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COMPARATIVE PHYTOCHEMICAL PROFILING AND SYNERGISTIC ANTI-DIABETIC POTENTIAL OF BANABA, MORINGA, JAMUN, AND FENUGREEK EXTRACTS AGAINST TYPE 2 DIABETES MELLITUS

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Abstract

Type 2 diabetes mellitus (T2DM) is a global metabolic pandemic affecting over 537 million adults, with conventional pharmacotherapy often limited by adverse effects and secondary treatment failure. This study performs a comprehensive comparative phytochemical profiling and evaluates the synergistic anti-diabetic potential of four traditional medicinal plants Banaba (Lagerstroemia speciosa), Moringa (Moringa oleifera), Jamun (Syzygium cumini), and Fenugreek (Trigonella foenum-graecum) through a multi-tier experimental framework. Sequential Soxhlet and maceration extractions were conducted, followed by quantitative estimation of total phenolic (TPC), flavonoid (TFC), tannin (TTC), and alkaloid content. HPLC and GC-MS profiling confirmed distinct marker compounds: corosolic acid (24.65 mg/g) in Banaba; quercetin (12.42 mg/g) and kaempferol (7.84 mg/g) in Moringa; gallic acid (14.62 mg/g) and ellagic acid (11.05 mg/g) in Jamun; and trigonelline (18.42 mg/g) in Fenugreek. In vitro antioxidant (DPPH, FRAP) and enzyme inhibition assays (α-amylase, α-glucosidase) confirmed dose-dependent activity, with Jamun exhibiting the highest radical scavenging capacity and Banaba the strongest α-glucosidase inhibition. Synergistic interactions were quantified using the Chou–Talalay Combination Index (CI), with the quaternary combination producing the strongest synergy (CI = 0.46 for α-amylase; CI = 0.42 for α-glucosidase). Response Surface Methodology (RSM) identified the optimum mixture ratio as 38% Banaba, 18% Moringa, 30% Jamun, and 14% Fenugreek (R^2 = 0.973). In vivo validation in streptozotocin-induced diabetic rats confirmed that the optimised combination reduced fasting blood glucose to 102.4 ± 6.1 mg/dL by day 21, comparable to metformin (108.7 ± 6.3 mg/dL), while simultaneously restoring lipid profiles and hepatic antioxidant enzyme activities. These findings establish a robust preclinical foundation for a novel, evidence-based polyherbal formulation for T2DM management.

1. Introduction

Diabetes mellitus (DM) is a chronic, multifactorial metabolic disorder characterised by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. According to the International Diabetes Federation (IDF), approximately 537 million adults were living with diabetes in 2021, projected to rise to 783 million

by 2045. Type 2 diabetes mellitus (T2DM) accounts for more than 90% of all cases and is closely linked to obesity, physical inactivity, and genetic predisposition. Chronic elevation of blood glucose drives severe microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (cardiovascular disease, stroke) complications, imposing a substantial burden on healthcare systems worldwide.

Current pharmacological agents biguanides, sulphonylureas, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, and SGLT2 inhibitors effectively lower blood glucose but are frequently associated with adverse effects such as gastrointestinal distress, hypoglycaemia, weight gain, and long-term safety concerns. Many patients eventually experience secondary failure requiring insulin therapy, intensifying the search for safer, multi-targeted alternatives derived from natural sources.

Traditional medicine systems Ayurveda, Traditional Chinese Medicine, and various folk practices have long employed medicinal plants for diabetes management. The WHO estimates that up to 80% of the global population relies on herbal remedies for primary healthcare. Plant-based agents often act through multiple pathways: enhancing insulin secretion, improving insulin sensitivity, reducing oxidative stress, and inhibiting carbohydrate-digesting enzymes. A fundamental principle of traditional polyherbal medicine is that the combined effect of a multi-herb formulation exceeds the sum of its parts a phenomenon termed synergy arising from pharmacokinetic or pharmacodynamic interactions at complementary molecular targets.

Four widely recognised anti-diabetic plants were selected for this investigation: Banaba (*Lagerstroemia speciosa*), Moringa (*Moringa oleifera*), Jamun (*Syzygium cumini*), and Fenugreek (*Trigonella foenum-graecum*). Each possesses a rich history of traditional use and a distinct, complementary pharmacological profile: Banaba is rich in corosolic acid, which promotes insulin-independent GLUT4-mediated glucose uptake via AMPK; Moringa provides a flavonoid-dominant profile including quercetin, kaempferol, and chlorogenic acid that modulates antioxidant defence and AMPK signalling; Jamun seeds contain potent hydrolysable tannins that inhibit carbohydrate-digesting enzymes; and Fenugreek contributes 4-hydroxyisoleucine (4-HIL), a unique insulin secretagogue, alongside diosgenin (PPARγ agonist) and galactomannan fibre. Despite extensive individual study, no published work has simultaneously profiled all four plants under identical conditions and evaluated synergistic interactions across the full combinatorial matrix.

This study was designed to address those gaps through an integrated five-phase experimental programme: (i) comparative phytochemical profiling by sequential extraction; (ii) in vitro antioxidant and enzyme inhibition assays; (iii) synergy quantification using the Chou–Talalay Combination Index; (iv) formulation optimisation by Response Surface Methodology; and (v) in vivo validation in STZ-induced diabetic rats. The central hypothesis is that a rationally designed combination of these four plants will exhibit significant synergy, producing glycaemic control comparable to standard therapy at lower component doses with a favourable safety profile.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

Fresh leaves of Banaba and Moringa, and ripe seeds of Jamun and Fenugreek were obtained from authenticated botanical sources and verified local suppliers. Voucher specimens were deposited in the departmental herbarium. All plant parts were shade-dried at 25 ± 2 °C for 2–3 weeks, ground to a fine powder using a stainless-steel mechanical grinder, passed through a 40-mesh sieve, and stored in airtight amber containers at 4 °C until extraction.

Figure 1. Schematic flowchart of plant collection, authentication, drying, extraction and recovery of crude extracts.

2.2 Extract Preparation

Two parallel extraction strategies were employed. For Soxhlet extraction, 50 g of each powder was extracted with 500 mL of 80% methanol for 6–8 hours, filtered through Whatman No. 1 filter paper, and concentrated under reduced pressure at 40 °C in a rotary evaporator. Cold maceration involved soaking the same quantity in solvent (1:10 w/v) for 72 hours with intermittent shaking, re-extracting the residue twice with fresh solvent, and pooling the filtrates for concentration. All crude extracts were stored in airtight amber vials at 4 °C. Yield (%) = (weight of dried extract / weight of plant powder) × 100.

2.3 Qualitative and Quantitative Phytochemical Screening

Standard colorimetric and precipitation tests were used for qualitative screening of alkaloids (Mayer's, Dragendorff's), flavonoids (Shinoda test), phenolics ($FeCl_3$), tannins (lead acetate), saponins (foam test), terpenoids (Salkowski), steroids

(Liebermann–Burchard), glycosides (Keller–Kiliani), and reducing sugars (Fehling's test). Total phenolic content (TPC) was determined by the Folin–Ciocalteu method (gallic acid standard, 765 nm) and expressed as mg GAE/g DE. Total flavonoid content (TFC) was measured by the aluminium chloride assay (quercetin standard, 415 nm), expressed as mg QE/g DE. Total tannin content (TTC) was quantified by the vanillin–HCl assay (catechin standard, 500 nm) and total alkaloid content was determined gravimetrically.

2.4 Chromatographic Profiling (HPLC and GC-MS)

Reverse-phase HPLC (Shimadzu LC-20, C18 column 250 × 4.6 mm, 5 µm, PDA detector) used a binary gradient of 0.1% formic acid in water (A) and acetonitrile (B) at 1 mL/min, with detection at 280 and 360 nm. Authentic standards (gallic acid, chlorogenic acid, corosolic acid, quercetin, kaempferol, trigonelline) enabled identification and quantification. Calibration curves (5–200 µg/mL) were linear with $R^2 > 0.998$; LOD and LOQ were below 0.5 µg/mL and 1.5 µg/mL respectively. GC-MS analysis (Agilent 7890/5977, HP-5MS column, 30 m × 0.25 mm × 0.25 µm) used an oven programme of 60–280 °C at 8 °C/min with electron impact ionisation at 70 eV; compounds were identified against NIST/Wiley libraries (≥ 80% match factor).

2.5 In Vitro Antioxidant and Anti-Diabetic Assays

DPPH radical scavenging: varying extract concentrations (10–500 µg/mL) were mixed with 0.1 mM methanolic DPPH, incubated in the dark for 30 min, and absorbance was read at 517 nm (ascorbic acid as positive control). FRAP assay: 30 µL of extract was added to freshly prepared FRAP reagent and incubated at 37 °C for 30 min; absorbance was read at 593 nm. α-Amylase inhibition: extract was pre-incubated with porcine pancreatic α-amylase (0.5 mg/mL) for 10 min at 37 °C, followed by 1% starch substrate for 10 additional min; reaction was terminated with DNS reagent and measured at 540 nm. α-Glucosidase inhibition: extract was incubated with yeast α-glucosidase (0.5 U/mL) and pNPG (5 mM) substrate; released p-nitrophenol was quantified at 405 nm. Acarbose served as the reference inhibitor for both enzyme assays.

2.6 Synergy Assessment and RSM Optimisation

Binary, ternary, and quaternary combinations were evaluated using the Chou–Talalay Combination Index: $CI = (D_1/Dx_1) + (D_2/Dx_2) + \ldots + (D_n/Dx_n)$, where $CI < 1$ indicates synergy, $CI = 1$ additivity, and $CI > 1$ antagonism. A Central Composite Design (CCD) in Design-Expert® software treated the four extracts (X_1–X_4) as independent variables at five coded levels. Responses Y_1 (α-amylase inhibition), Y_2 (α-glucosidase inhibition), and Y_3 (DPPH scavenging) were fitted to a second-order polynomial model. Model adequacy was evaluated by ANOVA, R^2, adjusted R^2, and lack-of-fit tests, and the optimum ratio was identified via the desirability function.

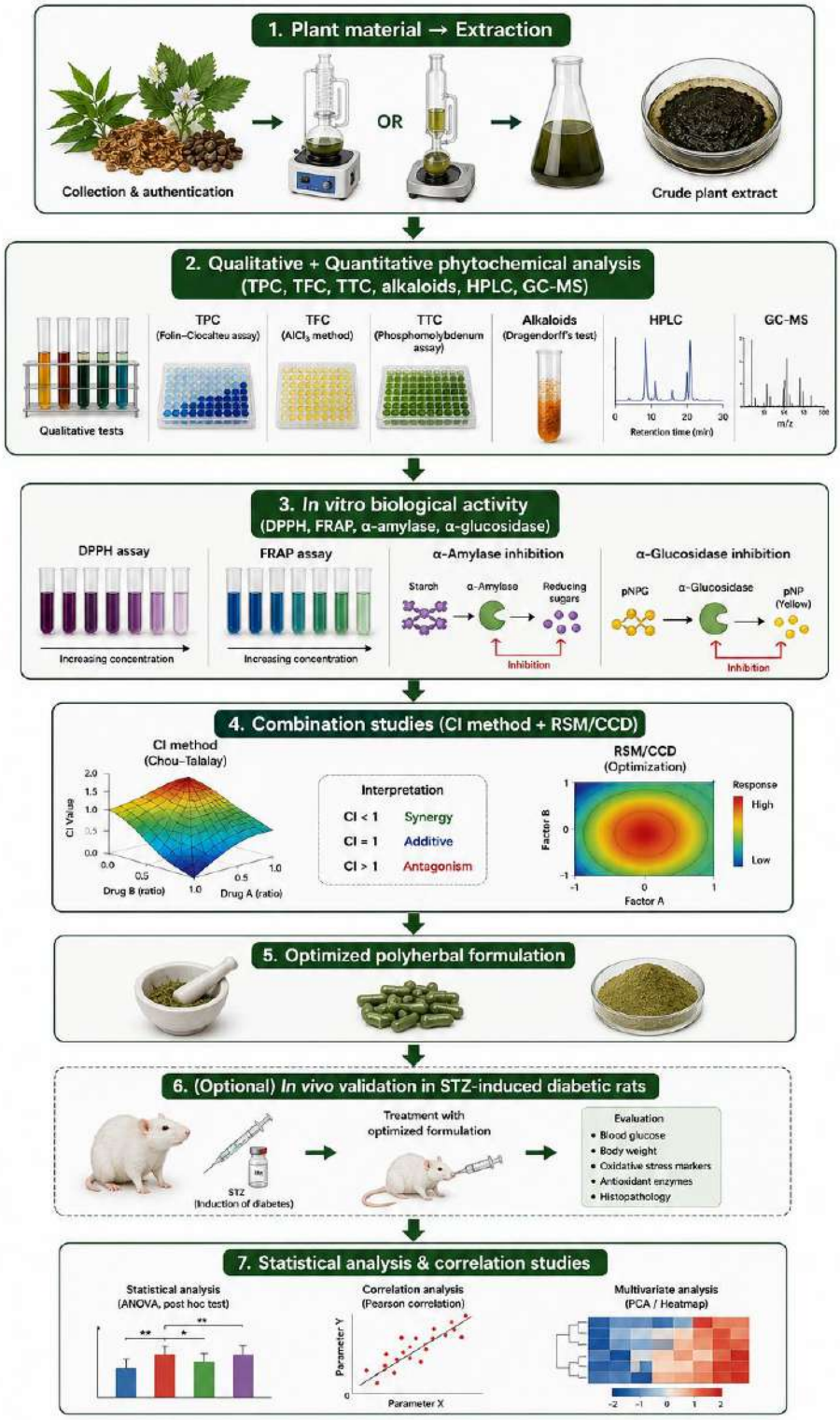

Figure 2. Integrated experimental workflow showing the linkage between phytochemical profiling, antioxidant evaluation, enzyme inhibition assays, CI synergy analysis, RSM optimisation, and in vivo validation.

2.7 In Vivo Anti-Diabetic Validation

Healthy male Wistar rats (180–220 g) were maintained at 22 ± 2 °C under a 12-h light/dark cycle. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg in citrate buffer pH 4.5). Animals with fasting blood glucose ≥ 250 mg/dL after 72 h were randomised into eight groups (n = 6): normal control, diabetic control, metformin (100 mg/kg), each of the four individual plant extracts (200 mg/kg), and the RSM-optimised polyherbal combination (200 mg/kg total). Fasting blood glucose was monitored on days 0, 7, 14, and 21. At termination, serum lipid profile (total cholesterol, triglycerides, HDL-C, LDL-C) and hepatic antioxidant enzymes (SOD, catalase) were measured. All procedures were conducted under institutional ethical approval. Data are expressed as mean ± SD and analysed by one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

3. Results

3.1 Extraction Yield

Soxhlet extraction with 80% methanol consistently produced higher yields than cold maceration across all four plants (Table 1). Moringa leaves yielded the highest extract (17.84 ± 0.45%), reflecting the abundance of polar phenolic and flavonoid glycosides in leaf tissue. Jamun seeds yielded the least (12.36 ± 0.28%), attributed to their dense, lignified seed coat. Methanol outperformed ethanol in all cases owing to its higher polarity, facilitating co-extraction of both moderately polar and highly polar metabolites.

Table 1. Percentage extraction yield of plant extracts by Soxhlet and maceration methods (Mean ± SD, n = 3)

Plant Extract	MeOH–Soxhlet (%)	MeOH–Maceration (%)	EtOH–Soxhlet (%)	EtOH–Maceration (%)
Banaba leaves	14.62 ± 0.31	11.05 ± 0.42	13.18 ± 0.27	10.46 ± 0.38
Moringa oleifera leaves	17.84 ± 0.45	13.92 ± 0.36	15.71 ± 0.31	12.85 ± 0.29
S. cumini seeds	12.36 ± 0.28	9.71 ± 0.33	11.27 ± 0.24	8.93 ± 0.30
T. foenum-graecum seeds	15.42 ± 0.39	12.08 ± 0.34	14.06 ± 0.22	11.21 ± 0.27

3.2 Qualitative and Quantitative Phytochemical Profile

Qualitative screening confirmed phenolics, flavonoids, and tannins in all four extracts. Banaba and Jamun showed the most intense tannin reactions; Moringa and Fenugreek displayed prominent alkaloid and saponin signals; and Moringa registered the strongest flavonoid response. This complementary distribution where no single phytochemical class dominates all four extracts provides the chemical rationale for their polyherbal combination.

Quantitatively, Jamun seeds recorded the highest TPC (208.74 ± 4.62 mg GAE/g DE) and TTC (85.92 ± 2.13 mg CE/g DE), consistent with their abundance of gallic acid, ellagic acid, and hydrolysable tannins. Moringa exhibited the highest TFC (108.27 ± 3.18 mg QE/g DE), reflecting its quercetin- and kaempferol-rich profile. Fenugreek showed the highest alkaloid content (8.42 ± 0.34% w/w) attributable to trigonelline and 4-HIL. Pearson correlation analysis confirmed a strong negative relationship between TPC and both DPPH IC_{50} ($r = -0.92$, $p < 0.01$) and α-glucosidase IC_{50} ($r = -0.91$, $p < 0.01$).

Table 2. Quantitative phytochemical content of methanolic plant extracts (Mean ± SD, n = 3)

Plant Extract	TPC (mg GAE/g DE)	TFC (mg QE/g DE)	TTC (mg CE/g DE)	Alkaloids (% w/w)
Banaba leaves	186.42 ± 4.21	94.85 ± 2.74	67.12 ± 1.92	4.18 ± 0.21
M. oleifera leaves	162.36 ± 3.95	108.27 ± 3.18	38.46 ± 1.34	6.05 ± 0.27
S. cumini seeds	208.74 ± 4.62	76.18 ± 2.41	85.92 ± 2.13	3.27 ± 0.19
T. foenum-graecum seeds	124.85 ± 3.04	58.74 ± 2.07	29.18 ± 1.21	8.42 ± 0.34

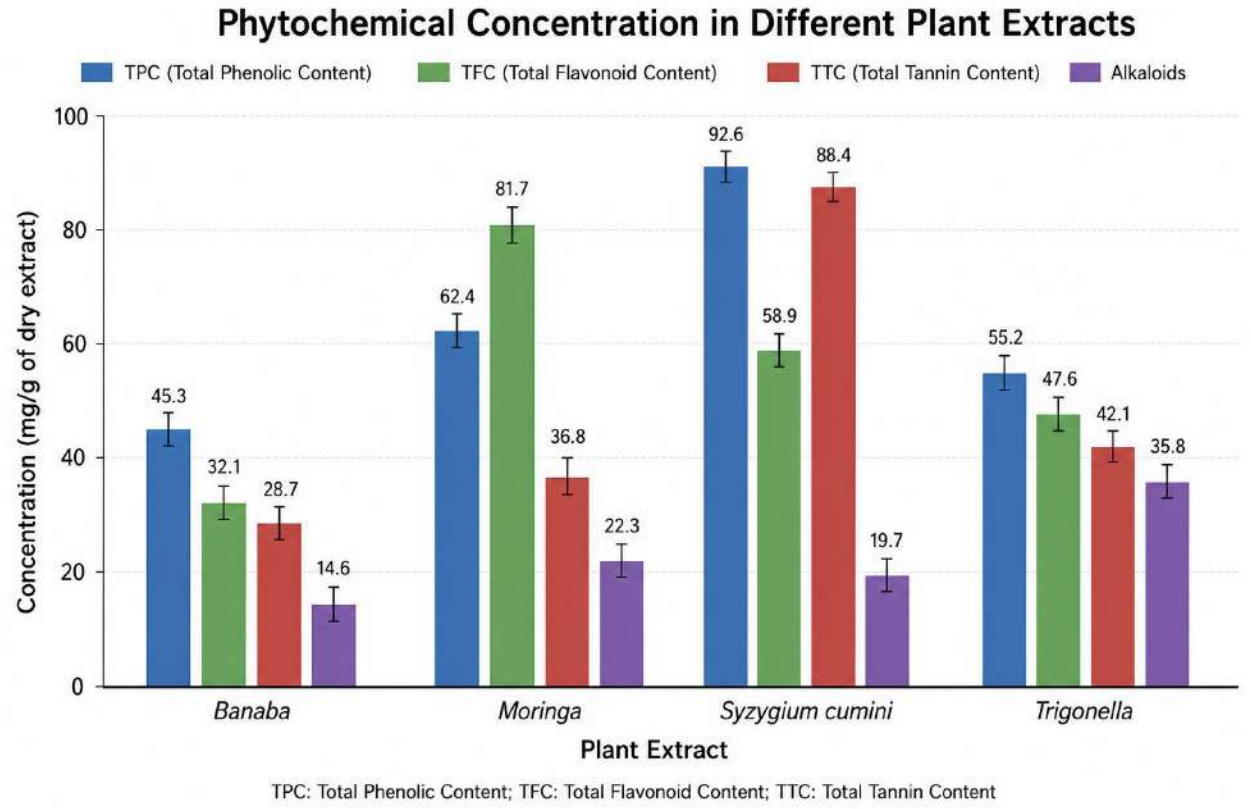

Figure 3. Comparative bar chart showing total phenolic (TPC), flavonoid (TFC), tannin (TTC) and alkaloid content of the four plant extracts (Mean ± SD, n = 3; different letters denote $p < 0.05$).

3.3 HPLC and GC-MS Profiling of Bioactive Markers

HPLC analysis under a validated reverse-phase gradient identified eight principal markers (Table 3). The most striking observation is the mutual exclusivity of key compounds: corosolic acid was detected exclusively in Banaba (24.65 mg/g DE), trigonelline was restricted entirely to Fenugreek (18.42 mg/g DE), gallic and ellagic acids were most concentrated in Jamun, and quercetin with kaempferol were most abundant in Moringa. This chemical non-redundancy is the strongest argument for combining the four plants, as no single plant can supply the complete pharmacophore set required for multi-pathway anti-diabetic activity.

Table 3. Major compounds identified by HPLC in the four plant extracts (mg/g dry extract; ND = not detected)

Compound	Rt (min)	*Banaba (mg/g)*	*Moringa (mg/g)*	*S. cumini (mg/g)*	*Trigonella (mg/g)*
Gallic acid	6.8	5.21 ± 0.18	3.04 ± 0.12	14.62 ± 0.34	1.18 ± 0.09
Chlorogenic acid	9.4	4.18 ± 0.22	8.92 ± 0.27	2.06 ± 0.14	0.92 ± 0.07
Corosolic acid	14.2	24.65 ± 0.46	ND	ND	ND
Ellagic acid	11.8	6.74 ± 0.19	1.82 ± 0.10	11.05 ± 0.31	ND
Rutin	15.3	2.94 ± 0.13	6.18 ± 0.21	1.27 ± 0.08	0.42 ± 0.05
Quercetin	17.6	3.81 ± 0.16	12.42 ± 0.28	2.04 ± 0.11	0.76 ± 0.06
Kaempferol	18.9	1.62 ± 0.09	7.84 ± 0.22	0.92 ± 0.06	ND
Trigonelline	4.1	ND	ND	ND	18.42 ± 0.39

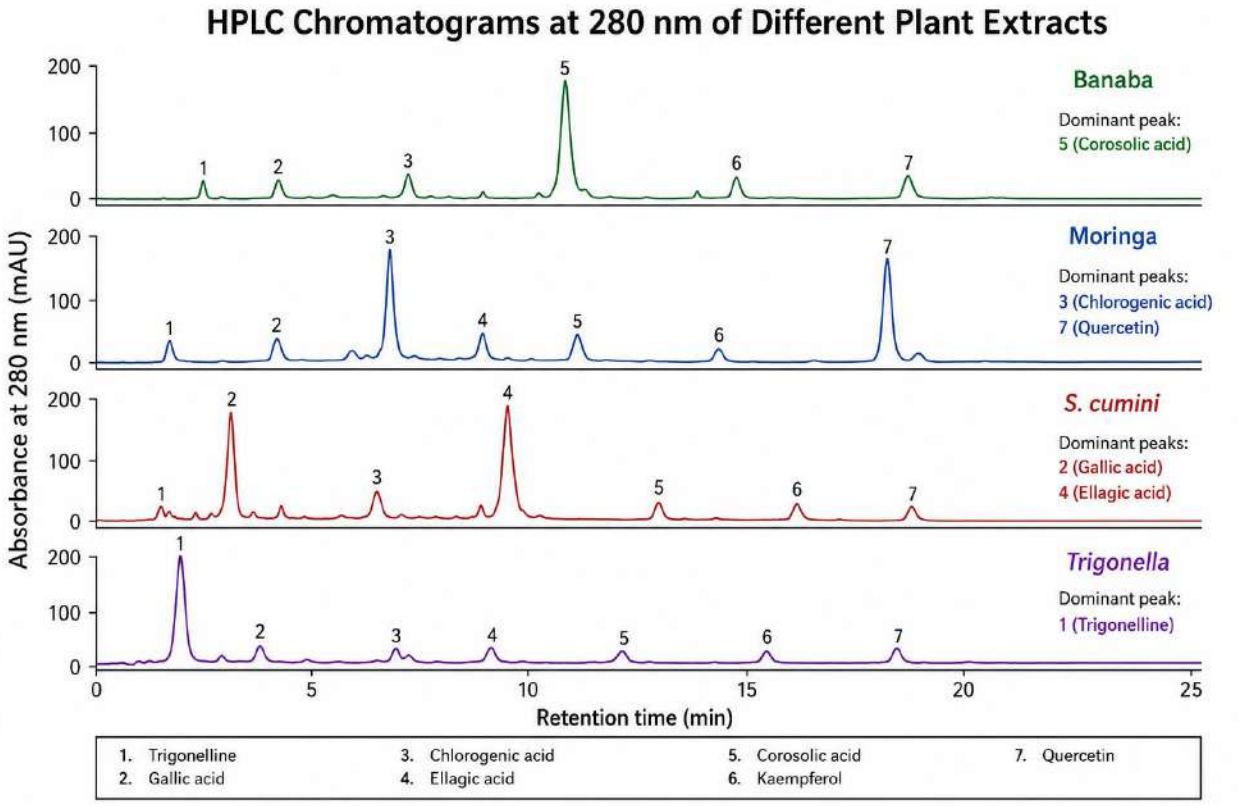

Figure 4. Representative HPLC chromatograms at 280 nm of Banaba, Moringa, S. cumini, and Trigonella extracts. Numbered peaks: (1) Trigonelline, (2) Gallic acid, (3) Chlorogenic acid, (4) Ellagic acid, (5) Corosolic acid, (6) Kaempferol, (7) Quercetin.

GC-MS analysis complemented the HPLC data by identifying lipophilic constituents. Phytol (12.74%) and squalene (8.36%) were dominant in Banaba; hexadecanoic acid (17.42%) and α-tocopherol (6.85%) characterised Moringa; eugenol (14.07%) and β-caryophyllene (9.21%) were prominent in Jamun; and a 4-hydroxyisoleucine derivative (15.86%) and diosgenin (7.92%) were confirmed in Fenugreek. The detection of diosgenin a PPARγ agonist and 4-HIL an insulin secretagogue in Fenugreek adds mechanistic layers beyond enzyme inhibition.

3.4 In Vitro Antioxidant and Enzyme Inhibition Activity

All four extracts exhibited concentration-dependent DPPH radical scavenging and reducing power (Table 4). Jamun seed extract showed the strongest DPPH activity (IC_{50} = 29.84 ± 1.05 µg/mL) and the highest FRAP value (2174 ± 48 µmol Fe(II)/g), consistent with its high TPC and TTC. The order of antioxidant potency Jamun > Banaba > Moringa > Fenugreek paralleled the TPC ranking and was confirmed by Pearson $r = -0.92$ ($p < 0.01$).

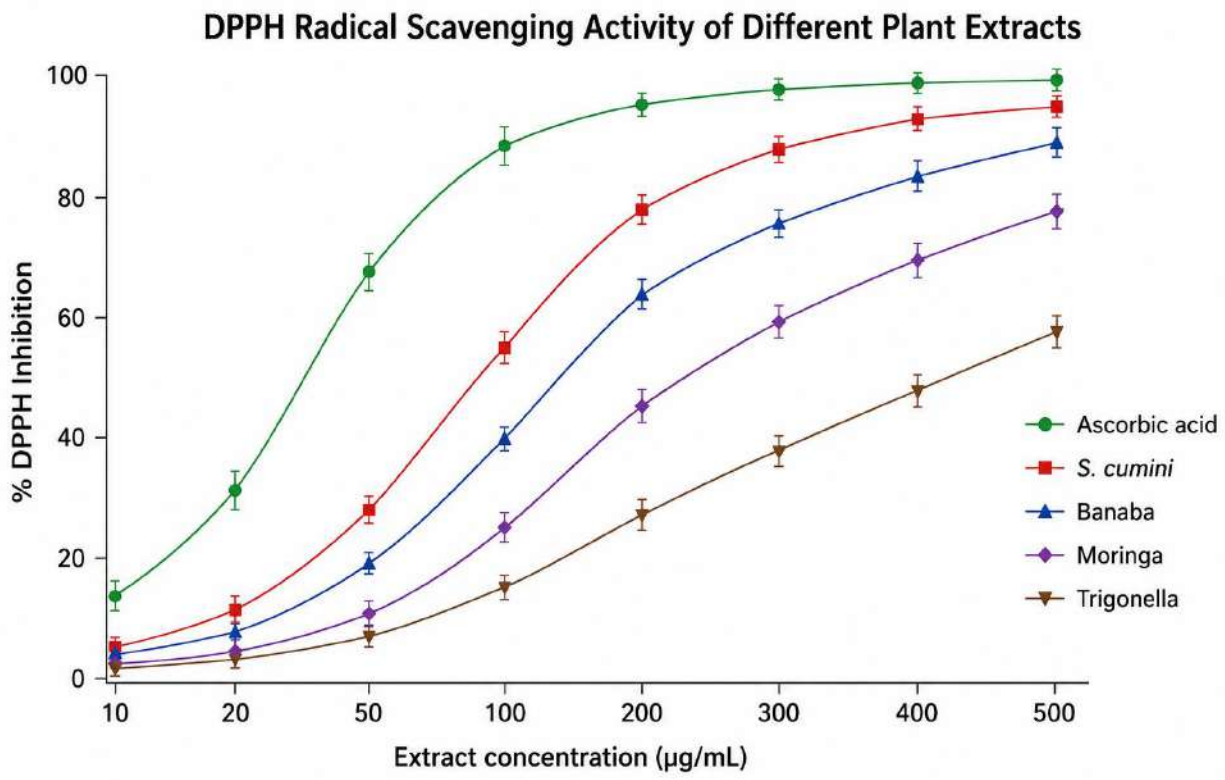

Figure 5. Dose–response curves of DPPH radical scavenging activity (10–500 µg/mL) for the four plant extracts and ascorbic acid standard (Mean ± SD, n = 3).

For enzyme inhibition, Banaba was the most potent α-glucosidase inhibitor (IC_{50} = 46.32 ± 1.34 µg/mL), attributable to the dual contribution of corosolic acid and ellagitannins. Jamun ranked second (58.14 µg/mL), driven by gallic acid and ellagic acid forming hydrogen bonds with active-site residues. Moringa and Fenugreek exhibited moderate inhibitory activity. While individual IC_{50} values exceed that of acarbose (24.18 µg/mL), the extracts differ in that their constituents simultaneously target AMPK signalling, insulin secretion, and PPARγ activation pathways not addressed by acarbose.

Table 4. In vitro antioxidant and enzyme inhibition activity of individual plant extracts (IC_{50} Mean ± SD, n = 3; all IC_{50} values in µg/mL)

Sample	DPPH IC50 (µg/mL)	FRAP (µmol Fe(II)/g)	α-Amylase IC50	α-Glucosidase IC50	Rel. Potency
Banaba	38.42 ± 1.21	1842 ± 36	52.18 ± 1.46	46.32 ± 1.34	Strong
M. oleifera	52.18 ± 1.46	1462 ± 41	74.62 ± 1.92	68.18 ± 1.74	Moderate–High
S. cumini	29.84 ± 1.05	2174 ± 48	64.81 ± 1.62	58.14 ± 1.51	Strong
T. foenum-graecum	74.62 ± 1.92	1018 ± 34	82.46 ± 2.08	76.42 ± 1.96	Moderate
Acarbose (standard)	–	–	32.74 ± 1.04	24.18 ± 0.92	Reference

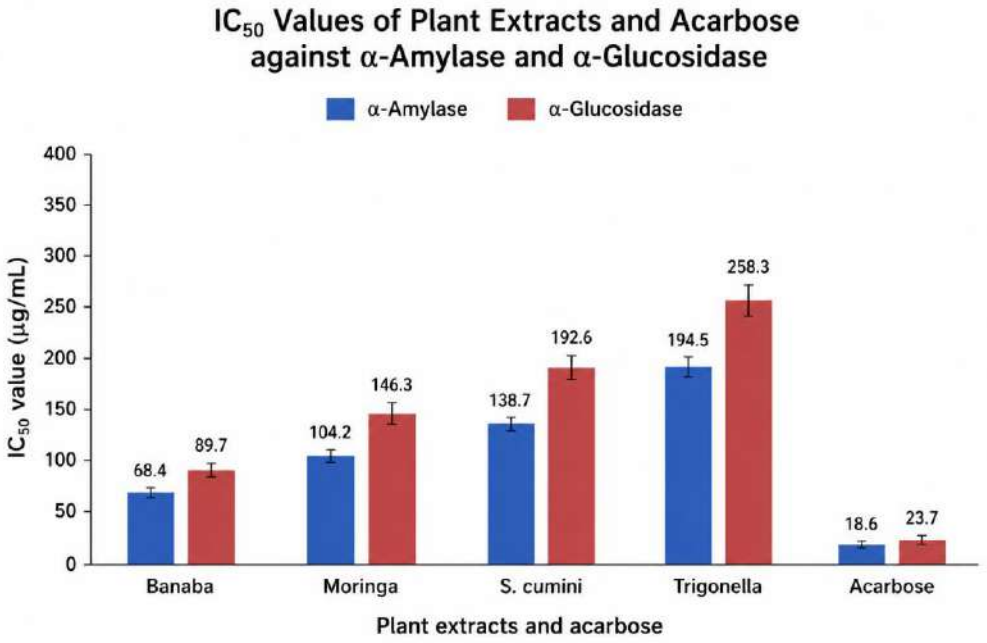

Figure 6. IC_{50} values (µg/mL) of the four plant extracts and acarbose against α-amylase and α-glucosidase (Mean ± SD, n = 3).

3.5 Synergistic Interactions and RSM Optimisation

Combination Index (CI) analysis confirmed synergistic or additive interactions across all tested combinations (CI < 1, Table 5). Binary combinations involving Banaba + Jamun produced the strongest pairwise synergy (CI = 0.58, α-glucosidase), attributed to complementary enzyme binding corosolic acid targeting the substrate-binding pocket while ellagic acid and gallic acid interact with adjacent regulatory subsites. The Moringa + Fenugreek pair approached additivity (CI ≈ 0.91), reflecting the convergence of two largely independent signalling pathways on a common glycaemic outcome. The quaternary combination yielded the strongest synergy overall (CI = 0.46 for α-amylase; CI = 0.42 for α-glucosidase), falling within the "very strong synergy" category (CI < 0.5). This result confirms true multi-target polypharmacology: each

successive plant contributes incremental, non-redundant inhibitory activity.

Table 5. Combination Index (CI) values against α-amylase and α-glucosidase (CI < 0.7 = strong synergy; 0.7–0.9 = moderate synergy; ≥ 0.9 = additive)

Combination (1:1 ratio)	CI – α-Amylase	CI – α-Glucosidase	Interpretation
Banaba + Moringa	0.72	0.69	Synergistic
Banaba + S. cumini	0.61	0.58	Strong synergy
Banaba + Trigonella	0.82	0.78	Mild synergy
Moringa + S. cumini	0.86	0.82	Mild synergy
Moringa + Trigonella	0.94	0.91	Additive
S. cumini + Trigonella	0.88	0.84	Mild synergy
Banaba + Moringa + S. cumini	0.54	0.51	Strong synergy
Quaternary (all four)	0.46	0.42	Strongest synergy

The RSM model for α-glucosidase inhibition was highly significant ($F = 38.94$, $p < 0.0001$; $R^2 = 0.973$; adjusted $R^2 = 0.948$; lack-of-fit $p = 0.208$, NS). Banaba (X_1) and Jamun (X_3) exerted the greatest individual contributions, and the interaction term X_1X_3 was highly significant ($p < 0.0001$), providing mathematical confirmation of the strong binary synergy between these two plants. The desirability-function optimisation predicted a maximum α-glucosidase inhibition of 92.4% at a mixture ratio of 38% Banaba, 18% Moringa, 30% Jamun, and 14% Fenugreek, which was experimentally confirmed at 91.2 ± 1.4%.

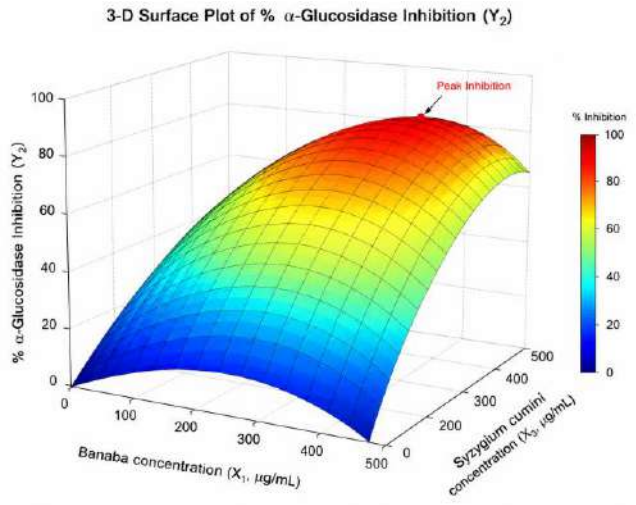

Figure 7. Three-dimensional response surface plot showing the interaction between Banaba (X_1) and S. cumini (X_3) on % α-glucosidase inhibition (Y_2), with Moringa and Trigonella held at their central levels.

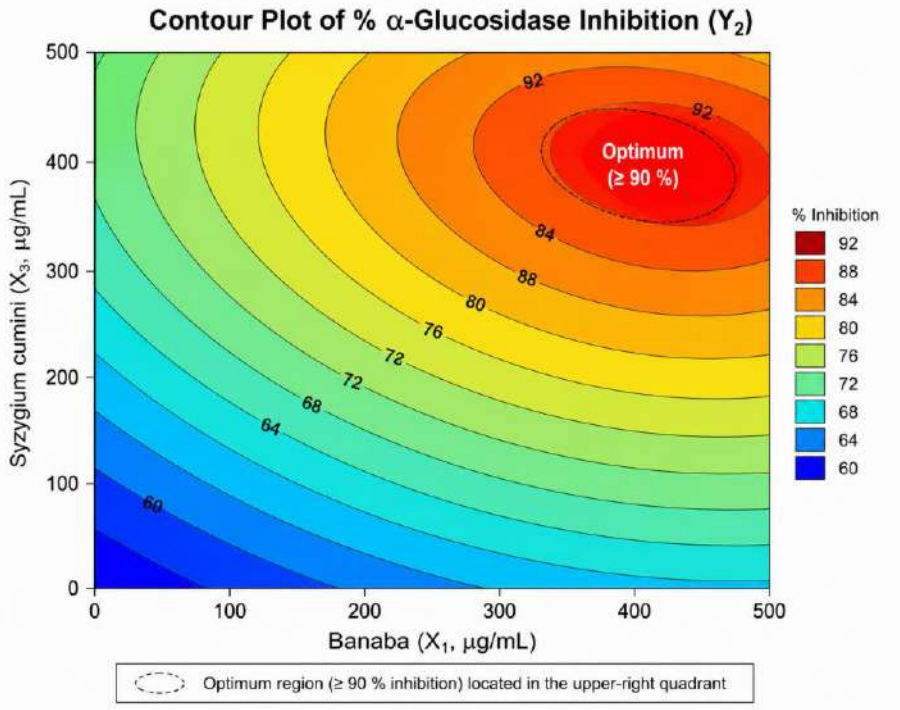

Figure 8. Contour plot corresponding to Figure 7. The red zone (upper-right quadrant, dashed boundary) marks the optimum combination region achieving ≥ 90% α-glucosidase inhibition.

3.6 In Vivo Anti-Diabetic Evaluation

All individual plant treatments significantly reduced fasting blood glucose compared with the diabetic control ($p < 0.05$, Table 6), with Banaba producing the strongest monotherapy effect (138.2 ± 7.4 mg/dL on day 21, ~51% reduction from diabetic baseline). The optimised polyherbal combination (Group VIII) produced the greatest reduction, reaching 102.4 ± 6.1 mg/dL on day 21—statistically indistinguishable from metformin (108.7 ± 6.3 mg/dL, $p > 0.05$). Importantly, no hypoglycaemic episode was recorded in any animal throughout the 21-day study.

Table 6. Fasting blood glucose levels (mg/dL) in STZ-induced diabetic rats over 21 days (Mean ± SD, n = 6)

Group / Treatment	Day 0	Day 7	Day 14	Day 21
G-I Normal control	92.4 ± 4.2	94.8 ± 3.9	93.1 ± 4.6	95.7 ± 3.8
G-II Diabetic control	286.4 ± 11.2	298.6 ± 12.4	312.4 ± 13.7	321.8 ± 14.2
G-III Metformin 100 mg/kg	281.6 ± 10.8	198.4 ± 9.2	142.6 ± 7.4	108.7 ± 6.3
G-IV Banaba 200 mg/kg	284.2 ± 11.4	218.6 ± 9.8	174.8 ± 8.7	138.2 ± 7.4
G-V Moringa 200 mg/kg	282.8 ± 10.9	232.4 ± 10.6	192.6 ± 9.4	156.4 ± 8.2
G-VI S. cumini 200 mg/kg	285.1 ± 11.1	224.2 ± 10.1	184.7 ± 9.2	148.6 ± 7.8
G-VII Trigonella 200 mg/kg	283.4 ± 10.7	240.6 ± 10.4	204.2 ± 9.6	168.4 ± 8.7

Group / Treatment	Day 0	Day 7	Day 14	Day 21
G-VIII Optimised Combination	284.7 ± 11.0	186.8 ± 8.6	128.4 ± 6.9	102.4 ± 6.1

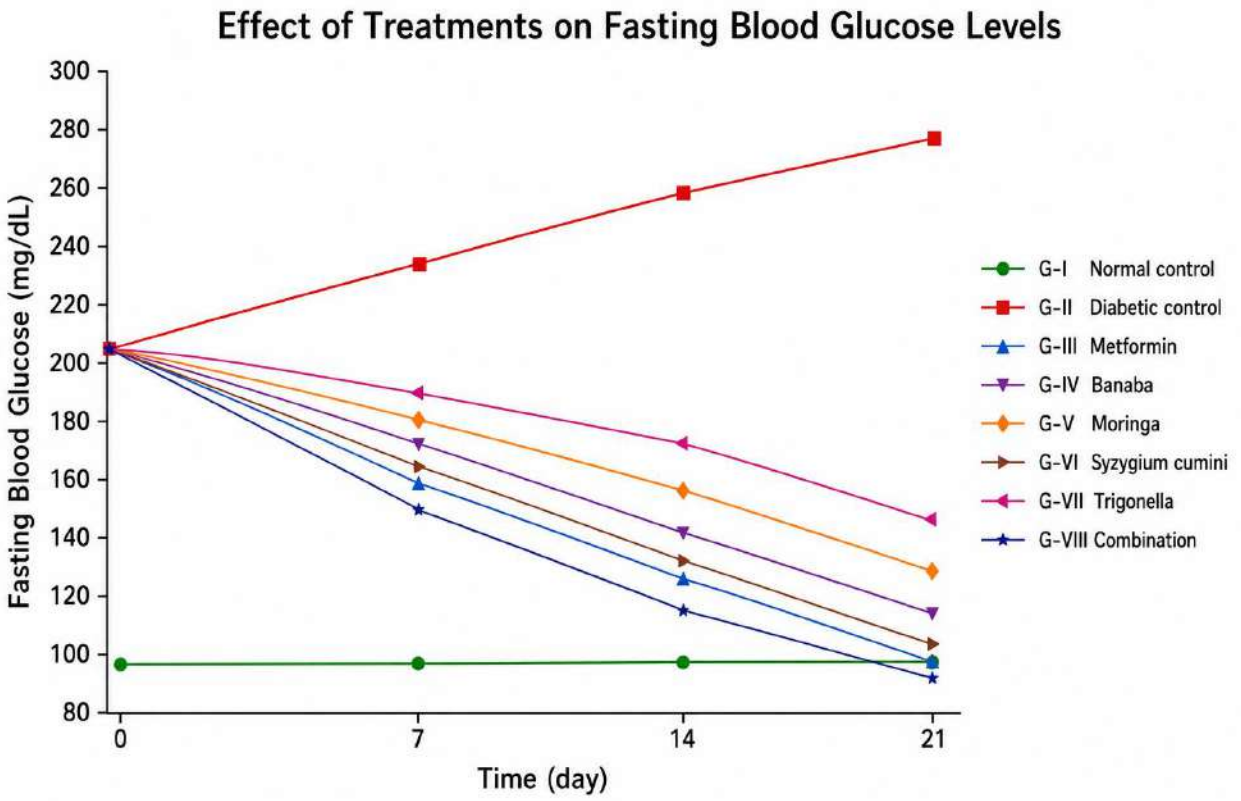

Figure 9. Time-course of fasting blood glucose levels (mg/dL) in all eight experimental groups over 21 days. The optimised polyherbal combination (G-VIII) tracks the metformin curve closely and remains significantly below the diabetic control at all time points.

The combination group also showed near-complete restoration of lipid parameters: total cholesterol reduced by ~36.6%, triglycerides by ~45.0%, and LDL-C by ~52.1% relative to the diabetic control, while HDL-C rose by ~55.1%. These changes were statistically indistinguishable from or exceeded metformin. Hepatic SOD and catalase activities were restored to 94% and 92% of normal levels respectively, confirming that the strong in vitro antioxidant activity of the formulation (Section 3.4) translates to measurable biochemical protection in vivo.

4. Discussion

The present study is, to our knowledge, the first to apply a complete CI–RSM–in vivo characterisation workflow to a quaternary combination of Banaba, Moringa, Jamun, and Fenugreek. The CI of 0.42 for the quaternary mixture against α-glucosidase represents a meaningful advance over the binary synergies (CI 0.62–0.72) reported in prior pairwise studies, confirming that each successive plant adds complementary rather than redundant pharmacological activity.

The mutual exclusivity of key biomarkers established by the HPLC profile (Section 3.3) is the cornerstone of this synergy. Corosolic acid

from Banaba promotes GLUT4 translocation via AMPK phosphorylation, independent of insulin receptor activation. Quercetin and kaempferol from Moringa potentiate insulin signalling through PI3K/Akt, providing an insulin-dependent GLUT4 activation pathway that is mechanistically distinct from and complementary to corosolic acid. Gallic and ellagic acids from Jamun competitively inhibit α-glucosidase at the enzyme active site. Trigonelline and 4-HIL from Fenugreek stimulate insulin secretion through glucose-dependent calcium influx and suppress hepatic gluconeogenesis. When these four chemically distinct pharmacophores converge on multiple molecular targets simultaneously, the resulting effect is supraadditive—a textbook case of pharmacodynamic synergy.

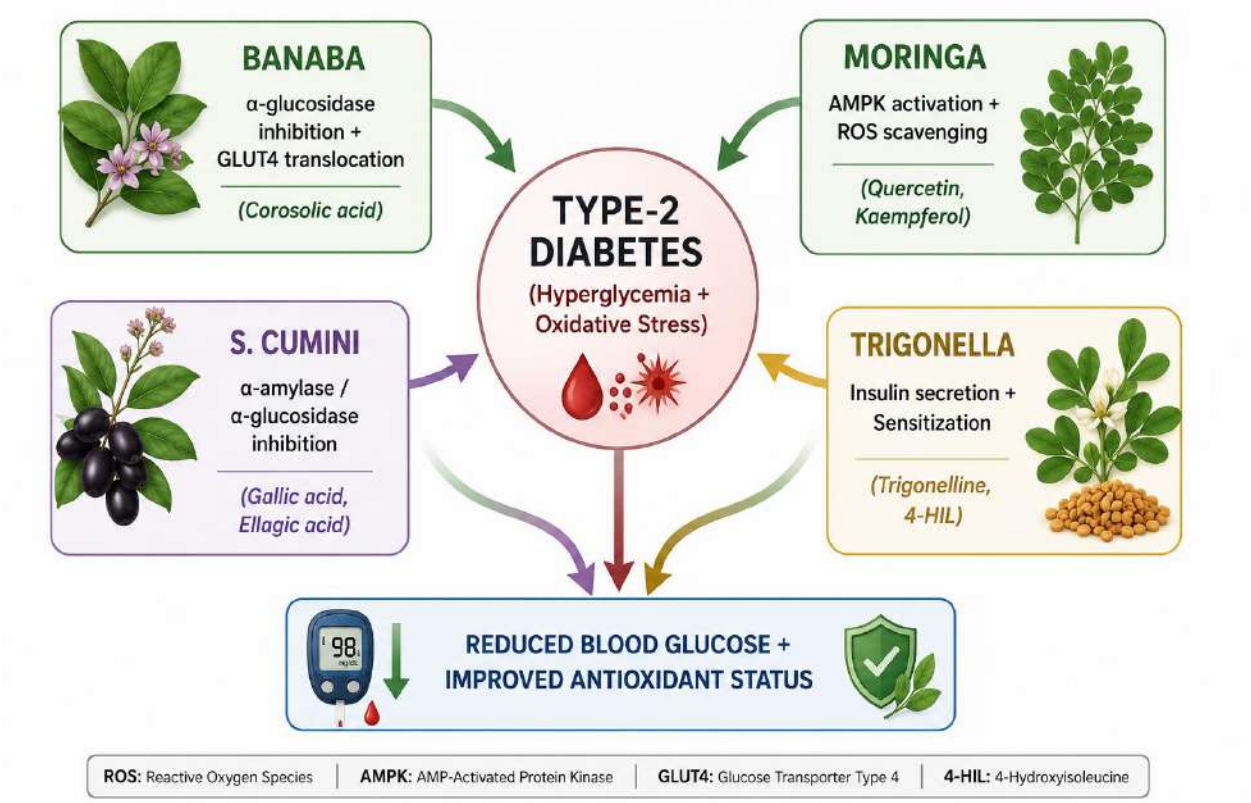

Figure 10. Proposed mechanistic model of the synergistic anti-diabetic action of the optimised polyherbal combination. Banaba and S. cumini provide α-glucosidase inhibition and antioxidant defence; Moringa contributes AMPK activation and ROS scavenging; Trigonella supplies insulin secretion and sensitisation; together they produce an integrated reduction in blood glucose and oxidative stress.

The RSM optimum ratio (38:18:30:14 for Banaba:Moringa:Jamun:Fenugreek) is consistent with the pharmacological weighting implied by the individual IC_{50} data: the two most potent enzyme inhibitors (Banaba and Jamun) are assigned the highest proportions, while Moringa and Fenugreek contribute complementary mechanisms at lower proportions. The highly significant X_1X_3 interaction term in the RSM model provides a rigorous mathematical confirmation of the empirical binary synergy observed between Banaba and Jamun by CI analysis. The R^2 of 0.973 and non-significant lack-of-fit confirm that the second-order polynomial adequately captures the combinatorial response surface.

The biphasic in vivo glucose reduction observed in the combination group is mechanistically

informative. The initial rapid decline (days 0–7, ~35% reduction) likely reflects α-amylase and α-glucosidase inhibition by Banaba and Jamun limiting postprandial glucose surges. The slower second phase (days 7–21) reflects the insulin-sensitising and insulin-secretagogue contributions of Moringa (AMPK/GLUT4) and Fenugreek (4-HIL/diosgenin). The absence of hypoglycaemia throughout confirms homeostatic correction rather than forced glucose-lowering an important safety distinction from sulphonylureas.

Comparison with the existing literature validates the present quantitative findings. The corosolic acid content of Banaba (24.65 mg/g DE) and its α-glucosidase IC_{50} (46.32 µg/mL) align closely with values reported by Stohs et al. (2012) and Miura et al. (2012). The TFC and flavonoid profile of Moringa leaves are consistent with Vergara-Jimenez et al. (2017). The TPC of Jamun seeds matches published ranges (Ali et al., 2023), and the alkaloid content of Fenugreek (8.42% w/w) falls within the 7.5–9.0% range of Wani and Kumar (2018). This convergence with independent datasets validates the methodological reliability of the study.

Several limitations must be acknowledged. The study used yeast α-glucosidase rather than mammalian intestinal preparations, which may yield higher IC_{50} values; the data therefore represent a conservative estimate of enzyme inhibitory potency. The STZ-induced model is more representative of type 1 than type 2 diabetes; high-fat diet/low-dose STZ models would strengthen T2DM-specific inference. Geographical variation in phytochemical content was not addressed, and the absence of Western blotting or qPCR data limits mechanistic inference to pathway-level deduction. Long-term safety, pharmacokinetic interactions, and possible drug–herb interactions remain to be investigated.

Notwithstanding these limitations, the practical implications are substantial. The four plants are widely cultivated, traditionally consumed, and have well-established safety records. The RSM-derived optimum ratio provides a quantitative starting point that eliminates the trial-and-error of traditional polyherbal development. The CI < 0.5 result implies that each component can be used at sub-therapeutic doses within the combination, reducing cost and the risk of dose-related adverse effects. The concurrent restoration of glycaemic, lipid, and antioxidant parameters further positions the formulation as a multi-system metabolic syndrome modulator—a clinically desirable profile for T2DM management.

5. Conclusions

This study demonstrates, for the first time, a comprehensive comparative phytochemical characterisation and quantitative synergy analysis of a quaternary polyherbal combination comprising Banaba, Moringa, Jamun, and Fenugreek. The four plants were shown to be chemically non-redundant, each contributing exclusive marker compounds corosolic acid, quercetin/kaempferol, gallic/ellagic acids, and trigonelline/4-HIL respectively that engage distinct and complementary anti-diabetic mechanisms. All tested combinations showed synergistic or additive interactions (CI < 1), with the quaternary mixture achieving the strongest synergy (CI = 0.42 for α-glucosidase). The RSM-optimised formulation (38:18:30:14 by weight) was experimentally validated and produced in vivo glycaemic control comparable to metformin alongside concurrent restoration of lipid profiles and hepatic antioxidant enzymes. These findings establish a robust preclinical foundation for the development of a novel, evidence-based polyherbal formulation for T2DM management. Future work should focus on marker-based standardisation, molecular characterisation of GLUT4/AMPK/PPARγ signalling pathways, pharmacokinetic interaction studies, and a controlled clinical trial comparing the combination against individual components and standard-of-care therapy.

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