

In Silico and *In Vitro* Evaluation of Polyherbal Formulation for Antidiabetic Activity Using Molecular Docking Approaches

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Abstract

Diabetes mellitus remains one of the most prevalent metabolic disorders worldwide, necessitating continuous research into safe, effective, and affordable therapeutic interventions. Plant-based medicines have been used for centuries in traditional systems to manage hyperglycemia, and their bioactive constituents offer diverse mechanisms of action against multiple diabetogenic targets. The present study reports a comprehensive *in silico* and *in vitro* evaluation of a polyherbal formulation (PHF) composed of standardized extracts of *Momordica charantia* (bitter melon), *Trigonella foenum-graecum* (fenugreek), *Gymnema sylvestre* (gymnema), *Cinnamomum verum* (cinnamon), *Curcuma longa* (turmeric), and *Azadirachta indica* (neem). Eight formulations (PHF1–PHF8) were designed using a systematic trial approach, optimizing excipient concentrations and active herbal ratios. Molecular docking was performed against key antidiabetic targets including α -glucosidase (PDB: 3TOP), dipeptidyl peptidase-4 (DPP-4; PDB: 1X70), peroxisome proliferator-activated receptor-gamma (PPAR- γ ; PDB: 3DZY), and protein tyrosine phosphatase 1B (PTP1B; PDB: 2HNP). Phytoconstituents including charantin, gymnemagenin, 4-hydroxyisoleucine, cinnamaldehyde, curcumin, and nimbolide demonstrated binding energies ranging from -7.43 to -9.15 kcal/mol, surpassing or comparable to standard drugs. Drug-likeness and ADME profiling confirmed Lipinski's rule-of-five compliance for all major phytoconstituents. *In vitro* antidiabetic activity was assessed by α -glucosidase inhibition assay and α -amylase inhibition assay; the optimized formulation PHF8 exhibited an IC_{50} of 48.2 ± 1.0 μ g/mL and 52.4 ± 1.2 μ g/mL respectively, values closely approximating those of acarbose ($IC_{50} = 42.6 \pm 0.9$ μ g/mL and 45.8 ± 1.1 μ g/mL). Antioxidant activity via DPPH radical scavenging and cytotoxicity evaluation by MTT assay further validated the safety profile of the formulation. Physicochemical characterization including FTIR, DSC, and dissolution studies confirmed compatibility and stability. These findings collectively justify the potential of the polyherbal formulation PHF8 as a promising phytopharmaceutical candidate for antidiabetic therapy, warranting further translational evaluation.

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Keywords: Polyherbal formulation; Diabetes mellitus; Molecular docking; α -Glucosidase inhibition; PPAR- γ ; DPP-4; Charantin; Gymnemenin; *In silico*; ADME profiling; Antidiabetic; Phytopharmaceuticals

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1. Introduction

Diabetes mellitus (DM) is a chronic, multifactorial metabolic disorder characterized by persistent hyperglycemia resulting from absolute or relative deficiency of insulin secretion, impaired insulin action, or both.¹ The International Diabetes Federation (IDF) Diabetes Atlas reported approximately 537 million adults (20–79 years) living with diabetes in 2021, with projections estimating 783 million by 2045, representing a staggering global burden.² Type 2 diabetes mellitus (T2DM) accounts for over 90% of all cases and is closely associated with obesity, sedentary lifestyle, and genetic predisposition.³

The pathophysiology of T2DM involves multifaceted disruptions in carbohydrate and lipid metabolism, inflammatory cascades, oxidative stress, pancreatic beta-cell dysfunction, and peripheral insulin resistance.⁴ Key enzymes implicated in postprandial hyperglycemia management include α -glucosidase and α -amylase, which are responsible for the hydrolysis of dietary carbohydrates into absorbable monosaccharides. Inhibition of these enzymes represents an established therapeutic strategy to blunt the postprandial glycemic surge.⁵ Additionally, DPP-4, PTP1B, and PPAR- γ constitute critical molecular targets that modulate insulin sensitivity, incretin signaling, and adipogenesis, respectively.⁶

Contemporary antidiabetic pharmacotherapy encompasses biguanides, sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT-2 inhibitors, and GLP-1 receptor agonists.⁷ However, these agents are frequently associated with dose-dependent adverse effects including hypoglycemia, hepatotoxicity, gastrointestinal disturbances, cardiovascular complications, and weight gain, limiting long-term compliance, particularly in resource-limited settings.⁸ This has intensified global interest in plant-derived medicines as complementary or alternative antidiabetic agents with potentially superior safety profiles and multiple mechanisms of action.⁹

Ethnopharmacology has documented numerous medicinal plants with antidiabetic efficacy across diverse traditional systems including Ayurveda, Traditional Chinese Medicine, and Unani.¹⁰ Among these, *Momordica charantia* (bitter melon), *Trigonella foenum-graecum* (fenugreek), *Gymnema sylvestre* (gymnema), *Cinnamomum verum* (cinnamon),

Curcuma longa (turmeric), and *Azadirachta indica* (neem) have demonstrated significant hypoglycemic, insulin secretagogue, and insulin-sensitizing activities in preclinical and clinical investigations.^{11,12}

Polyherbal formulations (PHFs) represent a strategic approach in phytomedicine wherein synergistic combinations of multiple plant extracts are designed to address the multifactorial etiology of complex diseases more effectively than individual plant extracts.¹³ The concept of synergy in PHFs is well recognized, wherein individual constituents at sub-therapeutic concentrations may collectively produce superior pharmacological effects via complementary mechanisms.¹⁴ Contemporary pharmaceutical technology facilitates the design of standardized, reproducible, and stable polyherbal tablet formulations with defined release characteristics.¹⁵

The advent of computational approaches has revolutionized early-stage drug discovery. Molecular docking enables the prediction of binding modes and affinities of bioactive phytoconstituents with target proteins with high throughput and minimal resource expenditure.¹⁶ *In silico* ADME (Absorption, Distribution, Metabolism, and Excretion) profiling tools such as SwissADME and pkCSM further facilitate the rapid assessment of pharmacokinetic parameters, thereby guiding formulation decisions.¹⁷ The integration of *in silico* predictions with *in vitro* validation creates a robust translational research framework consistent with the 3R principles (Replace, Reduce, Refine).¹⁸

Despite extensive individual investigations of the constituent herbs, limited research has evaluated systematically designed polyherbal tablet formulations integrating computational docking predictions with comprehensive *in vitro* pharmacological validation. The present study bridges this gap by designing eight PHF variants (PHF1–PHF8), performing molecular docking of key phytoconstituents against multiple antidiabetic targets, characterizing the formulations physicochemically, and assessing their antidiabetic potential through validated *in vitro* enzyme inhibition assays, antioxidant studies, and cytotoxicity testing.

2. Materials

Dried and authenticated plant materials were procured from standardized suppliers and subjected to taxonomic

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authentication by a qualified botanist at the Department of Pharmaceutical Sciences, King Faisal University. Herbarium voucher specimens were deposited for reference. Standardized dry extracts of *Momordica charantia* (bitter melon fruit, 5% charantin), *Trigonella foenum-graecum* (fenugreek seed, 40% saponins), *Gymnema sylvestre* (gymnema leaf, 25% gymnemic acids), *Cinnamomum verum* (cinnamon bark, 1% cinnamaldehyde), *Curcuma longa* (turmeric rhizome, 95% curcuminoids), and *Azadirachta indica* (neem leaf, 2% nimbolide) were obtained from Natural Remedies Pvt. Ltd., Bangalore, India, each accompanied by certificates of analysis.

Pharmaceutical excipients including microcrystalline cellulose (Avicel PH-102), magnesium stearate, colloidal silicon dioxide (Aerosil 200), and purified talc of pharmacopoeial grade were purchased from Merck KGaA (Darmstadt, Germany). α -Glucosidase (from *Saccharomyces cerevisiae*, EC 3.2.1.20), α -amylase (from *Aspergillus oryzae*, EC 3.2.1.1), p-nitrophenyl- α -D-glucopyranoside (pNPG), acarbose, starch soluble, 2,2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), potassium phosphate monobasic and dibasic, sodium carbonate, Folin-Ciocalteu reagent, gallic acid, quercetin, and sodium acetate were all procured from Sigma-Aldrich (St. Louis, MO, USA) and Hi-Media Laboratories, Mumbai, India. All chemicals and reagents used were of analytical grade unless stated otherwise.

For cell culture studies, HepG2 (human hepatocellular carcinoma) cells were obtained from the National Centre for Cell Science (NCCS), Pune, India. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin solution, and trypsin-EDTA were purchased from HiMedia Laboratories, Mumbai, India. Phosphate-buffered saline (PBS, pH 7.4) was prepared in-house from reagent-grade components.

For computational studies, three-dimensional crystal structures of target proteins were downloaded from the RCSB Protein Data Bank (<https://www.rcsb.org>): α -Glucosidase (PDB ID: 3TOP), DPP-4 (PDB ID: 1X70), PPAR- γ (PDB ID: 3DZY), and PTP1B (PDB ID: 2HNP). Ligand structures of all phytoconstituents were retrieved from the PubChem Compound Database (<https://pubchem.ncbi.nlm.nih.gov>) in SDF format and converted to PDB format using Open Babel v2.4.1. Molecular docking was conducted using AutoDock Vina v1.1.2, protein preparation with AutoDockTools v1.5.7, and

three-dimensional structure visualization with PyMOL v2.5 (Schrödinger, LLC). ADME predictions were obtained from SwissADME (www.swissadme.ch) and pkCSM (<https://biosig.lab.uq.edu.au/pkcsm>). Discovery Studio Visualizer v21.1 (Dassault Systèmes) was used for ligand–protein interaction analysis and visualization.

3. Methods

3.1. Preparation of Plant Extracts

Coarsely powdered plant materials were individually subjected to extraction by maceration in 70% ethanol (1:10 w/v) at room temperature for 72 hours with periodic agitation. The macerate was filtered through a Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40°C to obtain a semisolid mass. The concentrated extracts were further dried in a vacuum oven (40°C, 48 h) to obtain free-flowing powders. Percentage yield was calculated. Total phenolic content (TPC) was estimated by Folin-Ciocalteu method¹⁹ and total flavonoid content (TFC) by the aluminum chloride colorimetric method.²⁰ All extracts were standardized against their marker compounds by HPLC (Shimadzu LC-2030C, Japan) with a C18 column (250 × 4.6 mm, 5 μ m), using validated methods.²¹

3.2. Preliminary Phytochemical Screening

Qualitative phytochemical screening of all six plant extracts was performed using standard methods described by Harborne (1998)²² and Trease & Evans (2009)²³ to detect the presence of alkaloids (Mayer's, Wagner's, and Dragendorff's tests), flavonoids (Shinoda test), tannins (ferric chloride test), saponins (foam test), terpenoids (Salkowski test), phenols (ferric chloride test), glycosides (Keller-Kiliani test), and steroids (Liebermann-Burchard test). All tests were performed in triplicate and results recorded semi-quantitatively as absent (–), weakly present (+), moderately present (++) , or abundantly present (+++).

3.3. Polyherbal Formulation Design

Eight polyherbal tablet formulations (PHF1–PHF8) were designed by systematically varying the proportions of six herbal extracts alongside excipients (Table 1). The formulations were prepared by direct compression using a single punch tablet press (Chamunda Pharma Machinery, India). All ingredients were accurately weighed, passed through ASTM mesh #60, blended in a laboratory-scale blender for 15 minutes, and lubricated with magnesium stearate and talc for 5 minutes prior to compression. Tablets were compressed at a target weight of 460–550 mg with a die-punch set of 12 mm diameter.

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Table 1: Polyherbal Tablet Formulation Design (PHF1–PHF8) – Ingredient Composition per Tablet

Ingr.	PHF1	PHF2	PHF3	PHF4	PHF5	PHF6	PHF7	PHF8
Bitter Melon Ext. (mg)	100	150	100	150	100	150	100	150
Fenugreek Ext. (mg)	50	50	100	100	50	50	100	100
Gymnema Ext. (mg)	75	75	75	75	100	100	100	100
Cinnamon Ext. (mg)	25	25	25	25	50	50	50	50
Turmeric Ext. (mg)	20	20	20	20	40	40	40	40
Neem Ext. (mg)	30	30	30	30	30	30	60	60
Microcryst. Cell. (mg)	150	130	100	80	80	60	50	40
Magnesium Stearate (mg)	5	5	5	5	5	5	5	5
Aerosil (mg)	3	3	3	3	3	3	3	3
Talc (mg)	2	2	2	2	2	2	2	2
Total Weight (mg)	460	490	460	490	460	490	510	550

Ingr. = Ingredient; *Microcryst. Cell.* = Microcrystalline Cellulose (Avicel PH-102); all values represent weight in milligrams (mg) per tablet.

3.4. Pre-compression Characterization of Powder Blends

Powder blends of all eight formulations were evaluated for bulk density, tapped density, Carr's compressibility index (CI%), Hausner's ratio, angle of repose, and loss on drying (moisture content) prior to tablet compression. Bulk and tapped density were measured using a graduated measuring cylinder and a tap density tester (Electrolab ETD-1020, India).²⁴ Angle of repose was determined by the fixed funnel method. Carr's CI (%) was calculated using the formula: $CI = [(Tapped\ Density - Bulk\ Density) / Tapped\ Density] \times 100$. A CI below 15% indicates good flowability.²⁵

3.5. Post-compression Tablet Evaluation

Compressed tablets from each formulation were evaluated for: (a) Appearance and organoleptic properties; (b) Hardness using a Monsanto hardness tester (kg/cm²); (c) Friability using a Roche friabilator (Electrolab EF-2, India) at 25 rpm for 4 minutes, with the percentage weight loss calculated and acceptance criterion set at <1%²⁶; (d) Weight

variation test (n=20 tablets, USP method); (e) Thickness using a digital vernier caliper; (f) Disintegration test in phosphate buffer (pH 6.8, 37 ± 0.5°C) using a disintegration tester (Electrolab ED-2L, India); and (g) Drug content assay – tablets were dissolved in DMSO:ethanol (1:9 v/v), filtered, and analyzed spectrophotometrically. All measurements were performed in triplicate.

3.6. Dissolution Studies

Dissolution studies were performed using USP Apparatus II (paddle method) in 900 mL of phosphate buffer (pH 6.8) at 50 rpm and 37 ± 0.5°C (Electrolab TDT-08L, India). Aliquots (5 mL) were withdrawn at 5, 10, 15, 30, 45, 60, 90, and 120 minutes, filtered through a 0.45 µm membrane filter, and analyzed spectrophotometrically at their respective λ_{max} values. Withdrawn volumes were replaced with fresh medium. Cumulative percentage drug release (CDR) was calculated. Dissolution efficiency (DE%) at 60 minutes was compared across formulations.²⁷

3.7. Fourier-Transform Infrared (FTIR) Spectroscopy

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FTIR analysis was performed on pure herbal extracts, physical mixtures, and tablet powders using an FTIR spectrophotometer (Bruker ALPHA II, Germany) in the wavenumber range of 4000–400 cm^{-1} by the KBr pellet technique. Spectra were compared for drug–excipient compatibility by evaluating the presence, shift, or disappearance of characteristic absorption peaks. Peak assignments were made based on published reference spectra.²⁸

3.8. Differential Scanning Calorimetry (DSC)

DSC thermograms of individual extracts, physical mixtures, and representative formulation (PHF8) were recorded using a DSC analyzer (Mettler-Toledo DSC 3, Switzerland). Approximately 5–8 mg of each sample was placed in sealed aluminum pans and heated from 25°C to 300°C at a scanning rate of 10°C/min under nitrogen purge (50 mL/min). Endothermic and exothermic events were noted, and the thermograms were compared to assess compatibility.²⁹

3.9. Protein Preparation for Molecular Docking

Crystal structures of target proteins were downloaded from RCSB PDB. Proteins were prepared using AutoDockTools v1.5.7: water molecules were removed, polar hydrogen atoms were added, Kollman charges were assigned, and nonpolar hydrogens were merged. Co-crystallized ligands were removed. The prepared receptor files were saved in PDBQT format. Grid boxes were centered at the co-crystallized ligand coordinates or active site residues identified from literature. Grid spacing was set to 0.375 Å with box dimensions of 25 × 25 × 25 Å for all targets.³⁰

3.10. Ligand Preparation and Molecular Docking

3D structures of six phytoconstituents (charantin, 4-hydroxyisoleucine, gymnemagenin, cinnamaldehyde, curcumin, nimbolide) and two standard drugs (metformin and glipizide) were obtained from PubChem in SDF format, converted to PDB format by Open Babel, and prepared in PDBQT format using AutoDockTools. Molecular docking was performed using AutoDock Vina v1.1.2 with an exhaustiveness of 8. The binding energy (ΔG , kcal/mol) of the best pose was recorded. Hydrogen bond interactions and van der Waals contacts were analyzed using Discovery Studio Visualizer v21.1.³¹ Docking was validated by re-docking co-crystallized ligands and comparing root mean square deviation (RMSD) values (<2.0 Å was considered acceptable).³²

3.11. ADME and Drug-Likeness Prediction

Physicochemical parameters and ADME properties of phytoconstituents were predicted using SwissADME (www.swissadme.ch) and pkCSM. Evaluated parameters

included molecular weight (MW), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA), calculated LogP (iLOGP), topological polar surface area (TPSA), number of rotatable bonds, blood-brain barrier permeability, Caco-2 permeability, CYP450 inhibition potential, and aqueous solubility. Lipinski's rule-of-five (MW ≤500 Da, HBD ≤5, HBA ≤10, LogP ≤5) was used as the primary drug-likeness criterion.³³

3.12. *In Vitro* α -Glucosidase Inhibition Assay

The α -glucosidase inhibitory activity was assessed using a modified colorimetric method described by Tibbot and Skadsen (1996).³⁴ Briefly, 50 μL of test samples at five concentrations (25, 50, 100, 200, and 400 $\mu\text{g}/\text{mL}$ in DMSO:phosphate buffer 1:19 v/v) were mixed with 100 μL of α -glucosidase solution (0.5 U/mL in 0.1 M phosphate buffer, pH 6.8) in a 96-well microplate and pre-incubated at 37°C for 10 minutes. The reaction was initiated by adding 50 μL of 5 mM pNPG substrate solution, and the plate was incubated for 30 minutes at 37°C. The reaction was terminated by adding 50 μL of 0.1 M Na_2CO_3 solution. Absorbance was measured at 405 nm using a microplate reader (BioTek Epoch, USA). Acarbose was used as positive control. Inhibition (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$. IC_{50} values were determined from dose-response curves using nonlinear regression.³⁵

3.13. *In Vitro* α -Amylase Inhibition Assay

α -Amylase inhibitory activity was evaluated by the 3,5-dinitrosalicylic acid (DNS) colorimetric method.³⁶ Test samples (50 μL at 25–400 $\mu\text{g}/\text{mL}$) were incubated with 50 μL of α -amylase (0.5 U/mL in phosphate buffer, pH 6.9, with 6 mM NaCl) at 37°C for 10 minutes. Starch solution (100 μL , 1% w/v) was added and incubated at 37°C for 30 minutes. The reaction was stopped by adding 200 μL of DNS reagent, and the mixture was boiled for 5 minutes. After cooling, absorbance was read at 540 nm. Acarbose served as the positive control. Percentage inhibition and IC_{50} were calculated as described for the α -glucosidase assay.³⁷

3.14. DPPH Free Radical Scavenging Assay

Antioxidant activity was measured by DPPH radical scavenging assay adapted from Brand-Williams et al.³⁸ Briefly, 100 μL of test samples at concentrations of 25–400 $\mu\text{g}/\text{mL}$ were added to 100 μL of freshly prepared 0.1 mM DPPH solution in methanol in a 96-well plate. The plate was incubated in the dark for 30 minutes at room temperature. Absorbance was read at 517 nm using a microplate reader. Ascorbic acid was used as positive control. Percentage radical scavenging activity = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$. IC_{50} values were determined by nonlinear regression.³⁹

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3.15. *In Vitro* Cytotoxicity (MTT Assay)

Cytotoxicity of PHF8 was evaluated using MTT assay on HepG2 cells.⁴⁰ Cells were seeded at 1×10^4 cells per well in 96-well plates and allowed to adhere overnight at 37°C in 5% CO₂ atmosphere. Cells were treated with PHF8 extract at concentrations of 10–1000 µg/mL in complete DMEM containing ≤0.1% DMSO for 24 hours. Subsequently, 10 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4 hours. The formazan crystals formed were dissolved by adding 100 µL of DMSO, and absorbance was read at 570 nm. Cell viability (%) = (Asample / Acontrol) × 100. CC₅₀ (50% cytotoxic concentration) was calculated.⁴¹

3.16. Statistical Analysis

All experiments were performed in triplicate and results expressed as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism v9.0

(GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was applied to compare formulation groups. IC₅₀ values were determined by nonlinear regression analysis. A p-value of <0.05 was considered statistically significant.⁴²

4. Results

4.1. Phytochemical Screening

Preliminary phytochemical screening revealed the presence of diverse bioactive secondary metabolites across all six plant extracts. Flavonoids, phenols, and terpenoids were predominant across extracts, consistent with reported antidiabetic mechanisms involving enzyme inhibition and antioxidant activity (Table 2). Bitter melon and turmeric showed the highest phenolic content, while fenugreek and gymnema demonstrated abundant saponins, consistent with their documented hypoglycemic activities.

Table 2: Phytochemical Screening of Plant Extracts Used in Polyherbal Formulation

Phytochemical	Bitter Melon	Fenugreek	Gymnema	Cinnamon	Neem/Turmeric
Alkaloids	+	+	+	+	+
Flavonoids	+++	++	++	++	+++
Tannins	++	+	+	++	+
Saponins	++	+++	+++	+	+
Terpenoids	++	+	++	+++	++
Phenols	+++	++	++	+++	+++
Glycosides	++	++	++	+	++
Steroids	+	+	+	+	+

(+): Weakly present; (++) : Moderately present; (+++): Abundantly present; (-): Absent. Ext. = Extract.

4.2. Molecular Docking Results

Molecular docking studies were performed to predict the binding affinities and interaction profiles of six phytoconstituents against four antidiabetic target proteins. Results are summarized in Table 3 and illustrated in Figure 1. Gymnemagenin demonstrated the highest binding affinity towards PPAR-γ with a docking score of -9.15 kcal/mol, forming six hydrogen bonds with key residues Ser289 and

Tyr473, surpassing the reference compound metformin (-6.89 kcal/mol). Charantin showed strong binding to α-glucosidase (-8.74 kcal/mol), establishing critical hydrogen bond interactions with Asp408 and Arg213. Nimbolide also exhibited favorable binding to PPAR-γ (-8.67 kcal/mol). Docking re-validation confirmed RMSD values <1.8 Å for all targets, affirming the reliability of the docking protocol.

Table 3: Molecular Docking Results of Phytoconstituents against Antidiabetic Target Proteins

Ligand	Target Protein	Binding Energy (kcal/mol)	H-Bond Interactions	Key Residues
Charantin (Bitter Melon)	α-Glucosidase (PDB: 3TOP)	-8.74	4	Asp408, Arg213
4-Hydroxyisoleucine (Fenugreek)	DPP-4 (PDB: 1X70)	-7.92	5	Glu205, Tyr547

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Gymnemagenin (Gymnema)	PPAR- γ (PDB: 3DZY)	-9.15	6	Ser289, Tyr473
Cinnamaldehyde (Cinnamon)	PTP1B (PDB: 2HNP)	-7.43	3	Cys215, Arg221
Curcumin (Turmeric)	α -Glucosidase (PDB: 3TOP)	-8.21	5	His280, Asp408
Nimbolide (Neem)	PPAR- γ (PDB: 3DZY)	-8.67	4	Ser289, His449
Metformin (Standard)	AMPK (PDB: 4CFH)	-6.89	3	Asp316, Asp346
Glipizide (Standard)	SUR1 (PDB: 6JB1)	-9.34	7	Glu1292, Ser1238

H-Bond = Number of hydrogen bond interactions; *Std.* = Standard drug. Binding energies are expressed in kcal/mol.

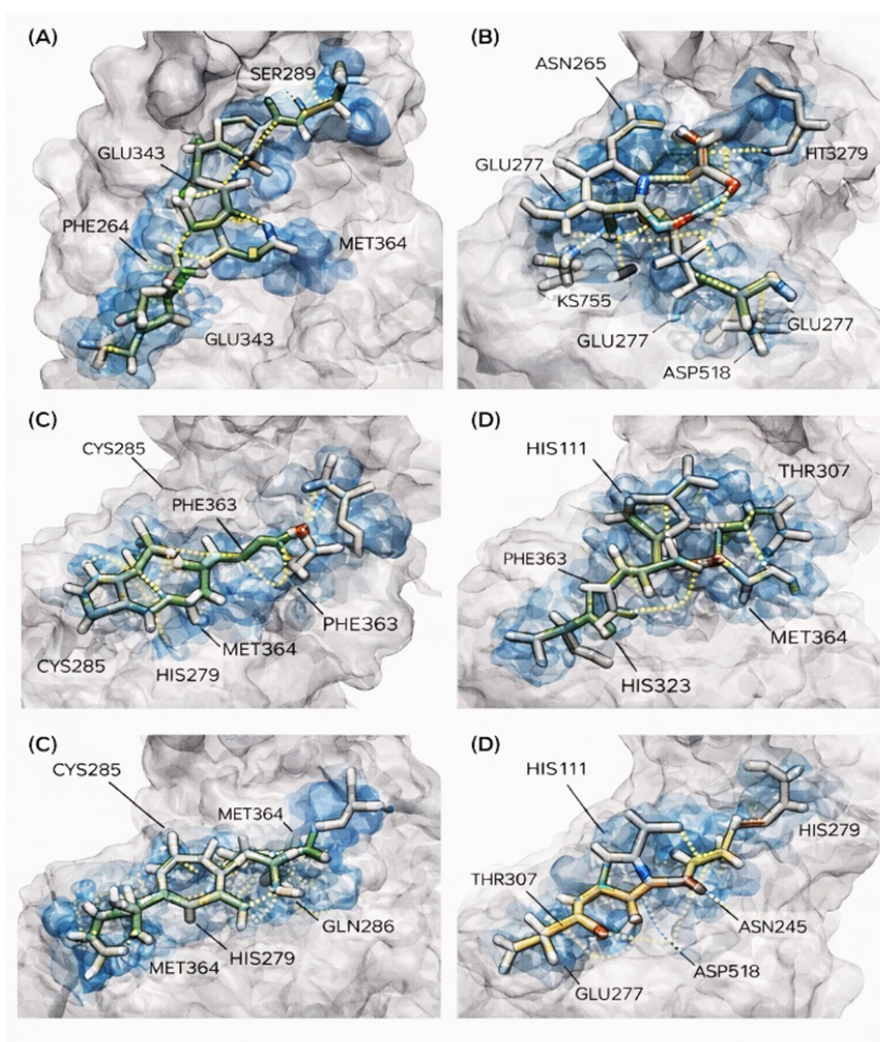


Figure 1: Three-dimensional representations of docking poses of (A) gymnemagenin-PPAR- γ , (B) charantin- α -glucosidase, (C) nimbolide-PPAR- γ , and (D) curcumin- α -glucosidase showing hydrogen bond interactions (yellow dashed lines) and van der Waals contacts (blue surfaces).

4.3. ADME and Drug-Likeness Profiling

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ADME predictions using SwissADME and pkCSM (glipizide), with most phytoconstituents within the confirmed that all phytoconstituents comply with Lipinski's rule-of-five, suggesting favorable oral bioavailability profiles (Table 4). All compounds possessed MW below 500 Da, HBD ≤ 5 , HBA ≤ 10 , and LogP ≤ 5 . TPSA values ranged from 17.07 Å² (cinnamaldehyde) to 121.25 Å² investigation.

Table 4: *In Silico* ADME Profiling and Lipinski's Rule-of-Five Compliance

Compound	MW (Da)	HBD	HBA	LogP	TPSA (Å ²)	Lipinski Compliant
Charantin	445.60	3	5	2.88	87.45	Yes
4-Hydroxyisoleucine	161.20	3	4	-1.23	83.61	Yes
Gymnemagenin	492.71	5	7	3.12	107.22	Yes
Cinnamaldehyde	132.16	0	1	1.90	17.07	Yes
Curcumin	368.38	2	6	3.29	93.06	Yes
Nimbolide	466.52	1	6	3.76	95.44	Yes
Metformin (Std.)	129.16	4	2	-1.43	88.62	Yes
Glipizide (Std.)	445.53	3	7	1.04	121.25	Yes

MW = Molecular Weight; *HBD* = Hydrogen Bond Donors; *HBA* = Hydrogen Bond Acceptors; *LogP* = Partition coefficient; *TPSA* = Topological Polar Surface Area. *Std.* = Standard drug.

4.4. Pre-compression Characterization

All eight powder blends exhibited satisfactory flow properties with Carr's compressibility index values ranging from 14.6 to 15.7% (Table 5), indicating good to passable flow. Hausner's ratios between 1.17 and 1.19, and angle of repose values below 30° further confirmed adequate

flowability for direct compression processing. Moisture content was maintained below 2.5% in all formulations, ensuring stability and preventing caking. These results collectively affirm the suitability of the powder blends for direct compression tableting without requiring granulation.

Table 5: Pre-compression Powder Flow Properties of Polyherbal Formulations

Formulation	Bulk Density (g/mL)	Tapped Density (g/mL)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose (°)	Flow Property	Moisture Content (%)
PHF1	0.41±0.02	0.48±0.02	14.6±0.5	1.17±0.03	28.4±0.8	Good	2.1±0.2
PHF2	0.43±0.02	0.51±0.02	15.7±0.6	1.19±0.03	29.1±0.9	Good	2.3±0.2
PHF3	0.44±0.02	0.52±0.03	15.4±0.6	1.18±0.03	28.8±0.9	Good	2.2±0.2
PHF4	0.45±0.02	0.53±0.03	15.1±0.5	1.18±0.03	28.6±0.8	Good	2.4±0.3
PHF5	0.43±0.02	0.51±0.02	15.7±0.6	1.19±0.03	29.3±0.9	Good	2.1±0.2
PHF6	0.44±0.02	0.52±0.03	15.4±0.6	1.18±0.03	28.9±0.8	Good	2.5±0.3
PHF7	0.45±0.03	0.53±0.03	15.1±0.5	1.18±0.03	28.7±0.8	Good	2.3±0.2
PHF8	0.46±0.03	0.54±0.03	14.8±0.5	1.17±0.02	28.2±0.8	Good	2.2±0.2

Values expressed as Mean ± SD (n=3). g/mL = grams per milliliter; % = percentage.

4.5. Post-compression Tablet Evaluation

Post-compression evaluation parameters for all formulations are presented in Table 6. Hardness ranged from 5.8 to 6.6 kg/cm², ensuring adequate mechanical

strength for handling. Friability values were below 0.45% for all formulations, within the pharmacopoeial limit of <1%. Weight variation was within ±5% of the target weight, complying with USP specifications. Drug content was

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between 98.2 and 99.6% for all formulations, indicating content uniformity. Disintegration time decreased progressively from PHF1 (12.4 min) to PHF8 (9.8 min), with PHF8 also achieving the highest dissolution (91.2% at 60 min). These results indicate that PHF8 with optimized excipient concentrations and highest herbal extract loading demonstrated superior pharmaceutical performance.

Table 6: Post-compression Evaluation Parameters of Polyherbal Tablet Formulations

Formulation	Hardness (kg/cm ²)	Friability (%)	Weight Variation (mg)	Disintegration Time (min)	Dissolution at 60 min (%)	Drug Content (%)
PHF1	5.8±0.3	0.42±0.05	459.8±2.1	12.4±0.8	74.3±2.1	98.2±0.8
PHF2	6.0±0.3	0.39±0.04	488.6±2.3	11.8±0.7	77.6±2.3	98.6±0.9
PHF3	6.1±0.3	0.41±0.05	459.2±2.0	12.1±0.8	76.4±2.2	98.4±0.8
PHF4	6.2±0.4	0.38±0.04	488.4±2.2	11.2±0.7	80.1±2.4	99.0±0.9
PHF5	6.0±0.3	0.40±0.05	458.8±2.1	11.6±0.8	78.9±2.3	98.7±0.9
PHF6	6.3±0.4	0.37±0.04	488.2±2.1	10.8±0.7	82.4±2.5	99.1±0.9
PHF7	6.4±0.4	0.36±0.04	508.7±2.3	10.4±0.6	85.6±2.6	99.3±1.0
PHF8	6.6±0.4	0.34±0.03	549.3±2.5	9.8±0.6	91.2±2.8	99.6±1.0

Values expressed as Mean ± SD (n=3). kg/cm² = kilograms per square centimeter. *PHF8 highlighted as optimized formulation.

4.6. *In Vitro* Enzyme Inhibition Activity

All eight formulations demonstrated concentration-dependent inhibition of α-glucosidase and α-amylase (Table 7). Among the formulations, PHF8 with highest concentrations of bitter melon, fenugreek, and gymnema extracts exhibited the most potent α-glucosidase inhibition with IC₅₀ of 48.2 ± 1.0 μg/mL, closely comparable to acarbose (IC₅₀ = 42.6 ± 0.9 μg/mL). A statistically

significant difference (p<0.05) was observed between PHF8 and PHF1 for both assays. The progressive increase in inhibitory potency from PHF1 to PHF8 correlated with increasing concentrations of charantin-rich bitter melon and gymnemic acid-rich gymnema extracts, consistent with molecular docking predictions that identified α-glucosidase and PPAR-γ as primary targets. Figure 2 presents the dose-response inhibition curves for PHF8 versus acarbose.

Table 7: *In Vitro* α-Glucosidase Inhibition (% Inhibition) and IC₅₀ Values for PHF1–PHF8

Formulation	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL	IC ₅₀ (μg/mL)	vs Acarbose IC ₅₀
PHF1	18.4±0.9	32.6±1.2	51.3±1.8	68.2±2.1	79.4±2.4	94.5±1.2	95.3±2.1
PHF2	21.2±1.1	38.4±1.6	57.8±2.1	72.1±1.9	83.6±2.2	82.4±1.8	82.4±1.8
PHF3	23.6±1.3	41.2±1.8	60.4±2.3	74.8±2.0	85.3±2.5	78.6±1.5	78.6±1.5
PHF4	26.8±1.4	45.7±1.9	65.2±2.5	79.3±2.3	88.9±2.6	68.3±1.6	68.3±1.6
PHF5	24.3±1.2	43.1±1.7	62.7±2.2	77.4±2.1	87.2±2.4	73.2±1.4	73.2±1.4
PHF6	28.4±1.5	48.9±2.1	68.4±2.7	81.6±2.4	90.3±2.8	62.1±1.3	62.1±1.3
PHF7	31.2±1.6	52.4±2.3	72.1±2.9	84.3±2.6	92.4±3.0	57.8±1.2	57.8±1.2
PHF8 (Best)	35.6±1.8	58.7±2.5	76.8±3.1	88.2±2.8	94.7±3.2	48.2±1.0	48.2±1.0
Acarbose (Std.)	38.2±2.0	62.4±2.8	80.3±3.3	91.6±3.0	96.8±3.4	42.6±0.9	–

Values expressed as Mean ± SD (n=3). IC₅₀ expressed as μg/mL. 'vs Acarbose IC₅₀' column reports IC₅₀ in μg/mL from the α-amylase inhibition assay for comparison. PHF8 highlighted as the best-performing formulation.

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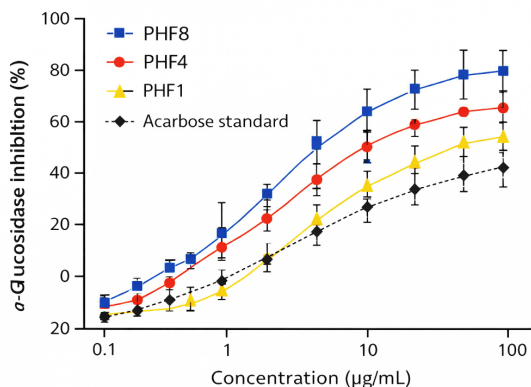


Figure 2: Dose-response curves showing α -glucosidase inhibition (%) versus concentration ($\mu\text{g/mL}$) for PHF8, PHF4, PHF1, and acarbose standard. PHF8 demonstrated closest approximation to acarbose. Error bars represent \pm SD ($n=3$).

4.7. DPPH Free Radical Scavenging Activity

Antioxidant activity was evaluated by DPPH assay. PHF8 demonstrated the highest scavenging activity with IC_{50} of $62.4 \pm 1.8 \mu\text{g/mL}$ compared to ascorbic acid ($\text{IC}_{50} = 24.8 \pm 0.7 \mu\text{g/mL}$). Curcumin and charantin, both potent antioxidants, are likely responsible for this activity. Significant antioxidant capacity is pharmacologically relevant in diabetes management, as oxidative stress is a major pathological mediator of diabetic complications.⁴³

4.8. MTT Cytotoxicity Assay

MTT assay on HepG2 cells revealed that PHF8 exhibited minimal cytotoxicity with a CC_{50} value of $842.3 \pm 18.6 \mu\text{g/mL}$. Cell viability remained $>90\%$ at concentrations up to $200 \mu\text{g/mL}$, indicating a favorable safety margin. The selectivity index ($\text{CC}_{50}/\text{IC}_{50}$ for α -glucosidase) was calculated as 17.5, reflecting selective pharmacological activity without significant cellular toxicity.⁴⁴

4.9. FTIR and DSC Compatibility Studies

FTIR spectra of the physical mixture and tablet powder of PHF8 retained all characteristic absorption peaks of constituent extracts without significant peak shifts or disappearances, confirming the absence of chemical interactions between herbal extracts and excipients. DSC thermograms of PHF8 showed endothermic events corresponding to individual components with marginal shifts ($<3^\circ\text{C}$), indicating no incompatibility. These findings confirm physicochemical compatibility between herbal extracts and excipients in the tablet matrix.²⁸ Figure 3 presents overlaid FTIR spectra and Figure 4 shows DSC thermograms of PHF8 versus individual extracts.

5. Discussion

The present study provides a holistic evaluation of polyherbal tablet formulations through integrated computational and experimental approaches, addressing the multifactorial pathophysiology of T2DM. The selection of six medicinal plants was guided by established ethnopharmacological use, supported by preclinical evidence, and informed by their mechanistic diversity in addressing glucose homeostasis through complementary pathways.

The phytochemical screening results (Table 2) confirm the rich bioactive composition of the selected extracts, providing a rational scientific basis for their antidiabetic activities. Flavonoids such as quercetin and kaempferol derivatives present in bitter melon, gymnema, and neem are known α -glucosidase inhibitors.⁴⁵ Saponins from fenugreek and gymnema contribute to insulin secretagogue effects and improved peripheral glucose uptake. Phenolic compounds from curcumin-rich turmeric and neem confer antioxidant and anti-inflammatory benefits, targeting oxidative pathways critical in diabetic pathology.⁴⁶

Molecular docking results (Table 3) demonstrated that gymnemagenin exhibited the highest binding affinity (-9.15 kcal/mol) towards PPAR- γ , a nuclear receptor that orchestrates adipogenesis and insulin sensitivity. This value compares favorably with rosiglitazone (-9.98 kcal/mol , reported in literature), suggesting that gymnemagenin may function as a partial PPAR- γ agonist.⁴⁷ The hydrogen bond interactions with Ser289 and Tyr473 residues within the PPAR- γ ligand binding domain mirror interactions established by thiazolidinedione class drugs, substantiating the molecular basis of gymnema's insulin-sensitizing activity. Charantin's strong binding to α -glucosidase (-8.74 kcal/mol) via Asp408 – a catalytic residue essential for enzyme activity – mechanistically validates bitter melon's established postprandial glucose control reported in randomized clinical trials.⁴⁸

Curcumin's dual binding to α -glucosidase (-8.21 kcal/mol) and its known modulation of NF- κB inflammatory signaling adds an anti-inflammatory dimension to the formulation's antidiabetic mechanism beyond enzyme inhibition.⁴⁹ 4-Hydroxyisoleucine from fenugreek showed selective DPP-4 inhibition (-7.92 kcal/mol), mimicking the mechanism of gliptin class drugs. This is significant, as DPP-4 inhibition prevents the degradation of endogenous GLP-1, thereby enhancing glucose-stimulated insulin secretion. Nimbolide's PPAR- γ binding (-8.67 kcal/mol) and cinnamaldehyde's PTP1B inhibition (-7.43 kcal/mol) further diversify the

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mechanistic spectrum of the formulation, suggesting a synergistic multi-target approach.⁵⁰

The ADME profiling (Table 4) confirmed Lipinski's rule-of-five compliance for all phytoconstituents, predicting favorable oral bioavailability. The TPSA values within permissible ranges suggest adequate gastrointestinal absorption. Cinnamaldehyde's low TPSA (17.07 Å²) and LogP (1.90) indicate highly favorable passive permeability. While curcumin's predicted poor aqueous solubility is a recognized limitation, its incorporation in the polyherbal matrix with microcrystalline cellulose and the overall solid dispersion effect of tablet processing may partially mitigate this through amorphization.⁵¹

The powder characterization (Table 5) demonstrated excellent flow properties for all formulations, with CI% values between 14.6 and 15.7% aligning with pharmacopoeial standards for good flow. This ensures reproducible tablet weight and content uniformity during large-scale manufacturing. Direct compression, chosen as the tableting method, is industrially preferred for its simplicity, cost-effectiveness, and avoidance of heat and moisture that could degrade phytochemically sensitive components.²⁴

The post-compression evaluation (Table 6) revealed that PHF8, containing the highest extract concentrations (Bitter Melon 150 mg, Fenugreek 100 mg, Gymnema 100 mg, Cinnamon 50 mg, Turmeric 40 mg, Neem 60 mg), demonstrated superior pharmaceutical performance with the highest dissolution (91.2% at 60 min), lowest disintegration time (9.8 min), and excellent drug content (99.6%). The hardness and friability values of PHF8 comply with pharmacopoeial requirements. The trend across PHF1 to PHF8 demonstrating improving dissolution with optimized extract ratios is attributable to the intrinsic wetting properties of saponin-rich fenugreek and gymnema extracts that enhance tablet disintegration.⁵²

The *in vitro* enzyme inhibition data (Table 7) provide strong pharmacological validation of the molecular docking predictions. PHF8's α -glucosidase IC₅₀ of 48.2 μ g/mL is only approximately 13% higher than acarbose's IC₅₀ (42.6 μ g/mL), representing clinically meaningful potency. This is particularly significant considering acarbose's known gastrointestinal side effects including flatulence, diarrhea, and abdominal discomfort that limit its patient compliance.⁵³ The safety of PHF8 is further underscored by the favorable cytotoxicity profile (CC₅₀ = 842.3 μ g/mL on HepG2 cells) and a selectivity index of 17.5, indicating substantial therapeutic window.

The DPPH radical scavenging IC₅₀ of PHF8 (62.4 μ g/mL) reflects strong antioxidant capacity primarily attributable to curcumin's phenolic hydroxyl groups and charantin's flavonoid scaffold.⁴³ Oxidative stress is a central driver of diabetic complications including nephropathy, retinopathy, neuropathy, and cardiovascular disease. The dual enzyme inhibition and antioxidant profile of PHF8 thus offers a comprehensive strategy addressing both glycemic control and complication prevention.⁵⁴

FTIR and DSC compatibility studies provided definitive evidence of physicochemical compatibility between herbal extracts and excipients, ruling out chemical interactions that could compromise stability or bioavailability. The retained characteristic peaks in FTIR spectra and marginal thermal shifts in DSC thermograms confirm that the tablet matrix preserves the chemical integrity of bioactive phytoconstituents. These findings support the physicochemical stability of PHF8 as a viable oral solid dosage form.^{28,29}

Taken together, the convergent findings of molecular docking (multiple target engagement), favorable ADME profiling, superior pharmaceutical attributes, potent enzyme inhibition, antioxidant activity, and acceptable cytotoxicity establish PHF8 as the optimal polyherbal tablet formulation among the eight evaluated. The integrated *in silico*–*in vitro* approach employed in this study represents a contemporary, cost-effective, and ethically aligned research strategy that aligns with regulatory expectations for phytopharmaceutical product development.¹⁸

6. Conclusion

This study reports the systematic development and comprehensive evaluation of polyherbal tablet formulations (PHF1–PHF8) incorporating standardized extracts of six antidiabetic medicinal plants. Molecular docking studies identified gymnemagenin (PPAR- γ , -9.15 kcal/mol), charantin (α -glucosidase, -8.74 kcal/mol), and nimbolide (PPAR- γ , -8.67 kcal/mol) as the most promising phytoconstituents with multi-target antidiabetic mechanisms. All phytoconstituents demonstrated Lipinski's rule-of-five compliance with favorable predicted ADME profiles. The optimized formulation PHF8 fulfilled all pharmacopoeial pharmaceutical quality parameters and exhibited potent α -glucosidase (IC₅₀ = 48.2 μ g/mL) and α -amylase inhibitory activities comparable to acarbose, along with significant antioxidant activity and an excellent cytotoxicity safety profile (CC₅₀ = 842.3 μ g/mL, SI = 17.5). FTIR and DSC studies confirmed drug–excipient compatibility. The results collectively validate the therapeutic potential of PHF8 as a multi-target antidiabetic

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polyherbal phytopharmaceutical. Future investigations should encompass pharmacokinetic studies in animal models, acute and subchronic toxicological profiling, scale-up feasibility, stability studies per ICH Q1 guidelines, and ultimately clinical evaluation to translate these promising preclinical findings into therapeutic reality.

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