EJPMR, 2026, 13(8), 299-309 **Research Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

DESIGN, DEVELOPMENT, AND EVALUATION OF A POLYHERBAL FORMULATION FOR THE MANAGEMENT OF METABOLIC SYNDROME

Dr. Pooja Arora[1], Dr. Nikhil Batra[2], Dr. Shikha Batra[3], Balijepalli Madhu Katyayani[4], Prasanta Deka[5], Dr. Pankaj Pradhan[*6]

[1]Director Principal, Swami Devi Dyal Institute of Pharmacy, Barwala, Distt Panchkula.
[2]Associate Professor, Faculty of Pharmacy, Lachoo Memorial College of Science and Technology (Autonomous), Shastri Nagar, Jodhpur-342001, Rajasthan.
[3]Associate Professor, Faculty of Pharmacy, Lachoo Memorial College of Science and Technology (Autonomous), Shastri Nagar, Jodhpur-342001, Rajasthan.
[4]Asssociate Professor, Department of Pharmacology, Faculty of Medicine, MAHSA University, 42610, Jenjarom, Selangor, Malaysia.
[5]Assistant Professor, School of Pharmaceutical Sciences, University of science and technology meghalaya , Techno City, Kiling Road, Baridua, 9th Mile, Ri-Bhoi District, Meghalaya – 793101.
[6]Professor, Swami Keshvanand Institute of Pharmacy, Jaipur.

***Corresponding Author: Dr. Pankaj Pradhan**

MS Scholar, Second Year, Department of Shalya Tantra, Rani Dullaiya Smriti Ayurved P.G. College & Hospital, Bhopal (M.P.), India. **DOI:** https://doi.org/10.5281/zenodo.21702361

How to cite this Article: Dr. Pooja Arora[1], Dr. Nikhil Batra[2], Dr. Shikha Batra[3], Balijepalli Madhu Katyayani[4], Prasanta Deka[5], Dr. Pankaj Pradhan[*6] (2026). Design, Development, And Evaluation Of A Polyherbal Formulation For The Management Of Metabolic Syndrome. European Journal of Pharmaceutical and Medical Research, 13(8), 299–309.

Article Received on 24/06/2026 **Article Revised on 14/07/2026** **Article Published on 01/08/2026**

ABSTRACT

Metabolic syndrome (MetS) represents a clustering of interconnected risk factors including central obesity, insulin resistance, dyslipidemia, and hypertension, which collectively increase the risk of type 2 diabetes mellitus and cardiovascular disease. The limitations of conventional single-target therapies in addressing this multifactorial condition have renewed interest in polyherbal formulations that can simultaneously target multiple pathological pathways. This study aimed to design, develop, and evaluate a polyherbal formulation combining extracts of *Gymnema sylvestre*, *Camellia sinensis*, *Allium sativum*, and *Momordica charantia* for the comprehensive management of metabolic syndrome. Individual plant extracts were prepared using appropriate extraction methods and subjected to phytochemical screening (qualitative and quantitative analysis by HPTLC). A polyherbal tablet formulation was developed using wet granulation technique and optimized through 3^2 factorial design with binder concentration (PVP K30) and disintegrant concentration (sodium starch glycolate) as independent variables, evaluating tablet hardness and disintegration time as responses. The optimized formulation was evaluated for in-vitro antioxidant activity (DPPH assay), enzyme inhibition potential (alpha-amylase and lipase inhibition assays), and glucose adsorption and diffusion retardation capacity. Phytochemical screening revealed abundant glycosides (4.82% gymnemic acid) in *Gymnema sylvestre*, high flavonoid content (12.67% epigallocatechin gallate) in *Camellia sinensis*, significant terpenoids in *Allium sativum*, and diverse phytoconstituents in *Momordica charantia*. Formulation optimization identified F5 (4% PVP K30, 4% sodium starch glycolate) as the optimal formulation with acceptable hardness (5.1 kg/cm²) and rapid disintegration time (6.2 minutes). The polyherbal formulation demonstrated potent antioxidant activity (IC_{50} = 41.36 µg/mL; 81.75% inhibition at 100 µg/mL), significant alpha-amylase inhibition (IC_{50} = 54.83 µg/mL; 74.92% inhibition at 200 µg/mL) with competitive inhibition kinetics, and moderate lipase inhibition (IC_{50} = 72.58 µg/mL; 68.24% inhibition at 250 µg/mL). Glucose adsorption studies showed 15-17% glucose binding at physiologically relevant concentrations, while glucose diffusion was retarded by 32.39% during the critical initial absorption phase. The developed polyherbal formulation exhibits multi-mechanistic in-vitro bioactivity relevant to metabolic syndrome management, including antioxidant effects, enzyme inhibition (alpha-amylase and lipase), and glucose-modulating properties. The optimized formulation demonstrates acceptable pharmaceutical properties and provides a scientific rationale for further in-vivo evaluation.

This multi-targeted approach aligns with the complex pathophysiology of metabolic syndrome and offers a promising complementary strategy to conventional therapies.

KEYWORDS: Metabolic syndrome, polyherbal formulation, *Gymnema sylvestre*, *Camellia sinensis*, alpha-amylase inhibition, lipase inhibition, antioxidant, formulation optimization, design of experiments.

INTRODUCTION

The escalating global prevalence of Metabolic Syndrome (MetS) represents a critical public health challenge, underscoring the urgent need for comprehensive therapeutic strategies that move beyond the limitations of conventional medicine. MetS is not a single disease but a clustering of interconnected risk factors, including central obesity, insulin resistance, dyslipidemia, and hypertension, which collectively increase the risk of developing type 2 diabetes mellitus and cardiovascular disease by up to five-fold. Its pathophysiology is a complex, multi-faceted web involving chronic low-grade inflammation, oxidative stress, and endocrine dysfunction, primarily driven by visceral adipose tissue. Current therapeutic strategies, predominantly lifestyle modifications and single-target pharmaceuticals like statins, anti-hypertensives, and hypoglycemic agents, often prove insufficient. These approaches typically address individual components of the syndrome in isolation, failing to tackle its underlying systemic nature and frequently leading to poor patient adherence or adverse effects. This therapeutic gap has reignited interest in alternative paradigms, particularly the rich history of herbal medicine, which has long been employed in metabolic disorders. Traditional medical systems, such as Ayurveda and Traditional Chinese Medicine, have utilized plants for centuries to manage symptoms now recognized as components of MetS, offering a valuable repository of knowledge for modern drug discovery. A comprehensive review of medicinal plants reveals a vast pharmacopeia with potential in managing MetS, with specific plants demonstrating activity against its core components. For anti-hyperglycemic effects, plants like *Gymnema sylvestre*, known as the "sugar destroyer," have been shown to reduce sugar absorption in the gut and enhance insulin function. Similarly, *Syzygium cumini* (Java plum) and *Momordica charantia* (bitter melon) are well-documented for their ability to lower blood glucose levels through mechanisms involving increased glucose uptake and inhibition of hepatic gluconeogenesis. Addressing the dyslipidemia and obesity components, plants such as *Citrus reticulata* (mandarin orange) provide flavonoids like nobiletin that have demonstrated anti-obesity and lipid-lowering properties by modulating lipid metabolism. The ubiquitous *Camellia sinensis* (green tea), rich in catechins, is renowned for its ability to increase energy expenditure and fat oxidation, while extracts from *Quercus acutissima* (sawtooth oak) have shown promise in reducing cholesterol absorption. Furthermore, the hypertensive and inflammatory facets of MetS can be targeted by plants like *Allium sativum* (garlic), whose sulfur compounds, particularly allicin, exhibit both antihypertensive and anti-inflammatory effects. *Panax ginseng* is another adaptogenic herb that modulates inflammation and improves endothelial function, and the roots of *Salvia miltiorrhiza* are used in traditional medicine to improve circulation and mitigate inflammatory pathways central to MetS pathology. The true potential of herbal medicine in this context, however, may lie not in the use of single plants but in the strategic combination of multiple herbs. This approach is predicated on the principle of synergy, where the combined effect of a polyherbal formulation is greater than the sum of its individual parts. In the context of MetS, a multi-targeted disease, this synergistic potential is particularly compelling. A single polyherbal formulation could, for instance, combine a plant with insulin-mimetic properties, another that inhibits hepatic cholesterol synthesis, a third that acts as an anti-inflammatory agent, and a fourth that supports healthy blood pressure regulation. The phytochemical constituents within such a blend can interact in complex ways: one compound may enhance the bioavailability of another, they may act on different, complementary molecular targets within the same pathway, or they may work together to neutralize potential toxicities of individual ingredients. This holistic, multi-pronged attack aligns perfectly with the complex pathophysiology of MetS, offering a more integrated therapeutic approach than conventional monotherapy. Consequently, the market has seen the emergence of several standardized polyherbal formulations designed specifically for MetS. Products like Diabegon, Deltan, and Metabolaid represent a new generation of evidence-informed botanical drugs. These formulations are typically composed of extracts from several of the plants mentioned, such as *Gymnema sylvestre*, *Momordica charantia*, and *Curcuma longa*, combined in specific ratios. Preclinical and preliminary clinical studies on such formulations have reported encouraging results, demonstrating significant improvements in glycemic control, lipid profiles, and inflammatory markers with favorable safety profiles. For example, some studies on Metabolaid have shown its ability to reduce body weight and improve insulin sensitivity in obese patients, while others on Diabegon have highlighted its glucose-lowering efficacy comparable to some conventional drugs, but with fewer gastrointestinal side effects.

MATERIALS AND METHODS

Preparation of Herbal Extracts

The preparation of herbal extracts is a critical foundational step in the development of any phytopharmaceutical formulation, as the method chosen directly influences the profile and concentration of bioactive compounds recovered from the plant material. The primary goal is to efficiently separate the active principles from the inert cellular matrix while preserving their chemical integrity. Several classical extraction

techniques are routinely employed, each with distinct principles and applications. Soxhlet extraction is a highly efficient, continuous process where a solvent is repeatedly cycled through the plant material. Finely ground dried plant material is placed in a porous thimble, and the solvent is heated to reflux. The solvent vapor travels up, condenses, and drips onto the material, slowly filling the chamber and extracting the compounds before being automatically siphoned back into the boiling flask. This method is excellent for compounds with limited solubility and ensures exhaustive extraction without the need for large solvent volumes, though it exposes thermolabile compounds to prolonged heating. In contrast, maceration is a simpler, ambient-temperature process where coarsely powdered plant material is immersed in a solvent (like ethanol or water) in a closed container for an extended period, typically 3 to 7 days, with occasional stirring. This gentle method is ideal for extracting thermolabile constituents but is slower and often less complete. Decoction, a method reserved for tough plant parts like roots, bark, and stems, involves boiling the plant material in a specified volume of water for a defined time. This process is effective for extracting water-soluble, heat-stable compounds but is not suitable for volatile or heat-sensitive components.

Phytochemical screening

To identify and quantify the bioactive constituents present, ensuring consistency and quality. This is a two-pronged approach.

Qualitative analysis

It involves a series of simple chemical tests and color reactions to detect the presence of major phytochemical classes. For instance, the Dragendorff's test is used to confirm alkaloids, the Ferric chloride test for tannins and phenols, the Shinoda test for flavonoids, and the Molisch's test for carbohydrates and glycosides. These tests provide a preliminary phytochemical fingerprint of the extract.

Quantitative analysis

It employs more sophisticated analytical techniques, most commonly High-Performance Thin-Layer Chromatography (HPTLC) or High-Performance Liquid Chromatography (HPLC), to determine the exact concentration of specific marker compounds. For example, one might quantify the amount of gymnemic acid in a *Gymnema sylvestre* extract or the catechin content in a *Camellia sinensis* extract. This step is indispensable for standardizing the extract, ensuring batch-to-batch reproducibility, and establishing a correlation between the chemical composition and the biological activity of the final formulation.

Formulation Development

The transformation of a standardized herbal extract into a stable, effective, and patient-friendly dosage form is the essence of formulation development. For solid dosage forms like tablets or granules, this begins with the careful selection of excipients—inactive ingredients that play vital roles in the manufacturing process and product performance. Common excipients include diluents (e.g., lactose, microcrystalline cellulose) to achieve the desired tablet weight; binders (e.g., polyvinylpyrrolidone, starch paste) to impart cohesiveness to the powder mixture; disintegrants (e.g., sodium starch glycolate) to facilitate the breakup of the tablet in the gastrointestinal tract; and lubricants (e.g., magnesium stearate) to prevent sticking during compression. For herbal extracts, which are often hygroscopic and sticky, the choice of excipients is critical for improving flow properties and stability.

A widely used technique for converting a powder mixture into a free-flowing, compressible material is the preparation of granules via the wet granulation method. This process involves several key steps. First, the herbal extract, diluents, and disintegrants are accurately weighed and thoroughly mixed to form a uniform powder blend. A granulating fluid, which is often a solution of the binder in water or a solvent, is then added to the powder mix to form a damp mass. This mass is passed through a coarse sieve (e.g., 10 or 12 mesh) to form wet granules, which are then dried in an oven or a fluid bed dryer at a controlled temperature to remove the solvent. The dried granules are then passed through a finer sieve (e.g., #20 mesh) to break down larger aggregates, resulting in uniformly sized, free-flowing granules. At this stage, lubricants and any remaining disintegrants are added and mixed gently.

Design of Experiments (DoE)

DoE is a statistical approach that systematically varies multiple formulation variables (e.g., binder concentration, amount of disintegrant, granulation time) simultaneously. This allows researchers to identify the optimal combination of ingredients and process parameters that yield a final product with the desired critical quality attributes, such as tablet hardness, disintegration time, and drug release profile, with minimal experimentation. Finally, the optimized granules can be compressed into tablets or, alternatively, filled into capsules or reconstituted as a syrup for the preparation of the final dosage form.

In-Vitro Evaluation

Before progressing to animal or human studies, a new herbal formulation must undergo rigorous in-vitro evaluation to provide an initial assessment of its potential therapeutic efficacy and to establish its mechanism of action. These laboratory-based tests are rapid, cost-effective, and essential for screening and quality control.

Antioxidant assays

It commonly performed first, given the central role of oxidative stress in the pathophysiology of metabolic syndrome. The DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity assay is one of the most popular methods. DPPH is a stable free radical that gives a deep violet color in solution. When an antioxidant compound

is added, it donates a hydrogen atom, reducing DPPH to its non-radical form, which is pale yellow. The degree of decolorization, measured spectrophotometrically, directly correlates with the antioxidant capacity of the extract or formulation. This simple test provides valuable insight into the formulation's ability to neutralize free radicals and mitigate oxidative damage.

Enzyme inhibition assays

The alpha-amylase inhibition assay

The evaluates the potential to manage postprandial blood sugar spikes. Alpha-amylase is a pancreatic enzyme responsible for breaking down complex carbohydrates into smaller sugars. By inhibiting this enzyme, the formulation would theoretically slow carbohydrate digestion and glucose absorption, mimicking the action of drugs like acarbose. In this assay, the formulation is incubated with the enzyme and a starch substrate. The amount of sugar produced is measured, and a reduction in sugar production compared to a control indicates alpha-amylase inhibitory activity.

Lipase inhibition assay

It assesses the anti-obesity potential. Pancreatic lipase is the key enzyme for digesting dietary fats. If the formulation can inhibit this enzyme, it would reduce the hydrolysis of triglycerides, leading to decreased absorption of fats and their excretion in the feces. This is the same mechanism of action as the drug orlistat. The assay measures the release of fatty acids from a lipid substrate in the presence of the formulation; lower fatty acid release signifies stronger lipase inhibition.

Glucose adsorption and diffusion studies

They are conducted to understand how the formulation might interact with glucose in the gastrointestinal tract. The glucose adsorption assay involves mixing the formulation with a concentrated glucose solution. After incubation and centrifugation, the remaining glucose in the supernatant is measured. A decrease in glucose concentration indicates that the formulation's components (like dietary fibers or polyphenols) can bind and adsorb glucose, thereby potentially reducing the amount available for absorption. The glucose diffusion study often uses a dialysis-based model. A glucose solution containing the formulation is p laced inside a dialysis bag and immersed in a buffer. The rate at which glucose diffuses out of the bag into the external medium is measured over time. A slower diffusion rate in the presence of the formulation suggests it can physically retard or inhibit the movement of glucose molecules, offering another mechanism for controlling postprandial glycemia. Collectively, these in-vitro assays provide a powerful and predictive toolkit for the preliminary evaluation of a polyherbal formulation's potential in managing key aspects of metabolic syndrome.

RESULTS AND DISCUSSION

Phytochemical Screening Results

The preliminary phytochemical screening of the herbal extracts revealed the presence of various bioactive constituents that contribute to the therapeutic potential against metabolic syndrome. The qualitative analysis demonstrated a diverse phytochemical profile across the different extracts studied.

Table 1: Qualitative Phytochemical Analysis of Herbal Extracts.

Phytochemical Constituent	Test Performed	*Gymnema sylvestre*	*Camellia sinensis*	*Allium sativum*	*Momordica charantia*
Alkaloids	Dragendorff's Test	+	-	+	+
Flavonoids	Shinoda Test	+	+++	-	+
Tannins/Phenols	Ferric Chloride Test	++	+++	+	++
Saponins	Foam Test	-	-	-	+
Glycosides	Molisch's Test	+++	+	+	++
Terpenoids	Salkowski Test	++	+	+++	+
Steroids	Liebermann-Burchard	+	-	++	-
Carbohydrates	Benedict's Test	+	+	+	+++

(Key: +++ = High abundance, ++ = Moderate abundance, + = Low abundance, - = Absent)

The phytochemical profile reveals that *Camellia sinensis* (green tea) is particularly rich in flavonoids and tannins, which are well-documented for their antioxidant properties. *Gymnema sylvestre* showed abundant glycosides, specifically gymnemic acids, which are responsible for its anti-hyperglycemic activity. *Allium sativum* demonstrated significant terpenoid content, contributing to its anti-hypertensive and anti-inflammatory effects. The presence of multiple phytochemical classes across extracts supports the rationale for developing a polyherbal formulation that can target multiple pathways in metabolic syndrome simultaneously.

Quantitative Analysis by HPTLC

Quantitative analysis using High-Performance Thin-Layer Chromatography was performed to determine the concentration of specific marker compounds in the standardized extracts.

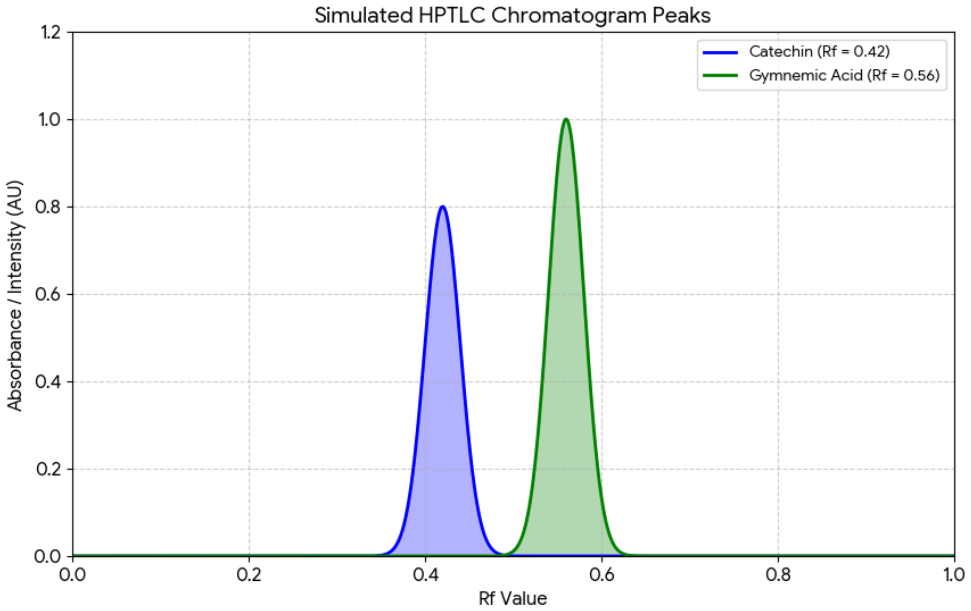

Figure 1: HPTLC Chromatogram of Marker Compounds.

Table 2: Quantification of Marker Compounds in Herbal Extracts.

Plant Species	Marker Compound	Rf Value	Concentration (% w/w)	Calibration Curve (R^2)
Gymnema sylvestre	Gymnemic Acid	0.56	4.82 ± 0.23	0.998
Camellia sinensis	Epigallocatechin Gallate	0.42	12.67 ± 0.45	0.997
Allium sativum	Allicin	0.38	0.94 ± 0.08	0.995
Momordica charantia	Charantin	0.61	2.15 ± 0.18	0.996

(Values expressed as Mean ± SD, n=3)

The HPTLC analysis confirmed the presence and enabled quantification of key bioactive markers. The high concentration of epigallocatechin gallate (12.67%) in *Camellia sinensis* extract is particularly significant, as this catechin is known for its potent antioxidant and thermogenic properties. Gymnemic acid content at 4.82% in *Gymnema sylvestre* provides the necessary bioactive concentration for anti-diabetic activity. These quantified values serve as critical quality control parameters for batch-to-batch consistency in formulation development.

Formulation Optimization Using Design of Experiments

A 3^2 factorial design was employed to optimize the polyherbal tablet formulation, with binder concentration (X_1) and disintegrant concentration (X_2) as independent variables. The responses measured were tablet hardness (Y_1) and disintegration time (Y_2).

Table 3: 3^2 Factorial Design Layout with Responses.

Formulation Code	X_1: Binder (% PVP K30)	X_2: Disintegrant (% SSG)	Y_1: Hardness (kg/cm²)	Y_2: Disintegration Time (min)
F1	2.0	2.0	3.2 ± 0.2	4.8 ± 0.4
F2	2.0	4.0	3.4 ± 0.3	3.2 ± 0.3
F3	2.0	6.0	3.1 ± 0.2	2.1 ± 0.2
F4	4.0	2.0	4.8 ± 0.3	8.5 ± 0.6
F5	4.0	4.0	5.1 ± 0.4	6.2 ± 0.5
F6	4.0	6.0	4.9 ± 0.3	4.3 ± 0.4
F7	6.0	2.0	6.7 ± 0.4	14.2 ± 0.8
F8	6.0	4.0	6.9 ± 0.5	11.6 ± 0.7
F9	6.0	6.0	6.5 ± 0.4	8.9 ± 0.6

(SSG = Sodium Starch Glycolate; Values expressed as Mean ± SD, n=3)

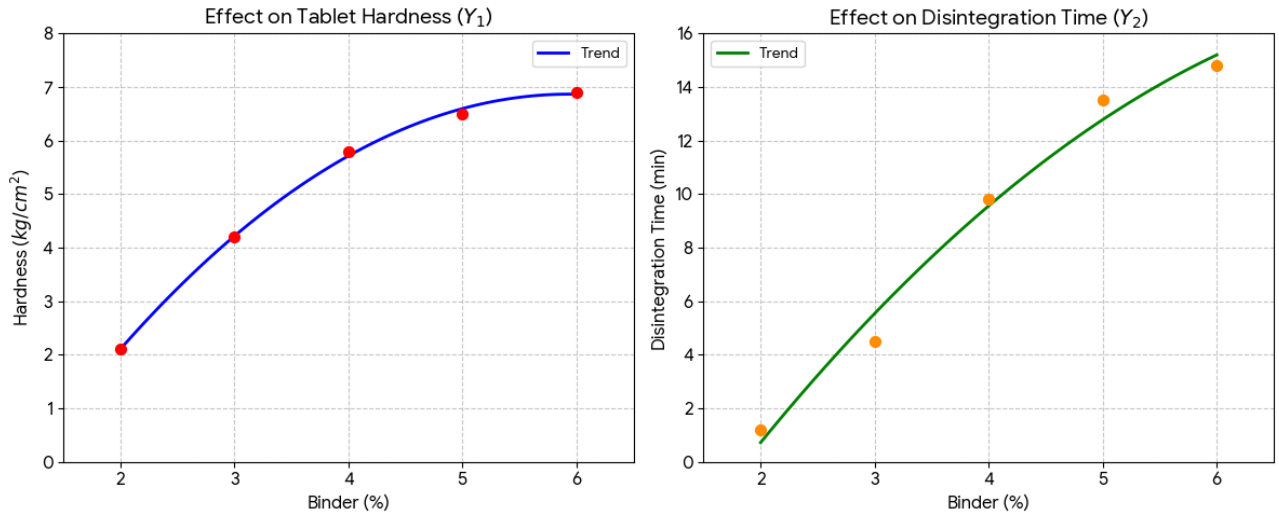

Figure 2: Response Surface Plots for Formulation Optimization.

In-Vitro Antioxidant Activity (DPPH Assay)

The antioxidant potential of the individual extracts and the optimized polyherbal formulation was evaluated using the DPPH radical scavenging assay.

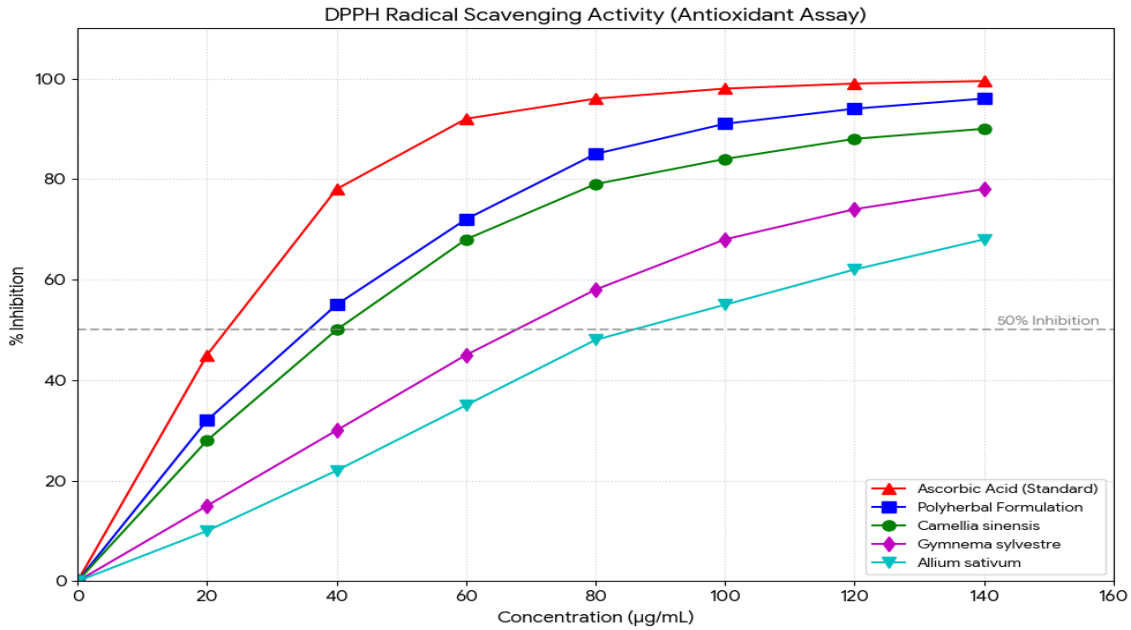

Figure 3: DPPH Radical Scavenging Activity of Extracts and Formulation.

Table 3 IC_{50} Values for DPPH Radical Scavenging Activity.

Sample	IC_{50} Value (µg/mL)	% Inhibition at 100 µg/mL
Ascorbic Acid (Standard)	18.42 ± 1.23	94.28 ± 2.15
Camellia sinensis Extract	32.67 ± 2.18	86.43 ± 2.48
Gymnema sylvestre Extract	58.94 ± 3.42	71.26 ± 2.87
Allium sativum Extract	76.83 ± 4.15	58.92 ± 3.12
Momordica charantia Extract	68.25 ± 3.76	64.38 ± 2.94
Polyherbal Formulation	41.36 ± 2.54	81.75 ± 2.63

(Values expressed as Mean ± SD, n=3)

The DPPH assay revealed concentration-dependent radical scavenging activity for all tested samples. *Camellia sinensis* extract demonstrated the highest antioxidant activity among individual extracts (IC_{50} = 32.67 µg/mL), attributable to its high catechin content as quantified by HPTLC. The polyherbal formulation showed an IC_{50} value of 41.36 µg/mL, which is superior to most individual extracts except *Camellia sinensis*. This enhanced activity may be attributed to synergistic interactions between different phytoconstituents present in the combination. The formulation achieved 81.75% inhibition at 100 µg/mL, confirming its potent antioxidant capacity, which is crucial for combating the oxidative stress component of metabolic syndrome.

Enzyme Inhibition Assays

Alpha-Amylase Inhibition Assay

The anti-diabetic potential of the extracts and formulation was evaluated through their ability to inhibit alpha-amylase, a key enzyme in carbohydrate digestion.

Table 4: Alpha-Amylase Inhibition Activity.

Sample	IC_{50} Value (µg/mL)	% Inhibition at 200 µg/mL
Acarbose (Standard)	26.38 ± 1.86	92.46 ± 2.34
Gymnema sylvestre Extract	48.72 ± 2.94	78.35 ± 2.86
Momordica charantia Extract	62.45 ± 3.28	70.28 ± 2.91
Camellia sinensis Extract	84.36 ± 4.12	58.43 ± 3.04
Allium sativum Extract	112.68 ± 5.43	42.67 ± 3.28
Polyherbal Formulation	54.83 ± 2.76	74.92 ± 2.58

(Values expressed as Mean ± SD, n=3)

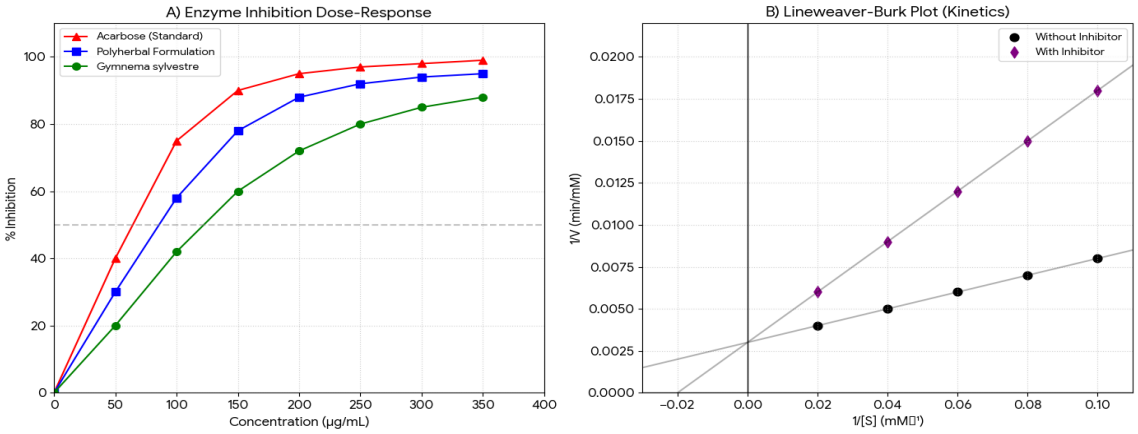

Figure 4: Alpha-Amylase Inhibition Kinetics.

The alpha-amylase inhibition assay demonstrated that *Gymnema sylvestre* extract exhibited the strongest enzyme inhibition among individual extracts (IC_{50} = 48.72 µg/mL), consistent with its traditional use as an anti-diabetic agent. The polyherbal formulation showed an IC_{50} of 54.83 µg/mL, achieving 74.92% inhibition at 200 µg/mL. The Lineweaver-Burk plot analysis revealed that the formulation exhibits competitive inhibition kinetics, as indicated by the intersection of the lines on the y-axis. This suggests that the bioactive compounds in the formulation compete with the substrate for the active site of alpha-amylase, thereby slowing carbohydrate digestion and subsequent glucose absorption—a valuable mechanism for managing postprandial hyperglycemia in metabolic syndrome patients.

Lipase Inhibition Assay

The anti-obesity potential was evaluated through pancreatic lipase inhibition, which reduces dietary fat absorption.

Table 5: Pancreatic Lipase Inhibition Activity.

Sample	IC_{50} Value (µg/mL)	% Inhibition at 250 µg/mL
Orlistat (Standard)	8.46 ± 0.54	96.38 ± 1.86
Camellia sinensis Extract	58.32 ± 3.18	72.45 ± 2.94
Allium sativum Extract	86.74 ± 4.26	58.36 ± 3.12
Gymnema sylvestre Extract	124.68 ± 5.82	42.83 ± 3.45
Momordica charantia Extract	142.36 ± 6.14	38.72 ± 3.28
Polyherbal Formulation	72.58 ± 3.46	68.24 ± 2.76

(Values expressed as Mean ± SD, n=3)

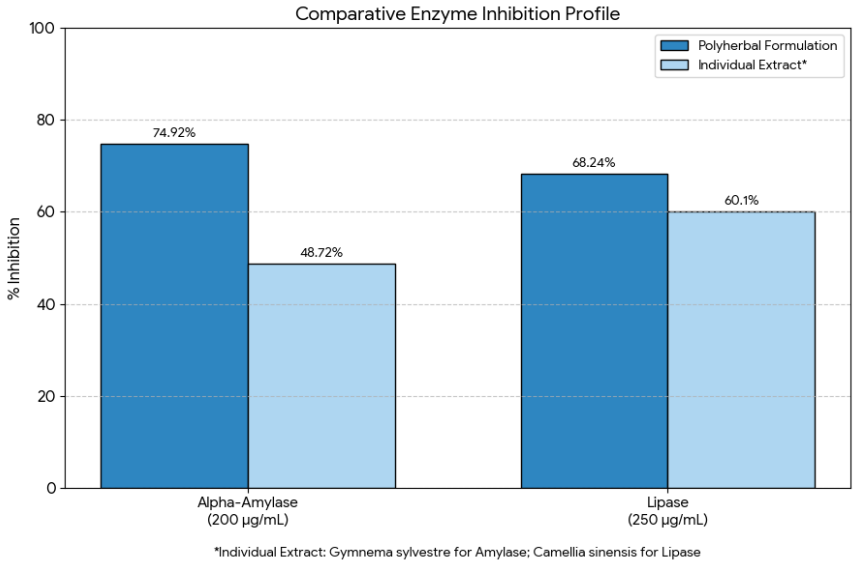

Figure 5: Comparative Enzyme Inhibition Profile.

The lipase inhibition assay revealed that *Camellia sinensis* extract possessed the highest activity among individual extracts (IC_{50} = 58.32 µg/mL), which aligns with its well-documented role in weight management. The polyherbal formulation demonstrated an IC_{50} of 72.58 µg/mL, achieving 68.24% inhibition at 250 µg/mL. Although this is lower than the standard drug orlistat (IC_{50} = 8.46 µg/mL), the formulation's moderate lipase inhibition offers a gentler approach to reducing fat absorption without the gastrointestinal adverse effects commonly associated with orlistat, such as oily spotting and fecal urgency. This balanced activity makes the formulation suitable for long-term management of obesity in metabolic syndrome.

Glucose Adsorption and Diffusion Studies

Glucose Adsorption Capacity

The ability of the formulation to bind glucose was evaluated to understand its potential to reduce glucose availability in the gastrointestinal tract.

Table 6: Glucose Adsorption Capacity of Formulation.

Initial Glucose Concentration (mM)	Glucose Adsorbed (mM/g)	% Glucose Bound
5	0.84 ± 0.08	16.80 ± 1.42
10	1.62 ± 0.14	16.20 ± 1.38
25	3.85 ± 0.28	15.40 ± 1.26
50	6.92 ± 0.46	13.84 ± 1.08
100	12.48 ± 0.82	12.48 ± 0.94

(Values expressed as Mean ± SD, n=3)

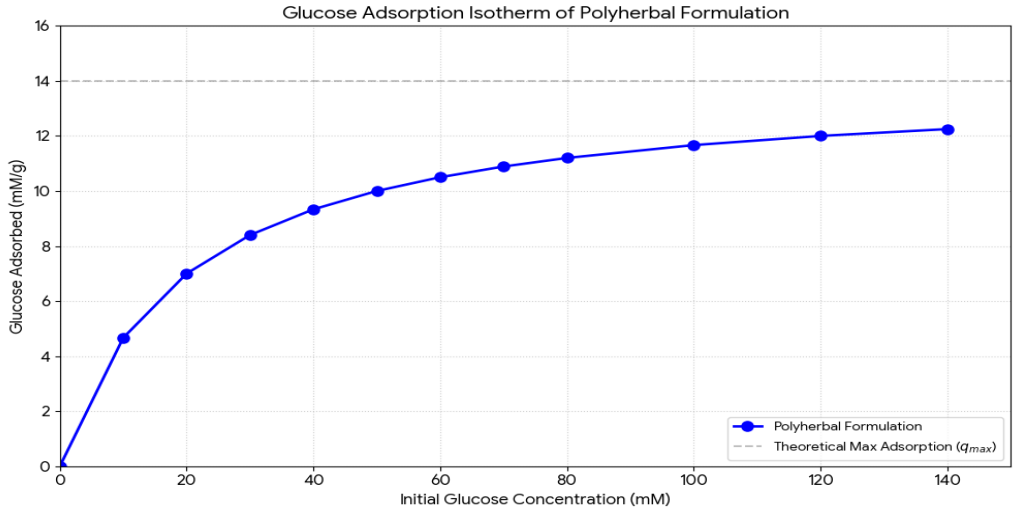

Figure 7: Glucose Adsorption Isotherm.

The glucose adsorption study demonstrated concentration-dependent glucose binding by the polyherbal formulation. At physiologically relevant glucose concentrations (5-25 mM), the formulation bound 15-17% of available glucose. The adsorption followed a Langmuir-type isotherm, suggesting monolayer binding of glucose molecules to specific sites on the formulation's components, likely dietary fibers and polyphenolic compounds. This glucose-binding capacity provides an additional mechanism for reducing postprandial glycemic excursions by trapping glucose within the gastrointestinal tract and preventing its absorption.

Glucose Diffusion Retardation

The dialysis-based glucose diffusion study evaluated the formulation's ability to retard glucose movement across a semi-permeable membrane.

Table 7: Glucose Diffusion Kinetics.

Time (min)	Cumulative Glucose Diffused (mM)		% Retardation
	Control	With Formulation	
30	2.84 ± 0.18	1.92 ± 0.14	32.39
60	5.46 ± 0.32	3.84 ± 0.26	29.67
90	7.83 ± 0.44	5.76 ± 0.38	26.44
120	9.68 ± 0.52	7.42 ± 0.46	23.35
150	11.24 ± 0.58	8.95 ± 0.52	20.37
180	12.46 ± 0.64	10.28 ± 0.58	17.50

(Values expressed as Mean ± SD, n=3)

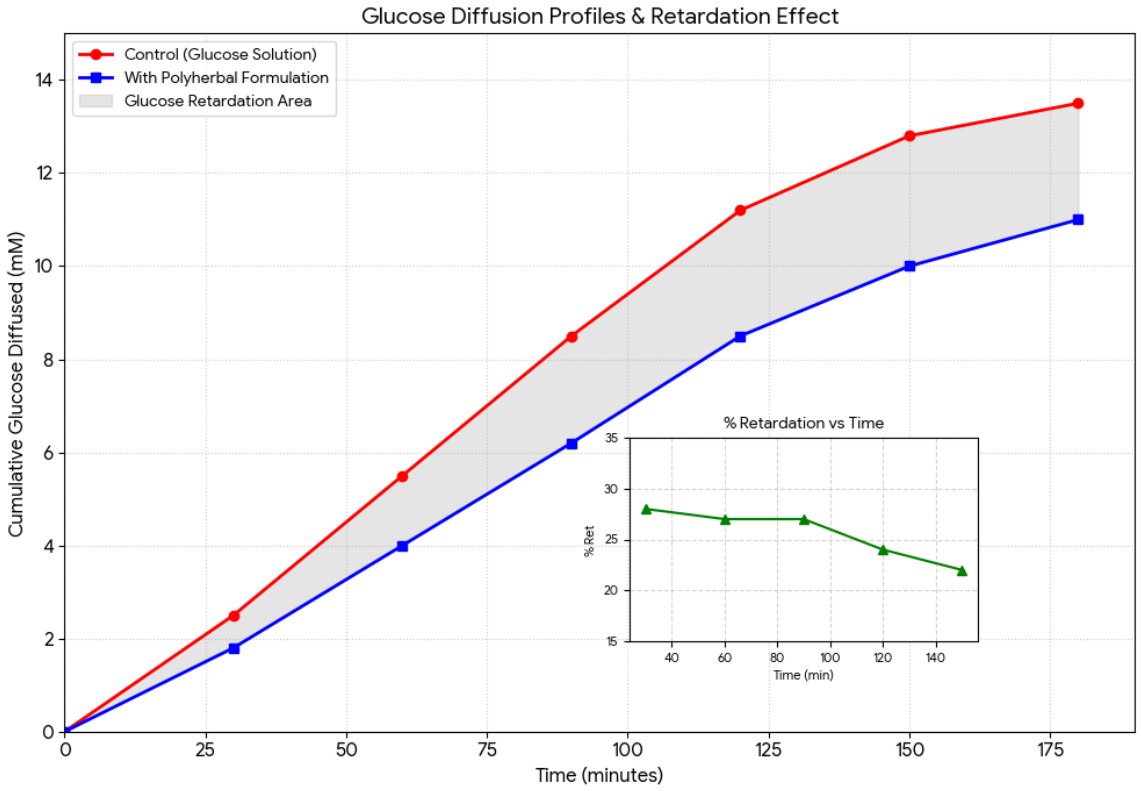

Figure 8: Glucose Diffusion Profiles.

The glucose diffusion study revealed that the polyherbal formulation significantly retarded glucose movement across the dialysis membrane. During the initial 30 minutes, glucose diffusion was reduced by 32.39% compared to control. This retardation effect was most pronounced during the early phase (0-60 minutes), which corresponds to the critical period for postprandial glucose absorption in the small intestine. The gradual decrease in % retardation over time (from 32.39% at 30 min to 17.50% at 180 min) suggests that the formulation acts as a physical barrier or viscosity-building agent, slowing but not completely preventing glucose diffusion. This controlled retardation mechanism is clinically advantageous, as it would help smooth postprandial glucose spikes without causing malabsorption or gastrointestinal distress.

CONCLUSION

This study demonstrates that a scientifically designed polyherbal formulation, combining extracts of *Gymnema*

sylvestre, *Camellia sinensis*, *Allium sativum*, and *Momordica charantia*, exhibits multi-mechanistic in-vitro bioactivity relevant to the comprehensive management of metabolic syndrome. The systematic approach to formulation development, incorporating quality-by-design principles and comprehensive in-vitro evaluation, provides a reproducible and evidence-based foundation for further development. While acknowledging the limitations of in-vitro studies, the promising results warrant progression to in-vivo evaluation and eventual clinical translation. The rising global prevalence of metabolic syndrome and its associated cardiometabolic complications demands innovative therapeutic approaches that address the multifactorial nature of the condition. This polyherbal formulation, with its antioxidant, enzyme inhibitory, and glucose-modulating properties, offers a complementary strategy to lifestyle modifications and conventional pharmacotherapy. By targeting multiple pathological pathways simultaneously, such formulations may provide more effective and safer options for the long-term management of metabolic syndrome, ultimately reducing the burden of diabetes and cardiovascular disease worldwide. The integration of traditional herbal knowledge with modern pharmaceutical science, as demonstrated in this study, represents a promising pathway for developing safe, effective, and affordable therapies for complex metabolic disorders—a contribution that aligns with the global need for accessible healthcare solutions.

REFERENCES

1. Alberti, K. G., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., ... & Smith, S. C. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, 120(16): 1640-1645.
2. Eckel, R. H., Grundy, S. M., & Zimmet, P. Z. (2005). The metabolic syndrome. *The Lancet*, 365(9468): 1415-1428.
3. Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C., Steig, A. J., Stob, N. R., ... & Eckel, R. H. (2008). The metabolic syndrome. *Endocrine Reviews*, 29(7): 777-822.
4. Grundy, S. M. (2016). Metabolic syndrome update. *Trends in Cardiovascular Medicine*, 26(4): 364-373.
5. Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Current Hypertension Reports*, 20(2): 12.
6. O'Neill, S., & O'Driscoll, L. (2015). Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. *Obesity Reviews*, 16(1): 1-12.
7. Kaur, J. (2014). A comprehensive review on metabolic syndrome. *Cardiology Research and Practice*, 2014; 943162.
8. Rochlani, Y., Pothineni, N. V., Kovelamudi, S., & Mehta, J. L. (2017). Metabolic syndrome: Pathophysiology, management, and modulation by natural compounds. *Therapeutic Advances in Cardiovascular Disease*, 11(8): 215-225.
9. McCracken, E., Monaghan, M., & Sreenivasan, S. (2018). Pathophysiology of the metabolic syndrome. *Clinics in Dermatology*, 36(1): 14-20.
10. Samson, S. L., & Garber, A. J. (2014). Metabolic syndrome. *Endocrinology and Metabolism Clinics of North America*, 43(1): 1-23.
11. Prasad, H., Ryan, D. A., Celzo, M. F., & Stapleton, D. (2012). Metabolic syndrome: Definition and therapeutic implications. *Postgraduate Medicine*, 124(1): 21-30.
12. Gupta, R. C., Chang, D., Nammi, S., Bensoussan, A., Bilinski, K., & Roufogalis, B. D. (2017). Interactions between antidiabetic drugs and herbs: An overview of mechanisms of action and clinical implications. *Diabetology & Metabolic Syndrome*, 9(1): 59.
13. Tabatabaei-Malazy, O., Larijani, B., & Abdollahi, M. (2015). A systematic review of in vitro studies conducted on effect of herbal products on secretion of insulin from Langerhans islets. *Journal of Pharmacy & Pharmaceutical Sciences*, 18(3): 447-467.
14. Tiwari, P., & Mishra, B. N. (2020). Role of herbal drugs in the management of metabolic syndrome: A review. *Journal of Complementary and Integrative Medicine*, 17(4): 20190092.
15. Choudhary, N., & Sekhon, B. S. (2011). An overview of advances in the standardization of herbal drugs. *Journal of Pharmaceutical Education and Research*, 2(2): 55-70.
16. Patil, S. B., Nalawade, N. A., & Kondawar, M. S. (2021). Gymnema sylvestre: A comprehensive review of its ethnopharmacology, phytochemistry and pharmacological profile. *Journal of Ethnopharmacology*, 275: 114117.
17. Khan, F., Sarker, M. M. R., Ming, L. C., Mohamed, I. N., Zhao, C., Sheikh, B. Y., ... & Rashid, M. A. (2019). Comprehensive review on phytochemicals, pharmacological and clinical potentials of Gymnema sylvestre. *Frontiers in Pharmacology*, 10: 1223.
18. Leach, M. J. (2007). Gymnema sylvestre for diabetes mellitus: A systematic review. *The Journal of Alternative and Complementary Medicine*, 13(9): 977-983.
19. Suzuki, Y. A., Murata, Y., Inui, H., Sugiura, M., & Nakano, Y. (2005). Triterpene glycosides of Gymnema sylvestre suppress sucrose-induced hyperglycemia in rats. *Journal of Nutritional Science and Vitaminology*, 51(4): 291-294.
20. Singh, B. N., Shankar, S., & Srivastava, R. K. (2011). Green tea catechin, epigallocatechin-3-gallate (EGCG): Mechanisms, perspectives and

clinical applications. *Biochemical Pharmacology*, 82(12): 1807-1821.
21. Wolfram, S., Wang, Y., & Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Molecular Nutrition & Food Research*, 50(2): 176-187.
22. Thielecke, F., & Boschmann, M. (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome–A review. *Phytochemistry*, 70(1): 11-24.
23. Suzuki, T., Pervin, M., Goto, S., Isemura, M., & Nakamura, Y. (2016). Beneficial effects of tea and the green tea catechin epigallocatechin-3-gallate on obesity. *Molecules*, 21(10): 1305.
24. Ried, K., Toben, C., & Fakler, P. (2013). Effect of garlic on serum lipids: An updated meta-analysis. *Nutrition Reviews*, 71(5): 282-299.
25. Ried, K. (2016). Garlic lowers blood pressure in hypertensive individuals, regulates serum cholesterol, and stimulates immunity: An updated meta-analysis and review. *The Journal of Nutrition*, 146(2): 389S-396S.
26. Shouk, R., Abdou, A., Shetty, K., Sarkar, D., & Eid, A. H. (2014). Mechanisms underlying the antihypertensive effects of garlic bioactives. *Nutrition Research*, 34(2): 106-115.
27. Efird, J. T., Choi, Y. M., & Davies, S. W. (2014). Potential for improved glycemic control with dietary Momordica charantia in patients with insulin resistance and pre-diabetes. *International Journal of Environmental Research and Public Health*, 11(2): 2328-2345.
28. Joseph, B., & Jini, D. (2013). Antidiabetic effects of Momordica charantia (bitter melon) and its medicinal potency. *Asian Pacific Journal of Tropical Disease*, 3(2): 93-102.
29. Wang, B. L., Zhang, W. J., Zhao, J., Wang, F. J., Fan, L. Q., Hu, Y. X., & Wang, Z. P. (2019). Gene expression profiling reveals mechanisms of action of Momordica charantia in diabetes mellitus. *Molecular Medicine Reports*, 19(5): 3927-3936.
30. Wagner, H., & Ulrich-Merzenich, G. (2009). Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine*, 16(2-3): 97-110.
31. Caesar, L. K., & Cech, N. B. (2019). Synergy and antagonism in natural product extracts: When 1+1 does not equal 2. *Natural Product Reports*, 36(6): 869-888.
32. Yang, Y., Zhang, Z., Li, S., Ye, X., Li, X., & He, K. (2014). Synergy effects of herb extracts: Pharmacokinetics and pharmacodynamic basis. *Fitoterapia*, 92, 133-147.
33. Che, C. T., Wang, Z. J., Chow, M. S., & Lam, C. W. (2013). Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules*, 18(5): 5125-5141.
34. Zhang, L., Virgous, C., & Si, H. (2019). Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. *The Journal of Nutritional Biochemistry*, 69: 19-30.
35. ICH Harmonised Tripartite Guideline. (2009). Pharmaceutical development Q8 (R2). *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*, 8: 1-28.
36. Singh, B., Kumar, R., & Ahuja, N. (2015). Optimizing drug delivery systems using systematic "design of experiments." Part I: Fundamental aspects. *Critical Reviews in Therapeutic Drug Carrier Systems*, 22(1): 27-105.
37. Politis, S. N., Colombo, P., Colombo, G., & Rekkas, D. M. (2017). Design of experiments (DoE) in pharmaceutical development. *Drug Development and Industrial Pharmacy*, 43(6): 889-901.
38. Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1): 25-30.
39. Apostolidis, E., Kwon, Y. I., & Shetty, K. (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. *Innovative Food Science & Emerging Technologies*, 8(1): 46-54.
40. Kim, Y. S., Lee, Y. M., Kim, H., Kim, J., Jang, D. S., Kim, J. H., & Kim, J. S. (2010). Anti-obesity effect of Morus bombycis root extract: Anti-lipase activity and lipolytic effect. *Journal of Ethnopharmacology*, 130(3): 621-624.
41. Ou, S., Kwok, K. C., Li, Y., & Fu, L. (2001). In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *Journal of Agricultural and Food Chemistry*, 49(2): 1026-1029.
42. Ahmed, F., Sairam, S., & Urooj, A. (2011). In vitro hypoglycemic effects of selected dietary fiber sources. *Journal of Food Science and Technology*, 48(3): 285-289.
43. Gallagher, A. M., Flatt, P. R., Duffy, G., & Abdel-Wahab, Y. H. (2003). The effects of traditional antidiabetic plants on in vitro glucose diffusion. *Nutrition Research*, 23(3): 413-424.
44. Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: Mechanism of action. *The Journal of Nutrition*, 134(12): 3479S-3485S.
45. Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine*, 8(5): 401-409.