

3.4. Systematic Qualitative Phytochemical Screenings

Standard qualitative tests were applied to the hydroalcoholic extracts to profile the secondary metabolites:

Table 2 : Major Phytochemicals and Their Antidiabetic Activities

Phytochemical Class	Representative Compounds	Major Pharmacological Effects
Flavonoids	Quercetin, Kaempferol, Rutin	Antioxidant, insulin sensitization



Alkaloids	Berberine, Magnoflorine	AMPK activation, glucose uptake
Phenolic acids	Gallic acid, Chlorogenic acid	Antioxidant, anti-inflammatory
Terpenoids	Curcumin, Ursolic acid	β -cell protection, PPAR- γ activation
Saponins	Gymnemic acid, Charantin	Insulin secretion, enzyme inhibition
Tannins	Catechin, Ellagitannins	α -Amylase inhibition, antioxidant
Glycosides	Salacinol, Kotalanol	α -Glucosidase inhibition

3.4.1. Alkaloids (Mayer's Test)

Alkaloids are naturally occurring basic nitrogen-containing compounds that react with alkaloidal reagents to produce insoluble complexes. Mayer's reagent is commonly employed for the qualitative identification of alkaloids in plant extracts. The formation of a cream or pale-yellow precipitate following the addition of Mayer's reagent indicates the presence of alkaloids.

Procedure

Approximately 2 mL of the hydroalcoholic plant extract was transferred into a clean test tube. The extract was acidified with a few drops of dilute hydrochloric acid and filtered when necessary to obtain a clear solution. Subsequently, 2–3 drops of Mayer's reagent were added slowly to the acidified extract. The contents were mixed gently and allowed to stand for 2–3 minutes. The mixture was then examined for the development of turbidity or precipitate.

Table 3 : observation table for alkaloids

Observation	Inference
Cream or pale-yellow precipitate formed	Alkaloids present
No precipitate formed	Alkaloids absent

3.4.2. Flavonoids (Shinoda Reduction Assay)

A 2 mL sample of the extract was mixed with small magnesium turnings, followed by the slow addition of 3 drops of concentrated Hydrochloric Acid (HCl) along the test tube wall. The formation of a deep pink, magenta, or orange color within 5 minutes indicates the reduction of flavones or flavonols into flavylum salts.

Flavonoids constitute one of the largest and most extensively studied classes of naturally occurring polyphenolic compounds, with more than 8,000 structurally diverse molecules identified in higher plants. They are widely distributed in fruits, vegetables, cereals, medicinal herbs, and beverages such as tea and wine, where they contribute to pigmentation, plant defense, and protection against environmental stress. In medicinal plants, flavonoids are considered among the principal bioactive constituents responsible for a broad spectrum of pharmacological activities, including antioxidant, anti-inflammatory,

antimicrobial, anticancer, cardioprotective, neuroprotective, and antidiabetic effects. Common flavonoids with well-documented antidiabetic potential include **quercetin, kaempferol, myricetin, rutin, luteolin, and apigenin**, all of which are frequently reported in medicinal plants used in traditional systems of medicine. These compounds exhibit remarkable therapeutic efficacy owing to their ability to modulate multiple molecular targets involved in glucose metabolism and insulin signaling.

The antidiabetic effects of flavonoids are mediated through several complementary molecular mechanisms that collectively improve glucose homeostasis. One of the most important mechanisms is the activation of **5'-adenosine monophosphate-activated protein kinase (AMPK)**, a key cellular energy sensor that enhances glucose uptake, suppresses hepatic gluconeogenesis, and promotes fatty acid oxidation. Flavonoids also facilitate the



translocation of **glucose transporter-4 (GLUT-4)** to the plasma membrane in skeletal muscle and adipose tissues, thereby increasing peripheral glucose utilization and improving insulin sensitivity. Furthermore, these compounds reduce oxidative stress by scavenging reactive oxygen species (ROS) and enhancing endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Through inhibition of the **nuclear factor-kappa B (NF- κ B)** signaling pathway, flavonoids suppress the production of pro-inflammatory cytokines including tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β), thereby reducing chronic inflammation associated with insulin resistance.

3.4.3. Deoxysugar Glycosides (Keller-Killiani Assay)

A 2 mL portion of the extract was dissolved in 2 mL of glacial acetic acid containing 1 drop of 5% ferric chloride (FeCl₃) solution. This mixture was carefully layered over 1 mL of concentrated sulfuric acid (H₂SO₄). The appearance of a reddish-brown or brown ring at the liquid interface, changing to a blue-green upper layer, indicates the presence of digitoxose deoxysugars. The Keller–Killiani test is a widely used qualitative phytochemical assay for the detection of cardiac glycosides, particularly those containing deoxysugar moieties such as digitoxose. The principle of the test is based on the reaction of deoxysugars with ferric ions in the presence of concentrated sulfuric acid, resulting in the formation of a characteristic colored complex. For the analysis, approximately 2 mL of the hydroalcoholic plant extract was mixed with 2 mL of glacial acetic acid containing one drop of 5% ferric chloride (FeCl₃) solution. Subsequently, 1 mL of concentrated sulfuric acid (H₂SO₄) was carefully added along the inner wall of the test tube

to form a separate lower layer without disturbing the upper solution. The development of a reddish-brown or brown ring at the junction of the two liquid layers, followed by the appearance of a bluish-green coloration in the upper acetic acid layer, indicated the presence of deoxysugar-containing glycosides. The absence of these characteristic color changes was considered a negative result, indicating that glycosides were not present in the extract. This test is routinely employed during preliminary phytochemical screening to identify glycosidic constituents in medicinal plants.

3.3.4 Tannins (Ferric Chloride Complexation Assay)

The Ferric Chloride test is a widely employed qualitative phytochemical assay for the detection of tannins, which are naturally occurring polyphenolic compounds known for their antioxidant, antimicrobial, and antidiabetic properties. The principle of the test is based on the ability of phenolic hydroxyl groups present in tannins to form colored coordination complexes with ferric ions (Fe³⁺). For the analysis, approximately 2 mL of the hydroalcoholic plant extract was transferred into a clean test tube, and 3 drops of freshly prepared 5% ferric chloride (FeCl₃) solution were added. The mixture was gently shaken and observed for the development of characteristic color changes. The appearance of a deep blue-black coloration indicated the presence of hydrolyzable tannins, whereas the formation of a greenish-black or dark green color confirmed the presence of condensed tannins (proanthocyanidins). The absence of any characteristic color change indicated that tannins were not present in the extract. This test is routinely used during preliminary phytochemical screening to identify tannin-containing medicinal plants and to support their quality evaluation.



3.3.5 Saponins (Froth Generation Assay)

The Froth Generation Test is a widely employed qualitative phytochemical assay for the detection of saponins, a class of naturally occurring glycosides characterized by their surface-active and foaming properties. The principle of the test is based on the ability of saponins to reduce the surface tension of aqueous solutions, resulting in the formation of a stable and persistent froth upon vigorous agitation. For the analysis, approximately 2 mL of the hydroalcoholic plant extract was transferred into a graduated test tube and diluted with 5 mL of deionized water. The mixture was shaken vigorously for 2 minutes to facilitate foam formation and subsequently allowed to stand undisturbed for 15 minutes. The persistence of a stable, honeycomb-like froth measuring at least 1 cm in height was considered indicative of the presence of saponin glycosides, whereas the absence of persistent foam or the rapid disappearance of froth indicated a negative result. Owing to its simplicity, rapidity, and reliability, the Froth Generation Test is routinely employed during preliminary phytochemical screening to identify saponin-containing medicinal plants.

3.3.6 Phenolic Formations (Ferric Chloride Phenolate Test)

The Ferric Chloride Test is a standard qualitative of phenolic compounds in medicinal plant extracts. The principle of the test is based on the formation of colored coordination complexes between ferric ions (Fe^{3+}) and the hydroxyl groups of phenolic compounds. These complexes produce characteristic color changes that indicate the presence of phenolic constituents. For the analysis, approximately 2 mL of the hydroalcoholic plant

extract was transferred into a clean test tube, followed by the addition of three drops of freshly prepared 5% ferric chloride (FeCl_3) solution. The mixture was gently shaken and observed for the development of characteristic color changes. The appearance of an intense blue, green, violet, or bluish-green coloration confirmed the presence of phenolic compounds, whereas the absence of any noticeable color change indicated a negative result. This assay is widely employed in preliminary phytochemical screening due to its simplicity, rapidity, and sensitivity, providing an effective method for the qualitative identification of phenolic constituents that contribute significantly to the antioxidant and therapeutic properties of medicinal plants.

3.4 Chromatographic Fingerprinting (TLC and HPTLC Protocols)

Thin-Layer Chromatography (TLC) and High-Performance Thin-Layer Chromatography (HPTLC) were performed using pre-coated silica gel 60 F₂₅₄ aluminum sheets 10 cm times 10cm, layer thickness 0.2 mm, Merck). Extracts and reference markers were applied as 6 mm bands using a CAMAG Linomat V applicator at a delivery rate of 150 nL/s.

Chromatographic separation was performed in a twin-trough developing chamber pre-saturated with targeted mobile phases for 20 minutes at 25°C. The migration distance was fixed at 80 mm. Developed plates were dried with hot air and analyzed under short-wave [254nm](#) and long-wave [366nm](#) ultraviolet light using a CAMAG Reprostar system.

Table 4: Plant profile and their protocols

Plant Profile Target	Quantitative Chemical Marker Reference	Optimized Mobile Solvent System Ratio (v/v)	Optical Detection Wavelength	Expected Retardation Factor (Rf) Range
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<i>Curcuma longa</i>	Curcumin	Chloroform : Methanol (95:5)	366nm	0.52 – 0.58
<i>Salacia reticulata</i>	Mangiferin	Ethyl Acetate : Formic Acid : Acetic Acid : Water (10:1.1:1.1:2.6)	254 nm	0.32 – 0.38
<i>Pterocarpus marsupium</i>	Epicatechin	Toluene : Ethyl Acetate : Formic Acid (5:4:1)	280 nm/388nm	0.40 – 0.46
<i>Tinospora cordifolia</i>	Alkaloid Matrix (Tinosporin)	Toluene : Ethyl Acetate : Diethylamine (7:2:1)	254 nm	0.28 – 0.35
<i>Coccinia grandis</i>	Quercetin	Toluene : Ethyl Acetate : Formic Acid (5:4:1)	366 nm	0.48 – 0.55

To visualize specific chemical zones, plates were treated with specialized derivatization reagents: Natural Product Reagent for structural flavonoids, Dragendorff's Reagent for alkaloid bands, Vanillin-Sulfuric Acid for triterpenoid segments, and Anisaldehyde-Sulfuric Acid for curcuminoid spots.

3.4.1. Scientific Basis for Combining Selected Medicinal Plants

The development of standardized polyherbal formulations for the management of type 2 diabetes mellitus (T2DM) is supported by the principle of phytotherapeutic synergy, in which multiple medicinal plants with complementary pharmacological activities act simultaneously on different pathological pathways involved in disease progression. Unlike monotherapy, polyherbal formulations provide a multitarget therapeutic strategy capable of addressing the complex pathophysiology of T2DM. *Gymnema sylvestre* primarily enhances pancreatic insulin secretion and promotes regeneration of β -cells, thereby improving endogenous glucose regulation. *Salacia reticulata* reduces postprandial hyperglycemia by inhibiting intestinal α -glucosidase and α -amylase enzymes, resulting in delayed carbohydrate digestion and glucose absorption. *Curcuma longa* exerts potent antioxidant and anti-inflammatory effects by suppressing oxidative stress and inflammatory mediators that contribute to insulin resistance and

diabetic complications. *Tinospora cordifolia* provides cytoprotective effects on pancreatic β -cells while enhancing insulin secretion and improving antioxidant defense mechanisms. *Pterocarpus marsupium* improves insulin sensitivity, facilitates glucose utilization, and supports pancreatic β -cell function. *Momordica charantia* exhibits insulin-mimetic activity and enhances peripheral glucose uptake, thereby contributing to improved glycemic control. *Syzygium cumini* suppresses carbohydrate-hydrolyzing enzymes and possesses antioxidant properties that help regulate postprandial blood glucose levels. *Trigonella foenum-graecum* delays intestinal glucose absorption through its high soluble fiber content while improving insulin sensitivity and overall glycemic control.

Collectively, these medicinal plants exert complementary and synergistic pharmacological effects by targeting multiple interconnected mechanisms involved in T2DM, including impaired insulin secretion, insulin resistance, excessive intestinal glucose absorption, oxidative stress, chronic inflammation, dyslipidemia, and postprandial hyperglycemia. Consequently, the integration of these botanicals into standardized polyherbal formulations offers a scientifically justified and holistic therapeutic approach with the potential to improve glycemic control, minimize disease progression, and reduce the risk of diabetes-associated complications. This multitarget strategy provides a strong



pharmacological foundation for the continued development and clinical evaluation of standardized polyherbal antidiabetic formulations.

3.5 Industrial Capsule Formulation Design Matrix

The polyherbal capsule formulation was prepared using standardized dried hydroalcoholic extracts of the selected medicinal plants following good pharmaceutical manufacturing practices to ensure uniformity and reproducibility. Initially, the dried extracts were passed through sieve No. 60 to obtain a uniform particle size distribution, thereby improving blend homogeneity and flow characteristics. The accurately weighed extracts were blended in predetermined proportions using a clean porcelain mortar to achieve a homogeneous powder mixture. Microcrystalline cellulose (MCC) was incorporated as an inert

diluent and filler to standardize the final capsule fill weight while enhancing compressibility and flow properties. Purified talc was added as a glidant to reduce interparticle friction and improve powder flow during capsule filling, whereas magnesium stearate was incorporated during the final 3 minutes of blending as a lubricant to minimize friction between the powder blend and processing equipment without causing over-lubrication that could adversely affect capsule performance. The optimized blend was mixed thoroughly to ensure uniform distribution of all components and subsequently filled into hard gelatin capsules (Size 0) using a manual capsule-filling machine. The prepared capsules were then stored in airtight containers under appropriate environmental conditions until further evaluation for quality control parameters and in vitro antidiabetic studies.

Table 5 : ingredients and their target batches

Generic Ingredient	Plant Source Matrix	Functional Category	Target Batch Mass (mg/Capsule)
<i>Coccinia grandis</i> Extract	Leaves	Active Ingredient	100
<i>Salacia reticulata</i> Extract	Roots	Active Ingredient	100
<i>Pterocarpus marsupium</i> Extract	Heartwood	Active Ingredient	100
<i>Tinospora cordifolia</i> Extract	Stem	Active Ingredient	50
<i>Curcuma longa</i> Extract	Rhizome	Active Ingredient	50
Microcrystalline Cellulose (MCC)	—	Inert Diluent / Binder	q.s. to 500
Magnesium Stearate	—	Hydrophobic Lubricant	5
Purified Talc	—	Glidant Matrix	5

3.6 Formulation Optimization Trials Matrix

Five distinct experimental batches (F1 to F5) were developed by systemically adjusting the internal

proportions of the plant extracts and excipients while keeping the final target capsule fill weight constant at 500 mg.

Table 6: Formulation trial batches

Formulation Component Ingredients (mg/Capsule)	F1	F2	F3 (Optimized)	F4	F5
<i>Coccinia grandis</i> Leaf Extract	95	100	90	105	110
<i>Salacia reticulata</i> Root Extract	95	105	120	100	95
<i>Pterocarpus marsupium</i> Wood Extract	105	100	110	95	100
<i>Tinospora cordifolia</i> Stem Extract	55	50	60	50	50



<i>Curcuma longa</i> Rhizome Extract	50	45	40	50	45
Microcrystalline Cellulose (MCC)	75	80	70	95	95
Magnesium Stearate Lubricant	5	5	5	5	5
Purified Talc Glidant	20	15	10	5	0
Total Composite Form Mass (mg)	500	500	500	500	500

3.7 Pre-Filling Powder Micromeritic Modeling

The pre-formulation evaluation of powder blends, commonly referred to as **micromeritic analysis**, is a critical quality control step in the development of solid dosage forms, particularly capsules and tablets. Micromeritic properties determine the flow behavior, packing characteristics, compressibility, and handling of powder mixtures during pharmaceutical manufacturing. Since polyherbal formulations comprise multiple plant extracts with varying particle sizes, densities, and moisture contents, evaluation of micromeritic characteristics is essential to ensure uniform die filling, consistent capsule weight, improved content uniformity, and reproducible product quality. Poor flowability and excessive cohesiveness may result in segregation of ingredients, weight variation, and inconsistent drug content, thereby affecting the therapeutic efficacy and quality of the final formulation. Therefore, standard pharmacopoeial micromeritic parameters are routinely assessed before capsule filling to optimize formulation performance.

3.7.1 Angle of Repose

The angle of repose is one of the most important micromeritic parameters used to evaluate the flowability of pharmaceutical powders before formulation into solid dosage forms. It is defined as the maximum angle formed between the surface of a freely flowing powder heap and the horizontal plane. The principle of the test is based on the relationship between gravitational forces and interparticle friction. Powders with lower angles of repose exhibit better flow characteristics due to reduced friction between particles, whereas higher angles indicate increased cohesiveness and poor

flowability. In pharmaceutical formulation, good flow properties are essential to ensure uniform die filling, consistent capsule weight, and reproducible content uniformity during manufacturing.

An angle of repose less than 30° indicates excellent flowability, 30–35° indicates good flow, 35–40° indicates fair flow, and values greater than 40° suggest poor flow properties. Consequently, the angle of repose serves as a simple, reliable, and widely accepted parameter for assessing the handling characteristics and suitability of powder blends for capsule filling and other pharmaceutical manufacturing processes.

3.7.2 Bulk Density

Bulk density is an important micromeritic parameter used to evaluate the packing characteristics of pharmaceutical powders before formulation into solid dosage forms. It is defined as the mass of a powder divided by its bulk volume, including the void spaces between particles. Bulk density is influenced by particle size, shape, porosity, moisture content, and packing arrangement. It is an essential parameter for determining capsule size, storage requirements, and the flow behavior of powder blends during pharmaceutical manufacturing. Powders with appropriate bulk density exhibit improved handling characteristics and contribute to uniform die filling and content uniformity.

An accurately weighed mass of the blend (M) was introduced into a clean, graduated 100 mL cylinder to record the initial un-compacted bulk volume (V_{bulk}):

$$\text{Bulk Density} = \frac{\text{Bulk Volume of Powder}}{\text{Mass of Powder}}$$



3.7.3 Tapped Density

Tapped density is an important micromeritic parameter used to evaluate the packing characteristics and compressibility of pharmaceutical powder blends. It is defined as the mass of a powder divided by its volume after mechanical tapping, which causes the particles to rearrange into a more compact arrangement by reducing the interparticle void spaces. Tapped density provides valuable information regarding the packing behavior of powders and is widely used in conjunction with bulk density to determine flowability, compressibility, and suitability for capsule filling or tablet compression. Variations between bulk density and tapped density reflect the degree of particle rearrangement and interparticle cohesion within the powder blend.

The cylinder was mechanically tapped using an automated tap density tester until a stable volume plateau was achieved (V_{tapped}):

$\text{Tapped Density} = \text{Tapped Volume of Powder} / \text{Mass of Powder}$

3.7.4 Hausner Ratio

The Hausner ratio is a widely accepted micromeritic parameter used to evaluate the flowability and cohesiveness of pharmaceutical powder blends. It is defined as the ratio of tapped density to bulk density and serves as an indirect measure of interparticle friction. The principle of the Hausner ratio is based on the extent of volume reduction that occurs when a powder is mechanically tapped. Powders exhibiting low interparticle friction undergo minimal volume reduction after tapping and therefore possess lower Hausner ratio values, indicating good flow characteristics. Conversely, higher Hausner ratio values suggest increased cohesiveness and poor flowability, which may adversely affect powder handling and dosage uniformity during pharmaceutical manufacturing.

The Hausner Ratio serves as an indicator of interparticle friction and powder flowability, calculated as:

Hausner Ratio = Tapped Density / Bulk Density

The polyherbal powder blend showed a Hausner Ratio of **1.18**, indicating **good flow properties** and low interparticle friction, making it suitable for capsule filling operations.

3.7.5 Carr's Compressibility Index

The compressibility percentage represents an indirect measure of flow dynamics, derived using the following equation:

$\text{Carr's Index (\%)} = [(\text{Tapped Density} - \text{Bulk Density}) / \text{Tapped Density}] \times 100$

The polyherbal powder blend exhibited a Carr's Index value of **16.36%**, indicating **good flow properties** and acceptable compressibility, making it suitable for capsule filling operations.

3.8 Post-Filling Evaluation Parameters

The quality, safety, and therapeutic performance of herbal capsules depend not only on the characteristics of the powder blend but also on the quality of the final filled dosage form. Following capsule filling, a series of post-filling evaluation tests are performed according to official pharmacopoeial standards such as the Indian Pharmacopoeia (IP), United States Pharmacopeia (USP), and British Pharmacopoeia (BP). These quality control parameters ensure batch-to-batch uniformity, compliance with regulatory requirements, and suitability of the formulation for commercial manufacturing.

3.8.1 Weight Variation Test

The weight variation test is a routine pharmacopoeial quality control procedure used to assess the uniformity of capsule fill weight. It provides an indirect measure of dose consistency and the efficiency of the capsule-filling process. Uniform capsule weight is essential to ensure that each unit delivers a consistent amount of the



formulation, thereby contributing to therapeutic efficacy and patient safety.

Twenty capsules from each experimental batch were randomly sampled and weighed individually on a calibrated analytical balance. The mean capsule weight was determined, and individual weights were compared against the mean value to confirm compliance with the permitted percentage deviation (pm 7.5%).

Acceptance limits are specified by pharmacopoeial standards and depend on the average capsule weight. For hard gelatin capsules, the formulation complies with the test if not more than two capsules deviate from the prescribed percentage limits and none exceeds twice the allowable deviation. A weight variation within $\pm 7.5\%$ is generally considered acceptable for capsules of appropriate fill weight, indicating good filling accuracy and batch uniformity.

3.8.2 Disintegration Performance

Disintegration Test

The disintegration test is an essential quality control parameter used to evaluate the ability of capsule formulations to break down into smaller particles under simulated physiological conditions. It provides an indication of the time required for the capsule shell to rupture and release its contents, thereby influencing the subsequent dissolution and bioavailability of the active constituents. For immediate-release hard gelatin capsules, rapid and complete disintegration is critical to ensure prompt drug release and therapeutic efficacy.

According to the Indian Pharmacopoeia (IP), United States Pharmacopeia (USP), and British Pharmacopoeia (BP), the test is performed using a standard disintegration test apparatus consisting of a six-tube basket-rack assembly. One capsule is placed in each tube, and the assembly is immersed in a suitable disintegration medium, typically 900

mL of purified water or simulated gastric fluid, maintained at $37 \pm 2^\circ\text{C}$ to simulate physiological conditions. The apparatus is operated at the specified frequency, allowing the basket to move vertically through the medium until complete disintegration of the capsules occurs.

The disintegration time is recorded as the time required for each capsule to break apart completely, with no palpable core remaining on the mesh screen, except for fragments of the capsule shell or other insoluble materials. In accordance with pharmacopoeial specifications, conventional hard gelatin capsules should completely disintegrate within 30 minutes, unless otherwise stated in the individual monograph. Compliance with this test confirms the suitability of the capsule formulation for efficient drug release and supports its overall pharmaceutical quality.

3.8.3 Analytical Content Uniformity

Content uniformity is a critical quality control parameter used to verify that each capsule contains the intended amount of active pharmaceutical ingredient or phytochemical marker within the specified pharmacopoeial limits. This test is particularly important for herbal capsule formulations, where variability in phytochemical composition may occur due to differences in raw materials, extraction processes, and manufacturing conditions. Ensuring uniform distribution of active constituents is essential for maintaining consistent therapeutic efficacy, safety, and batch-to-batch reproducibility.

Ten individual capsules were sampled from each batch, and their internal powder contents were collected and dissolved in the hydroalcoholic solvent matrix. The samples were filtered and analyzed using UV-Visible spectrophotometry to confirm that the active marker concentrations remained within the acceptable pharmacopoeial range 85% to 105%.



3.8.4 Critical Moisture Analysis

Moisture content is an important quality control parameter for herbal capsule formulations, as excessive moisture can adversely affect the physical stability, flow properties, microbial quality, and chemical integrity of phytoconstituents. Appropriate moisture levels are essential to maintain capsule shell integrity, prevent degradation of active compounds, minimize microbial proliferation, and ensure an acceptable shelf life.

Moisture content is commonly determined using the Loss on Drying (LOD) method, as described in the Indian Pharmacopoeia (IP), United States Pharmacopoeia (USP), and British Pharmacopoeia (BP). In this procedure, a known quantity of the capsule contents is accurately weighed and dried in a hot air oven maintained at $105 \pm 2^\circ\text{C}$ until a constant weight is achieved. The decrease in weight corresponds to the moisture and other volatile substances lost during the drying process. Moisture content was evaluated by drying a known weight of the capsule powder in a hot air oven at 105°C until a stable weight was reached. The percentage weight loss was calculated to verify compliance with the specified maximum limit ($< 5.0\%$)

4. In-Vitro Antidiabetic Enzyme Kinetics

4.1 Porcine Pancreatic alpha-Amylase Inhibition Assay (DNSA Method)

The alpha-amylase inhibitory activity was evaluated using the 3,5-Dinitrosalicylic Acid (DNSA) colorimetric method. Porcine pancreatic alpha-amylase (1 U/mL) was dissolved in a 0.02 M sodium phosphate buffer solution (pH 6.9), containing 0.006 M NaCl. Experimental concentrations of the polyherbal extract (10 to 100mg/ml) were prepared.

A 200 μL volume of the extract was combined with 200 μL of the enzyme solution and pre-

incubated at 37°C for 10 minutes to allow the phytoconstituents to interact with the enzyme active site. The enzymatic reaction was initiated by adding 200 μL of a 1% w/v soluble starch solution, followed by incubation at 37°C for 15 minutes.

The reaction was stopped by adding 400 μL of the DNSA reagent, and the mixture was heated in a boiling water bath for 5 minutes to drive the reduction of DNSA into 3-amino-5-nitrosalicylic acid by the free reducing sugars. After cooling to room temperature, the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. A control solution without the plant extract and a blank solution without the enzyme were evaluated concurrently. The percentage inhibition was calculated using the equation below, and the IC_{50} value was determined via non-linear regression analysis:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

4.2 Intestinal alpha-Glucosidase Inhibition Assay (pNPG Method)

The alpha-glucosidase inhibition assay was performed using p-nitrophenyl-alpha-D-glucopyranoside (pNPG) as the substrate. Intestinal alpha-glucosidase (1 U/mL) was prepared in a .1 M phosphate buffer matrix (pH6.8).

A 50 μL volume of the polyherbal extract at various concentrations (10 to 100 $\mu\text{g/mL}$) was pre-incubated with 100 μL of the enzyme solution at 37°C for 10 minutes. The reaction was initiated by adding 50 μL of a 5m pNPG substrate solution, and the mixture was incubated at 37°C for 20 minutes. The reaction was terminated by adding 1 mL of a 0.1M sodium carbonate Na_2CO_3 solution, which shifts the pH and stops enzymatic activity. The absorbance of the released yellow p-nitrophenol product was measured at 405nm using a spectrophotometer.



Control and blank samples were evaluated using the same procedure, and the percentage inhibition was calculated as:

5. Critical Academic Discussion

5.1 Phytochemical Screening Profiling

Qualitative screenings confirmed that the hydroalcoholic solvent system successfully extracted a broad spectrum of secondary metabolites from the five plant sources.

Table 7: Phytochemical screening profiling

Phytochemical Class Group	<i>Coccinia grandis</i> Leaves	<i>Salacia reticulata</i> Roots	<i>Pterocarpus marsupium</i> Wood	<i>Tinospora cordifolia</i> Stem	<i>Curcuma longa</i> Rhizome
Alkaloids (Mayer's)	–	–	–	+	–
Flavonoids (Shinoda)	++	+	+	+	++
Glycosides (Keller)	+	++	+	+	+
Tannins (FeCL ₃)	+	+	++	–	+
Saponins (Froth)	+	+	–	+	–
Phenolics (Complex)	++	++	+	+	++
Terpenoids / Diterpenes	+	–	+	++	+
Total Polyphenols Matrix	++	++	++	+	++

(–) Not Detected; (+) Detected; (++) Strongly Detected

The chemical profile of *Tinospora cordifolia* was notable for its diverse constituents, showing positive results across almost all assessed classes, including secondary alkaloids and diterpenoids. *Curcuma longa* and *Coccinia grandis* exhibited high concentrations of structural flavonoids and phenolic matrices, which are known to support cellular antioxidant pathways. *Pterocarpus marsupium* demonstrated high concentrations of condensed tannins, which can form non-covalent hydrophobic bonds with enzyme surfaces, contributing to the inhibition of carbohydrate-hydrolyzing enzymes.

5.2 Pre-Filling Micromeritic Flow Characteristics

The composite powder blend containing the five extracts mixed with excipients displayed highly

favorable micromeritic properties. An angle of repose of 28.2⁰C falls within the optimal pharmacopoeial range 25⁰C to 30⁰C, indicating excellent free-flowing properties. This flowability is supported by the bulk density 0.46g/mL and tapped density 0.55 g/mL, yielding a Carr's Compressibility Index of 16% and a Hausner ratio of 1.18. These values suggest minimal interparticle friction and low cohesiveness, which help prevent mechanical bridging or uneven die filling during high-speed capsule manufacturing.

5.3 Post-Filling Evaluation Parameters

All five manufactured batches (F1 to F5) met the strict quality control criteria outlined in the Indian Pharmacopoeia.



Table 8: Evaluation parameters

Batch Series Identity	Mean Capsule Weight (mg)	Weight Deviation Percentage	Disintegration Time Duration (min)	Residual Moisture Retention (%)	Content Uniformity Assay (%)	Quality Pass/Fail Status
F1	500	0.80%	8.5 ± 0.3	4.1 ± 0.09	97.8 ± 0.65	Pass
F2	503	0.99%	8.1 ± 0.2	4.4 ± 0.11	96.5 ± 0.72	Pass
F3	499	0.60%	7.8 ± 0.2	3.9 ± 0.08	98.5 ± 0.5	Pass
F4	504	1.19%	8.3 ± 0.3	5.0 ± 0.12	95.8 ± 0.81	Pass
F5	501	0.80%	8.8 ± 0.4	4.7 ± 0.10	97.2 ± 0.69	Pass

The weight variation test results demonstrated excellent uniformity, with all five batches falling well within the $\pm 7.5\%$ limits. This uniformity confirms that the powder blend possessed consistent flow properties during manufacturing.

The disintegration times ranged between 7.8 and 8.8 minutes, significantly faster than the maximum allowable pharmacopoeial limit of 30 minutes. This rapid breakdown is attributed to the optimized inclusion of Microcrystalline Cellulose (MCC), which acts as both a dry binder and a disintegrant by facilitating rapid water absorption and swelling within the capsule shell.

Batch F3 exhibited the lowest moisture retention at 3.9%, which helps protect against moisture-induced aggregation and limits microbial growth, supporting long-term formulation stability. Content uniformity values remained tightly grouped 95.8% to 98.5%, verifying consistent mixing and distribution of the active herbal extracts across all manufactured units.

5.4 Enzyme Inhibition Kinetics and Synergy Analysis

Inhibition of carbohydrate-digesting enzymes represents one of the most important therapeutic mechanisms by which medicinal plants exert

antihyperglycemic effects in the management of type 2 diabetes mellitus (T2DM). This approach primarily targets the intestinal enzymes α -amylase and α -glucosidase, which are responsible for the digestion and absorption of dietary carbohydrates. α -Amylase catalyzes the hydrolysis of complex starch molecules into smaller oligosaccharides, whereas α -glucosidase, located at the brush border of the small intestine, further hydrolyzes these oligosaccharides into glucose for intestinal absorption. Inhibition of these enzymes delays carbohydrate digestion, slows glucose absorption, and attenuates postprandial blood glucose excursions, thereby improving overall glycemic control. Several phytoconstituents have been identified as potent natural inhibitors of these enzymes, including salacinol and kotalanol from *Salacia reticulata*, gymnemic acids from *Gymnema sylvestre*, as well as tannins, flavonoids, and catechins present in various medicinal plants. These bioactive compounds reduce the rate of glucose release into the bloodstream without causing abrupt fluctuations in blood glucose levels, thereby offering an effective strategy for the management of postprandial hyperglycemia. Consequently, inhibition of α -amylase and α -glucosidase constitutes a key pharmacological



mechanism underlying the antidiabetic efficacy of standardized polyherbal formulations and contributes significantly to their therapeutic potential in T2DM management.

5.4.1 Porcine Pancreatic α -Amylase Inhibition

The polyherbal formulation exhibited strong, concentration-dependent inhibitory effects on porcine pancreatic α -amylase across the tested concentration range 20 to 100 $\mu\text{g/mL}$.

[α -Amylase Inhibition Assay Profile]

The formulation achieved a maximum inhibition of 82% at 100 $\mu\text{g/mL}$. Regression analysis established an IC_{50} value of 52.00 $\mu\text{g/mL}$. While slightly less potent than the pure chemical reference drug Acarbose $\text{IC}_{50} = 40.00\mu\text{g/mL}$, this level of inhibition is notable for a complex, crude plant extract matrix.

The formulation demonstrated higher inhibitory activity against intestinal α -glucosidase than against α -amylase.

[α -Glucosidase Inhibition Assay Profile]

Inhibition increased from 32% at 20 $\mu\text{g/mL}$ to 88% at the maximum concentration of 100 $\mu\text{g/mL}$. The calculated IC_{50} value was 44.00 $\mu\text{g/mL}$ comparing favorably with Acarbose $\text{IC}_{50} = 33.33\mu\text{g/mL}$.

This specific inhibitory profile—showing stronger activity against α -glucosidase relative to α amylase—is clinically advantageous. Moderate inhibition of α -amylase combined with potent inhibition of α -glucosidase slows the final step of oligosaccharide digestion. This reduces the accumulation of undigested starch in the lower bowel, potentially minimizing the gastrointestinal side effects, such as flatulence and bloating, commonly observed with synthetic inhibitors like Acarbose.

5.4.2 Intestinal α -Glucosidase Inhibition

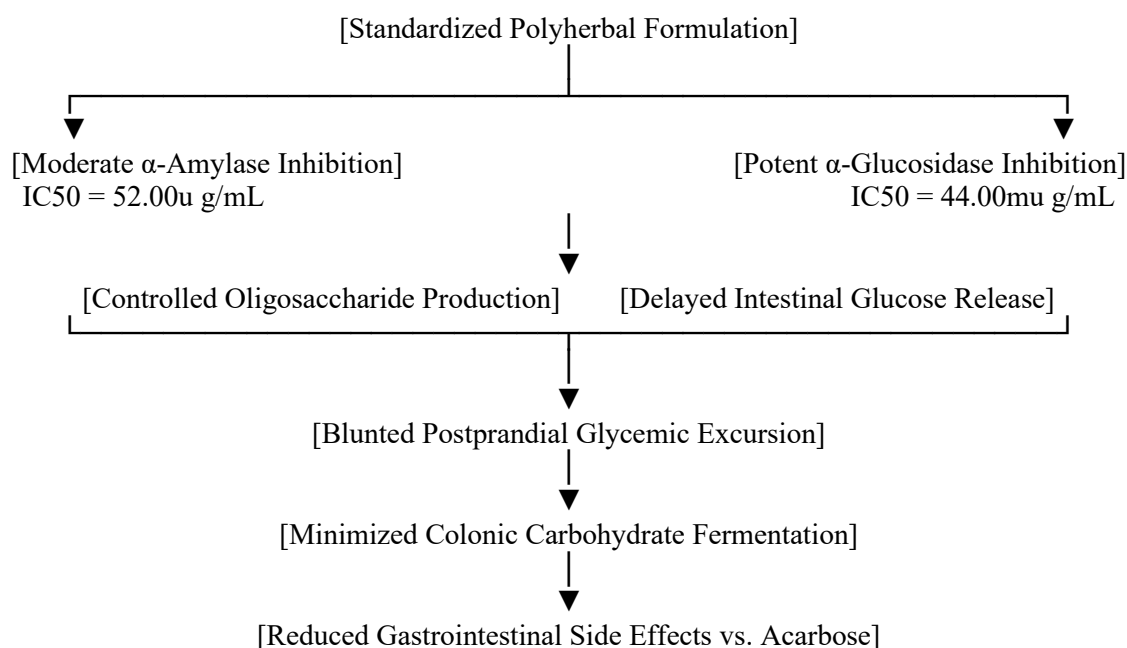


Fig 1: Flowchart for standardized polyherbal formulation

5.5 Mechanisms of Phytochemical Synergy

The high enzyme-inhibitory performance of Batch F3 is likely due to its optimized extract ratio,



which contains the highest proportion of *Salacia reticulata* extract (120 mg) among all tested configurations. This composition enables synergistic interactions between several classes of phytoconstituents:

- **Competitive Active-Site Blocking:** Thiosugar sulfonium sulfates (salacinol and kotalanol) from *Salacia reticulata* mimic the oxocarbenium ion transition state of carbohydrate substrates, binding directly to the active sites of intestinal α -glucosidases to block substrate access.
- **Allosteric / Non-Competitive Binding:** Structural flavonoids (such as quercetin from *Coccinia grandis* and curcumin from *Curcuma longa*) interact with allosteric or secondary binding pockets on the enzymes, altering their conformation and reducing catalytic efficiency.
- **Steric Hindrance via Complexation:** Condensed tannins and polyphenols from *Pterocarpus marsupium* form non-covalent, hydrophobic bonds and hydrogen-bonding networks with the surface amino acid residues of the enzymes, causing structural precipitation and further reducing enzyme activity.

6. Future Outlook

This research successfully demonstrates the design, formulation scaling, quality control standardization, and in-vitro therapeutic validation of a standardized polyherbal capsule formulation for managing diabetes mellitus. The experimental work systematically advanced through extraction kinetics, quantitative pre-filling micromeritic profiling, and post-filling pharmacopoeial validation.

FUTURE PERSPECTIVES

The growing global burden of type 2 diabetes mellitus (T2DM) has intensified the need for safe,

effective, and affordable therapeutic alternatives. Standardized polyherbal formulations have emerged as promising candidates owing to their multitarget pharmacological actions and favorable safety profiles. However, translating these formulations from traditional practice to evidence-based clinical therapeutics requires substantial advances in scientific research, pharmaceutical development, and regulatory frameworks. Future investigations should therefore focus on establishing robust scientific evidence to support the quality, efficacy, and safety of polyherbal antidiabetic formulations.

One of the foremost priorities is the development of globally standardized polyherbal formulations through rigorous quality control and standardization protocols. Variability in plant species, geographical origin, cultivation conditions, harvesting time, and extraction methods can significantly influence phytochemical composition and therapeutic efficacy. The establishment of internationally accepted quality standards, validated analytical methods, and standardized manufacturing practices will ensure batch-to-batch consistency and improve the reproducibility of clinical outcomes.

Advances in phytochemical research are expected to facilitate the identification of novel bioactive compounds and phytochemical biomarkers responsible for the therapeutic effects of medicinal plants. Modern analytical techniques, including high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC–MS/MS), gas chromatography–mass spectrometry (GC–MS), and nuclear magnetic resonance (NMR) spectroscopy, can provide comprehensive phytochemical fingerprinting and support the discovery of new antidiabetic constituents. The identification of reliable biomarkers will further strengthen quality



assurance and aid in the standardization of herbal formulations.

Emerging omics technologies, particularly metabolomics, proteomics, transcriptomics, and genomics, offer unprecedented opportunities to elucidate the complex molecular mechanisms underlying the therapeutic actions of polyherbal medicines. Integration of these technologies with systems biology approaches can reveal interactions between multiple phytoconstituents and biological targets, enabling a deeper understanding of synergistic pharmacological effects. Such comprehensive molecular profiling will facilitate the development of mechanism-based herbal therapeutics and improve the prediction of therapeutic responses.

Artificial intelligence (AI), machine learning, and computational pharmacology are expected to revolutionize herbal drug discovery and formulation development. AI-assisted algorithms can analyze large phytochemical databases, predict herb-herb interactions, optimize formulation compositions, identify novel therapeutic targets, and accelerate the screening of bioactive compounds. Computational tools may also facilitate network pharmacology studies, molecular docking, and pharmacokinetic modeling, thereby reducing the time and cost associated with conventional drug development.

Future research is also likely to promote the transition toward personalized herbal medicine and precision phytotherapy. Individual differences in genetic makeup, metabolic profile, gut microbiota composition, environmental factors, and lifestyle significantly influence treatment outcomes in patients with T2DM. Personalized polyherbal formulations tailored to specific patient characteristics may enhance therapeutic efficacy while minimizing adverse effects. The integration of pharmacogenomics and precision medicine with traditional herbal therapy represents an

innovative approach for individualized diabetes management.

Despite encouraging preclinical and preliminary clinical findings, there remains a critical need for large-scale, multicenter, randomized controlled clinical trials to establish the long-term efficacy and safety of standardized polyherbal formulations. Future clinical studies should include diverse populations, longer follow-up periods, standardized outcome measures, and comprehensive safety assessments. Additionally, investigations into herb-drug interactions, pharmacokinetics, pharmacodynamics, and long-term toxicity are essential to facilitate the safe integration of herbal medicines with conventional antidiabetic therapies.

Sustainable pharmaceutical manufacturing should also be prioritized through the adoption of environmentally friendly extraction and processing technologies. Green extraction techniques, including supercritical fluid extraction, ultrasound-assisted extraction, microwave-assisted extraction, and enzyme-assisted extraction, can improve extraction efficiency while minimizing solvent consumption, environmental impact, and production costs. These technologies are expected to enhance the quality, purity, and sustainability of herbal products.

Nanotechnology represents another promising area for future research. Nano-herbal formulations, such as phytosomes, liposomes, polymeric nanoparticles, nanoemulsions, and solid lipid nanoparticles, have demonstrated the potential to improve the solubility, stability, bioavailability, targeted drug delivery, and controlled release of phytoconstituents. Such advanced drug delivery systems may significantly enhance the therapeutic effectiveness of herbal medicines while reducing dosage requirements and systemic adverse effects.



The global acceptance of herbal medicines will also depend on the harmonization of international regulatory guidelines governing quality, safety, efficacy, manufacturing, and post-marketing surveillance. Collaborative efforts among regulatory agencies, academic institutions, pharmaceutical industries, and healthcare organizations are necessary to establish uniform standards for the registration and commercialization of standardized herbal products. Furthermore, robust pharmacovigilance systems and the generation of real-world evidence through electronic health records, patient registries, and observational studies will provide valuable information regarding the long-term safety, effectiveness, and utilization patterns of polyherbal formulations in routine clinical practice.

Overall, the future of standardized polyherbal formulations lies in the successful integration of traditional medicinal knowledge with modern pharmaceutical sciences, advanced analytical technologies, systems biology, artificial intelligence, nanotechnology, and evidence-based clinical research. Such multidisciplinary approaches have the potential to transform herbal medicine into a scientifically validated, globally accepted, and patient-centered therapeutic platform for the prevention and management of type 2 diabetes mellitus and other chronic metabolic disorders.

CONCLUSION

The global increase in the prevalence of type 2 diabetes mellitus has highlighted the urgent need for safe, effective, and affordable therapeutic strategies that address the complex pathophysiology of the disease. Standardized polyherbal formulations have emerged as promising alternatives to conventional antidiabetic therapies because of their multitarget mechanisms of action, synergistic phytochemical interactions,

and comparatively favorable safety profiles. The medicinal plants discussed in this review, including *Coccinia grandis*, *Salacia reticulata*, *Pterocarpus marsupium*, *Tinospora cordifolia*, and *Curcuma longa*, contain diverse bioactive constituents capable of improving glycemic control through inhibition of α -amylase and α -glucosidase enzymes, enhancement of insulin sensitivity, protection of pancreatic β -cells, reduction of oxidative stress, and modulation of inflammatory pathways.

This review further emphasizes that the therapeutic success of polyherbal formulations depends not only on the selection of appropriate medicinal plants but also on rigorous quality standardization, phytochemical characterization, and adherence to established pharmaceutical and regulatory guidelines. Advanced analytical techniques such as HPLC, HPTLC, LC-MS/MS, metabolomics, and systems biology have significantly improved the scientific validation and reproducibility of herbal medicines, facilitating their transition from traditional remedies to evidence-based therapeutics.

Despite encouraging preclinical and emerging clinical evidence, further well-designed multicenter randomized clinical trials are essential to establish long-term efficacy, safety, pharmacokinetic behavior, herb-drug interactions, and optimal dosage regimens. The integration of artificial intelligence, network pharmacology, nanotechnology-based drug delivery systems, and precision phytotherapy is expected to accelerate the development of next-generation standardized herbal medicines.

Overall, standardized polyherbal formulations represent a scientifically rational and holistic approach for the management of type 2 diabetes mellitus. By combining traditional medicinal knowledge with modern pharmaceutical sciences, these formulations have the potential to become effective complementary or adjunct therapeutic



options that improve patient outcomes while supporting the global movement toward evidence-based, personalized, and sustainable healthcare.

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