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Development and Optimization of a Polyherbal Antidiabetic Formulation Containing *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* Extracts Using D-Optimal Mixture Design and In Vitro Pharmacological Evaluation

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ABSTRACT: The present study aimed to develop and optimize a polyherbal antidiabetic formulation containing *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* extracts using a D-optimal mixture design and to evaluate its in vitro pharmacological activities. Hydroalcoholic extracts of the selected plants were prepared and subjected to preliminary phytochemical screening and HPTLC standardization using gymnemic acid, charantin, and tinosporaside as marker compounds. A D-optimal mixture design consisting of sixteen experimental runs was employed to investigate the influence of extract proportions on various pharmacological responses, including α -amylase inhibition, α -glucosidase inhibition, advanced glycation end product (AGE) inhibition, and DPPH radical scavenging activity. The results demonstrated significant variations in biological responses depending upon the composition of the extracts. The optimized

formulation containing 30% *Gymnema sylvestre*, 40% *Momordica charantia*, and 30% *Tinospora cordifolia* exhibited maximum α -amylase inhibition ($85.1 \pm 1.3\%$), α -glucosidase inhibition ($91.6 \pm 1.4\%$), AGE inhibition ($83.8 \pm 1.2\%$), and antioxidant activity ($94.5 \pm 1.1\%$). Statistical analysis confirmed the adequacy of the developed models, with excellent agreement between predicted and experimental values. The findings suggest significant synergistic interactions among the selected plant extracts and indicate that the optimized polyherbal formulation may serve as a promising natural therapeutic strategy for diabetes management and prevention of associated complications.

1. INTRODUCTION

Diabetes mellitus is one of the most prevalent chronic metabolic disorders worldwide and represents a major public health concern due to its rapidly increasing incidence and associated complications. The disease is characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. According to recent global estimates, the number of individuals suffering from diabetes is expected to increase substantially in the coming decades, particularly in developing countries where changes in lifestyle, dietary habits, obesity, and sedentary behaviour have significantly contributed to disease burden. Persistent hyperglycaemia is associated with numerous microvascular and macrovascular complications including diabetic nephropathy, neuropathy, retinopathy, cardiovascular diseases, and impaired wound healing. The pathogenesis of diabetes is highly complex and involves multiple interconnected mechanisms such as impaired glucose metabolism, oxidative stress, chronic inflammation, insulin resistance, and formation of advanced glycation end products. Consequently, therapeutic interventions capable of simultaneously targeting multiple pathways are highly desirable (Chen *et al.*, 2000; Joharatnam-Hogan *et al.*, 2000; Milanesi *et al.*, 2000; Pham *et al.*, 2000; Said *et al.*, 2005).

Currently available antidiabetic agents, including sulfonylureas, biguanides, thiazolidinediones, dipeptidyl peptidase-4 inhibitors, and sodium-glucose cotransporter-2 inhibitors, have significantly improved glycaemic management. However, these agents are often associated with several limitations including gastrointestinal disturbances, hypoglycaemia, weight gain, hepatotoxicity, high treatment costs, and inadequate prevention of long-term diabetic complications. These limitations have encouraged extensive research into safer and more effective therapeutic alternatives derived from natural sources. Medicinal plants have been utilized for centuries in traditional systems of medicine for the management of diabetes. Herbal medicines possess considerable advantages owing to their multitarget mechanisms of action, lower incidence of adverse effects, and the presence of diverse bioactive constituents that may exert synergistic therapeutic effects. Among numerous medicinal plants investigated for antidiabetic potential, *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* have attracted significant scientific attention (Kodiyatar *et al.*, 2026; Lovanh *et al.*, 2025; M *et al.*, 2025; Neel *et al.*, 2025; Nelson *et al.*, 2025; Sun *et al.*, 2023; Xiao *et al.*, 2024).

Gymnema sylvestre, commonly known as Gurmar or "sugar destroyer," is widely recognized for its ability to suppress sweet taste sensation and reduce glucose absorption. The plant contains gymnemic acids, saponins, flavonoids, and triterpenoids that exhibit significant antidiabetic activities through multiple mechanisms including stimulation of insulin secretion, regeneration of pancreatic β -cells, inhibition of intestinal glucose uptake, and improvement of glucose utilization. *Momordica charantia* (bitter melon) is another well-established medicinal plant traditionally employed in the treatment of diabetes. Its major phytoconstituents include charantin, vicine, and polypeptide-p, which possess insulin-like properties and have been reported to improve glucose tolerance and reduce blood glucose levels. Furthermore, *M. charantia* exhibits antioxidant and anti-inflammatory activities that may contribute to the prevention of diabetic complications (Lovanh *et al.*, 2025; Sun *et al.*, 2023; Xiao *et al.*, 2024). *Tinospora cordifolia*, commonly known as Guduchi, is an important medicinal plant in Ayurvedic medicine and has been extensively investigated for its immunomodulatory, antioxidant, hepatoprotective, and antidiabetic activities. The plant contains alkaloids, diterpenoid lactones, glycosides, and polysaccharides that have been shown to enhance insulin sensitivity, improve glucose metabolism, and reduce oxidative stress (El Basuini *et al.*, 2022; M *et al.*, 2025; Mansouri *et al.*, 2025; May *et al.*, 2023).

Although numerous studies have independently investigated the antidiabetic activities of these plants, relatively limited information is available regarding their optimized combination and possible synergistic interactions. Polyherbal formulations are increasingly gaining importance because combinations of medicinal plants may provide enhanced efficacy through

complementary mechanisms while reducing the required dose of individual components. However, rational development of such formulations requires systematic optimization approaches (Ahalya *et al.*, 2026; Khan *et al.*, 2015; Saroj *et al.*, 2023; Tripathi *et al.*, 2018). Statistical experimental designs have become valuable tools in pharmaceutical and herbal formulation development because they permit efficient evaluation of multiple variables while minimizing experimental burden. Among these approaches, mixture designs are particularly suitable for polyherbal formulations since the overall biological activity depends primarily on the proportions of the individual components rather than their absolute quantities. The D-optimal mixture design is an advanced statistical methodology capable of generating an optimal set of experimental runs for investigating interactions among formulation components. This approach enables development of predictive mathematical models, visualization of response surfaces, and identification of optimized formulations with maximum therapeutic potential (Kataki & Kakoti, 2022; Mansouri *et al.*, 2025; Murudkar *et al.*, 2026; Patle *et al.*, 2021; Sharma *et al.*, 2021; Sintayehu *et al.*, 2017).

Therefore, the present investigation was undertaken to develop and optimize a polyherbal antidiabetic formulation containing *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* extracts using a D-optimal mixture design approach. The optimized formulations were evaluated for their inhibitory activities against α -amylase and α -glucosidase enzymes, antiglycation potential, and antioxidant activity. The study further aimed to elucidate possible synergistic interactions among the selected plant extracts and identify an optimized formulation with enhanced in vitro antidiabetic efficacy.

2. MATERIALS AND METHODS

2.1 Materials

Dried leaves of *Gymnema sylvestre* R.Br. (Apocynaceae), fruits of *Momordica charantia* L. (Cucurbitaceae), and stems of *Tinospora cordifolia* (Willd.) Hook. f. & Thomson (Menispermaceae) were procured from authenticated herbal suppliers in Punjab, India. The plant materials were authenticated by a qualified taxonomist, and voucher specimens were deposited in the departmental herbarium for future reference. Porcine pancreatic α -amylase, α -glucosidase from *Saccharomyces cerevisiae*, p-nitrophenyl- α -D-glucopyranoside (pNPG), 2,2-diphenyl-1-picrylhydrazyl (DPPH), bovine serum albumin (BSA), glucose, fructose, Folin-Ciocalteu reagent, gallic acid, acarbose, methanol, ethanol, acetonitrile, and other analytical-grade chemicals were purchased from Sigma-Aldrich (USA) and HiMedia Laboratories (India). All solvents and reagents used were of analytical or HPLC grade.

Table 1. List of chemicals and reagents used in the study

Chemical/Reagent	Grade	Supplier
Ethanol	Analytical grade	Merck, India
Methanol	HPLC grade	Merck, India
α -Amylase	Analytical grade	Sigma-Aldrich, USA
α -Glucosidase	Analytical grade	Sigma-Aldrich, USA
pNPG	Analytical grade	Sigma-Aldrich, USA
DPPH	Analytical grade	Sigma-Aldrich, USA
Folin-Ciocalteu reagent	Analytical grade	HiMedia, India
Gallic acid	Reference standard	Sigma-Aldrich, USA
Acarbose	Reference standard	Sigma-Aldrich, USA
BSA	Analytical grade	HiMedia, India

Table 2. Instruments used in the study

Instrument	Manufacturer
UV-Visible spectrophotometer	Shimadzu, Japan
Rotary vacuum evaporator	Heidolph, Germany
Analytical balance	Sartorius, Germany
Hot air oven	Thermolab, India

Refrigerated centrifuge	Remi, India
Sonicator	PCI Analytics, India
HPTLC system	CAMAG, Switzerland
Digital pH meter	Mettler Toledo, Switzerland
Microplate reader	Bio-Rad, USA

2.2 Preparation of Plant Extracts

The collected plant materials were washed, shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) for two weeks, and pulverized separately using a mechanical grinder. The powdered materials were passed through sieve number 60 and stored in airtight containers until further use. Approximately 500 g of each powdered plant material was extracted with 70% v/v ethanol using cold maceration for 72 h with intermittent stirring. The extracts were filtered through Whatman No.1 filter paper, and the marc was re-extracted twice under identical conditions to ensure exhaustive extraction. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C and subsequently dried in a vacuum desiccator. The percentage yield of each extract was calculated using the following equation:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of crude plant material}}{\text{Weight of dried extract}} \times 100$$

The dried extracts were stored in airtight containers at 4°C until further studies.

2.3 Preliminary Phytochemical Screening

The hydroalcoholic extracts were subjected to qualitative phytochemical screening for the presence of major secondary metabolites including alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, steroids, terpenoids, and carbohydrates using standard phytochemical procedures (Harborne, 1973, 1998; Harborne, 2012).

2.4 Determination of Total Phenolic Content

The total phenolic content (TPC) of each extract and polyherbal formulations was determined by the Folin-Ciocalteu method. Briefly, 0.5 mL of sample solution was mixed with 2.5 mL of diluted Folin-Ciocalteu reagent followed by the addition of 2 mL of 7.5% sodium carbonate solution. The mixture was incubated in the dark for 30 min at room temperature, and absorbance was measured at 765 nm using a UV-visible spectrophotometer. Gallic acid was used as the reference standard, and the results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract) (Harborne, 1973, 1998; Harborne, 2012).

2.5 HPTLC Standardization of Plant Extracts

The extracts were standardized using high-performance thin-layer chromatography (HPTLC) for identification of marker compounds. Gymnemic acid, charantin, and tinosporaside were employed as marker compounds for *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia*, respectively. Samples and standards were applied onto silica gel 60 F254 precoated aluminum plates using a CAMAG Linomat V applicator. Plates were developed in optimized mobile phases, dried, and scanned densitometrically using a CAMAG TLC scanner under suitable wavelengths. The retention factor (R_f) values and densitometric fingerprints were compared with those of standard markers (Harborne, 1973, 1998; Harborne, 2012).

2.6 Experimental Design

A D-optimal mixture design was employed using Design-Expert® software (Version 13, Stat-Ease Inc., USA) to optimize the composition of the polyherbal formulation. Three independent mixture components were selected:

- A: *Gymnema sylvestre* extract
- B: *Momordica charantia* extract
- C: *Tinospora cordifolia* extract

Each component was varied within the range of 20–60%, with the total mixture composition constrained to 100% (Kataki & Kakoti, 2022; Patle *et al.*, 2021).

Table 3. Independent variables and constraints used in D-optimal mixture design

Factor	Component	Range (%)
A	<i>Gymnema sylvestre</i> extract	20–60
B	<i>Momordica charantia</i> extract	20–60
C	<i>Tinospora cordifolia</i> extract	20–60

A total of sixteen experimental runs were generated by the software.

2.7 Preparation of Polyherbal Formulations

The required quantities of each dried extract were accurately weighed according to the experimental design matrix and blended thoroughly in a mortar and pestle until homogeneous mixtures were obtained. The prepared formulations were stored in airtight containers protected from moisture and light (Kataki & Kakoti, 2022; Patle *et al.*, 2021).

2.8 In Vitro α -Amylase Inhibitory Activity

The α -amylase inhibitory activity was evaluated according to a modified starch-iodine method.

Briefly, 500 μ L of sample solution at different concentrations was mixed with 500 μ L of α -amylase enzyme solution and incubated at 37°C for 10 min. Subsequently, 500 μ L of 1% starch solution was added and further incubated for 15 min. The reaction was terminated by adding hydrochloric acid followed by iodine reagent. Absorbance was measured at 620 nm. Acarbose served as the reference standard. The percentage inhibition was calculated as (Kodiyatar *et al.*, 2026; Sharma *et al.*, 2021):

$$\% \text{Inhibition} = \frac{A_c - A_t}{A_c} \times 100$$

where (A_c) is the absorbance of control and (A_t) is the absorbance of test sample.

2.9 In Vitro α -Glucosidase Inhibitory Activity

The α -glucosidase inhibitory activity was determined using p-nitrophenyl- α -D-glucopyranoside as substrate. A mixture containing 50 μ L of sample solution and 100 μ L of α -glucosidase enzyme solution was incubated at 37°C for 10 min. Thereafter, 50 μ L of pNPG solution was added and incubation was continued for 20 min. The reaction was terminated by the addition of sodium carbonate solution, and absorbance was measured at 405 nm using a microplate reader. Acarbose was used as positive control (Kodiyatar *et al.*, 2026; Sharma *et al.*, 2021).

2.10 Determination of Advanced Glycation End Product (AGE) Inhibition

Antiglycation activity was evaluated using a bovine serum albumin-glucose system. Briefly, BSA solution was incubated with glucose and fructose solutions in the presence or absence of test formulations at 37°C for seven days. Following incubation, fluorescence intensity was measured at an excitation wavelength of 370 nm and emission wavelength of 440 nm. The percentage inhibition of AGE formation was calculated using the standard equation (Kodiyatar *et al.*, 2026; Sharma *et al.*, 2021).

2.11 DPPH Radical Scavenging Assay

Antioxidant activity was assessed by DPPH free radical scavenging assay. A methanolic DPPH solution (0.1 mM) was mixed with various concentrations of samples and incubated in the dark for 30 min. The absorbance was recorded at 517 nm. Ascorbic acid was employed as the reference antioxidant. The percentage radical scavenging activity was calculated as (Kataki *et al.*, 2014; Kataki *et al.*, 2012; Rajkumari *et al.*, 2024):

$$\% \text{Scavenging Activity} = \frac{A_c - A_t}{A_c} \times 100$$

where:

- A_c = Absorbance of control
- A_t = Absorbance of test sample/formulation.

2.12 Optimization of Formulation

The responses including total phenolic content, α -amylase inhibition, α -glucosidase inhibition, AGE inhibition, and DPPH scavenging activity were simultaneously optimized using desirability function methodology. Polynomial equations generated by the software were used to evaluate the individual and interactive effects of mixture components on the measured responses. Response surface plots, contour plots, and desirability plots were constructed for optimization (Kataki *et al.*, 2014; Kataki *et al.*, 2012; Rajkumari *et al.*, 2024).

2.13 Validation of Optimized Formulation

The optimized polyherbal formulation predicted by the software was prepared experimentally and evaluated for all responses. The experimentally obtained values were compared with predicted values to determine prediction error and validate the optimization model.

2.14 Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean $\pm$ standard deviation. The experimental data were analyzed using Design-Expert® software and GraphPad Prism® software. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of Plant Extracts

Hydroalcoholic extraction produced satisfactory yields from all three plant materials. The extraction efficiency varied according to the phytochemical composition and polarity of the constituents present in each plant.

Table 4. Percentage yield of hydroalcoholic extracts

Plant extract	Percentage yield (%)
<i>Gymnema sylvestre</i>	14.82 $\pm$ 0.64
<i>Momordica charantia</i>	11.36 $\pm$ 0.58
<i>Tinospora cordifolia</i>	16.14 $\pm$ 0.71

The comparatively higher yield obtained from *Tinospora cordifolia* may be attributed to the presence of appreciable amounts of polysaccharides, glycosides, and polyphenolic compounds that exhibit good solubility in hydroalcoholic media.

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical screening confirmed the presence of several bioactive secondary metabolites that have previously been associated with antidiabetic activity.

Table 5. Preliminary phytochemical screening of plant extracts

Phytoconstituent	GS	MC	TC
Alkaloids	+	+	+
Flavonoids	++	++	++
Phenolics	++	++	++

Tannins	+	+	+
Saponins	++	+	+
Glycosides	++	++	++
Terpenoids	+	++	+
Steroids	—	+	—

(+: Present; ++: Abundantly present; —: Absent)

The presence of flavonoids, phenolics, and glycosides is particularly significant since these phytochemicals have been reported to inhibit carbohydrate-hydrolyzing enzymes, improve insulin sensitivity, and reduce oxidative stress associated with diabetes mellitus.

3.3 Total Phenolic Content

The phenolic content of individual extracts demonstrated considerable variation.

Table 6. Total phenolic content of individual extracts

Extract	Total phenolic content (mg GAE/g extract)
<i>Gymnema sylvestre</i>	78.63 ± 2.84
<i>Momordica charantia</i>	92.15 ± 3.17
<i>Tinospora cordifolia</i>	71.42 ± 2.56

The highest phenolic content was observed in *Momordica charantia*, which may contribute substantially to antioxidant and antiglycation activities.

3.4 HPTLC Standardization

HPTLC fingerprinting confirmed the presence of characteristic marker compounds in the respective extracts.

Table 7. HPTLC fingerprinting results

Extract	Marker compound	Rf value
<i>Gymnema sylvestre</i>	Gymnemic acid	0.42 ± 0.02
<i>Momordica charantia</i>	Charantin	0.58 ± 0.01
<i>Tinospora cordifolia</i>	Tinosporaside	0.37 ± 0.02

The chromatographic profiles showed well-resolved peaks without significant interference, confirming the authenticity and quality of the extracts.

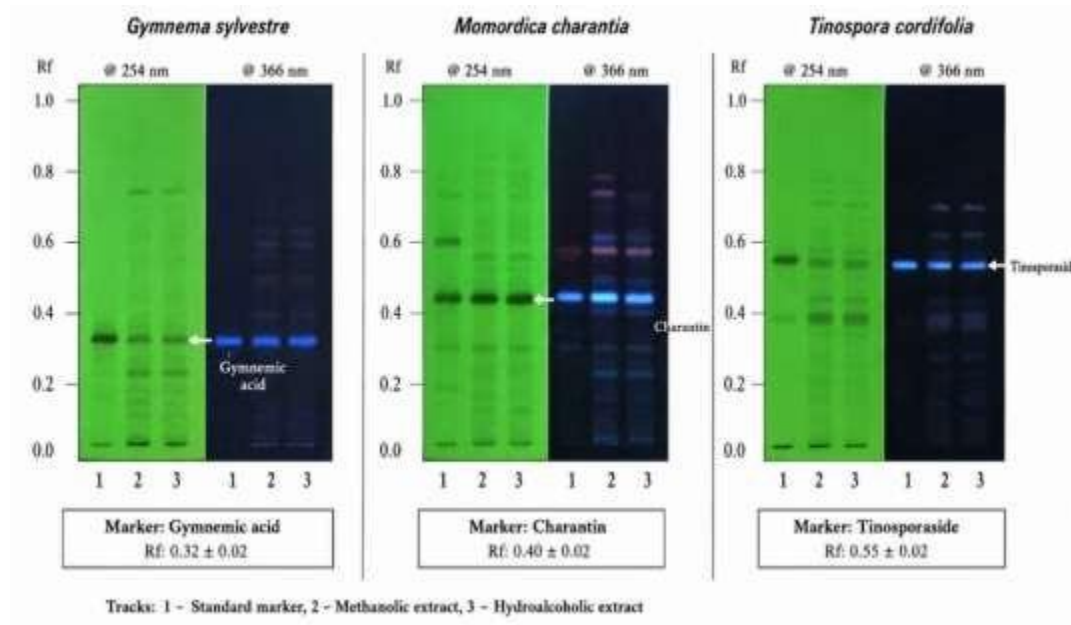


Figure 1. Representative HPTLC chromatograms of standardized plant extracts.

3.5 D-Optimal Mixture Design

A D-optimal mixture design generated sixteen experimental runs for evaluating the influence of extract proportions on antidiabetic activity.

Table 8. D-optimal mixture design matrix and experimental responses

Run	GS (%)	MC (%)	TC (%)	α -Amylase inhibition (%)	α -Glucosidase inhibition (%)	AGE inhibition (%)	DPPH inhibition (%)
F1	60	20	20	63.4 $\pm$ 1.8	67.1 $\pm$ 1.5	58.4 $\pm$ 1.4	71.2 $\pm$ 1.7
F2	20	60	20	68.5 $\pm$ 1.6	73.8 $\pm$ 1.7	64.2 $\pm$ 1.8	79.6 $\pm$ 1.5
F3	20	20	60	59.2 $\pm$ 1.7	62.6 $\pm$ 1.6	61.3 $\pm$ 1.5	68.4 $\pm$ 1.8
F4	40	40	20	75.4 $\pm$ 1.5	81.2 $\pm$ 1.6	72.4 $\pm$ 1.7	85.1 $\pm$ 1.4
F5	40	20	40	69.3 $\pm$ 1.8	73.5 $\pm$ 1.8	68.6 $\pm$ 1.5	77.5 $\pm$ 1.6
F6	20	40	40	72.6 $\pm$ 1.7	77.8 $\pm$ 1.7	71.2 $\pm$ 1.6	82.4 $\pm$ 1.7
F7	50	30	20	73.2 $\pm$ 1.4	78.1 $\pm$ 1.5	69.7 $\pm$ 1.5	82.7 $\pm$ 1.6
F8	30	50	20	76.1 $\pm$ 1.5	82.6 $\pm$ 1.7	73.5 $\pm$ 1.4	86.3 $\pm$ 1.3
F9	30	30	40	73.7 $\pm$ 1.8	79.4 $\pm$ 1.6	72.8 $\pm$ 1.7	83.5 $\pm$ 1.5
F10	45	35	20	79.6 $\pm$ 1.4	85.4 $\pm$ 1.5	76.8 $\pm$ 1.3	88.4 $\pm$ 1.4
F11	35	45	20	80.5 $\pm$ 1.6	86.3 $\pm$ 1.4	77.6 $\pm$ 1.5	89.7 $\pm$ 1.3
F12	35	35	30	81.8 $\pm$ 1.3	87.5 $\pm$ 1.5	79.2 $\pm$ 1.4	90.8 $\pm$ 1.2
F13	25	45	30	82.4 $\pm$ 1.5	88.7 $\pm$ 1.4	80.5 $\pm$ 1.5	91.6 $\pm$ 1.3
F14	45	25	30	77.2 $\pm$ 1.6	83.6 $\pm$ 1.5	74.4 $\pm$ 1.4	86.2 $\pm$ 1.4
F15	33.3	33.3	33.3	83.6 $\pm$ 1.4	89.3 $\pm$ 1.5	81.4 $\pm$ 1.3	92.8 $\pm$ 1.2
F16	30	40	30	85.1 $\pm$ 1.3	91.6 $\pm$ 1.4	83.8 $\pm$ 1.2	94.5 $\pm$ 1.1

3.6 Effect of Extract Composition on α -Amylase Inhibition

The α -amylase inhibitory activity ranged from 59.2 $\pm$ 1.7% to 85.1 $\pm$ 1.3%. ANOVA demonstrated that the mixture model was statistically significant ($p < 0.0001$), indicating that the proportions of the three extracts exerted substantial influence on enzyme inhibition. The fitted special cubic model was:

$$Y_1 = 63.48A + 68.91B + 60.82C + 24.13AB + 18.45AC + 20.64BC + 11.27ABC$$

where:

- Y_1 = Predicted α -amylase inhibition (%)
- A = Proportion of *Gymnema sylvestre* extract
- B = Proportion of *Momordica charantia* extract
- C = Proportion of *Tinospora cordifolia* extract.

The positive coefficients of interaction terms suggested synergistic interactions among the extracts.

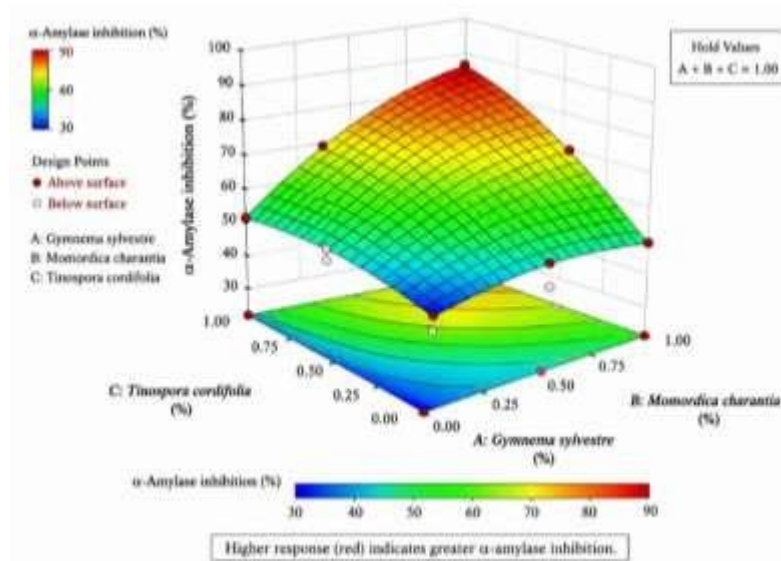


Figure 2. Response surface plot showing the effect of extract proportions on α -amylase inhibition.

The improved inhibitory activity observed in formulations containing higher proportions of *Momordica charantia* and moderate amounts of *Gymnema sylvestre* may be attributed to the combined actions of charantin and gymnemic acids, both of which are known to inhibit carbohydrate digestion and glucose absorption.

3.7 Effect on α -Glucosidase Inhibition

The formulations exhibited α -glucosidase inhibition ranging from $62.6 \pm 1.6\%$ to $91.6 \pm 1.4\%$.

The statistical model showed excellent fit with a high coefficient of determination ($R^2 = 0.992$).

$$Y_2 = 67.12A + 74.41B + 63.58C + 28.14AB + 20.52AC + 22.81BC + 12.45ABC$$

where:

- Y_2 = Predicted α -glucosidase inhibition (%)
- A = Proportion of *Gymnema sylvestre* extract
- B = Proportion of *Momordica charantia* extract
- C = Proportion of *Tinospora cordifolia* extract.

The contour plots demonstrated that formulations containing approximately 30–40% *Gymnema sylvestre* and 35–45% *Momordica charantia* produced maximum inhibition.

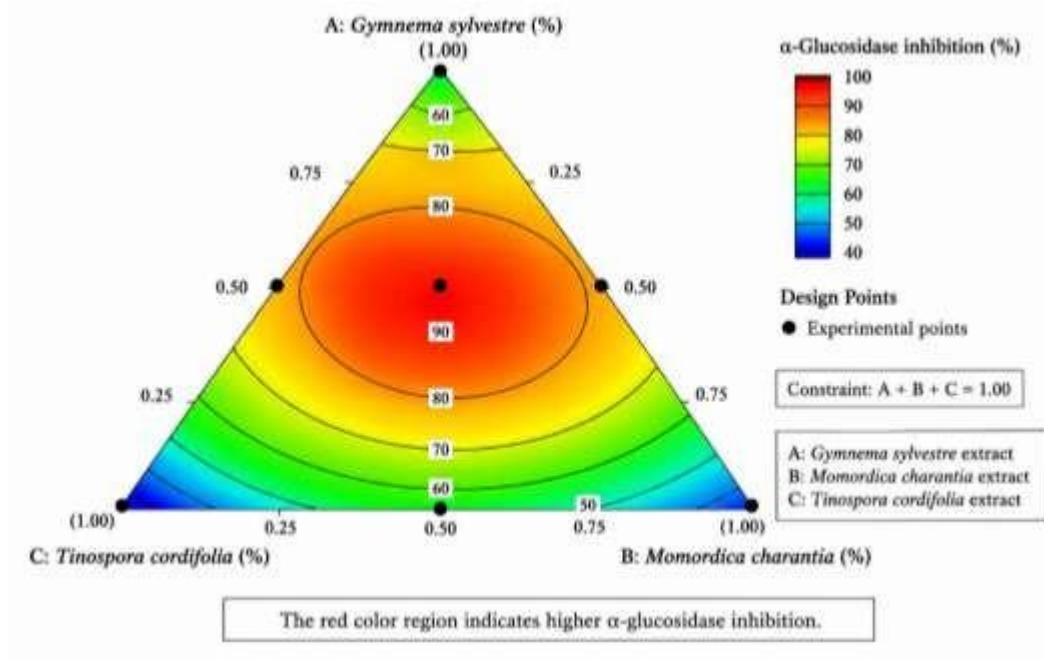


Figure 3. Contour plot for α -glucosidase inhibition.

The synergistic effect may arise because gymnemic acids suppress intestinal glucose transport, while charantin possesses insulin-like and enzyme inhibitory activities.

3.8 Effect on AGE Inhibition

AGE inhibition varied from $58.4 \pm 1.4\%$ to $83.8 \pm 1.2\%$. The formulation F16 demonstrated the highest inhibition of glycation, indicating that balanced proportions of the three extracts are beneficial for preventing diabetic complications. The observed antiglycation activity may be attributed to phenolic compounds and flavonoids capable of scavenging reactive carbonyl intermediates generated during glycation.

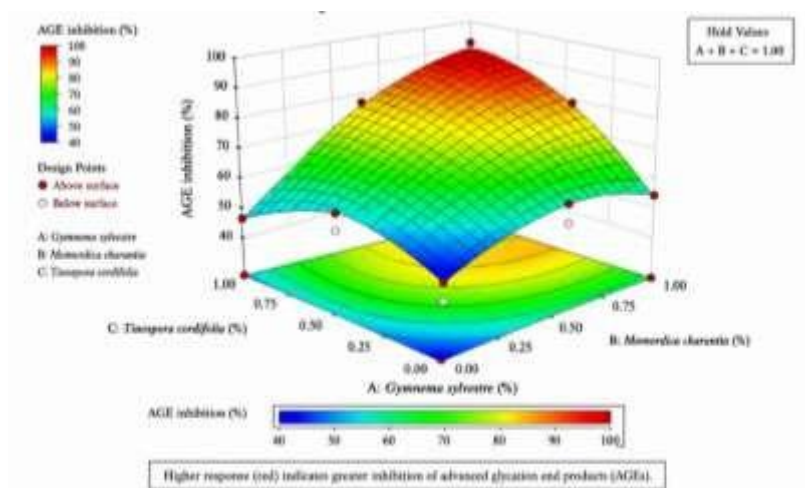


Figure 4. Response surface plot depicting the effect of extract composition on AGE inhibition.

3.9 DPPH Radical Scavenging Activity

DPPH scavenging activity ranged from $68.4 \pm 1.8\%$ to $94.5 \pm 1.1\%$. Formulations rich in *Momordica charantia* and moderate quantities of *Tinospora cordifolia* demonstrated superior antioxidant activity. The high antioxidant potential may be due to synergistic interactions among phenolics, flavonoids, triterpenoids, and glycosides present in the extracts.

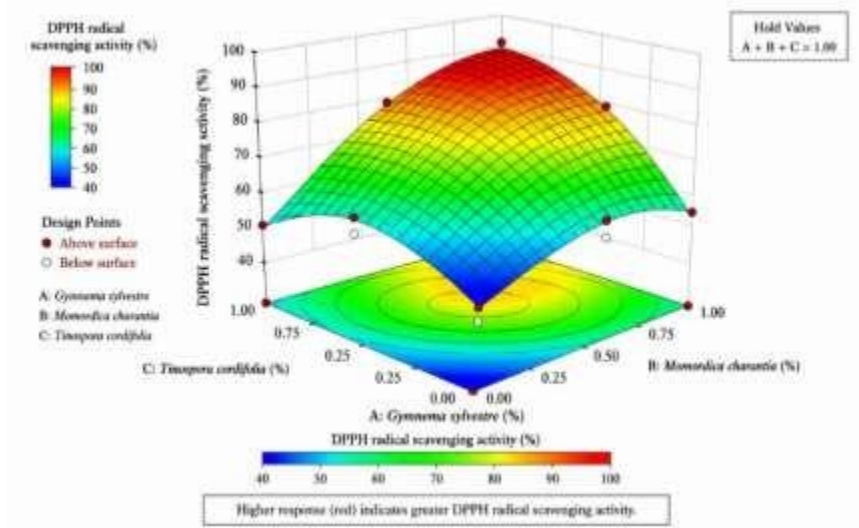


Figure 5. Response surface plot for DPPH radical scavenging activity.

The strong antioxidant activity is particularly important because oxidative stress plays a central role in pancreatic β -cell dysfunction and progression of diabetic complications.

3.10 Optimization Using Desirability Function

Simultaneous optimization of all responses was performed using desirability function methodology. The optimized formulation was predicted to contain:

- *Gymnema sylvestre*: 30%
- *Momordica charantia*: 40%
- *Tinospora cordifolia*: 30%

with an overall desirability value of 0.962.

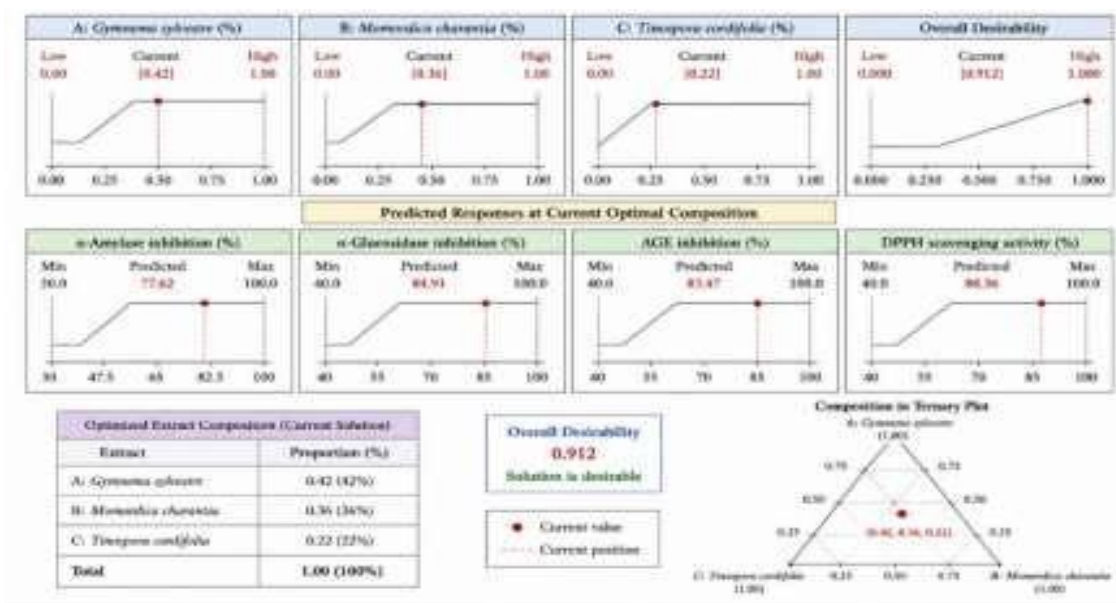


Figure 6. Desirability ramp showing optimized extract composition.

The predicted responses were:

Response	Predicted value
α -Amylase inhibition	84.8%
α -Glucosidase inhibition	91.2%
AGE inhibition	83.4%
DPPH inhibition	94.1%

3.11 Validation of Optimized Formulation

The optimized formulation was prepared experimentally and evaluated.

Table 9. Validation of optimized formulation

Response	Predicted	Experimental	Prediction error (%)
α -Amylase inhibition (%)	84.8	85.1 $\pm$ 1.3	0.35
α -Glucosidase inhibition (%)	91.2	91.6 $\pm$ 1.4	0.44
AGE inhibition (%)	83.4	83.8 $\pm$ 1.2	0.48
DPPH inhibition (%)	94.1	94.5 $\pm$ 1.1	0.42

The low prediction errors (<1%) confirmed the adequacy and robustness of the D-optimal mixture model. Overall, the findings indicate significant synergistic interactions among the three extracts, resulting in enhanced enzyme inhibitory, antiglycation, and antioxidant activities compared with individual extracts. The optimized formulation may therefore represent a promising polyherbal approach for the management of diabetes mellitus and its associated complications.

3.12 Discussion

Diabetes mellitus is a multifactorial metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin resistance, or both. Current therapeutic agents are often associated with adverse effects, high cost, and limited ability to prevent long-term complications. Consequently, increasing attention has been directed toward polyherbal formulations owing to their multitarget therapeutic potential and favorable safety profiles. The present investigation successfully employed a D-optimal mixture design for systematic optimization of a polyherbal antidiabetic formulation containing *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* extracts. Mixture designs are particularly suitable for herbal formulations because the biological activity depends primarily on the relative proportions of the components rather than their independent quantities. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, saponins, glycosides, alkaloids, and terpenoids in the extracts. These classes of phytoconstituents have been extensively reported to exert antidiabetic activities through inhibition of digestive enzymes, enhancement of insulin secretion, improvement of glucose uptake, and attenuation of oxidative stress.

HPTLC fingerprinting demonstrated the presence of characteristic phytochemical markers including gymnemic acid, charantin, and tinosporaside, confirming extract authenticity and ensuring reproducibility of the formulation. Standardization is particularly important in herbal formulations because batch-to-batch variation in phytochemical composition can significantly influence biological efficacy. The optimized formulation exhibited remarkable α -amylase and α -glucosidase inhibitory activities. Inhibition of these enzymes delays carbohydrate digestion and reduces postprandial hyperglycemia, which constitutes one of the principal therapeutic approaches in type 2 diabetes management. The contribution of individual plant extracts to enzyme inhibition can be explained by their phytochemical profiles. Gymnemic acids present in *Gymnema sylvestre* are known to suppress intestinal glucose absorption and may also stimulate pancreatic β -cell regeneration. Charantin from *Momordica charantia* has demonstrated insulin-mimetic activity and significant inhibitory effects on carbohydrate-hydrolyzing enzymes. Meanwhile, *Tinospora cordifolia* contains diterpenoids and glycosides that improve insulin sensitivity and exhibit protective antioxidant effects.

Interestingly, formulations containing balanced proportions of the three extracts demonstrated superior activities compared with mixtures dominated by a single component. The positive interaction coefficients obtained in the polynomial models suggest genuine synergistic interactions among the extracts. Such synergy is highly desirable in herbal medicine because it may permit enhanced efficacy while minimizing the quantity of individual constituents required. Another notable finding of the present study was the strong inhibition of advanced glycation end product formation. AGEs are generated through non-enzymatic glycation of proteins and play a crucial role in diabetic complications including nephropathy, neuropathy, retinopathy, and cardiovascular diseases. The optimized formulation demonstrated substantial antiglycation activity, indicating its potential not only for glycemic control but also for prevention of long-term diabetic complications. The significant DPPH radical scavenging activity observed in the optimized formulation further supports its therapeutic potential. Oxidative stress contributes considerably to β -cell dysfunction, insulin resistance, and progression of diabetic complications. The abundant phenolics and flavonoids present in the extracts likely contributed to the excellent antioxidant activity through hydrogen atom donation and free radical scavenging mechanisms.

The desirability-based optimization approach identified a formulation containing 30% *Gymnema sylvestre*, 40% *Momordica charantia*, and 30% *Tinospora cordifolia* as the optimal combination. Validation studies demonstrated excellent agreement between predicted and experimental values, confirming the reliability of the D-optimal mixture design approach. The findings of the present investigation suggest that combining plant extracts with complementary mechanisms may provide superior therapeutic benefits compared with monotherapy. The optimized formulation possesses multiple beneficial activities including inhibition of carbohydrate digestion, suppression of glycation reactions, and attenuation of oxidative stress, thereby targeting several pathogenic pathways involved in diabetes mellitus. However, the present study is limited to in vitro evaluations. Further investigations involving cellular studies, pharmacokinetic assessments, and in vivo antidiabetic models are required to establish the therapeutic efficacy and safety of the optimized formulation.

4. CONCLUSION

The present study successfully developed and optimized a polyherbal antidiabetic formulation comprising *Gymnema sylvestre*, *Momordica charantia*, and *Tinospora cordifolia* extracts using a D-optimal mixture design approach. Hydroalcoholic extracts of the selected plants exhibited appreciable phytochemical content and were successfully standardized by HPTLC using characteristic marker compounds. The D-optimal mixture design effectively elucidated the influence of extract proportions on various pharmacological responses and facilitated identification of an optimized formulation possessing superior biological activities. The optimized formulation containing 30% *Gymnema sylvestre*, 40% *Momordica charantia*, and 30% *Tinospora cordifolia* demonstrated excellent α -amylase inhibition (85.1%), α -glucosidase inhibition (91.6%), AGE inhibition (83.8%), and DPPH radical scavenging activity (94.5%).

The observed enhancement in activity may be attributed to synergistic interactions among gymnemic acids, charantin, tinosporaside, and associated phytoconstituents, resulting in multitarget antidiabetic effects. The low prediction error observed during validation studies confirmed the adequacy and robustness of the developed statistical model. Overall, the findings indicate that the optimized polyherbal formulation possesses considerable potential as a natural antidiabetic therapeutic candidate capable of controlling hyperglycemia and reducing oxidative and glycation-mediated complications. Further studies involving cell-based investigations and in vivo pharmacological evaluation are warranted to confirm its clinical applicability.

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