RESEARCH PAPER

"Integrated Phytochemical and Pharmacological Evaluation of Polyherbal Extract for Experimental Diabetes"

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ABSTRACT

Background: Diabetes mellitus is associated with hyperglycemia, oxidative stress, and dyslipidemia. Herbal medicines rich in bioactive phytoconstituents may provide a safer therapeutic approach. This study evaluates the phytochemical profile, antioxidant potential, antidiabetic activity, and lipid-modulatory effects of a polyherbal extract.

Aim: To investigate the phytochemical constituents, in vitro antioxidant activity, in vivo antidiabetic efficacy, and serum lipid profile modulation of a polyherbal formulation prepared from Momordica charantia, Gymnema sylvestre, Aegle marmelos, and Coccinia grandis.

Materials and Methods: Plant materials were authenticated, shade-dried, powdered, and extracted using 70% ethanol via Soxhlet extraction. Physicochemical constants and preliminary phytochemical screening were performed using standard methods. Antioxidant activity was evaluated using DPPH, ABTS radical scavenging assays, and total antioxidant capacity. Antidiabetic activity was assessed in streptozotocin-induced diabetic Wistar rats treated orally with polyherbal extract (200 and 400 mg/kg) for 30 days. Blood glucose levels were monitored periodically, and serum lipid parameters were analyzed using biochemical kits. Data were statistically analyzed using one-way ANOVA followed by Tukey's post hoc test.

Results: Physicochemical analysis confirmed acceptable quality of raw plant materials. Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, saponins, steroids, and diterpenes. The polyherbal extract showed dose-dependent antioxidant activity in DPPH, ABTS, and total antioxidant assays. In vivo studies demonstrated a significant reduction in blood glucose levels in treated groups compared to diabetic control. Lipid profile analysis showed improvement in total cholesterol, triglycerides, LDL, VLDL, and HDL levels.

Conclusion: The polyherbal extract exhibited significant antioxidant, antidiabetic, and hypolipidemic activities, likely due to synergistic effects of phytoconstituents. The formulation shows potential as a natural therapeutic agent for diabetes management.

Keywords: Polyherbal Extract; Diabetes Mellitus; Momordica Charantia; Gymnema Sylvestre; Aegle Marmelos; Coccinia Grandis; Antioxidant Activity; Antidiabetic Activity; Streptozotocin; Lipid Profile; Phytochemicals.

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. [1,2] It is one of the major global health challenges, with a rapidly increasing prevalence due to sedentary lifestyle, unhealthy dietary habits, and genetic predisposition. Prolonged hyperglycemia is associated with severe complications such as neuropathy, nephropathy, retinopathy, cardiovascular disorders, and dyslipidemia, significantly affecting the quality of life of patients.[3,4]

Oxidative stress plays a key role in the pathogenesis and progression of diabetes by promoting pancreatic β-cell dysfunction and insulin resistance.[5,6] Excessive generation of reactive oxygen species (ROS) further aggravates cellular damage and metabolic imbalance. Therefore, therapeutic

strategies targeting both hyperglycemia and oxidative stress are considered essential in diabetes management.[7,8]

Although synthetic antidiabetic drugs are widely used, they are often associated with adverse effects, high cost, and limited long-term efficacy. This has led to an increasing interest in herbal medicines and polyherbal formulations, which are believed to offer a safer and more holistic approach due to their multi-targeted actions and minimal side effects.[9]

Medicinal plants such as *Momordica charantia* (bitter melon), *Gymnema sylvestre*, *Aegle marmelos* (bael), and *Coccinia grandis* (ivy gourd) have been traditionally used in Ayurveda and folk medicine for the management of diabetes. These plants are rich in bioactive phytoconstituents including flavonoids, saponins, alkaloids, and phenolic compounds, which exhibit antioxidant, hypoglycemic, and lipid-lowering properties.[10]

Considering the complementary mechanisms of these plants, the present study aims to develop and evaluate a polyherbal extract for its phytochemical composition, antioxidant activity, antidiabetic potential, and its effect on lipid profile in streptozotocin-induced diabetic experimental models. The study also attempts to explore the synergistic therapeutic potential of the selected medicinal plants in managing diabetes mellitus and its associated complications.

MATERIALS AND METHOD

Plant Material and Extraction

The plant *materials—Momordica charantia, Gymnema sylvestre, Aegle marmelos, and Coccinia grandis*—were procured from available sources, and a qualified botanist from the institute's Department of Botany authenticated them. The materials were shade-dried and coarsely powdered. The powdered samples were packed into filter paper thimbles and subjected to extraction using a Soxhlet apparatus. A round-bottom flask containing 70% ethanol as the solvent was connected to the apparatus, and the extraction was carried out under controlled heating using a water bath. The solvent was continuously refluxed over a specified period to ensure efficient extraction. The extracts were then concentrated and stored at room temperature for further use.[11]

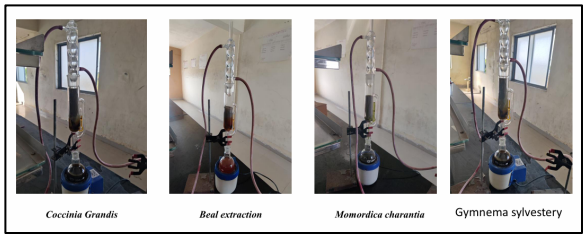

FIG 1: *Momordica charantia, Gymnema sylvestre, Aegle marmelos, and Coccinia grandis Extraction*

Physicochemical constants

The physicochemical constants of the powdered drug were determined using standard procedures. Moisture content was evaluated by the loss on drying method, wherein accurately weighed powdered samples were placed in pre-weighed evaporating dishes, dried in an oven at 105°C for one hour, cooled in a desiccator, and reweighed until a constant weight was obtained. Total ash value was determined by incinerating 2 g of powdered plant material in a silica crucible, gradually increasing the temperature until a white residue free from carbon was formed, followed by cooling in a desiccator and weighing to constant weight. Acid-insoluble ash was determined by treating the total ash with 25 ml of dilute hydrochloric acid, simmering for 5 minutes, filtering through ash-free filter paper, washing with hot water, and igniting the residue to constant weight. Water-soluble ash was determined by boiling the total ash with 25 ml of water, filtering, washing the insoluble matter, igniting the residue, and calculating the difference between total ash and the insoluble residue (WHO, 2011). Alcohol-soluble extractive value was determined by macerating 4 g of powdered drug with 100 ml ethanol for 24 hours with intermittent shaking, filtering, evaporating 25 ml of filtrate to dryness, and weighing after desiccation to constant weight. Similarly, water-soluble extractive value was determined using water as the solvent in place of ethanol, following the same procedure.[12]

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract was carried out using standard qualitative tests to detect the presence of major secondary metabolites. Carbohydrates were identified by Molisch's test and Benedict's test, showing characteristic violet ring and color change, respectively. Proteins and amino acids were confirmed by Biuret test, Ninhydrin test, cysteine test, and precipitation test, indicating violet coloration or precipitate formation. Fats and fixed oils were detected using Sudan red test, spot test, and

saponification test. Saponins were confirmed by foam and haemolytic tests, while alkaloids were identified using Dragendorff's test, Mayer's test, Wagner's test, and Hager's test based on precipitate formation. Flavonoids were detected by Shinoda test, alkaline reagent test, and zinc hydrochloride test, producing characteristic color changes. Phenolic compounds were confirmed using ferric chloride test, while triterpenoids were identified by Liebermann–Burchard test and sulfur powder test. Lignins were detected using phloroglucinol-HCl and thionine tests, and tannins were confirmed by vanillin-HCl and gelatin tests, indicating the presence of diverse phytoconstituents in the extract.[13]

In Vitro Antioxidant Activity

The in vitro antioxidant activity of individual plant extracts—Momordica charantia, Gymnema sylvestre, Aegle marmelos, Coccinia grandis—along with the polyherbal extract and standard Ascorbic acid was evaluated using DPPH radical scavenging assay, ABTS radical scavenging assay, and total antioxidant capacity assay. Various concentrations (0.1, 0.5, and 1.0 mg/mL) of each extract were prepared. In the DPPH assay, the decrease in absorbance at 517 nm after reaction with the stable DPPH radical indicated scavenging activity. The ABTS assay involved the generation of $ABTS^{+}$ radicals and measurement of absorbance reduction at 734 nm. Total antioxidant capacity was determined using the phosphomolybdenum method and expressed as mg ascorbic acid equivalents (AAE)/g of extract. All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation.[14]

In Vivo Antidiabetic Activity

The antidiabetic activity of the polyherbal extract was evaluated using Streptozotocin-induced diabetes in Wistar rats. Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ), and animals with fasting blood glucose levels above 250 mg/dL were considered diabetic. The animals were divided into five groups: normal control, positive control (diabetic untreated), standard drug-treated group, and two test groups receiving polyherbal extract at doses of 200 mg/kg and 400 mg/kg body weight. Treatments were administered orally for 30 days. Blood glucose levels were measured at regular intervals (Day 0, 6, 12, 18, 24, and 30) using a glucometer. The results were expressed as mean ± SD, and statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test to determine significance.[15]

Serum Lipid Profile Analysis

At the end of the experimental period, blood samples were collected from all groups of animals for the estimation of serum lipid parameters. Serum was separated by centrifugation and analyzed for total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) using standard biochemical assay kits. The study groups included normal control, positive control, standard-treated group, and polyherbal extract-treated groups (200 mg/kg and 400 mg/kg). Lipid parameters were calculated using established formulas where required, and results were expressed as mean ± SD. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparison test to evaluate the effect of treatments on lipid metabolism.[16]

RESULT AND DISCUSSION

Physicochemical analysis of powdered herbs

The physicochemical evaluation of the selected medicinal plants revealed acceptable quality parameters suitable for further pharmacological investigation. Moisture content was found to be within the permissible range (6.50–8.50%), indicating proper drying and reduced risk of microbial contamination. *Momordica charantia* showed relatively higher moisture content (8.50±0.90), while *Coccinia grandis* exhibited the lowest (6.50±0.80), suggesting better stability. Total ash values ranged from 5.75±0.90 to 9.20±1.10, reflecting the total inorganic content and confirming the purity of the plant materials.

Table 1: Physicochemical Analysis of Powdered Herbs

Plant species	Moisture	Ash	Acid-insol. ash	Water-sol. ash	Water ext.	Ethanol ext.
Gymnema sylvestre	6.70 ±0.90	6.90 ±1.00	5.80 ±0.70	7.10 ±0.80	16.50 ±1.20	23.03 ±2.62
Mom	8.50	9.20	7.80	8.00	15.50	22.00

ordica charantia	±0.90	±1.10	±1.00	±1.20	±1.20	±2.00
Aegle marmelos	7.00 ±0.80	8.50 ±0.95	7.00 ±0.75	7.20 ±0.85	15.80 ±1.10	21.50 ±2.20
Coccinia grandis	6.50 ±0.80	5.75 ±0.90	4.80 ±0.70	6.25 ±0.75	12.75 ±1.00	20.50 ±2.00

Acid-insoluble ash and water-soluble ash values further indicated minimal contamination with siliceous matter and presence of water-soluble minerals. Alcohol-soluble extractive values were higher than water-soluble extractives in all plants, with *Gymnema sylvestre* showing the highest ethanol extract (23.03±2.62), indicating the presence of more alcohol-soluble phytoconstituents such as alkaloids and flavonoids. Overall, these findings confirm the suitability of the selected plant materials for extraction and pharmacological studies.

Physicochemical analysis of powdered herbs

Preliminary phytochemical analysis revealed the presence of major classes of bioactive compounds in all four medicinal plants. Carbohydrates, proteins, alkaloids, fats and oils, steroids, diterpenes, and saponins were consistently present in all extracts, while glycosides, tannins, flavonoids, phenols, and amino acids were not detected in significant amounts. The strong presence of saponins (++ level) across all plants suggests a key role in antidiabetic and hypolipidemic activity, as saponins are known to modulate glucose metabolism and lipid absorption. Alkaloids and steroids further contribute to metabolic regulation and antioxidant defense. The uniform phytochemical profile among the plants supports the rationale for combining them in a polyherbal formulation to achieve synergistic therapeutic effects.

Table 2: Phytochemical investigation

S. No	Phyto-constituent	*M. charantia*	*G. sylvestre*	*A. marmelos*	*C. grandis*
1	Proteins	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Fats & oils	+	+	+	+
4	Alkaloids	+	+	+	+
5	Glycosides	−	−	−	−
6	Tannins	−	−	−	−
7	Resins	−	−	−	−
8	Flavonoids	−	−	−	−
9	Steroids	+	+	+	+
10	Amino acids	−	−	−	−
11	Phenols	−	−	−	−
12	Diterpenes	+	+	+	+
13	Saponins	++	++	++	++

FTIR

FTIR spectroscopy confirmed the presence of various functional groups associated with bioactive phytoconstituents in all plant extracts. Broad peaks corresponding to O–H stretching indicated the presence of alcohols and phenolic compounds, while C–H stretching bands suggested aliphatic structures. Carbonyl (C=O) and aromatic functional groups further confirmed the presence of flavonoids, terpenoids, and other secondary metabolites. These functional groups are known to contribute to antioxidant and antidiabetic activities through redox modulation and enzyme inhibition. The similarity in FTIR spectra among the plant extracts indicates the presence of common phytochemical frameworks, supporting their combined use in a polyherbal formulation.

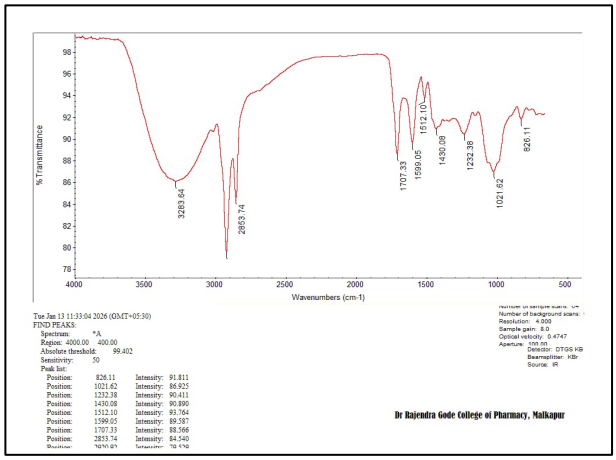

Fig 2: FTIR of *Beal extraction*

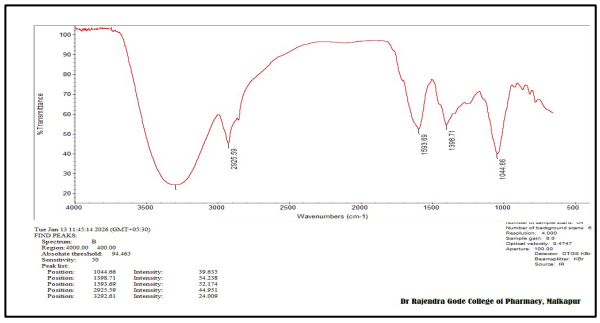

Fig 3: FTIR of Gymnema sylvestery

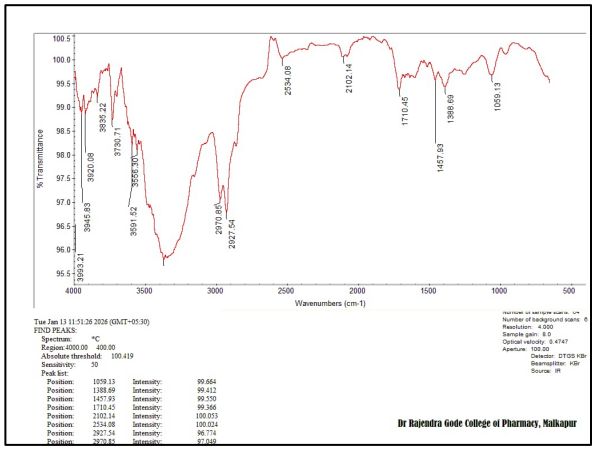

Fig 4: FTIR of *Coccinia Grandis*

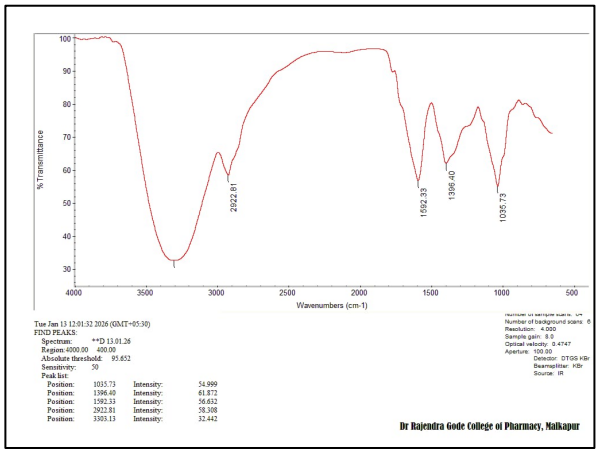

Fig 5: FTIR of *Momordica charantia*

IN VITRO ANTI-OXIDANT ACTIVITY

The antioxidant evaluation demonstrated a concentration-dependent increase in free radical scavenging activity for all extracts in DPPH, ABTS, and total antioxidant capacity assays. Among individual extracts, *Gymnema sylvestre* showed the highest antioxidant activity, followed by *Momordica charantia*, *Aegle marmelos*, and *Coccinia grandis*. However, the polyherbal extract exhibited significantly higher antioxidant activity than individual extracts at all tested concentrations, indicating synergistic interactions among phytoconstituents. At 1.0 mg/mL, the polyherbal extract showed 55% DPPH inhibition and 65% ABTS scavenging activity, which was substantially higher than individual plants but lower than ascorbic acid. The enhanced antioxidant activity may be attributed to the combined presence of saponins, alkaloids, and other secondary metabolites, which collectively neutralize reactive oxygen species and reduce oxidative stress.

Table 3: In Vitro Anti-Oxidant Activity

Sample	Concentration (mg/mL)	DPPH Radical Scavenging (%)	ABTS Radical Scavenging (%)	Total Antioxidant Capacity (mg AAE/g)
Momordica charantia	0.1	20 ± 1.50	30 ± 2.00	15 ± 1.00
	0.5	30 ± 2.00	40 ± 2.50	18 ± 1.20
	1.0	40 ± 2.50	50 ± 3.00	20 ± 1.50
Gymnema sylvestre	0.1	25 ± 2.00	35 ± 2.50	18 ± 1.20
	0.5	35 ± 2.50	45 ± 3.00	22 ± 1.50
	1.0	45 ± 3.00	55 ± 3.50	25 ± 2.00
Aegle marmelos (Bael)	0.1	22 ± 1.80	32 ± 2.20	17 ± 1.10
	0.5	32 ± 2.20	42 ± 2.80	20 ± 1.30
	1.0	42 ± 2.80	52 ± 3.30	24 ± 1.50
Coccinia grandis (Ivy Gourd)	0.1	18 ± 1.20	28 ± 1.80	14 ± 0.90
	0.5	28 ± 1.80	38 ± 2.30	18 ± 1.10
	1.0	38 ± 2.30	48 ± 2.80	22 ± 1.30
Polyherbal extract	0.1	35 ± 3.00	45 ± 3.50	25 ± 2.00
	0.5	45 ± 3.50	55 ± 4.00	30 ± 2.20
	1.0	55 ±	65 ±	35 ±

		4.00	4.50	2.50
Ascorbic acid	0.1	42 ± 2.93	56 ± 3.14	32.5 ± 1.18
	0.5	63 ± 1.76	76 ± 1.02	52.6 ± 2.07
	1.0	76 ± 1.86	89 ± 2.83	64.4 ± 1.06

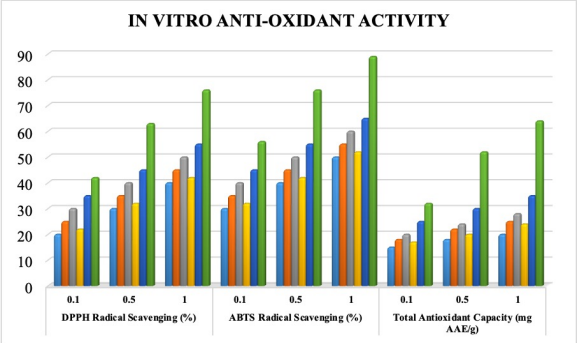

Fig 6: IN VITRO ANTI-OXIDANT ACTIVITY

IN VIVO ANTIDIABETIC ACTIVITY:

EFFECTS ON BLOOD GLUCOSE IN STZ INDUCED DIABETIC RATS AT DAY 0-30 DAYS

The streptozotocin-induced diabetic rat model demonstrated significant hyperglycemia in the positive control group, confirming successful induction of diabetes. Treatment with the polyherbal extract resulted in a marked reduction in blood glucose levels over 30 days. The higher dose (400 mg/kg) showed greater efficacy compared to the lower dose (200 mg/kg), indicating dose-dependent antidiabetic activity. At day 30, the 400 mg/kg group reduced glucose levels to 176.20±24.10 mg/dL compared to 311.50±22.90 mg/dL in the diabetic control group. The standard drug group also showed significant glucose reduction, validating the model. The observed hypoglycemic effect may be due to improved insulin secretion, enhanced glucose uptake, and inhibition of carbohydrate-digesting enzymes, potentially mediated by phytoconstituents such as saponins and alkaloids.

Table 4: Effects on blood glucose in STZ induced diabetic rats at day 0-30 days

Group	Treatment	Day 0	Day 6	Day 12	Day 18	Day 24	Day 30
1	**Poly-herbal extract 400 mg/kg**	91.05 ± 4.10	265.84 ± 28.9	222.10 ± 22.95 ***	219.45 ± 23.10 ***	216.90 ± 22.88 **	176.20 ± 24.10
2	**Poly-herbal extract 200 mg/kg**	89.95 ± 4.32	271.20 ± 15.8	234.85 ± 21.96 **	237.10 ± 22.10 **	239.60 ± 22.20	200.30 ± 25.10
3	**Standard Group**	88.75 ± 3.60	112.30 ± 33.9	207.80 ± 32.85	210.25 ± 33.10	183.40 ± 32.95 **	106.15 ± 17.30
4	**Positive control**	91.60 ± 3.05	263.40 ± 19.90 ###	274.85 ± 18.20 ###	277.10 ± 18.45 ###	284.30 ± 18.10 ###	311.50 ± 22.90 ###
5	**Normal control**	91.80 ± 2.05	94.20 ± 4.10	92.60 ± 3.55	92.10 ± 3.60	93.10 ± 3.50	92.70 ± 2.70

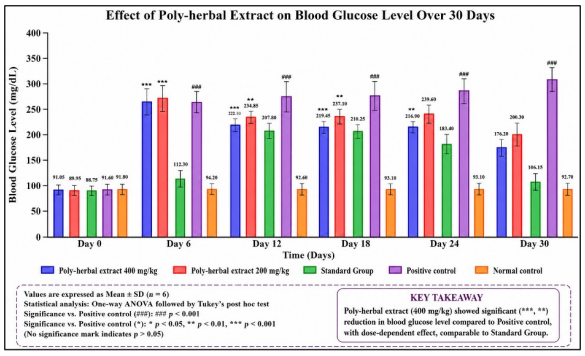

Fig 7: Effects on blood glucose in STZ induced diabetic rats at day 0-30 days

SERUM LIPID PROFILE

Diabetic control animals exhibited elevated levels of total cholesterol, triglycerides, LDL, and VLDL, along with reduced HDL levels, indicating diabetic dyslipidemia. Treatment with the polyherbal extract significantly improved lipid parameters in a dose-dependent manner. The 400 mg/kg dose showed the

most favorable lipid profile, with reduced cholesterol (87.12±2.35 mg/dL), triglycerides (74.62±4.10 mg/dL), LDL (49.8±3.28 mg/dL), and increased HDL (22.4±1.36 mg/dL) compared to diabetic control. These effects were comparable to the standard drug group. The lipid-lowering activity may be attributed to enhanced lipid metabolism, inhibition of cholesterol synthesis, and improved insulin sensitivity. The results confirm the cardioprotective potential of the polyherbal formulation in diabetes-associated dyslipidemia.

Table 5: Effects of test compounds on Blood glucose in STZ induced diabetic rats onTotal Cholesterol (mg/dl)

Group	Treatment	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
5	**Poly-herbal extract 400 mg/kg**	87.12 ± 2.35	74.62 ± 4.10	25.1 ± 1.12	49.8 ± 3.28	22.4 ± 1.36
4	**Poly-herbal extract 200 mg/kg**	86.03 ± 2.58	80.12 ± 4.28	27.2 ± 1.88	50.7 ± 3.54	21.6 ± 1.43
3	**Standard Group**	88.20 ± 9.85	76.35 ± 5.88	21.6 ± 2.32	48.9 ± 5.42	23.1 ± 1.44
2	**Positive control**	92.35 ± 5.78	83.65 ± 5.22	29.6 ± 3.44	54.7 ± 4.51	22.2 ± 1.95
1	**Normal control**	89.85 ± 7.40	93.10 ± 4.30	20.4 ± 3.18	47.9 ± 3.72	26.3 ± 2.65

Conclusion

The present study demonstrates that the polyherbal extract formulated from *Momordica charantia*, *Gymnema sylvestre*, *Aegle marmelos*, and *Coccinia grandis* possesses significant pharmacological potential. The phytochemical screening confirmed the presence of diverse bioactive constituents such as alkaloids, saponins, steroids, flavonoids, and diterpenes, which may be responsible for the observed biological activities. The extract exhibited notable dose-dependent antioxidant activity in DPPH, ABTS, and total antioxidant assays, indicating its strong free radical scavenging potential. In vivo evaluation in streptozotocin-induced diabetic rats revealed a significant reduction in blood glucose levels following treatment with the polyherbal formulation, with the higher dose showing better efficacy. Furthermore, the extract improved serum lipid parameters, including total cholesterol, triglycerides, LDL, VLDL, and HDL levels, suggesting a beneficial role in correcting diabetes-associated dyslipidemia. Overall, the findings suggest that the polyherbal formulation exerts synergistic antidiabetic, antioxidant, and hypolipidemic effects. These results support its potential use as a complementary therapeutic approach for managing diabetes mellitus and its associated metabolic complications. However, further mechanistic studies and clinical validation are required to establish its safety profile and therapeutic applicability in humans.

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