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Polyherbal emulsion; Diabetes mellitus; Oil-in-water emulsion; Azadirachta indica; Syzygium aromaticum; Allium sativum; Bombax ceiba; GC–MS; Streptozotocin; Antidiabetic activity.

Formulation and Comprehensive Evaluation of a Polyherbal Oil-in-Water Emulsion with Antidiabetic Activity in Streptozotocin-Induced Diabetic Rats

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ABSTRACT: Diabetes mellitus and dyslipidemia are closely associated metabolic disorders that increase the risk of cardiovascular and other chronic complications. Although synthetic drugs are effective, adverse effects, treatment costs, and limitations of combination therapy have increased interest in polyherbal alternatives. However, scientifically optimized herbal emulsions integrating multiple bioactive oils remain scarce. The aim was to develop and optimize an oil-in-water (O/W) polyherbal emulsion containing oils of Azadirachta indica, Syzygium aromaticum, Allium sativum, and Bombax ceiba, characterize its phytochemical composition by GC–MS, and evaluate its antidiabetic activity. Nine formulations (F1–F9) were prepared by wet-gum emulsification and optimized using a factorial design. F5 was selected based on physicochemical characteristics and evaluated in STZ-induced diabetic Wistar rats (n = 6/group). Animals received PHF (200 or 400 mg/kg) orally for 28 days. Blood glucose and body weight were assessed, and GC–MS characterized the major phytoconstituents. Statistical significance was set at $p < 0.05$. The optimized PHF produced significant, dose-dependent antihyperglycemic activity, markedly reducing fasting blood glucose compared with disease controls ($p < 0.05$). GC–MS identified pharmacologically important constituents, including eugenol, β-caryophyllene, eugenyl acetate, diallyl sulfur compounds, lupeol, β-sitosterol, and fatty acids, supporting potential synergistic and insulin-sensitizing effects. The optimized polyherbal O/W emulsion demonstrated significant antidiabetic activity and excellent acute safety. Its complementary phytoconstituents suggest multitarget therapeutic potential. Further pharmacokinetic, mechanistic, chronic toxicity, and clinical studies are warranted.

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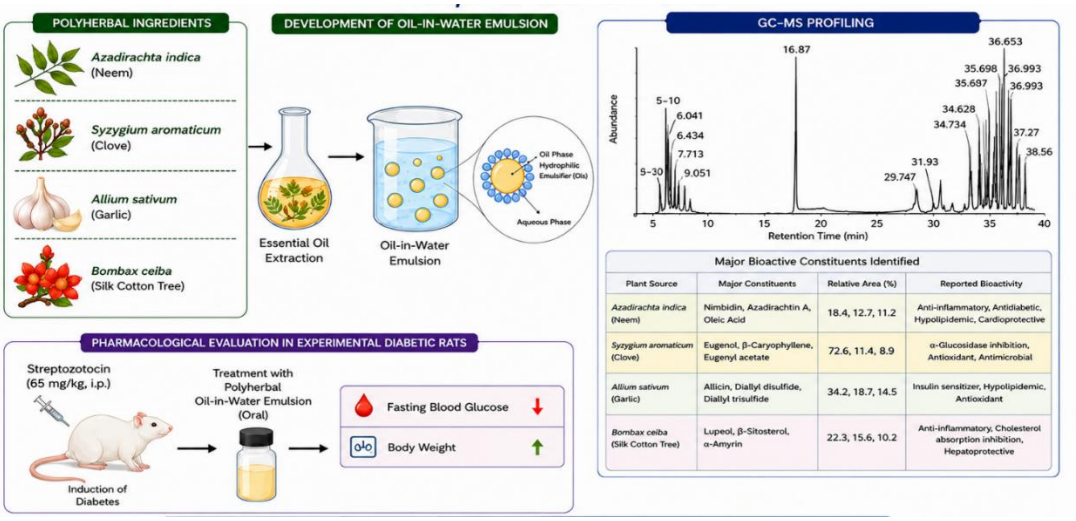
Graphical Abstract



1. INTRODUCTION

Diabetes mellitus (DM) is a prevalent metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin deficiency, or defective insulin signaling [1]. Associated dyslipidemia, including elevated triglycerides and LDL cholesterol with reduced HDL cholesterol, further increases cardiovascular and microvascular complications [2]. Therefore, therapeutic approaches targeting both glucose and lipid abnormalities may provide greater clinical benefits than single-target interventions [3,4].

Conventional antidiabetic drugs include biguanides, sulfonylureas, thiazolidinediones, and SGLT-2 inhibitors, while dyslipidemia is commonly managed with statins, fibrates, and bile acid sequestrants. Despite their efficacy, adverse effects and the burden of lifelong polypharmacy have heightened interest in plant-derived therapies [5]. Polyherbalism, particularly in Ayurveda, combines medicinal plants to achieve complementary or synergistic effects, potentially improving bioavailability and modulating glucose metabolism, lipid homeostasis, oxidative stress, inflammation, and tissue protection [6,7].

Azadirachta indica (Neem), Syzygium aromaticum (Clove), Allium sativum (Garlic), and Bombax ceiba possess complementary antidiabetic and antihyperlipidemic properties. A. indica contains limonoids, flavonoids, and polyphenols that are associated with improved insulin sensitivity, reduced gluconeogenesis, and decreased oxidative stress and inflammation [8]. S. aromaticum, particularly its eugenol-rich oil, exhibits antioxidant, α-glucosidase/α-amylase inhibitory, and pancreatic β-cell protective effects. A. sativum contains organosulfur compounds with hypoglycemic, hypolipidemic, antioxidant, and cardioprotective activities [9]. B. ceiba contains triterpenoids, phytosterols, flavonoids, and phenolics with antihyperglycemic, hepatoprotective, nephroprotective, anti-inflammatory, and lipid-lowering effects [10].

However, limited studies have investigated their rational combination within a standardized pharmaceutical formulation. Previous herbal formulations have often lacked systematic optimization, comprehensive physicochemical characterization, reproducible manufacturing, and integrated biological validation [11,12]. Moreover, herbal oils exhibit poor aqueous solubility, volatility, oxidative susceptibility, and variable gastrointestinal absorption. Optimized oil-in-water (O/W) emulsions can improve dispersion, interfacial surface area, physical stability, dose uniformity, gastrointestinal absorption, and potentially oral bioavailability of lipophilic phytoconstituents [13,14].

Accordingly, the present study systematically developed and optimized a polyherbal O/W emulsion containing oils of *A. indica, S. aromaticum, A. sativum, and B. ceiba* using factorial experimental design [15]. GC–MS was employed to characterize major phytoconstituents and provide a chemical basis for the observed pharmacological effects [16]. The study integrates formulation development, phytochemical profiling, toxicological assessment, and pharmacological evaluation, thereby bridging traditional herbal knowledge with evidence-based formulation development [17,18]. The objective was to develop and optimize a stable polyherbal emulsion, characterize its phytochemical constituents, and evaluate its antidiabetic efficacy in experimentally induced diabetic rats, with potential application as an adjunctive approach for diabetes-associated dyslipidemia.

Natural Resources for Human Health
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2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Streptozotocin (STZ; ≥98% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Metformin hydrochloride, of pharmaceutical grade, was obtained from certified pharmaceutical suppliers and used as a reference standard for the antidiabetic respectively. Tween 80 (polyoxyethylene sorbitan monooleate) served as the non-ionic surfactant, and propylene glycol served as the co-surfactant to enhance emulsion stability and improve the dispersion of lipophilic phytoconstituents. Gum acacia (Acacia senegal) served as the primary natural emulsifying agent, and sodium benzoate was incorporated as an antimicrobial preservative. Carboxymethylcellulose sodium (CMC-Na) served as the suspending vehicle for oral administration. Analytical-grade n-hexane, methanol, ethanol, chloroform was also used. All chemicals and reagents used throughout the study were of analytical or HPLC grade, and freshly prepared solutions were used whenever required.

2.2 Plant Material

Fresh, mature seeds of *Azadirachta indica* A. Juss. (Neem), dried flower buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry (Clove), fresh bulbs of *Allium sativum* L. (Garlic), and healthy leaves of *Bombax ceiba* L. were obtained from authenticated herbal suppliers in Meerut district, Uttar Pradesh, India, during the appropriate harvesting season. The collected plant materials were carefully examined and cleaned to remove foreign matter, dust, and visibly damaged portions before further processing. Botanical authentication was conducted independently by taxonomists at the National Institute of Science Communication and Policy Research (NIScPR), New Delhi, and the Department of Botany, Chaudhary Charan Singh University, Meerut, India. Voucher specimens of all plant materials were deposited for future reference under Voucher No. NIScPR/RHMD/Consult/2023/4594-95-1-2-4.

2.3 Extraction of Herbal Oils

2.3.1 Neem seed oil

Coarsely powdered neem seeds (100 g) were placed in a Soxhlet thimble. A solvent mixture of ethanol (300 mL) and n-hexane (200 mL) was used in a round-bottom flask. Extraction was performed for 6 h at 60–80°C. The oil–solvent mixture was cooled, collected, and concentrated under reduced pressure using a rotary evaporator at 40°C for 90 min to yield solvent-free oil[19].

2.3.2 Clove flower buds oil

Washed and dried clove flower buds were powdered and sieved through a 0.5-mm mesh. Accurately weighed coarse powder (60 g) was placed in a Soxhlet apparatus with n-hexane (300 mL) and extracted for 6 h at 70°C. The essential oil was collected, evaporated at 40°C for 90 min using a rotary evaporator, and stored in amber bottles at 4°C[20].

2.3.2 Garlic bulbs oil

Fresh garlic bulbs (200 g) were crushed, dissolved in 100 mL of distilled water, and diluted to 2 L. The mixture was hydrodistilled using a Clevenger apparatus for 90 min. The essential oil was concentrated under reduced pressure with a rotary evaporator at 40°C for 90 min [21].

2.3.4 Bombax ceiba leaves oil

Bombax ceiba leaves collected from the Partapur region, Meerut (U.P.) were shade-dried for 40 days. The coarsely powdered dried material (250 g) was refluxed with methanol (500 mL) for 6 h using a reflux condenser. The extract was collected, filtered, and concentrated at 40°C for 90 min[22]

2.4 Preliminary Phytochemical Screening

Preliminary phytochemical screening of each extracted oil was conducted to investigate secondary metabolites according to established protocols, including tests for alkaloids (Wagner's test), flavonoids, phenols, tannins (Braymer's test), saponins (foam test), steroids (Salkowski test), glycosides (Liebermann's test), terpenoids, carbohydrates (Molisch's test), reducing sugars, and anthraquinones (Borntrager's test)[23].

2.5 Experimental Design for Polyherbal Formulation Development

A systematic optimization strategy based on Design of Experiments (DoE) principles was adopted to develop a stable oil-in-water (O/W) polyherbal emulsion with desirable physicochemical characteristics and improved oral delivery. Initially, screening experiments were conducted to identify suitable ranges for total oil concentration, surfactant concentration, co-surfactant concentration, and emulsifying agent. These studies evaluated visual appearance, emulsion stability, ease of emulsification. Following the screening phase, a factorial experimental design was constructed using Minitab Statistical Software (Version 14, Minitab Inc., USA). Nine experimental formulations (F1–F9) were generated by varying the proportions of oils and excipients according to the factorial design matrix. Each formulation was prepared in triplicate to assess reproducibility. The optimized formulation (F5) was selected based on a comprehensive physicochemical evaluation, demonstrating superior physical stability, a homogeneous appearance, acceptable viscosity, and desirable emulsification characteristics throughout the storage period.

2.6 Preparation of Polyherbal Oil-in-Water (O/W) Emulsion

Polyherbal oil-in-water emulsions were prepared using a modified wet-gum emulsification method. Gum acacia was dispersed in approximately 40 mL purified distilled water and stirred until hydrated. Sodium benzoate and propylene glycol were incorporated into the aqueous phase, which was maintained at 60 ± 2°C. Separately, specified quantities of *Azadirachta indica*, *Syzygium aromaticum*, *Allium sativum*, and *Bombax ceiba* oils were mixed with Tween 80 and maintained at 60 ± 2°C. The oil phase was gradually added to the aqueous phase under stirring at approximately 1000 rpm for 15 min, followed by homogenization to reduce globule size [24]. The emulsion was cooled gradually, and the volume was adjusted with purified water. Formulations were filled into sterilized amber glass containers and stored at 25 ± 2°C away from light and humidity. Among nine formulations, F5 showed the most favorable physicochemical characteristics and was selected for further evaluation. [25].

2.7 Evaluation of Formulations F1–F9

2.7.1 Organoleptic evaluation

Colour, odour, appearance and phase separation of all nine formulations were evaluated physically and observed weekly for 30 days [26].

2.7.2 Determination of homogeneity

Prepared emulsions were inspected for clarity and colour and evaluated for foreign particles by microscopical examination. The shake flask test was performed by shaking samples for 10–15 s and observing for phase separation upon setting [27].

2.7.3 Determination of pH

pH of formulations was measured using a calibrated digital pH meter (Systronics S.No. 5257). One milliliter of each emulsion was diluted with 10 mL distilled water; the average of three readings was recorded [28].

2.7.4 Determination of viscosity

Viscosity was determined using a Brookfield digital viscometer with spindle no. 4 at 700 rpm. The average of three determinations was recorded [29].

2.7.5 Globule size and zeta potential analysis

Particle size, polydispersity index (PDI) and zeta potential of all formulations were determined using an Anton Paar Litesizer 500 analyzer at 25°C with a scattering angle of 90°. Samples were diluted 1000-fold with distilled water and placed in quartz cuvettes prior to analysis. Each measurement was performed in triplicate [30].

2.8 Experimental Animals

Healthy adult male Wistar albino rats (140–180 g; 8–10 weeks) were obtained from the Central Animal House Facility, Swami Vivekanand Subharti University, Meerut, India, and acclimatized for seven days under standardized conditions (22 ± 2°C, 55 ± 10% relative humidity, 12 h light/dark cycle), with food and filtered water ad libitum. All procedures complied with CPCSEA guidelines and were approved by the IAEC, Translam Institute of Pharmaceutical Education and Research, Meerut (Approval No. IAEC/PH-25/TIPER/223). Animals were randomly assigned to experimental groups using computer-generated randomization, and histopathological assessments were performed by investigators blinded to treatment allocation.

2.9 Streptozotocin-Induced Experimental Diabetes

Experimental diabetes mellitus was induced using streptozotocin (STZ), a selective pancreatic β-cell cytotoxin. STZ was freshly prepared immediately before administration by dissolving it in freshly prepared ice-cold 0.1 M citrate buffer (pH 4.5) under light-protected conditions. Overnight-fasted rats received a single intraperitoneal injection of STZ at 65 mg/kg body weight. Following STZ administration, animals received 5% glucose solution for 24 hours to minimize initial hypoglycemic mortality. Seventy-two hours after induction, fasting blood glucose was measured using a validated glucometer from tail-vein blood samples. Rats exhibiting fasting blood glucose concentrations ≥250 mg/dL were considered diabetic and included in the study, whereas animals failing to meet the induction criterion were excluded. Animals were randomly assigned into five experimental groups (n = 6 per group) [31]:

S. No	Group		Dose
1.	Group I	Normal control	(distilled water, 10 mL/kg, p.o)
2.	Group II	Normal control	(distilled water, 10 mL/kg, p.o)
3.	Group III	Standard treatment	(Metformin, 100 mg/kg/day, p.o)
4.	Group IV	Polyherbal formulation	(200 mg/kg/day, p.o)
5.	Group V	Polyherbal formulation	(200 mg/kg/day, p.o)

Body weight and fasting blood glucose were recorded at baseline and weekly intervals (Days 0, 7, 14, 21, and 28)

2.10 GC–MS Analysis

Phytochemical profiling was performed using gas chromatography–mass spectrometry (Agilent GC-MS 7890).

2.10.1 Sample Preparation

Samples were diluted to 1% (v/v) using HPLC-grade n-hexane and filtered through sterile 0.45 µm PTFE syringe filters prior to injection.

2.10.2 Instrumentation

GC–MS analysis was performed using an Agilent 7890B gas chromatograph coupled with a 5977B mass selective detector and an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium (99.999%) was used as the carrier gas at 1.0 mL/min. Constituent abundance was determined from normalized peak-area percentages without correction factors. Representative chromatograms and mass spectra were provided as supplementary figures. Thermolabile compounds, including allicin, azadirachtin A, and nimbidin, were considered tentatively identified based on spectral-library matching because of potential degradation during GC analysis. Definitive identification would require LC–MS/MS, HRMS, or NMR confirmation

2.11 Statistical Analysis

All experimental data were expressed as mean ± standard error of the mean (SEM) for six animals per group (n = 6). Variables measured repeatedly over time (fasting blood glucose, body weight) were analyzed using two-way analysis of variance (ANOVA) with treatment and time as independent factors Statistical analyses were conducted using GraphPad Prism Version 10.0 (GraphPad Software Inc., San Diego, CA, USA). Differences were considered statistically significant at $p < 0.05$.

3. RESULTS

3.1 Phytochemical Screening of Individual Herbal Oils

The percentage yield of neem seeds oil, clove flower bud oil, garlic bulb oil and *Bombax ceiba* leaves oil was 42.52%, 53.16%, 1.00% and 10.00%, respectively. Preliminary phytochemical screening revealed the presence of multiple bioactive compounds with established therapeutic potential. Neem oil showed strong presence of flavonoids, phenols, carbohydrates and anthraquinones. Garlic oil exhibited alkaloids, saponins, triterpenoids, cardiac glycosides and steroids. Clove oil contained alkaloids, tannins, saponins, glycosides and terpenoids. *Bombax ceiba* leaf oil revealed alkaloids, tannins, saponins, carbohydrates

and reducing sugars. Diverse phytochemical profiles across the selected oils validate their potential for synergistic therapeutic application in polyherbal formulations (Table 1).

S.No.	Phytochemical Constituent	Clove oil	Garlic oil	Neem oil	*B. ceiba* oil
1.	Alkaloids	+	+	−	+
2.	Flavonoids	−	−	+++	−
3.	Phenols	−	−	+++	−
4.	Tannins	++	−	−	+
5.	Saponins	+	+	−	+
6.	Steroids	−	+	+	−
7.	Glycosides / Cardiac glycosides	+	+	−	−
8.	Terpenoids / Triterpenoids	+	+	−	−
9.	Carbohydrates	−	−	+++	+
10.	Reducing sugars	−	−	−	+
11.	Anthraquinones	−	−	+++	−

Note: + Present, - Absent

Table 1. Preliminary qualitative phytochemical screening of the essential oils from Syzygium aromaticum (Clove), Allium sativum (Garlic), Azadirachta indica (Neem), and Bombax ceiba used in the polyherbal formulation:

3.2 Optimization and Physicochemical Characterization of the Polyherbal Oil-in-Water Emulsion

Nine experimental oil-in-water (O/W) polyherbal emulsions (F1–F9) were successfully prepared using the wet-gum emulsification technique by varying herbal oils, surfactant, co-surfactant, emulsifying agent, preservative, and aqueous phase concentrations. Formulations were evaluated for appearance, homogeneity, ease of emulsification, and physical stability immediately after preparation and during preliminary storage. Lower surfactant concentrations produced slight creaming and reduced homogeneity, whereas optimized surfactant-to-oil ratios improved dispersion and stability. Tween 80 and propylene glycol enhanced emulsification and minimized phase separation, while gum acacia provided additional steric stabilization.

Comparative factorial evaluation (Table 2) identified F5 as the optimized formulation, containing balanced proportions of *Azadirachta indica*, *Syzygium aromaticum*, *Allium sativum*, and *Bombax ceiba* oils with optimized excipients. F5 exhibited a smooth, uniform, creamy appearance without aggregation, creaming, cracking, or phase separation. It also showed acceptable consistency, pleasant appearance, and excellent redispersibility. No oil leakage, sedimentation, or irreversible instability was observed during preliminary and accelerated stability studies, confirming satisfactory physical robustness

S.No.	Code	Clove oil (mL)	Garlic oil (mL)	Neem oil (mL)	B. ceiba oil (mL)	Total oils (mL)	Tween 80 (mL)	Prop. Glycol (mL)	Sod. Benzoate (g)	Gum Acacia (g)	Dist. Water (mL)
1.	**F1**	0.6	0.6	0.6	0.6	2.4	3.6	3.0	0.06	0.20	51.0
2.	**F2**	0.9	0.9	0.6	0.6	3.0	4.2	3.0	0.06	0.25	48.9
3.	**F3**	0.6	0.6	0.9	0.9	3.0	3.0	3.6	0.09	0.20	48.9
4.	**F4**	1.2	1.2	1.2	1.2	4.8	4.8	2.4	0.06	0.30	47.4
5.	**F5**	1.5	1.5	1.5	1.5	6.0	3.0	2.0	0.002	0.20	49.0
6.	**F6**	1.2	0.6	1.2	0.6	3.6	3.6	3.0	0.06	0.25	49.8
7.	**F7**	0.9	0.6	0.9	0.6	3.0	3.6	3.6	0.06	0.20	48.9
8.	**F8**	0.6	0.6	0.6	0.6	2.4	3.0	3.0	0.03	0.50	51.0
9.	**F9**	0.6	0.6	0.6	0.6	2.4	2.4	4.2	0.12	0.20	50.4

Table 2: Composition of Polyherbal Formulation

3.3 Organoleptic Evaluation

All nine formulations (F1–F9) were evaluated for colour, odour, appearance and phase separation. Formulations F1–F5 showed uniform appearance with no visible phase separation, whereas slight turbidity to opacity was observed in F6–F9. Homogeneity studies revealed that F1–F5 were uniformly homogeneous with no evidence of aggregation or creaming, whereas F6 and F8 showed slight non-uniformity. These observations suggest that formulation stability is enhanced at optimal surfactant concentrations (Table 3).

Formulation	Homogeneity	Colour	Odour	Appearance	Phase Separation
F1	Homogeneous	Light brown	Herbal	Opaque	No
F2	Slightly homogeneous	Brownish	Strong herbal	Opaque	No
F3	Homogeneous	Greenish	Mild herbal	Opaque	No
F4	Slightly homogeneous	Translucent	Herbal	Slightly translucent	No
F5	Homogeneous	Milky white	Pleasant herbal	Opaque	No
F6	Slightly homogeneous	Very light brown	Characteristic	Translucent	No
F7	Homogeneous	Greenish	Moderately herbal	Translucent	Slight
F8	Slightly heterogeneous	Light brown	Herbal	Semi-clear	Slight
F9	Homogeneous	Light brown	Herbal	Slightly turbid	Moderate

Table 3. Organoleptic evaluation of polyherbal oil-in-water emulsion formulations (F1–F9) based on homogeneity, colour, odour, appearance, and phase stability.

3.4 pH and Viscosity (F1–F9)

The pH of all nine formulations ranged between 6.20 and 6.60, with no significant variation during the evaluation period, suggesting good formulation stability and suitability for oral administration. Viscosity measurements confirmed acceptable flow properties for all formulations. Among all, F5 demonstrated optimum viscosity, indicating an equilibrium between structural integrity and followability (Table 4) (Figure 1, Figure 2).

Formulation	pH	Viscosity (cP)
F1	6.20	120±5
F2	6.25	125±6
F3	6.30	130±7
F4	6.35	118±5

Natural Resources for Human Health
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F5	6.60	115±4
F6	6.40	122±5
F7	6.40	127±7
F8	6.50	124±5
F9	6.55	129±6

Table 4. *pH and viscosity of the developed polyherbal oil-in-water emulsion formulations (F1–F9):*

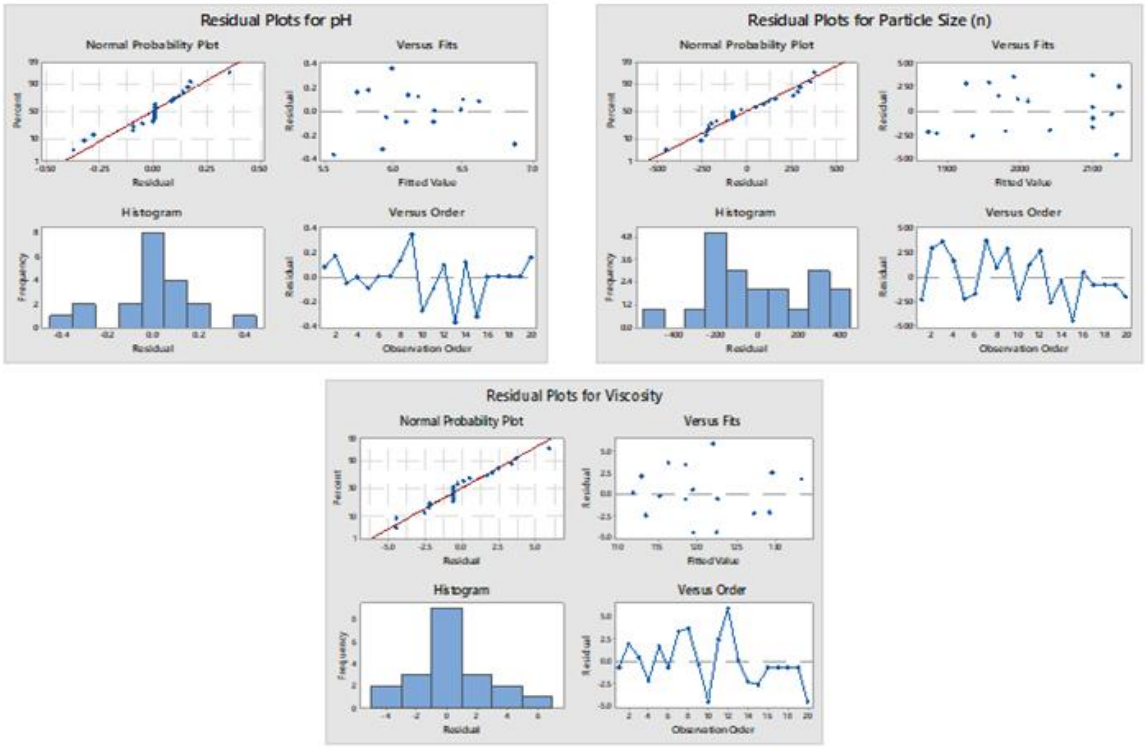

Figure 1: Residual Plots for pH, Viscosity and Particle size

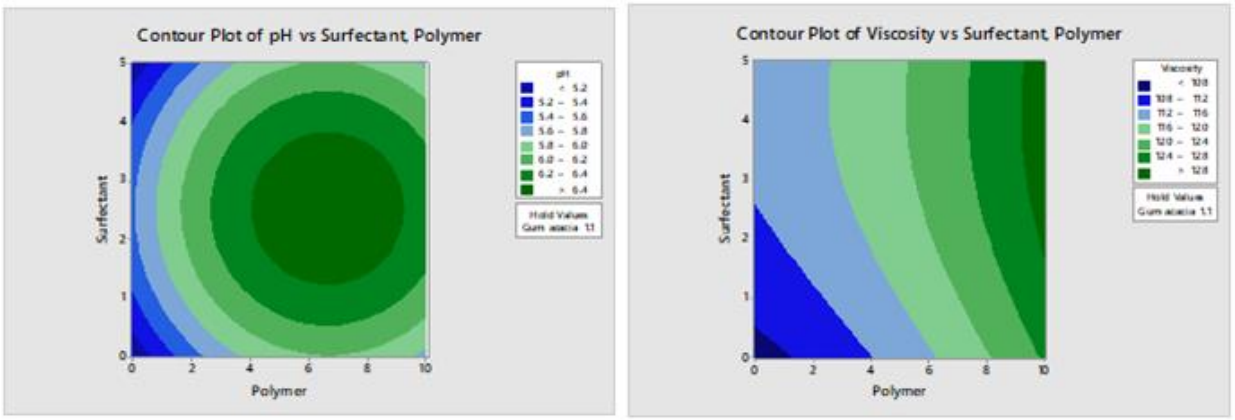

Figure 2: Contour Plots of pH and Viscosity

Natural Resources for Human Health
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3.5 Globule Size and Zeta Potential (F1–F9)

Globule size decreased with increasing surfactant concentration. Formulation F5 exhibited the most uniform globule size, indicating efficient emulsification and improved physical stability. Zeta potential analysis demonstrated that F5 possessed a negative surface charge, indicating electrostatic stabilization. The observed zeta potential suggests sufficient repulsive forces between droplets, reducing the risk of aggregation (Table 5) (Figure 3).

Formulation	Globule Size (nm)	Zeta Potential (mV)
F1	1653.5	-12.2
F2	1034.9	-17.5
F3	895.3	-10.4
F4	8151	-0.3
F5	420.2	-30.3
F6	674.8	-15.1
F7	510.4	-28.6
F8	380.2	-33.4
F9	312.6	-41.2

Table 5. Globule size and zeta potential of the developed polyherbal oil-in-water emulsion formulations (F1–F9):

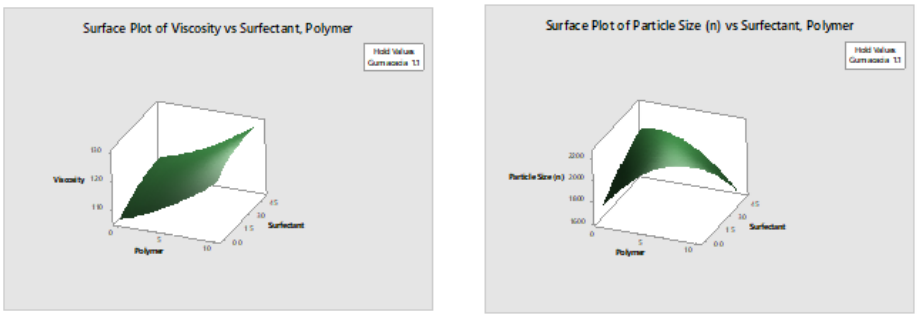

Figure 3: Surface Plots of Viscosity and Particle Size

3..6 Antidiabetic Activity

3.6.1 Effect of the Polyherbal Formulation on Fasting Blood Glucose Levels

The antihyperglycemic activity of optimized PHF was evaluated in STZ-induced diabetic Wistar rats over 28 days. STZ administration produced marked hyperglycemia, confirming successful diabetes induction. Repeated oral administration of PHF produced a progressive, dose-dependent reduction in fasting blood glucose compared with the diabetic control group. PHF 200 mg/kg produced a gradual glucose-lowering effect, whereas PHF 400 mg/kg demonstrated a more pronounced reduction. By Day 28, the high-dose PHF group showed blood glucose levels approaching those of the metformin-treated group. These findings indicate sustained antihyperglycemic activity and improved glycemic regulation, with greater efficacy at the higher dose. Data are presented in Table 6 and Figure 4.

Natural Resources for Human Health
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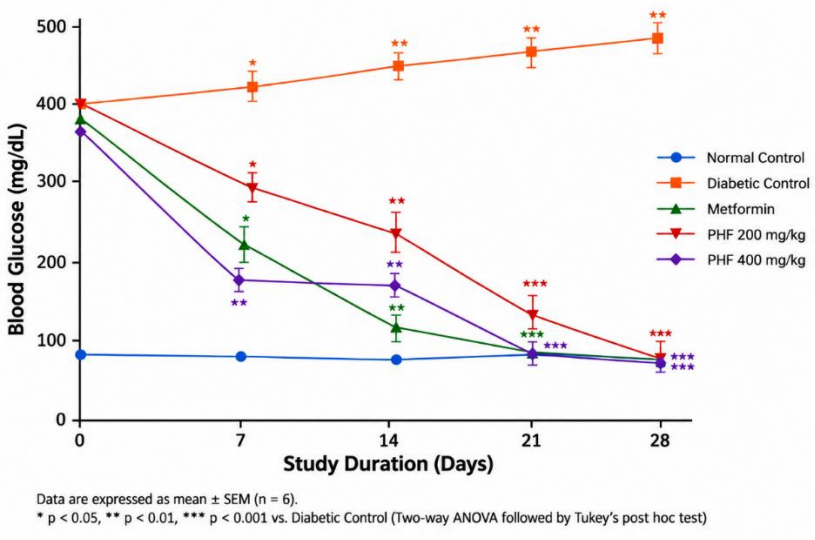

Figure 4. Effect of polyherbal formulation (PHF) on blood glucose levels in streptozotocin-induced diabetic rats over a 28-day treatment period**:** Blood glucose was measured on Days 0, 7, 14, 21, and 28 (mean ± SEM, n=6). Normal controls remained stable (81–89 mg/dL), whereas diabetic controls increased from 398 ± 5.23 to 480 ± 11.45 mg/dL. Metformin reduced glucose to 89.4 ± 6.54 mg/dL by Day 28. PHF 200 mg/kg reduced glucose from 398.2 ± 12.92 to 86.2 ± 5.72 mg/dL ($P < 0.01$), while PHF 400 mg/kg produced the greatest reduction, from 378.2 ± 11.02 to 82.2 ± 4.62 mg/dL ($P < 0.001$). Two-way repeated-measures ANOVA showed significant treatment, time, and interaction effects ($P < 0.001$)..

3.6.2 Effect of the Polyherbal Formulation on Body Weight

Body weight was monitored over 28 days as an indicator of metabolic status and overall health. Baseline body weights showed no significant differences among groups, confirming appropriate randomization. The diabetic control group exhibited progressive weight loss, consistent with STZ-induced insulin deficiency, impaired glucose utilization, and increased catabolism. PHF treatment attenuated diabetes-associated we.

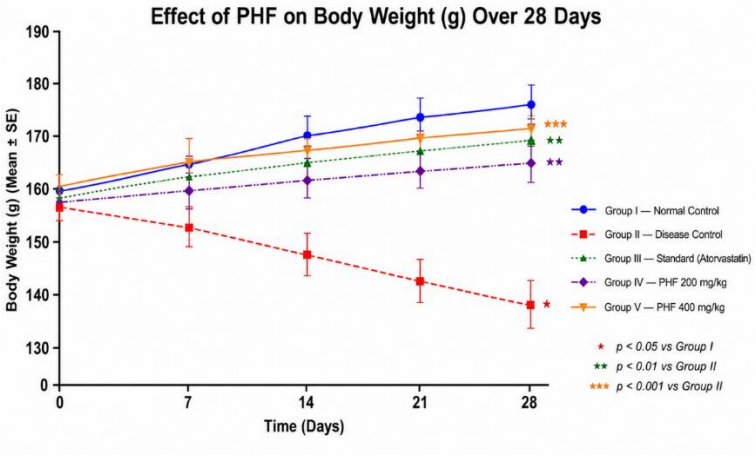

Figure 5: Effect of polyherbal formulation (PHF) on body weight in experimental animals during the 28-day treatment period. Data are expressed as mean ± SE (n = 6). Disease controls showed progressive weight loss, from 157.9 ± 4.1 g at baseline to 142.5 ± 3.5 g on Day 28. Atorvastatin increased body weight to 170.6 ± 3.8 g ($P < 0.01$). PHF 200 mg/kg improved weight to 166.3 ± 3.9 g ($P < 0.01$), while PHF 400 mg/kg produced the greatest increase (171.4 ± 4.1 g; $P < 0.001$), demonstrating dose-dependent protection comparable to atorvastatin.

Results are presented as mean ± SE (n = 6). We used two-way repeated-measures ANOVA to assess the effects of treatment, time, and their interaction, followed by Tukey's multiple-comparison post hoc test. Statistical significance was accepted at P <

0.05. The analysis indicated significant effects of treatment ($P < 0.001$), time ($P < 0.001$), and treatment × time interaction ($P < 0.001$), confirming that both atorvastatin and PHF significantly prevented disease-induced body weight loss in a dose-dependent manner

3.4 GC-MS Phytochemical Characterization

GC–MS analysis of the polyherbal essential oil formulation revealed a diverse phytochemical profile with distinct early, intermediate, and late elution regions (Figure 6). Early peaks (5–10 min) were associated with volatile sulfur-containing compounds and oxygenated monoterpenoids, while prominent peaks at 16.87 and 36.653 min indicated major constituents. *Syzygium aromaticum* oil was characterized by eugenol (72.6%), β-caryophyllene (11.4%), and eugenyl acetate (8.9%), contributing antioxidant and anti-inflammatory effects. Allium sativum contained allicin (34.2%), diallyl disulfide (18.7%), and diallyl trisulfide (14.5%), which may improve insulin sensitivity and reduce oxidative stress (Table 8).

Late-eluting constituents were mainly associated with *Azadirachta indica* and *Bombax ceiba*. Neem oil contained nimbidin (18.4%), azadirachtin A (12.7%), and oleic acid (11.2%), whereas *Bombax ceiba* contained lupeol (22.3%), β-sitosterol (15.6%), and α-amyrin (10.2%). These compounds possess reported hypoglycemic, antioxidant, anti-inflammatory, hepatoprotective, and lipid-modulating activities. Overall, the complementary phenolic, organosulfur, triterpenoid, and phytosterol constituents suggest potential synergistic effects against diabetes and dyslipidemia, supporting the formulation's observed pharmacological activities.

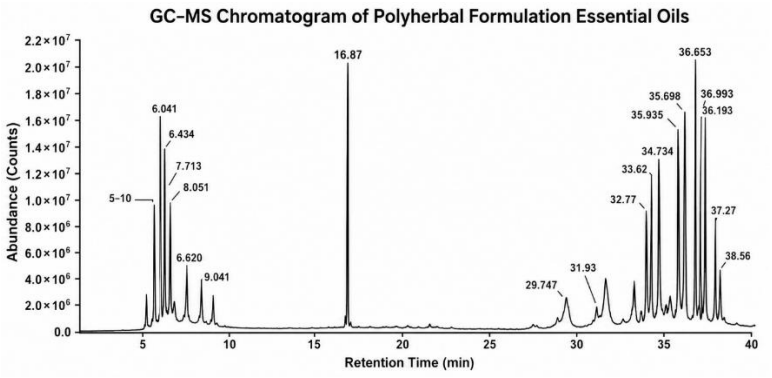

Figure 6: The GC–MS chromatogram of the polyherbal essential oil formulation containing Azadirachta indica, Syzygium aromaticum, Allium sativum, and Bombax ceiba showed a complex phytochemical profile. Early peaks (5–10 min) mainly represented volatile organosulfur compounds from garlic. The major peak at 16.87 min corresponded predominantly to eugenol-rich clove oil. Intense peaks between 34 and 38 min, particularly at 36.653 min, were associated with triterpenoids, phytosterols, and limonoids from Bombax ceiba and Azadirachta indica. Overall, the profile reflects the formulation's diverse bioactive constituents..

S. NO	Essential Oil (Plant)	Relative Area (%)	Reported Bioactivity
1.	*Azadirachta indica* (Neem)	18.4	Anti-inflammatory, antidiabetic
		12.7	Hypoglycaemic, antioxidant
		11.2	Cardioprotective, hypolipidaemic
2.	*Syzygium aromaticum* (Clove)	72.6	α-Glucosidase inhibition, antioxidant
		11.4	Anti-inflammatory, CB2 agonist
		8.9	Antioxidant, antimicrobial

3.	*Allium sativum* (Garlic)	34.2	Insulin sensitizer, HMG-CoA inhibition
		18.7	Hypolipidaemic, antioxidant
4.	*Bombax ceiba*	14.5	Antidiabetic, Antihyperlipidemic
		22.3	Anti-inflammatory, antidiabetic
		15.6	Cholesterol absorption inhibition
		10.2	Hypolipidaemic, hepatoprotective

Table 8: Major Bioactive Constituents Identified in the Essential Oils Used for Polyherbal Formulation and Their Reported Pharmacological Activities:

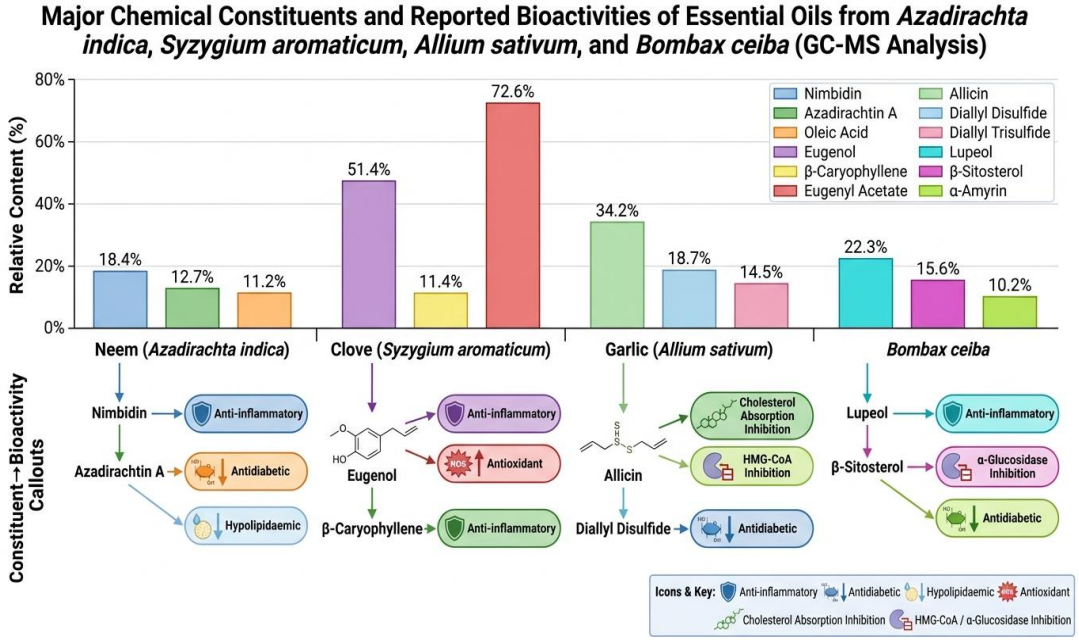

Figure 7: Major chemical constituents and reported bioactivities of essential oils from Azadirachta indica, Syzygium aromaticum, Allium sativum, and Bombax ceiba, identified by GC–MS analysis.

4. DISCUSSION

The present study developed and optimized a polyherbal oil-in-water (O/W) emulsion containing essential oils of *Azadirachta indica, Syzygium aromaticum, Allium sativum,* and *Bombax ceiba,* and demonstrated significant antidiabetic activity in streptozotocin (STZ)-induced diabetic rats. The study integrated formulation development, physicochemical characterization, GC–MS profiling, and pharmacological evaluation, providing evidence for the therapeutic potential of the optimized formulation.

The four medicinal plants were selected for their complementary pharmacological properties. Their combined phytochemicals may simultaneously target hyperglycemia, oxidative stress, inflammation, and dysregulated lipid metabolism, supporting the multi-target advantages of polyherbal therapy. Among the nine formulations, F5 demonstrated the most favorable overall physicochemical properties, including homogeneity, acceptable viscosity and pH, appropriate globule size, zeta potential, and absence of phase separation. Although F8 and F9 showed smaller globules and more negative zeta potentials, their slight-to-moderate phase separation reduced their suitability. F5 therefore provided the best balance of stability and pharmaceutical acceptability and may enhance dispersion and oral bioavailability of lipophilic phytoconstituents.

Preliminary phytochemical screening indicated the presence of diverse bioactive classes, including alkaloids, flavonoids, phenols, tannins, saponins, steroids, glycosides, and terpenoids. GC–MS further demonstrated chemical complexity, with prominent peaks at approximately 16.87 min and 34–38 min. Major identified constituents included eugenol, β-caryophyllene, eugenyl acetate, allicin, diallyl disulfide, diallyl trisulfide, nimbidin, azadirachtin A, lupeol, β-sitosterol, α-amyrin, and oleic acid. These compounds are associated with antioxidant, anti-inflammatory, hypoglycemic, insulin-sensitizing, hepatoprotective, and lipid-modulating activities, supporting potential synergistic pharmacological effects.

PHF treatment produced marked, dose-dependent antihyperglycemic activity in STZ-induced diabetic rats. The 400 mg/kg dose produced glucose-lowering effects approaching those of metformin, suggesting improved glucose homeostasis. The activity may involve enhanced insulin sensitivity, inhibition of intestinal carbohydrate digestion, modulation of hepatic glucose metabolism, and attenuation of oxidative stress. PHF also significantly attenuated diabetes-associated body weight loss, particularly at the higher dose, potentially reflecting improved nutrient utilization, insulin action, and reduced protein catabolism.

The observed efficacy may result from complementary actions of individual phytoconstituents. Eugenol may inhibit α-glucosidase and oxidative damage, garlic organosulfur compounds may improve insulin sensitivity, while nimbidin, azadirachtin A, lupeol, α-amyrin, and β-sitosterol may contribute anti-inflammatory, hepatoprotective, insulin-sensitizing, and lipid-modulating effects. Thus, simultaneous modulation of multiple pathological pathways may underlie the therapeutic benefits of PHF.

A major strength of the study is the integration of formulation optimization, phytochemical characterization, and biological validation. However, mechanistic conclusions remain based largely on reported activities of identified compounds. LC–MS/MS confirmation, pharmacokinetic studies, molecular pathway investigations, chronic toxicity assessment, and clinical studies are required to establish therapeutic translation. Overall, the optimized PHF represents a promising multifunctional phytopharmaceutical for improving glucose homeostasis and metabolic health. **5. Conclusion**

The present study successfully developed and optimized a stable polyherbal oil-in-water emulsion containing *Azadirachta indica, Syzygium aromaticum, Allium sativum,* and *Bombax ceiba* oils. Among nine formulations, F5 demonstrated the most favorable physicochemical properties, including excellent homogeneity, suitable pH and viscosity, satisfactory globule size, zeta potential, and physical stability. GC–MS analysis identified several bioactive constituents, including eugenol, allicin, β-caryophyllene, lupeol, β-sitosterol, α-amyrin, nimbidin, azadirachtin A, and oleic acid, supporting its therapeutic potential. Pharmacological evaluation demonstrated significant, dose-dependent antihyperglycemic activity and restoration of body weight in STZ-induced diabetic rats. The effects may involve improved insulin sensitivity, reduced oxidative stress and inflammation, β-cell protection, and improved metabolic regulation. The higher PHF dose showed efficacy comparable to metformin. Overall, F5 represents a promising multifunctional herbal delivery system for diabetes management. Further studies should investigate phytochemical standardization, molecular mechanisms, pharmacokinetics, long-term safety, and clinical efficacy.

Declarations

Ethics Approval and Consent to Participate

All experimental procedures were performed in accordance with the OECD guidelines and applicable ethical standards for animal experimentation. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Translam Institute of Pharmaceutical Education and Research, Meerut, India (Ref. No. IAEC/PH-25/TIPER/223 (Approval Date: April 26, 2025). Thirty Wistar rats per model were used in the study for a duration of three months.

Competing Interests

The authors declare no relevant financial or non-financial interests.

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Authors' Contributions

[To be completed per CRediT taxonomy: Conceptualization; Methodology; Formal analysis and investigation; Writing — original draft preparation; Writing — review and editing; Supervision.]

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