Research Article

Collection, Standardization And Phytochemical Evaluation Of Momordica Charantia Against Diabetes Mellitus

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Abstract:

The present research work include the Anti-Diabetic, Anti-Oxidant and Anti-Hyperlipidemic activity of Momordica Charantia, focuses on the phytochemical investigation and antidiabetic potential of this same plant. Here in this study the collection and authentication of seeds were done and then finally extraction process of Momordica Charantia seeds plants was completed by maintaining temperature for various solvents. After extraction in various solvents and identification through applying various tests the presence was confirmed.Successive extraction of the plant materials was carried out, followed by detailed phytochemical screening. The extracts were found to contain several bioactive constituents, including tannins, phenolic compounds, carbohydrates, glycosides, terpenoids, proteins, amino acids, and flavonoids. Phenolic compounds and flavonoids, in particular, are widely recognized for their therapeutic value in controlling hyperglycemia and combating oxidative stress. The ethanolic extracts of Momordica Charantia showed a strong presence of these beneficial phytochemicals.The findings of the present investigation demonstrate that the ethanolic extracts of Momordica Charantia exhibit significant antidiabetic effects in streptozotocin (STZ)-induced diabetic rats. Their beneficial activity appears to be associated with improved glucose tolerance, enhanced utilization of glucose, and restoration of depleted liver glycogen stores at both tested doses (200 and 400 mg/kg).

1. Introduction

1.1 Medicinal plants

For thousands of years, humans have depended on plants not only as a food source but also for treating various ailments. Natural products have made substantial contributions to the advancement of modern medicine and remain highly relevant in drug discovery today. Historically, digitalis glycosides were introduced in the 18th century for managing heart diseases, while willow bark was widely utilised to relieve pain as well as fever. Broad-scale studies on occurrence of medicinal flora reveal it grows along a wide range of habitats and landscapes. In India, nearly 70% of medicinal flora are located in tropical regions, mainly within forest ecosystems of Western as well as Eastern Ghats, the Vindhyas, Chhota Nagpur Plateau, Aravallis, as well as the Himalayas. Out of the 386 plant families along with 2,200 genera known to include medicinal species, a few families contribute the majority. These include Asteraceae, Euphorbiaceae, Lamiaceae, Fabaceae, Rubiaceae, Poaceae, Acanthaceae, Rosaceae, as well as Apiaceae. Among them, the Asteraceae family stands out, with the largest representation, contributing about 419 species used for medicinal purposes (Gupta et al., 2025). Nearly 90% of the medicinal plants utilized by industries are still sourced directly from nature. Although more than 800 species are employed in industrial production, fewer than 20 percent are actually cultivated on a commercial scale. In addition, over 70% of these plants are collected through destructive harvesting methods, since different anatomical sections like roots, bark, woody tissue, stems, or, in the case of herbs, the complete plant are commonly used (Singh and Kumar, 2021).

1.2 Diabetes mellitus

It is endocrinal metabolic condition marked by elevated blood glucose levels and disturbances in the metabolism of lipids, carbohydrates, and proteins, which in turn heightens the risk of developing cardiovascular complications (Daisy et al., 2009). DM has emerged as a significant global health issue because of its rapidly increasing occurrence. It impacts individuals across all age groups, economic levels, and demographic sectors in almost every part of the world. The highest occurrence of the disease is observed in low- and middle-income countries (Sidahmed et al., 2023). At present, numerous countries are approaching a global diabetes epidemic, which is advancing rapidly across the globe (Selvin and Juraschek, 2020). Poorly managed diabetes often leads to persistent high blood sugar levels (hyperglycemia), which, over time, can cause significant damage to various body systems, affecting blood vessels as well as nerve tissues (Bishu et al., 2019). Persistent hyperglycemia in DM leads to damage, impaired function, and eventual failure of multiple organs and tissues, contributing to onset of

microvascular disorders like retinopathy, nephropathy, cardiovascular disorders as well as neuropathy (Harding et al., 2019). A hyperglycemic state, combined with impaired insulin function, hyperlipidemia, and oxidative stress, facilitates the onset of both microvascular and macrovascular disorders. These include conditions like diabetic retinopathy, neuropathy, cardiovascular diseases, foot ulcers, and even limb amputations (Control and Trial, 2005). Early indicators of hyperglycemia may include polydipsia, increased appetite, excessive need to urinate, headaches, blurry sight, fatigue, unintended loss of weight, recurrent vaginal yeast or skin infections, and delayed wound-healing or sores- many of which are also associated with prolonged elevated blood sugar levels (Tegegne et al., 2024). Despite some variations in classification, four primary types of diabetes are widely recognized across the literature: (1) Type 1 DM, resulting from the immune-mediated destruction of pancreatic β-cells; (2) Type 2 DM, characterized by a progressive decline in insulin secretion, often associated with insulin resistance; (3) Gestational DM, which is first identified in the mid to late stages of pregnancy; and (4) Other diabetes types resulting from distinct factors, such as drug/chemical- influenced diabetes as well as monogenic forms, including neonatal diabetes as well as MODY (Tegegne et al., 2024; Leslie et al., 2016).

2. Materials and methods

2.1 COLLECTION AND AUTHENTICATION OF PLANT MATERIAL

Seeds of Momordica Charantia were procured from local market and this plant parts were authenticated by a botanist at the Department of Botany of University. The authenticated plants were used for preparation of extracts.

2.2 Extract Preparation:

All the plant materials were washed, dried in the shade at room temperature, and then ground into a fine powder using an electric grinder. The powder was stored in airtight containers. It was also passed through sieve no. 44 before being kept for extraction. Cleaned and powdered seeds of Momordica Charantia were used for the extraction work. About 500 grams of the powdered material was evenly placed in a Soxhlet apparatus. Extraction was done using different solvents, starting from non-polar to polar, such as petroleum ether, chloroform, acetone, and ethanol. All solvents were purified before use. The extraction was performed through continuous hot percolation for 72 hours. Aqueous extraction was done separately using the cold maceration method (Harborne, 1998).

2.3 Solvent Extraction:

- Plant powder (50 g) extracted with 500 mL 70% ethanol, utilizing a Soxhlet apparatus for 6-8 hours.
- Extracts were filtered through filter paper (Whatman No. 1) as well as concentrated via rotary evaporator at 40°C.
- Concentrated extracts were kept at 4°C until further use, all solvent used at various temperature are shown in table no. 1.

Table 1 : Showing the temperature of various Solvent used

Sr.No.	Solvent Used	Required Temperature
1	Petroleum Ether	(60-80˚c)
2	Chloroform	(55-56˚c)
3	Acetone	(55-56˚c)
4	Alcohol 90% v/v	(75-78˚c)
5	Distilled Water	(40-45˚c)

Extraction of plant material using different solvents

- **Acetone Extract of the seeds of** Momordica Charantia:
  The marc remaining after chloroform extraction was dried and further extracted with acetone (55–56 °C) for up to 72 hours. Upon completion, the solvent was removed through distillation, resulting in a dark brownish-green residue, which was then stored in a desiccator.
- **Ethanol Extract of the seeds of** Momordica Charantia:

The marc remaining after acetone extraction was dried and subsequently extracted with 95% ethanol (75–78 °C) for 72 hours. After the extraction process, the solvent was removed by distillation, yielding dark greenish-yellow extracts. These extracts were placed in a desiccator to eliminate excess moisture and then stored in airtight glass containers for further use.

5.3 IDENTIFICATION OF PHYTOCHEMICAL ACTIVE CONSTITUENTS

5.3.1 Preliminary phytochemical analysis

Extracts obtained (Acetone and Ethanol) went through preliminary phytochemical screening to detect the presence of bioactive compounds (Kokate et al., 1990; Basset et al., 1985).

5.3.2 Tests for Alkaloids:

a) Dragendorff's Test: About 2 mg of each extract was dissolved in 5 mL of distilled water, followed by the addition of 2 M hydrochloric acid until the solution became acidic. One milliliter of Dragendorff's reagent was then added. Orange/red ppt indicates alkaloid presence.

5.3.3 Tests for Carbohydrates:

a) Anthrone Test: Approximately 2 mg extract was mixed with 10 mL distilled water, shaken thoroughly, filtered. Filterate was concentrated and treated with 2 mL of Anthrone reagent. The development of a green or blue coloration indicated the presence of reducing sugars in the sample.

5.3.4 Test for Glycosides:

Molisch's Test: Approximately 2 mg of the extract was mixed with 10 mL of distilled water, shaken thoroughly, and filtered. The filtrate was concentrated and treated with 2–3 drops of Molisch's reagent, followed by the careful addition of 2 mL of concentrated sulfuric acid along the side of the test tube to form a distinct layer. The appearance of a reddish-violet ring at the interface indicated the presence of glycosides.

5.3.5 Tests for Proteins and free Amino Acids

plant extract in a small quantity was dissolved in a few millilitres of distilled water, then subjected to these qualitative tests:

1. Million's test: Million's reagent was added to extract solution. Red coloration indicated protein as well as free amino acid presence.

5.3.6 Tests for Fixed oils:

a) Spot Test: A small portion of the extract was pressed between two clean filter papers. The appearance of a translucent oil stain on the paper confirmed the presence of fixed oils.

5.4 PHARMACOLOGICAL SCREENING

Experimental animals:

The experimental study was conducted using healthy male Wistar albino rats, aged between 8 to 10 weeks and weighing between 180 to 220 grams and they kept under controlled environmental conditions with a temperature of 25 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. The rats were provided with free access to standard laboratory chow and clean drinking water throughout the experimental period.

Induction of Diabetes:

Experimental diabetes was induced in overnight-fasted Wistar albino rats by administering streptozotocin (STZ), a well-known diabetogenic agent. STZ was freshly prepared immediately before use by dissolving it in cold 0.1 M citrate buffer (pH 4.5) to ensure stability and accuracy of dosing. Each rat received a single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight. Following STZ administration, the rats were provided with 5% glucose solution in their drinking water for the next 24 hours to prevent drug-induced hypoglycemia, which may occur due to sudden and rapid insulin release from the damaged pancreatic β-cells. After 72 hours of STZ injection, fasting blood glucose (FBG) levels were determined using a glucometer by collecting a small drop of blood from the tail

vein. The rats exhibiting fasting blood glucose levels equal to or greater than 250 mg/dL were considered diabetic and selected for inclusion in the experimental study. These diabetic rats were then stabilized and maintained under standard laboratory conditions before initiating treatment with the respective plant extracts and standard drug.

Experimental groups:
Diabetes induction of experimental animals were randomly allocated into five groups, each consisting of six rats. A separate group of non-diabetic rats was maintained as the normal control group. In total, five groups, each containing six animals, were used for the experimental study. The treatment was continued for a period of 28 consecutive days as per the following grouping plan:
Group I (Normal Control): Non-diabetic rats received 1% DMSO and 1% Tween 80 orally.
Group II (Diabetic Control): Diabetic rats received 1% DMSO and 1% Tween 80 orally.
Group III (Standard): Diabetic rats given metformin (150 mg/kg bw; orally).
Group IV: Diabetic rats given ethanolic extract of Momordica Charantia (200 mg/kg bw; orally).
Group V: Diabetic rats given ethanolic extract of Momordica Charantia at (400 mg/kg bw; orally).
All treatments were given once a day by oral gavage for the full 28-day experimental period.

Sample collection:
Before initiating the experiment, the fasting blood glucose (FBG) levels of all rats were recorded. Blood samples were collected weekly through tail vein puncture throughout the study period. During the 28 days of continuous drug administration, blood glucose levels were monitored on days 0, 7, 14, 21, and 28. On the 28th day, following an overnight fast, blood samples were obtained from the retro-orbital plexus under mild ether anesthesia. Collected blood was transferred into clean, dry test tubes and allowed to clot at room temperature for 30 minutes. The samples were then centrifuged at 2000 rpm for 10 minutes, and the clear serum obtained was used for the estimation of various biochemical parameters. For glucose and insulin analysis, blood was collected in tubes containing a mixture of potassium oxalate and sodium fluoride (1:3). Liver tissues were carefully excised, blotted, weighed, and stored at –80°C for the determination of antioxidant enzyme activities.

Biochemical analysis:
Blood glucose levels were determined using the glucose oxidase-peroxidase (GOD-POD) method. Serum insulin concentrations were analyzed using a microplate ELISA Kit (Diasorin, Saluggia, Italy). Liver glycogen content was determined following standard procedure (Reitman and Frankel, 1957). Total protein content was estimated using the method of Lowry et al. (1951). Serum urea and creatinine levels were determined using the diacetyl monoxime method (Wybenga et al., 1971) and Jaffe's method (Slot, 1965), respectively. Liver homogenates were prepared in ice-cold 10% potassium chloride solution and used for the assessment of antioxidant enzyme activities- superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as described by Rotruck et al. (1973).

Biochemical parameter investigated in liver tissue:

a) Estimation of Plasma Thiobarbituric Acid Reactive Substances (TBARS)
Lipid peroxidation levels in liver tissue were determined using the thiobarbituric acid (TBA) reaction as described by Ohkawa et al. (1979).
Reagents:
1. Trichloroacetic acid (TCA) – 15% solution
2. Hydrochloric acid (HCl) – 0.25 N
3. Thiobarbituric acid (TBA) – 0.38% prepared in hot distilled water
4. TCA–TBA–HCl reagent – freshly prepared by mixing reagents 1, 2, and 3 in the ratio of 1:1:1
5. Stock standard (4.8 mM) – prepared by diluting 0.079 ml of 1,1,3,3-tetramethoxypropane to 100 ml
6. Working standard – obtained by diluting the stock solution to achieve a concentration of 4.8 nM/ml
Procedure :
Liver tissue homogenates were made using Tris–HCl buffer (pH 7.5). To 1 ml of the homogenate, 2 ml of freshly prepared TCA-TBA-HCl reagent was added and mixed well. The mixture was heated in a boiling water bath for 15

minutes to develop the color. After cooling, the samples were centrifuged for 10 minutes, and the absorbance of the clear supernatant was read at 535 nm using a reagent blank. Standard solutions (2–10 nM) were processed in the same way to create the calibration curve. The results were reported as nmol of TBARS per 100 g of wet tissue. Here is a simple and clear paraphrased version: Liver tissue homogenates were made using Tris–HCl buffer (pH 7.5). To 1 ml of the homogenate, 2 ml of freshly prepared TCA-TBA-HCl reagent was added and mixed well. The mixture was heated in a boiling water bath for 15 minutes to develop the color. After cooling, the samples were centrifuged for 10 minutes, and the absorbance of the clear supernatant was read at 535 nm using a reagent blank. Standard solutions (2–10 nM) were processed in the same way to create the calibration curve. The results were reported as nmol of TBARS per 100 g of wet tissue.

b) Measurement of Lipid Peroxidation

Lipid peroxidation levels in liver tissue were determined following the method of Ohkawa et al. (1979).

Reagents :

1. Sodium dodecyl sulfate (SDS) – 4% solution
2. Acetic acid – 20% solution
3. Hydrochloric acid (HCl) – 1.27 M (pH 3.5)
4. Thiobarbituric acid (TBA) – 0.8% (pH 7.4)
5. Tetraethoxypropane – used as a standard

Procedure

The extent of lipid peroxidation, indicated by the formation of thiobarbituric acid reactive substances (TBARS) such as malondialdehyde (MDA), was measured in liver homogenates. To 1 ml of the sample, 0.2 ml of 4% (w/v) SDS, 1.5 ml of 20% acetic acid (adjusted to pH 3.5 with 1.27 M HCl), and 1.5 ml of 0.8% TBA (pH 7.4) were added. The reaction mixture was then heated in a water bath at 85°C for one hour. After cooling, the samples were centrifuged at 1200 g for 10 minutes, and the absorbance of the resulting pink-colored supernatant was measured at 532 nm against a reagent blank. Tetraethoxypropane was used to prepare the standard calibration curve. Results were expressed as millimoles of MDA formed per 100 g of wet tissue.

Results and Discussion

6.1 OVERVIEW

This work provides a comprehensive presentation of the experimental findings obtained from the study of Momordica Charantia. The chapter is structured to systematically describe the outcomes of the extraction procedures, preliminary phytochemical investigations, and pharmacological evaluations conducted on this plant extracts. Initially, the extraction process for this plant was assessed, and the percentage yield of the respective extracts was calculated to determine the efficiency of the extraction methods employed. Following this, a detailed phytochemical screening was carried out to identify the presence of various bioactive constituents, including alkaloids, flavonoids, glycosides, saponins, tannins, phenols, and other secondary metabolites, which are known to contribute to the therapeutic properties of the plants. Subsequently, the pharmacological activities of the extracts were evaluated, focusing on their anti-diabetic, anti-hyperlipidemic, and antioxidant potential. The anti-diabetic activity was assessed through relevant in vivo models, measuring parameters such as blood glucose levels, glucose tolerance, and insulin modulation. The anti-hyperlipidemic effect was determined by evaluating lipid profile parameters, including total cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels. Additionally, the antioxidant potential of the extracts was examined using standard assays to measure their ability to scavenge free radicals and reduce oxidative stress markers.

All experimental data were carefully analyzed using appropriate statistical methods to evaluate the significance of the observed effects in treated groups compared to control groups. This statistical evaluation helped in confirming the reliability and reproducibility of the results, thereby providing a solid foundation for interpreting the pharmacological efficacy of the studied plant extracts. The findings are presented in a structured format, including tables, figures, and descriptive analyses, allowing for a clear understanding of the therapeutic potential and biochemical characteristics of Momordica Charantia.

6.2 PERCENTAGE YIELD OF EXTRACTS

Successive solvent extraction of each plant yielded extracts of varying colors, textures, and quantities depending on the solvent polarity. The results are summarized in Table 6.1.

Solvent.Extraction:Observation:

Both the aqueous and ethanolic extracts of Momordica Charantia, exhibited the highest percentage yields among the different solvents tested. This observation suggests that these solvents are particularly effective in extracting a larger proportion of the plant material. The higher extractive yields in water and ethanol can be attributed to the greater solubility of polar bioactive constituents, including phenolic compounds, glycosides, and flavonoids, in polar solvents. These phytochemicals are known for their hydrophilic nature, which allows them to dissolve more readily in aqueous and alcoholic media compared to non-polar solvents. Consequently, the results indicate that aqueous and ethanolic extraction methods are more efficient for isolating the biologically active constituents responsible for the medicinal properties of these plants. Also, the higher yields observed in aqueous and ethanolic extracts can be attributed to the superior solubility of polar bioactive compounds, such as carbohydrates, glycosides, and alkaloids, in these solvents. Polar solvents are more effective at breaking down plant cell walls and solubilizing hydrophilic constituents, resulting in a greater recovery of biologically active compounds. In contrast, non-polar solvents yielded lower extractive values, likely due to their limited ability to dissolve these polar phytochemicals. These findings indicate that water and ethanol are the most suitable solvents for extracting the primary pharmacologically active constituents from these plants. The data also provide a basis for selecting solvents in subsequent phytochemical and pharmacological evaluations, ensuring optimal isolation of compounds responsible for anti-diabetic, anti-hyperlipidemic, and antioxidant activities.

Table 6.1: Extractive values of Momordica Charantia seeds.

Plant Name and Part Used	Solvent Used	Color of Extract	Yield (% w/w)
Momordica charantia (Fruits)	Petroleum ether	Pale yellow	4.5
	Chloroform	Green	3.1
	Acetone	Brownish black	3.6
	Ethanol	Greenish brown	5.7
	Aqueous	Dark brown	6.9

6.3 PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of Momordica charantia, extracts have bioactive constituents, including alkaloids, flavonoids, saponins, glycosides, tannins, and phenolic compounds. The qualitative composition and relative abundance of these phytochemicals varied based on extraction solvent used. Polar solvents such as water and ethanol were found to extract higher concentrations of hydrophilic compounds like carbohydrates and glycosides, , whereas non-polar solvents preferentially extracted lipophilic constituents. These variations highlight the influence of solvent polarity on the solubility and extractability of specific phytochemical classes. The results of this screening provide insight into the chemical profile of each plant and form the basis for understanding their pharmacological activities, as different bioactive compounds contribute to anti-diabetic, anti-hyperlipidemic, and antioxidant effects. The results are summarized in Table 6.2.

Observation:

The preliminary phytochemical screening demonstrated that all three plants- Momordica charantia contained substantial amounts of alkaloids, flavonoids, and phenolic compounds. These classes of bioactive constituents are widely recognized for their therapeutic potential, particularly in exerting antidiabetic and antioxidant effects through mechanisms such as enhancement of insulin sensitivity, modulation of carbohydrate metabolism, and scavenging of free radicals. In addition, Momordica charantia exhibited a notably high content of saponins, which have been reported to contribute to lipid-lowering, hypoglycemic, and cardioprotective activities.

Table 6.3: Preliminary phytochemical screening of various extracts of Momordica charantia mature fruits

Phytochemical Class	Test Method	Petroleum Ether	Chloroform	Acetone	Ethanol	Aqueous

Alkaloids	Mayer's Test	-	+	+	+	+
	Dragendorff's Test	-	+	+	+	+
Flavonoids	Shinoda Test	-	+	+	+	+
	Alkaline Reagent Test	-	+	+	+	+
Saponins	Froth Test	-	-	+	+	+
Glycosides	Keller-Killiani Test	-	+	+	+	+
	Borntrager's Test	-	+	+	+	+
Tannins	Ferric Chloride Test	-	+	+	+	+
	Gelatin Test	-	+	+	+	+
Phenols	Ferric Chloride Test	-	+	+	+	+
	Lead Acetate Test	-	+	+	+	+
Terpenoids	Salkowski Test	+	+	+	+	+
Steroids	Liebermann-Burchard Test	+	+	+	+	+

6.4 PHARMACOLOGICAL STUDIES

- **Screening of Antidiabetic Activity**

6.4.1 Hypoglycemic Study in Normal Fasted Rats (Acute) :

In normal fasted rats, a single oral administration of ethanolic extracts of M. charantia, at doses of 200 mg/kg and 400 mg/kg did not produce any significant reduction in blood glucose levels up to the 3rd hour of observation. Similarly, the standard drug metformin did not exhibit any marked hypoglycemic effect at the 3rd hour, and glucose levels remained within the normal range. These findings are summarized in **Table 6.5**.

Table 6.5: Hypoglycemic effect of ethanolic extracts of Momordica charantia on blood glucose level in normal rats.

Groups	**Treatment**	**Blood Glucose Level (mg/dL)**			
		0 hr	**1 hr**	**2 hr**	**3 hr**
I	Normal Control (1% DMSO + 1% Tween 80)	81.16 ± 0.62	78.83 ± 0.65	79.50 ± 0.80	78.00 ± 0.40
II	M. charantia extract (200 mg/kg)	80.33 ± 0.56	80.16 ± 1.01	79.50 ± 1.70	78.50 ± 1.33*
III	M. charantia extract (400 mg/kg)	82.83 ± 1.02	79.16 ± 1.66	80.83 ± 1.10	81.00 ± 1.13*

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
*p < 0.05 compared with respective normal control.

6.4.2 Effect on Oral Glucose Tolerance Test (OGTT)

Administration of glucose (2 g/kg) led to a sharp increase in blood glucose levels in normal rats within 30 minutes of glucose loading. In animals treated with the extracts or standard drug, the glucose levels peaked at 30 minutes and gradually returned to near-normal levels by 120 minutes. The maximum antihyperglycemic effects observed were as follows:

• Ethanolic extract of M. charantia (200 & 400 mg/kg): from 80.40 ± 1.14 to 90.85 ± 1.97 and 79.05 ± 1.72 to 92.60 ± 3.45, respectively.

Extract-treated groups exhibited a significant reduction in blood glucose levels compared to the control group. Based on the oral glucose tolerance test results, it was concluded that the 400 mg/kg dose of each extract demonstrated the maximum improvement in glucose tolerance. These results are detailed in Table 6.6.

Table 6.6: Effect of ethanolic extracts of Momordica charantia on blood glucose level in OGTT in normal rats.

Groups	Treatment	Blood Glucose Level (mg/dL)			
		0 min	30 min	60 min	120 min
I	Normal Control (1% DMSO + 1% Tween 80)	83.10 ± 0.92	84.20 ± 1.18	87.10 ± 0.77	91.85 ± 1.10
II	Standard (metformin)	81.45 ± 1.43	127.40 ± 1.22*	89.75 ± 2.84*	84.90 ± 2.95***
III	M. charantia extract (200 mg/kg)	80.40 ± 1.14	129.60 ± 2.16	109.65 ± 1.15*	90.85 ± 1.97**
IV	M. charantia extract (400 mg/kg)	79.05 ± 1.72	124.95 ± 2.65*	104.35 ± 1.75*	92.60 ± 3.45**

All the values are represented as mean±SEM n=6 in each group.
Data analysed by one-way ANOVA followed by Student-Newman-Keuls multiple comparisons evaluation.
*p < 0.05 compared with respective normal control.

6.4.3 Effect on blood glucose levels in diabetic rats

The impact of repeated oral administration of ethanolic extracts of M. charantia and S. cumini on fasting blood glucose levels was evaluated in STZ-induced diabetic rats at 0, 1st, 3rd, 6th, and 8th hours after treatment. The findings are summarized in Table 6.7. The diabetic control group showed a marked increase in blood glucose levels. In contrast, the standard drug metformin significantly reduced glucose levels from 278.38 ± 3.65 to 88.68 ± 3.24 mg/dL after 8 hours.

Treatment with the extracts at 200 mg/kg and 400 mg/kg doses also produced significant antihyperglycemic effects. The reductions observed were as follows:

- M. charantia (200 & 400 mg/kg): from 261.36 ± 4.67 to 139.36 ± 2.26 and 277.36 ± 3.57 to 98.26 ± 3.55

These results demonstrate that all three extracts significantly reduced elevated blood glucose levels in diabetic rats, with the higher dose (400 mg/kg) showing the most pronounced effect. These results are detailed in Table 6.7.

Table 6.7: Effect of solvent extracts of Momordica charantia on blood glucose levels (mg/dL) of STZ-induced rats after single dose treatment

Groups	Treatment	Blood Glucose Level (mg/dL)				
		0 hr	1st hr	3rd hr	6th hr	8th hr
I	Normal Control (1% DMSO + 1% Tween 80)	85.21 ± 0.45	90.21 ± 2.35	91.05 ± 2.80	88.59 ± 2.56	87.12 ± 3.15
II	Diabetic Control (STZ + vehicle)	261.68 ± 4.13#	275.32 ± 3.67#	281.58 ± 5.78#	296.38 ± 3.65#	298.50±3.89#
III	Standard (metformin)	278.38 ± 3.65	195.37 ± 3.58*	159.84 ± 4.80*	133.26 ± 6.36**	88.68 ± 3.24***
IV	M. charantia extract (200 mg/kg)	261.36 ± 4.67	232.67 ± 3.24*	200.25 ± 3.67*	175.26 ± 5.04*	139.36±2.26**
V	M. charantia extract (400 mg/kg)	277.36 ± 3.57	215.48 ± 5.63*	191.47 ± 4.75*	145.37 ± 4.22*	98.26 ± 3.55**

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
#p < 0.05 compared with respective normal control.
*p < 0.05 compared with respective diabetic control.

6.4.4 Effect on blood glucose levels in diabetic rats after 28 days of treatment

In experimental models, diabetes mellitus can be induced through partial pancreatectomy or by administration of diabetogenic agents such as streptozotocin, alloxan, dithizone, or anti-insulin serum. Streptozotocin (STZ), a naturally occurring nitrosourea compound produced by Streptomyces achromogenes, is commonly employed due to its selective toxicity toward pancreatic β-cells. A single intraperitoneal injection of STZ at a dose of 50 mg/kg body weight leads to β-cell necrosis within 48–72 hours, resulting in persistent hyperglycemia. In the present study, STZ was used to induce diabetes in Wistar rats to assess the antidiabetic potential of the ethanolic extracts over a 28-day treatment period. The effect of repeated oral administration of M. charantia on fasting blood glucose levels was evaluated on the 0th, 7th, 14th, 21st, and 28th days after induction of diabetes and compared with the normal and diabetic control groups. The data are summarized in Table 6.8. STZ-induced diabetic rats exhibited a marked elevation in blood glucose levels compared to normal rats, rising from 255.31 ± 6.84 to 298.85 ± 8.46 mg/dL by the 28th day. Treatment with the ethanolic extracts of M. charantia at 200 mg/kg and 400 mg/kg significantly reduced blood glucose levels relative to the diabetic control group. The observed reductions were as follows:

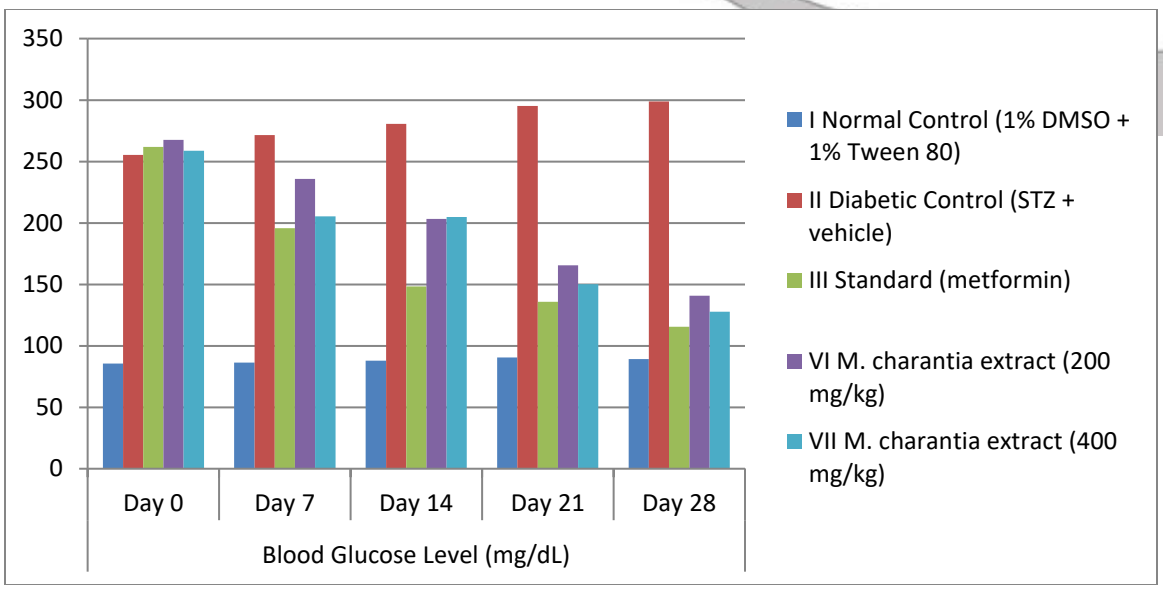
- M. charantia (200 & 400 mg/kg): from 267.54 ± 7.15 to 140.73 ± 4.57 and 258.82 ± 7.38 to 127.88 ± 3.56

Similarly, the metformin showed a significant decline in glucose levels from 261.85 ± 6.56 to 115.54 ± 3.24 mg/dL after 28 days of treatment. Overall, both the higher extract dose (400 mg/kg) and metformin produced a highly significant reduction in blood glucose levels, demonstrating a clear dose-dependent antihyperglycemic effect.

Table 6.8: Effect of ethanolic solvent extracts of Momordica charantia on blood glucose levels (mg/dL) of STZ-induced diabetic rats after single dose treatment.

Groups	Treatment	Blood Glucose Level (mg/dL)				
		Day 0	Day 7	Day 14	Day 21	Day 28
I	Normal Control (1% DMSO + 1% Tween 80)	85.52 ± 4.21	86.26 ± 3.81	88.01 ± 4.11	90.65 ± 4.35	89.17 ± 3.95
II	Diabetic Control (STZ + vehicle)	255.31 ± 6.84	271.41 ± 8.93#	280.72 ± 7.24#	295.12 ± 9.33#	298.85 ± 8.46#
III	Standard (metformin)	261.85 ± 6.56	195.63 ± 5.44*	148.34 ± 4.85**	135.73 ± 3.92***	115.54 ± 3.24***
VI	M. charantia extract (200 mg/kg)	267.54 ± 7.15	235.83 ± 6.47*	203.23 ± 5.95**	165.63 ± 5.04**	140.73 ± 4.57**
VII	M. charantia extract (400 mg/kg)	258.82 ± 7.38	205.23 ± 5.66*	204.85 ± 4.76**	150.13 ± 4.22**	127.88 ± 3.56**

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
#p < 0.05 compared with respective normal control.
*p < 0.05 compared with respective diabetic control.



- **Biochemical Parameters**

6.4.5 Effect on Serum Lipid Profile

In the STZ-induced diabetic group, a marked alteration in lipid metabolism was observed, as evidenced by a significant rise in total triglycerides (85.42 ± 3.21 to 165.25 ± 6.42 mg/dL), cholesterol (83.56 ± 4.27 to 151.47 ± 5.81 mg/dL), LDL (27.13 ± 2.06 to 65.38 ± 4.95 mg/dL), and VLDL (20.28 ± 0.64 to 63.65 ± 1.28 mg/dL), along with a notable decrease in HDL levels (44.36 ± 2.31 to 25.17 ± 1.43 mg/dL) compared to the normal control group. Treatment with Momordica charantia, and at 200 mg/kg and 400 mg/kg for 28 days significantly ameliorated these alterations in a dose-dependent manner.

The extracts produced a marked reduction in total cholesterol, triglycerides, LDL, and VLDL, while HDL levels increased significantly, suggesting an overall improvement in the lipid profile. These findings are summarized in Table 6.9.

Table 6.9: Effect of ethanolic solvent extracts of Momordica charantia on serum lipid profile.

Group	Treatment	Serum Triglycerides (mg/dl)	Serum Total Cholesterol (mg/dl)	Serum LDL (mg/dl)	Serum HDL (mg/dl)	Serum VLDL (mg/dl)
I	Normal Control (1% DMSO + 1% Tween 80)	85.42 ± 3.21	83.56 ± 4.27	27.13 ± 2.06	44.36 ± 2.31	20.28 ± 0.64
II	Diabetic Control (STZ + vehicle)	165.25 ± 6.42$^{\#}$	151.47 ± 5.81$^{\#}$	65.38 ± 4.95$^{\#}$	25.17 ± 1.43$^{\#}$	63.65 ± 1.28$^{\#}$
III	Standard (Metformin 150 mg/kg)	90.72 ± 3.54	93.64 ± 4.03*	28.46 ± 2.44	43.18 ± 2.10	21.34 ± 0.72***
IV	M. charantia extract (200 mg/kg)	127.47 ± 5.11*	115.36 ± 5.28*	33.73 ± 3.72*	31.85 ± 1.87**	29.69 ± 1.06*
V	M. charantia extract (400 mg/kg)	94.56 ± 4.54*	96.89 ± 4.06*	30.47 ± 2.83*	40.24 ± 1.96*	23.11 ± 0.84*

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
$^{\#}p < 0.05$ compared with respective normal control.
*p < 0.05 compared with respective diabetic control.

6.4.6 Effect on serum insulin, liver glycogen, and glycated hemoglobin

HbA1c (glycated hemoglobin) serves as a reliable biomarker for assessing the long-term glycemic control in diabetes mellitus. It reflects the extent of non-enzymatic glycation of hemoglobin due to prolonged exposure to elevated blood glucose levels, thereby correlating with the chronic complications of diabetes. In the present study, the STZ-induced diabetic rats exhibited a significant reduction in serum insulin levels (from 133.46 ± 1.65 to 64.78 ± 2.89 µU/mL) and liver glycogen content (from 19.43 ± 1.56 to 10.74 ± 0.34 mg/g tissue), accompanied by a marked increase in HbA1c (from 7.92 ± 0.23 to 12.85 ± 0.40 %).

Following 28 days of oral treatment with Momordica charantia (200 mg/kg and 400 mg/kg), there was a significant improvement in biochemical parameters. Serum insulin levels increased to 93.37 ± 2.58, 98.42 ± 1.68, 102.05 ± 1.56 (200 mg/kg) and 110.65 ± 1.34, 119.34 ± 1.23, 120.89 ± 1.09 µU/mL (400 mg/kg), while liver glycogen levels rose to 13.29 ± 0.18, 14.74 ±0.22, 15.45 ± 0.12 (200 mg/kg) and 16.58 ± 0.36, 17.84 ± 0.20, 18.92 ± 0.85 mg/g tissue (400 mg/kg). Simultaneously, a significant reduction in HbA1c was observed, decreasing to 10.18 ± 0.57, 10.84 ± 0.61, 9.63 ± 0.21 % (200 mg/kg) and 9.79 ± 0.23, 8.09 ± 0.34, 8.57 ± 0.72 % (400 mg/kg), respectively, when compared with diabetic control rats. These results are presented in Table 6.10.

Table 6.10: Effect of ethanolic solvent extracts of Momordica charantia on serum insulin, glycated haemoglobin and liver glycogen.

Group	Treatment	Serum insulin (µU/mL)	Glycated haemogloin (%)	Liver glycogen (mg/gm)
I	Normal Control (1% DMSO + 1% Tween 80)	133.46 ± 1.65	7.92 ± 0.23	19.43 ± 1.56
II	Diabetic Control (STZ + vehicle)	64.78 ± 2.89$^{\#}$	12.85 ± 0.40$^{\#}$	10.74 ± 0.34$^{\#\#}$
III	Standard (Metformin 150 mg/kg)	126.61 ± 2.08*	7.67 ± 0.33**	18.58 ± 0.67*
IV	M. charantia extract (200 mg/kg)	98.42 ± 1.68*	10.84 ± 0.61**	14.74 ±0.22*
V	M. charantia extract (400 mg/kg)	119.34 ± 1.23*	8.09 ± 0.34**	17.84 ± 0.20*

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
$^{\#}p < 0.05$ compared with respective normal control.
*p < 0.05 compared with respective diabetic control.

6.4.7 Effect on in vivo antioxidant parameters

The antioxidant enzymes SOD, CAT, and GPx play a critical role in cellular defense against oxidative stress. SOD converts superoxide radicals into hydrogen peroxide, which is further decomposed by CAT and GPx to non-toxic products, preventing oxidative injury. In diabetes, excessive glucose levels may glycate and inactivate these enzymes, enhancing oxidative stress and promoting lipid peroxidation. In STZ-induced diabetic rats, there was a significant reduction in liver SOD (8.46 ± 0.32 to 3.24 ± 0.18 U/mg protein), CAT (62.48 ± 2.14 to 38.63 ± 1.36 nM H_2O_2 decomposed/min/gm), and GPx (6.27 ± 0.25 to 2.14 ± 0.12 U/mg protein) compared with normal rats.

Treatment with Momordica charantia (200 and 400 mg/kg), as well as metformin, significantly restored these antioxidant enzyme levels toward normal, reflecting enhanced free radical scavenging activity and activation

of the antioxidant defense system. Conversely, diabetic rats showed an increase in lipid peroxidation markers, TBARS (1.24 ± 0.06 to 3.56 ± 0.18 nmoles/g protein) and LPO (1.86 ± 0.08 to 4.92 ± 0.22 nmoles/g protein), which were significantly reduced after extract and standard treatment, confirming their antioxidant and cytoprotective effects. The results are presented in Table 6.11.

Table 6.11: Effect of ethanolic solvent extracts of Momordica charantia on antioxidant marker levels.

Group	Treatment	SOD (U/mg protein)	CAT (nM H_2O_2 decomposed/min/gm)	LPO (nmoles/g protein)	TBARS (nmoles/g protein)	GPx (U/mg protein)
I	Normal Control (1% DMSO + 1% Tween 80)	8.46 ± 0.32	62.48 ± 2.14	1.86 ± 0.08	1.24 ± 0.06	6.27 ± 0.25
II	Diabetic Control (STZ + vehicle)	3.24 ± 0.18$^{\#}$	38.63 ± 1.36$^{\#}$	4.92 ± 0.22$^{\#}$	3.56 ± 0.18$^{\#\#}$	2.14 ± 0.12$^{\#\#\#}$
III	Standard (Metformin 150 mg/kg)	7.28 ± 0.28*	56.31 ± 2.03*	2.34 ± 0.10**	1.62 ± 0.07***	5.41 ± 0.21**
IV	M. charantia extract (200 mg/kg)	5.12 ± 0.20*	41.83 ± 1.79*	3.85 ± 0.17**	2.58 ± 0.12***	3.68 ± 0.15**
V	M. charantia extract (400 mg/kg)	6.54 ± 0.26*	50.73 ± 1.88*	2.73 ± 0.13**	1.89 ± 0.09***	4.86 ± 0.19**

All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
$^{\#}p < 0.05$ compared with respective normal control.
$^{*}p < 0.05$ compared with respective diabetic control.

6.4.8 Effect on hepatic parameters

SGOT, SGPT, and ALP are reliable biochemical indicators of liver function. In the STZ-induced diabetic rats, marked hepatic damage was evident, as reflected by significant increases in SGOT, SGPT, and ALP levels. These elevations can be attributed to the leakage of hepatic enzymes from damaged liver cells into the bloodstream, indicating STZ-induced hepatotoxicity. Following 28 days of oral administration of Momordica charantia (200 and 400 mg/kg), there was a significant reduction in SGOT, SGPT and ALP enzyme levels- indicating a hepatoprotective effect of the extracts against diabetic liver injury. Additionally, a decline in total protein levels was observed in diabetic rats, suggesting altered protein metabolism due to enhanced proteolysis and reduced synthesis. Treatment with the extracts restored total protein levels towards normal, demonstrating their ability to improve hepatic function. The findings are summarized in Figure 6.1.

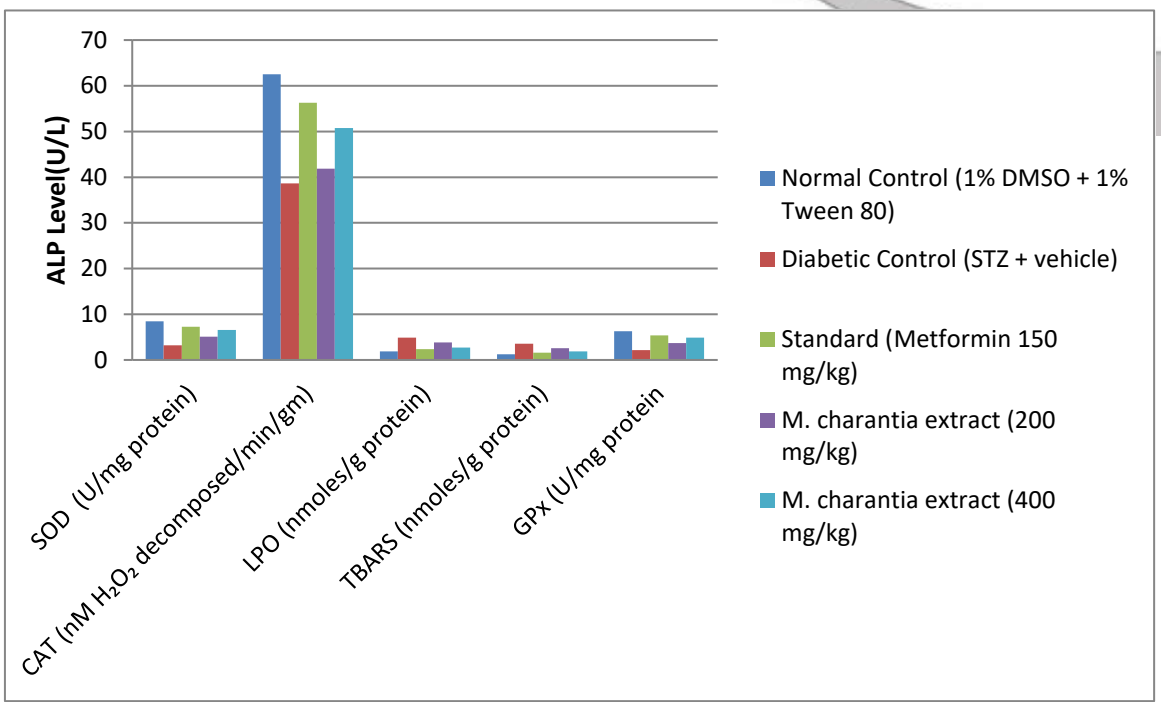


Figure 6.1: Effect of ethanolic solvent extracts of Momordica charantia on hepatic parameters.
All the values are expressed as mean ±SEM n=6 in each group.
Data analyzed by One-way ANOVA followed by Student-Newman-Keuls multiple comparisons test.
$^{\#}p < 0.05$ compared with respective normal control.
*$p < 0.05$ compared with respective diabetic control.

CONCLUSION:

The present thesis, titled "Evaluation of Anti-Diabetic, Anti-Oxidant and Anti-Hyperlipidemic Activity of Selected Medicinal Plant," focuses on the phytochemical investigation and antidiabetic potential of three traditionally used Indian medicinal plants: the seeds of Momordica charantia. This plants have long been employed by local communities and tribal groups across India for managing diabetes and related ailments. After proper botanical authentication, all three species were selected for systematic evaluation of their antidiabetic properties.

STZ administration produced a clear diabetic state in rats, characterized by marked hyperglycemia, excessive thirst (polydipsia), increased food intake (polyphagia), loss of body weight, dyslipidemia, and elevated oxidative stress. Treatment with ethanolic extracts of Momordica charantia for 28 days effectively countered these abnormalities. The extracts significantly reduced fasting blood glucose levels toward normal values, normalized lipid parameters, improved body weight, and stabilized food and water intake. They also rejuvenated the antioxidant defence system, indicating a strong ability to reduce oxidative stress. These results suggest that the extracts not only possess antihyperglycemic activity but also confer antioxidant protection and may help preserve pancreatic β-cell function.

Based on these outcomes, it can be inferred that the ethanolic extracts of Momordica charantia hold considerable promise for the management of diabetes mellitus. Their utility is particularly relevant in countries like India, where conventional antidiabetic medications may not be easily accessible or affordable for a large portion of the population. The traditional use of these plants, combined with the scientific evidence from this study, underscores their potential value as cost-effective therapeutic options. Future investigations should focus on isolating and characterizing the specific bioactive constituents responsible for the observed antidiabetic effects. Additionally, detailed mechanistic studies and well-designed clinical trials are necessary to establish their efficacy and safety in humans. Such research could contribute greatly to developing novel plant-based therapies for diabetes and other disorders associated with oxidative stress.

References:

1. Daisy P, Jasmine R, Ignacimuthu S, Murugan E. A novel steroid from Elephantopusscaber L. an ethnomedicinal plant with antidiabetic activity. Phytomedicine. 2009 Mar 1;16(2-3):252-7.
2. Sidahmed S, Geyer S, Beller J. Socioeconomic inequalities in diabetes prevalence: the case of South Africa between 2003 and 2016. BMC Public Health. 2023 Feb 14;23(1):324.
3. Selvin E, Juraschek SP. Diabetes epidemiology in the COVID-19 pandemic. Diabetes care. 2020 Aug 1;43(8):1690-4.
4. Bishu KG, Jenkins C, Yebyo HG, Atsbha M, Wubayehu T, Gebregziabher M. Diabetes in Ethiopia: a systematic review of prevalence, risk factors, complications, and cost. Obesity Medicine. 2019 Sep 1;15:100132.
5. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019 Jan;62(1):3-16.
6. Tegegne BA, Adugna A, Yenet A, Yihunie Belay W, Yibeltal Y, Dagne A, Hibstu Teffera Z, Amare GA, Abebaw D, Tewabe H, Abebe RB. A critical review on diabetes mellitus type 1 and type 2 management approaches: from lifestyle modification to current and novel targets and therapeutic agents. Frontiers in Endocrinology. 2024 Oct 18;15:1440456
7. Toren E, Burnette KS, Banerjee RR, Hunter CS, Tse HM. Partners in crime: beta-cells and autoimmune responses complicit in type 1 diabetes pathogenesis. Frontiers in immunology. 2021 Oct 7;12:756548..
8. Roep BO, Thomaidou S, Van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the β-cell (do not blame the immune system?). Nature Reviews Endocrinology. 2021 Mar;17(3):150-61.
9. Ruze R, Liu T, Zou X, Song J, Chen Y, Xu R, Yin X, Xu Q. Obesity and type 2 diabetes mellitus: connections in epidemiology, pathogenesis, and treatments. Frontiers in endocrinology. 2023 Apr 21;14:1161521.
10. Dludla PV, Mabhida SE, Ziqubu K, Nkambule BB, Mazibuko-Mbeje SE, Hanser S, Basson AK, Pheiffer C, Kengne AP. Pancreatic β-cell dysfunction in type 2 diabetes: Implications of inflammation and oxidative stress. World journal of diabetes. 2023 Mar 15;14(3):130.
11. Hurtado MD, Vella A. What is type 2 diabetes?. Medicine. 2019 Jan 1;47(1):10-5.
12. Holt RI. Diagnosis, epidemiology and pathogenesis of diabetes mellitus: an update for psychiatrists. The British Journal of Psychiatry. 2004 Apr;184(S47):s55-63.
13. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.
14. KoKate CK, Purohit AP, Gokhale SB(1990) Text book of Pharmacognosy,29th edn., Nirali Prakashan,Pune pp-123.
15. Basset,J.,Denny,J.JefferyJ.H. and Mendham ,J.Vogel's test book of quantitative inorganic analysis,4th edition,ELBS- Longman, Essex,UK,1985,196.
16. Del Prato S. Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. Diabetic medicine. 2009 Dec;26(12):1185-92.
17. Safavi M, Foroumadi A, Abdollahi M. The importance of synthetic drugs for type 2 diabetes drug discovery. Expert opinion on drug discovery. 2013 Nov 1;8(11):1339-63.
18. Bharathi A, Roopan SM, Vasavi CS, Munusami P, Gayathri GA, Gayathri M. In Silico Molecular Docking and In Vitro Antidiabetic Studies of Dihydropyrimido [4, 5-a] acridin-2-amines. BioMed research international. 2014;2014(1):971569
19. Bibi S, Sakata K. Current status of computer-aided drug design for type 2 diabetes. Current computer-aided drug design. 2016 Jun 1;12(2):167-77..
20. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Frontiers in endocrinology. 2017 Jan 24;8:6
21. Modak M, Dixit P, Londhe J, Ghaskadbi S, Devasagayam TP. Indian herbs and herbal drugs used for the treatment of diabetes. Journal of clinical biochemistry and nutrition. 2007;40(3):163-73..
22. Mandlik RV, Desai SK, Naik SR, Sharma G, Kohli RK. Antidiabetic activity of a polyherbal formulation (DRF/AY/5001). Indian journal of Experimental biology. 2009 Aug 1;46(8):599-606.
23. Patel SS, Shah RS, Goyal RK. Antihyperglycemic, antihyperlipidemic and antioxidant effects of Dihar, a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. Indian Journal of Experimental Biology. 2009 Jul 1;47(7):564..

24. Leslie RD, Palmer J, Schloot NC, Lernmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. Diabetologia. 2016 Jan;59(1):13-20.
25. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, Marco A, Shekhawat NS, Montales MT, Kuriakose K, Sasapu A. Clinical review of antidiabetic drugs: implications for type 2 diabetes mellitus management. Frontiers in endocrinology. 2017 Jan 24;8:6
26. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28(1):56-63. doi:10.1093/ajcp/28.1.56.
27. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-275.
28. Wybenga DR, Pileggi VJ, Dirstine PH, Di Giorgio J. [Exact title unknown — commonly cited method for urea/urease assay in biochemical studies]. Anal Biochem. 1971
29. Note: Wybenga et al. 1971 is widely used for urea analysis but full bibliographic details vary in databases and are not easily found in open sources at the moment. Many papers cite it as a standard method.
30. Slot C. Plasma creatinine determination. A new and specific Jaffe reaction method. Scand J Clin Lab Invest. 1965;17(4):381-387.
31. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-590.
32. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358.