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Integrated Pharmacological and Pharmacokinetic Assessment of Gymnema sylvestre as an Adjunct to Glimepiride Therapy in Diabetes"

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ABSTRACT

The present study investigated the pharmacodynamic and pharmacokinetic interactions between Gymnema sylvestre aqueous extract and glimepiride in streptozotocin (STZ)-induced diabetic rats. Phytochemical analysis confirmed the presence of flavonoids, saponins, tannins, glycosides, alkaloids, and phenolic compounds, with quercetin identified as a major bioactive constituent by HPTLC. In vitro studies demonstrated dose-dependent inhibition of hepatic CYP3A activity by the extract, indicating potential herb drug interaction. In vivo, G. sylvestre extract produced significant, dose-dependent reductions in blood glucose levels and improved oral glucose tolerance, comparable to glimepiride. The extract also restored antioxidant enzymes (SOD, CAT, and GSH), reduced lipid peroxidation, and normalized elevated liver enzymes (AST and ALT), indicating strong antioxidant and hepatoprotective effects. Combination therapy with glimepiride further enhanced antihyperglycemic and protective outcomes. Pharmacokinetic evaluation revealed altered glimepiride disposition in the presence of the extract, with reductions in Cmax, AUC, and half-life. These findings suggest that Gymnema sylvestre is a promising adjunct in diabetes management, though careful consideration of herb–drug interactions is required..

Keywords: *Gymnema sylvestre; Glimepiride; Streptozotocin-induced diabetes; Herb–drug interaction; Antihyperglycemic activity; Antioxidant enzymes; Pharmacokinetics; CYP3A inhibition*

INTRODUCTION

Diabetes mellitus is a chronic metabolic condition marked by sustained hyperglycemia due to impairments in insulin production, insulin action, or both. The illness causes long-term problems with the kidneys, eyes, nerves, and heart, which is a huge health problem around the world (1). Even though there are a lot of synthetic antidiabetic medicines on the market, their drawbacks like side effects, high costs, and poor glycemic control have led to the hunt for safer and more effective alternatives from natural sources (2).

Celosia argentea (Amaranthaceae), also called "silver cockscomb," is a traditional medicinal plant that is used a lot in Asia and Africa. Folklore medicine has used different elements of the plant to cure things like inflammation, liver problems, and metabolic problems. Phytochemical studies show that the plant has a lot of flavonoids, alkaloids (3), phenolics, and saponins, many of which can act as antioxidants and lower blood sugar levels. Recent experimental studies indicate that extracts of Celosia argentea can enhance glucose utilisation, safeguard pancreatic β-cells from oxidative stress, and regulate essential metabolic enzymes implicated in carbohydrate metabolism (4).

2. Material and Method

2.1 Collection of plants

Gymnema sylvestre leaves collected from Botanical garden CCS University, Meerut, U.P., India.

2.2 Authentication of Plants

The Celosia argentea seeds were verified by Prof. Dr. Vijay Malik from the Department of Botany at Chaudhary Charan Singh University in Meerut, Uttar Pradesh, India. The plant material was taken from its natural habitat and sent for taxonomic study. Dr. Malik, a seasoned taxonomist with a focus on medicinal plants, confirmed the species' authenticity by looking at its morphology, using regional floristic keys, and comparing it to authorised herbarium specimens. The confirmed voucher

specimens were put in the departmental herbarium (Voucher No. CCSU/2025/13) so that they might be used again later. This validation ensures that further pharmacological or phytochemical research using these species is scientifically valid and reliable.

2.3 Extraction of Gymnema sylvestre leaves

The dried and finely powdered leaves of Gymnema sylvestre were extracted using 70% ethanol in a Soxhlet apparatus. Approximately 50 g of the plant powder was placed in a Whatman filter paper thimble and loaded into the extractor chamber. About 500 mL of 70% ethanol (v/v) was added to a round-bottom flask attached to the Soxhlet setup. The extraction process was carried out for 6–8 hours and continued until the siphon tube showed a clear solvent, indicating complete extraction of the major phytoconstituents. The obtained extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator at 40–50°C to remove the solvent. The resulting crude extract was stored in an airtight amber container at 4°C until further use. This ethanolic extract was subsequently subjected to phytochemical screening and evaluation of its antidiabetic potential.(5).

2.4 Rout of administration

The desiccated ethanolic extract was dispersed in a 0.2% sodium carboxymethyl cellulose (Na CMC) solution, which functioned as the suspending agent. The prepared suspension was orally supplied to the designated treatment groups

2.5 Determination of Percentage Yield

To find out the extraction yield, the weight of the raw powdered material was measured before extraction. Then, the sample was put through solvent extraction with an appropriate solvent, like ethanol, methanol, or water, depending on what the study needed. After the extraction procedure was finished, the mixture was filtered, and the filtrate was concentrated to get rid of surplus solvent. After that, the concentrated extract was dried in a hot air oven or under reduced pressure until it reached a consistent weight. The final weight of the dried extract was recorded, and the usual formula was used to figure out the % yield (6).

$$\text{Percentage yield} = \left(\frac{\text{Weight of dried extract}}{\text{Weight of raw material used}}\right) \times 100$$

2.6 Determination of physical properties of extracts

2.6.1 Determination total Ash value

To find the total ash value, a set amount of the coarsely powdered, air-dried plant material was put in a silicon crucible that had already been lit and tared. The sample was burned over a low flame until it was completely blackened. The crucible was then moved to a muffle furnace that was kept at 500–600 °C and burned until the ash was a uniform white or grey colour, which showed that all of the organic materials had been removed. After the crucible was burned, it was taken out and put in a desiccator to cool off so it wouldn't soak up moisture. Then, it was weighed again, and the procedure of lighting, cooling, and weighing it again was repeated until the weight stayed the same (7).

The following calculation was used to compute the total ash content:

Total Ash (%) = (Weight of Ash / Weight of Sample) × 100

2.6.2 Examination of water Insoluble Ash

In order to find out how much acid-insoluble ash there was, a few drops of weak hydrochloric acid were added to the entire ash that had already been collected. Then, for about five minutes, the ash was gently heated with 25 mL of 2N HCl in the same silica crucible. We filtered the mixture through an ash-free filter paper, and then we cleaned both the crucible and the residue on the filter paper with hot water to make sure that all of the soluble parts were gone. The filter paper and the rest of the residue were put back into the original crucible and burned until all the carbonaceous stuff was gone, leaving a uniform white or grey ash. The crucible was put in a desiccator to cool down, and then it was weighed. We kept heating, cooling, and weighing the object until it reached a steady weight (8).

The acid-insoluble ash content was calculated using the following formula:

Acid Insoluble Ash (%) = (Weight of Acid Insoluble Ash / Weight of Sample) × 100

2.6.3 Examination of Water soluble Ash

To find the water-soluble ash value, the complete ash from the sample was put into a silica crucible and cooked with enough distilled water. The mixture was cooked long enough for the water-soluble parts to fully dissolve. Then, it was filtered to get rid of the residue that couldn't be dissolved. The ash-free filter paper was carefully used to gather the residue and put it

back in the original crucible. They set fire to this residue till it turned into white ash. After it was lit, the crucible was put in a desiccator to cool down and keep it from absorbing moisture. Then it was weighed to get an accurate measurement (9).

Through the subsequent calculation, we determined the fraction of ash that may dissolve in water:

$$\text{Water-Soluble Ash (\%)} = \left(\frac{\text{Weight of Ash}}{\text{Weight of Sample}}\right) \times 100$$

2.7 Phytochemical Examination

2.7.1 Chemical test for Carbohydrate

Monosaccharides are the simplest form of carbohydrates. Disaccharides, oligosaccharides, and polysaccharides are more complicated forms made up of two or more monosaccharide units joined by glycosidic linkages. When you add a little bit of hydrochloric or sulfuric acid to these complicated carbohydrates, they break down into their simpler sugar parts by hydrolysis. Most monosaccharides are optically active because they have one or more chiral carbon atoms, which lets them change the direction of polarised light. They also have aldehyde or ketone functional groups that take part in chemical reactions that are unique to them and cause colour changes. These reactions are the basis for a number of qualitative tests that can find and sort carbs. These tests are commonly used in food testing, clinical labs, and phytochemical research to find and tell apart different kinds of sugars (10).

Molisch's Test

A generic test called Molisch's test looks for carbs. For this test, a few drops of Molisch's reagent (an alcohol solution of α-naphthol) are mixed with the plant extract. Then, carefully add concentrated sulfuric acid to the side of the test tube. A violet or purple ring at the point where the two layers meet means that the reaction is positive, which means that carbs are present (11).

Fehling’s Solution Test

Fehling’s test is a classical qualitative assay used to detect reducing sugars. It involves the use of two solutions: Fehling’s A, containing copper (II) sulphate, and Fehling’s B, composed of sodium potassium tartrate in an alkaline medium. When equal volumes of these solutions are mixed and added to the plant extract, followed by heating in a water bath, reducing sugars if presentreduce the copper (II) ions to copper(I) oxide. This reaction produces a characteristic reddish-brown precipitate of cuprous oxide (Cu_2O), confirming the presence of reducing carbohydrates. The test relies on the ability of free aldehyde or ketone groups, commonly present in monosaccharides, to act as reducing agents. The intensity of the coloured precipitate also gives a rough indication of the amount of reducing sugar present in the sample (12).

Benedict’s test

The Benedict's test is employed to identify decreasing sugars in a sample. Upon heating the plant extract with Benedict’s reagent, any present reducing sugars convert Cu^{2+} ions to Cu^{+}, leading to the development of a green precipitate of cuprous oxide (Cu_2O). The green hue signifies a comparatively low concentration of reducing sugars in the extract (13).

2.7.2 Test for protein

Millon Test

The Millon test is a qualitative technique employed to identify phenolic amino acids, particularly tyrosine, inside proteins. In this experiment, Millon’s reagent, including mercuric and mercurous ions in nitric acid, is introduced to the plant extract. Heating results in the formation of a red or brick-red hue or precipitate in the presence of tyrosine or other phenolic-containing proteins. The alteration in hue verifies the existence of proteins containing phenolic groups in the sample (14).

Biuret Test

The Millon test is a qualitative assay employed to identify phenolic amino acids, particularly tyrosine, in proteins. In this experiment, Millon’s reagent, comprising mercuric and mercurous ions in nitric acid, is introduced to the plant extract. Heating results in the formation of a red or brick-red colour or precipitate in the presence of tyrosine or other phenolic-containing proteins. The alteration in colour verifies the existence of proteins containing phenolic groups in the sample (15).

2.7.3 Test for Alkaloid

Mayer's Test

Mayer's test is used to find alkaloids. Adding Mayer's reagent to the extract makes a white or cream-colored precipitate, which means that alkaloid compounds are present in the sample (16).

Test of Dragendorff

We added water to Dragendorff's reagent and extract. The extract has alkaloid in it, which makes a bright yellow precipitate form (17).

Wagner's Test

People often use Wagner's test to find alkaloids in plant extracts. When Wagner's reagent, which is iodine dissolved in potassium iodide, is added to the aqueous extract, a dark brown or reddish-brown precipitate shows that alkaloid compounds are present (18).

Hager's Test

Hager's Test is a method for finding alkaloids in plant extracts that is based on how good they are. For this test, Hager's reagent, which is a saturated solution of picric acid in water, is added to the extract. The formation of a yellow precipitate indicates the presence of alkaloids due to the formation of insoluble picrate complexes (19).

2.7.4 Test for Glycoside

Bornträger's Test

In Bornträger's Test, the powdered extract is heated with a weak hydrochloric acid solution to break glycosidic bonds. After being filtered, the hydrolysate was mixed with benzene to pull out aglycones. Then, ammonia solution was added. The ammoniacal layer turning pink to red means that anthraquinone glycosides are present (20).

Test for Saponins

Foam test

The extract was shaken hard with water to find saponins. The formation of stable foam that lasted for at least 10 minutes proved that saponin compounds were present (21).

2.7.5 Test for Steroids

Liebermann Burchard Test

To treat the extract, chloroform and acetic anhydride were used. To find steroids, concentrated sulfuric acid was used. The appearance of a violet to blue ring at the junction indicated the presence of steroidal compounds.

Salkowski Tests

The chloroform solution from the extract was mixed with concentrated sulfuric acid and left to sit. The red colour showed that steroidal compounds were present (22).

2.7.6 Test for Flavonoids

Shinoda Test

The ethanolic extract was mixed with magnesium ribbon and then hydrochloric acid. The reddish-brown colour that formed showed that flavonoids were present (23).

2.7.7 Test for Ammonia

A solution of the extract has been made, and a piece of filter paper was put into it. After that, ammonia vapours were let into the paper. The yellow dot on the paper showed that flavonoids were present (24).

2.7.8 Test for Lead Acetate

We put lead acetate solution on the plant extract. A yellow precipitate formed, which meant that flavonoids were present (25).

2.7.9 Test for Phenolic Compounds with Ferric Chloride

We used a mix of ethanol and ferric chloride to treat the extract. The presence of phenolic chemicals was indicated by a bluish-green or dark blue colour (26).

2.8 Examination of total soluble phenolic content in Celosia argentea seed extract

We used a modified Folin–Ciocalteu colorimetric method to find out how much total phenolic content (TPC) was in the aqueous seed extract of Celosia argentea. In this test, the Folin–Ciocalteu reagent works as an oxidizing agent by reacting with phenolic compounds to make a blue-colored complex. A diluted extract was combined with the Folin–Ciocalteu reagent, and then sodium carbonate solution was added to speed up the reaction. Using distilled water, the final volume was set to 25 mL. A UV-visible spectrophotometer was used to measure the absorbance of the solution that was made after incubation at 750 nm. A standard calibration curve was made with gallic acid at a concentration of 50 to 200 µg/mL. We

figured out how much phenolic was in the extract by comparing its absorbance to the gallic acid standard curve. The results were given in milligrams of gallic acid equivalents (mg GAE) per gram of extract (27).

2.9 Standardization examination of phytoconstituents present in extract using HPTLC

2.9.1 Analysis of Gallic acid in Celosia argentea seed extract

In order to make the right test solutions, the sample and standard were mixed with methanol. These were put on HPTLC plates that had already been coated with silica gel as the stationary phase. The chromatographic analysis was performed under optimal conditions to enable the separation and identification of components based on their Rf values. This was done with the right detection reagents and ultraviolet light (28).

2.10 *In Vitro* Examination of CYP3A Activity on Liver Microsome

2.10.1 High fat diet and sterptozzotocin induce diabetes in Rats

Wistar rats, weighing 150–200 g, were made insulin-resistant by being fed a high-fat diet for two weeks. After an overnight fast, diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) at a dosage of 33 mg/kg body weight. Before giving the STZ solution, it was made fresh in 0.1 M citrate buffer (pH 4.5). The rats were given a 20% glucose solution to stop them from getting very low blood sugar after the STZ injection. Seventy-two hours after the injection, animals whose fasting blood glucose levels were above 200 mg/dL were classified as diabetic and chosen for participation in the experimental study groups (29).

2.10.2 Examination of pharmacokinetic and pharmacodynamics interaction between Celosia argentea seed extract and Glimepiride

There were six Wistar rats in each group, and they were randomly assigned to groups. Each rat weighed between 150 and 200 grams. For 14 days, each animal got the treatments that were meant for them. The table below shows how the treatment groups were divided.

Table 1: Animal groups to study interaction between Gymnema sylvestre leaves extract and Glimepiride

Groups	Treatment	Dose
I	Normal Saline	1 mg/kg p.o.
II	Disease control	STZ 1 mg/kg p.o.
III	Glimepiride	5 mg/kg p.o.
IV	Gymnema sylvestre leaves extract	200 mg/kg p.o.
V	Gymnema sylvestre leaves extract	400 mg/kg p.o.
VI	Gymnema sylvestre leaves extract + Glimepiride	200 mg/kg p.o. + 5 mg/kg p.o.
VII	Gymnema sylvestre leaves extract + Glimepiride	400 mg/kg p.o. + 5 mg/kg p.o.

Gymnema sylvestre leaves extract was suspended in 0.2% carboxymethyl cellulose (CMC) and delivered via oral route. Glimepiride was delivered to all subjects by oral gavage one hour subsequent to the subcutaneous therapy.

2.10.3 Examination of pharmacokinetic interaction study parameters

Wistar rats weighing 180–250 g were used to investigate the pharmacokinetic interaction. As described in Section 3.5, the animals received oral administration of the plant extract and the standard drug once daily for 14 days. On the 15th day, a single oral dose of glimepiride (5 mg/kg) was administered. Blood samples were collected from the retro-orbital sinus at 1, 2, 4, 8, and 12 hours post-dose under light anaesthesia. The samples were transferred into heparinised tubes and centrifuged at 3,000 × g to obtain plasma, which was stored at −20°C until further analysis.

Glimepiride concentrations in plasma were quantified using LC–MS. Pharmacokinetic parameters such as maximum plasma concentration (Cmax), area under the concentration–time curve (AUC_0–t), elimination half-life (t½), time to reach Cmax (Tmax), and mean residence time (MRT) were calculated to evaluate potential herb–drug interactions. (30).

2.13 Examination of Pharmacodynamic integration study parameter

2.13.1 Measuring Blood Sugar

Blood glucose levels were checked on the 14th day of the study. At 1, 2, 4, and 8 hours after treatment, blood samples were taken from the tail vein. An Accu-Chek glucometer was used to find out how much glucose was in the blood (31).

2.13.2 Antioxidant enzyme assay

2.13.2.1 LPO, GSH SOD assay

On the fourteenth day, the liver was taken out of the test animals and quickly cleaned with normal saline to get rid of blood and other debris. We got rid of the extra moisture by blotting the tissue with filter paper. After that, a homogenizer mixed the liver with Tris buffer (pH 7.4) at 4°C. The homogenate was spun in a centrifuge, and the clear supernatant that was left over was used to measure the activity of antioxidant enzymes (32).

2.13.2.2 Catalase Analysis

Liver tissue was homogenised in cold phosphate buffer (pH 7.0) to prepare a 10% w/v homogenate. The homogenate was centrifuged at 4°C, and the resulting supernatant was collected for the catalase assay. Catalase activity was determined by measuring the rate of hydrogen peroxide (H_2O_2) decomposition, with the reaction monitored spectrophotometrically at 240 nm (33).

2.13.2.3 Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) is an important antioxidant enzyme found in all aerobic cells, mostly in the cytoplasm and mitochondria. It is very important for protecting cells from oxidative stress because it helps break down harmful superoxide anions (O_2^-), which are reactive oxygen species that are made during aerobic metabolism. SOD speeds up the process of turning superoxide radicals into hydrogen peroxide (H2O2) and molecular oxygen, which helps protect cells from damage (34).

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

2.13.2.4 Catalase (CAT) Examination

We used a reaction mixture of tissue homogenate and hydrogen peroxide (H_2O_2) in phosphate buffer (pH 7.0) to measure catalase activity. We used a blank with only phosphate buffer to fix the baseline. We started the reaction by adding H_2O_2 to the sample mixture. Then, we used a UV–Visible spectrophotometer to watch the decrease in absorbance at 240 nm. Every three seconds, we wrote down the absorbance readings and the time (Δt) it took for the optical density to reach 0.45. The rate at which the absorbance went down was the same as the rate at which catalase broke down H2O2 (35).

2.13.2.5 Reduced Glutathione (GSH)

We used a spectrophotometer to measure the drop in DTNB to find out how much reduced glutathione (GSH) was present. The tissue supernatant was combined with an EDTA solution and stored on ice to avoid degradation. Adding distilled water and 50% trichloroacetic acid (TCA) to the proteins caused them to precipitate. They were then incubated on ice and centrifuged at 4°C to get a clear supernatant. We measured the volume of the supernatant and put it in a new test tube, where we mixed it with 0.4 M Tris buffer (pH 8.9). Then, DTNB, which was made at 0.1 M in pure ethanol, was added to the mix. A yellow complex formed in 2 to 3 minutes, and a UV–Visible spectrophotometer measured its absorbance at 412 nm. The color's brightness was directly related to how much reduced glutathione was in the sample (36).

2.13.2.6 Lipid Peroxidation

To measure malondialdehyde (MDA), we first mixed tissue homogenate with saline and trichloroacetic acid (TCA) to make proteins fall out of solution. Then, the mixture was spun in a centrifuge to separate the proteins from the supernatant. When thiobarbituric acid (TBA) was added to the clear supernatant and heated for an hour, it turned pink and formed the MDA–TBA complex. A spectrophotometer measured the intensity of the pink color that resulted, which showed the concentration of MDA (37).

2.14 Assessment of Biochemical Parameters

On the 14th day of treatment, blood samples were taken from the test animals to check the levels of AST, ALT, and ALP in their serum. The serum was separated using standard methods and then analyzed with a Randox autoanalyzer, following the manufacturer's instructions for finding these biochemical parameters (38).

2.14.1 Alkaline Phosphatase (ALP)

First, double distilled water was sucked in to clean the system. Then, the flow cell mode was used to calibrate it. The option for the ALP (Alkaline Phosphatase) test was chosen from the run test screen. We set the spectrophotometer to zero with water before looking at the samples (39).

2.14.2 Alanine Aminotransferase (ALT)

The first step was to aspirate double-distilled water, and then the analyzer was set up in flow cell mode. The instrument panel showed that the ALT testing option had been chosen. To make sure the baseline was set correctly before looking at the samples, double-distilled water was used as a blank.

We used the formula U/L = 1746 × (−ΔA at 340 nm per minute) to find out how active alanine aminotransferase (ALT) is. This calculation is based on how much the absorbance changes every minute at 340 nm. The enzyme's activity level is shown by the drop in NADH absorbance. The negative sign means that the absorbance went down because NADH was changed into NAD^+ during the enzymatic reaction (40).

2.14.3 Oral Glucose Tolerance Test

As outlined in section 3.5, the rats received the corresponding treatments. The animals were not given any food for the night before the experiment. After thirty minutes of treatment, a glucose load of 2 g/kg was given by mouth. Blood samples were taken from all groups at 0, 30, 60, and 120 minutes after giving them glucose. Next, blood glucose levels were checked to see how the treatment affected glucose tolerance (41).

2.15 Statistical Analysis

Pharmacokinetic and pharmacodynamic data were analyzed using one-way ANOVA, followed by Dunnett's post hoc multiple comparison test to evaluate the significance of differences between the treatment groups and the control.

3. Results and discussion

3.1 Pharmacokinetic and pharmacodynamics interaction of Gymnema sylvestre and Glimepiride

The phytochemical analysis of the aqueous extract of Gymnema sylvestre leaves revealed the consistent presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenols, and carbohydrates in all three samples. These constituents support the plant's traditional use in managing metabolic and inflammatory disorders. The detection of saponins and flavonoids is particularly significant, as these compounds are known for their antioxidant, antidiabetic, and lipid-lowering activities. The presence of tannins and phenols indicates strong free-radical scavenging and possible hepatoprotective effects, while alkaloids and glycosides may contribute to glucose-modulating and antimicrobial properties. The absence of steroids and fixed oils reflects the selective extraction of polar compounds by the aqueous solvent. Overall, the findings validate the medicinal relevance of G. sylvestre and provide a foundation for further isolation and bioactivity studies.

Table 2: Initial phytochemical analysis of the aqueous extract from Gymnema sylvestre leaves (n=3).

Phytochemical Constituents	Observation (n=3)	Result
Alkaloids	Present in all tests	✓
Tannins	Present in all tests	✓
Saponins	Present in all tests	✓
Flavonoids	Present in all tests	✓
Glycosides	Present in all tests	✓
Phenols	Present in all tests	✓
Carbohydrates	Present in all tests	✓
Steroids	Absent in all tests	–
Fixed oils	Absent in all tests	–

3.2 Physical Constants of Aqueous Extract of Gymnema sylvestre

The physical constants of the aqueous extract of Gymnema sylvestre indicate good quality and purity. Total ash (7.82%), acid-insoluble ash (1.62%), and water-soluble ash (4.35%) suggest minimal contamination and presence of essential minerals. The extract's yield (12.47%) reflects efficient extraction of bioactive compounds like saponins, flavonoids, glycosides, and tannins. Overall, these values confirm the extract's reliability for further phytochemical and pharmacological studies.

Table 3: Physical Constants of Aqueous Extract of *Gymnema sylvestre* Leaves (n=3)

Parameter	Observation (% w/w, Mean ± SD)
Total Ash	7.82 ± 0.14
Acid Insoluble Ash	1.62 ± 0.08
Water Soluble Ash	4.35 ± 0.11
% Yield of Extract	12.47 ± 0.22

3.3 Total flavonoid content of Gymnema sylvestre

The total flavonoid content of Gymnema sylvestre was measured using the aluminium chloride colorimetric method, which forms yellow complexes with flavonoids detectable at 415 nm. Using a quercetin standard curve (40–200 µg/100 µL), flavonoid levels were quantified and expressed as quercetin equivalents (% w/w). Results indicated a high flavonoid content, highlighting G. sylvestre as a rich source of bioactive compounds.

Table 4: Total flavonoid content of Gymnema sylvestre

Extract	Part used	Total flavonoid content
Content	Leaves	39.08

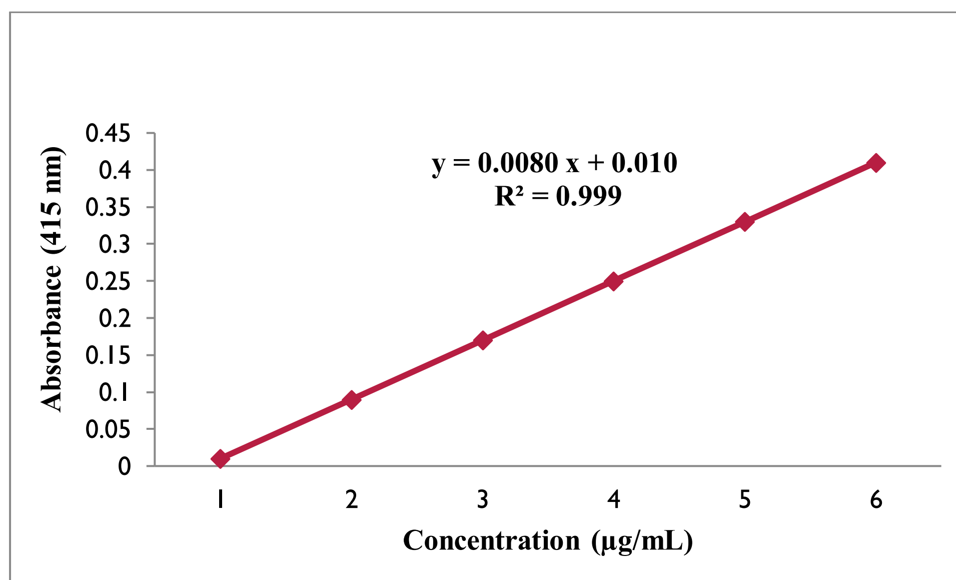

Figure 1: Total flavonoid content of Gymnema sylvestre

3.4 HPTLC profile of aqueous extract of Gymnema sylvestre

HPTLC analysis of the aqueous extract of Gymnema sylvestre provided a reliable method for identifying and quantifying its phytoconstituents. Using silica gel 60 F254 plates and quercetin as a standard, the extract showed a band with an Rf value of 0.481, confirming the presence of quercetin. Quantification revealed 9.3% w/w quercetin, a bioactive flavonoid with antioxidant, anti-inflammatory, and antidiabetic properties. This fingerprinting method supports standardization, quality control, and reproducibility of G. sylvestre extracts for future research and therapeutic use.

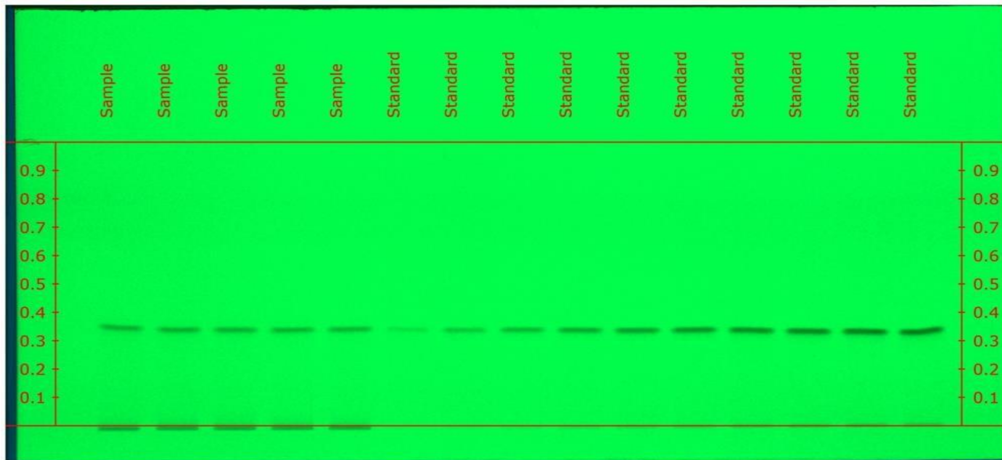

Figure 2: HPTLC chromatogram of *Gymnema sylvestre* aqueous extract and Quercetin standard at 254 nm, showing the presence of similar bands indicating the presence of Quercetin in the extract

3.5 Invitro investigation evaluated the in vitro effects of Gymnema sylvestre extract on CYP3A activity using rat liver microsomes

This study evaluated the in vitro effects of Gymnema sylvestre extract on CYP3A activity using rat liver microsomes. Results showed that a lower dose (50 µg) had minimal effect, whereas a higher dose (100 µg) significantly inhibited CYP3A, comparable to the reference inhibitor dexamethasone. Quercetin, a major constituent of G. sylvestre, is known to modulate CYP3A4 and P-glycoprotein pathways, which may explain the observed dose-dependent inhibition. Since CYP3A enzymes metabolize many drugs, including antidiabetics like glimepiride, concurrent use of the extract may alter drug metabolism and efficacy. These findings highlight the importance of assessing herb drug interactions for G. sylvestre, particularly with CYP3A4 or CYP2C9 substrates. Further mechanistic studies, including enzyme kinetics and in vivo validation, are warranted to clarify the role of quercetin and other bioactive components in this interaction.

Table 5: Effect of Gymnema sylvestre extract on CYP3A activity in liver microsome (n=6)

Treatment	Tween (5%)	Betamethasone (10 µM)	Gymnema sylvestre extract (100 µg)	Gymnema sylvestre extract (150 µg)
Mean	382.82 ± 4.52	231.14± 4.60***	331 ± 4.67***	262 ± 4.87***
% Change	---	41.09	14.4	34.84

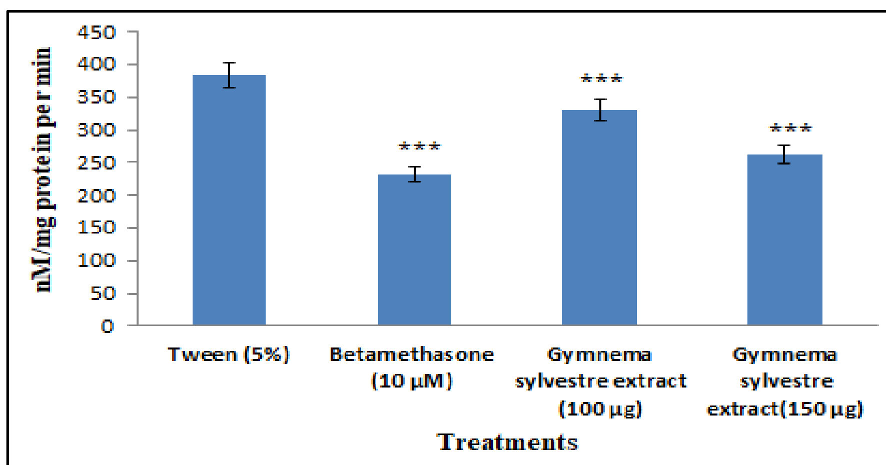

Figure 3: Gymnema sylvestre extract (100–150 µg) significantly inhibited CYP3A activity in liver microsomes, similar to betamethasone (10 µM).

3.6 Pharmacodynamic interaction of *Gymnema sylvestre* extract and Glimepiride

The study evaluated the antihyperglycemic effects of Gymnema sylvestre extract alone and in combination with glimepiride in STZ-induced diabetic rats. The diabetic control group maintained elevated blood glucose levels, while the normal group showed stable glucose homeostasis. Glimepiride significantly reduced glucose levels within 1 hour, reaching near-normal levels by 4–8 hours. G. sylvestre extract (200 and 400 mg/kg) also lowered glucose in a dose-dependent manner, with the 400 mg/kg dose showing marked reductions by 2 hours, lasting up to 8 hours, indicating insulin-mimetic or insulin-secretagogue activity. Combination therapy of extract and glimepiride produced a greater glucose-lowering effect than either alone, with the 400 mg/kg extract plus glimepiride showing significant synergism and near-normal glucose levels within 4–8 hours. These results highlight the potent antihyperglycemic properties of G. sylvestre and its potential to enhance sulfonylurea efficacy in diabetes management.

Table 6: Results of *Gymnema sylvestre* extract on glucose level in Streptozotocine Induced diabetic rats

Treatment groups	1 hr	2 hr	4 hr	8 hr
Normal group	92.46 ± 0.45***	96.6±0.87***	45.61±1.06***	93.4±0.99***
Diabetic control (STZ)	249.61±2.32***	246.78±2.34***	251.62±2.03***	248.21±3.19***
Glimepiride	211.73 ± 6.3[a]	139.7±4.23[c]	93.87±1.23[c]	100.42±1.99[c]
Gymnema sylvestre extract (200 mg/kg)	240.23±3.45	233.72±3.52***	180.42±1.02***	112.28±4.43*
Gymnema sylvestre extract (400 mg/kg)	242.37±5.44***	129.2±4.83***	169.21±2.48***	110.3±2.34*
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	241.35±4.83***	130.18±4.83[c]	111.3±1.96**	90.47±2.88**
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	223.4± 2.49[b]	127.36±1.45*	101.13±2.52[c]***	91.97±1.45*

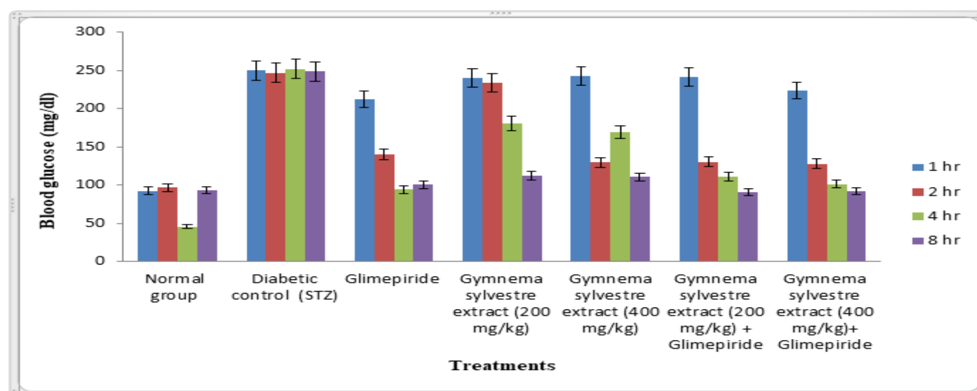

Figure 4: *Gymnema sylvestre* extract significantly lowered blood glucose in diabetic rats in a dose-dependent manner, showing notable differences versus both diabetic control and glimepiride groups (*P < 0.05 to ***P < 0.001).

3.7 Outcome of Gymnema sylvestre extract on SOD level in diabetic rats

STZ-induced diabetic rats showed reduced SOD activity, reflecting oxidative stress and impaired antioxidant defense. Glimepiride partially restored SOD (+37%), while Gymnema sylvestre extract improved SOD in a dose-dependent manner. Combination therapy nearly matched glimepiride's effect, indicating synergistic antioxidant and antihyperglycemic benefits, likely via free radical scavenging and enhanced antioxidant enzyme activity.

Table 7: **Gymnema sylvestre extract and glimepiride improved SOD activity in STZ-induced diabetic rats (mean ± S.E.M., n = 6; analyzed by One-way ANOVA with Dunnett's test).**

Treatment groups	SOD (U/min/mg of protein)	% Change in SOD level
Normal group	19.65 ± 1.45***c	57.89%
Diabetic control (STZ)	8.99±1.87**	---
Glimepiride	13.44 ± 1.32[a]	37.21%
Gymnema sylvestre extract (200 mg/kg)	10.23±1.24[c]	16.23%
Gymnema sylvestre extract (400 mg/kg)	11.23±1.99[c]	24.45%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	12.34±41.34**c	34.54%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	13.34± 2.34**c	37.60%

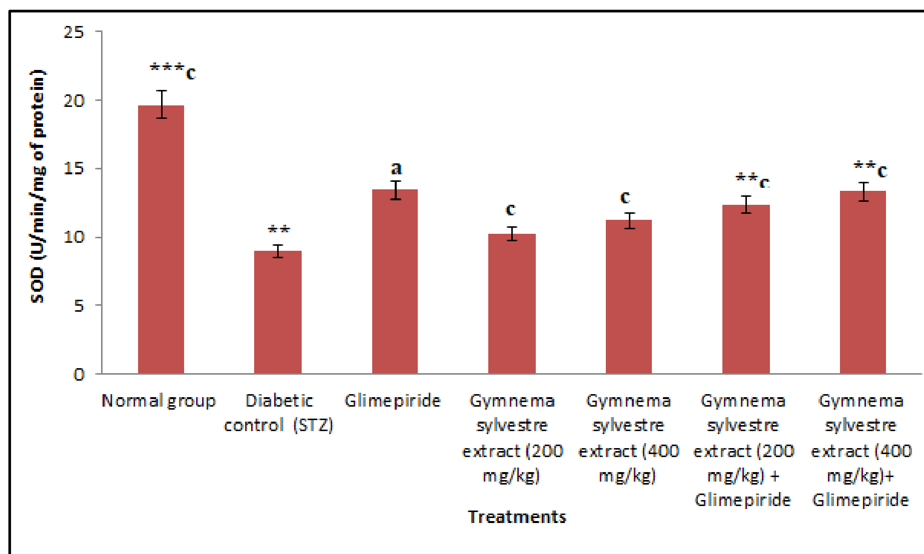

Figure 5: **Effect of Gymnema sylvestre extract and glimepiride, alone or combined, on SOD activity in STZ-induced diabetic rats. Data are mean ± S.E.M. (n = 6); statistical significance determined by One-way ANOVA with Dunnett's test.**

3.8 Effect of Gymnema sylvestre extract on catalase level in diabetic rats

STZ-induced diabetes significantly reduced catalase (CAT) activity, reflecting elevated oxidative stress. Glimepiride restored CAT (+38%), while Gymnema sylvestre extract enhanced activity dose-dependently. Combination therapy (400 mg/kg + glimepiride) showed moderate improvement (+28%), indicating the extract provides antioxidant protection and may serve

as an adjunct in diabetes management, though its effect on CAT is less pronounced than on SOD.

Table 8: **Effect of Gymnema sylvestre extract and glimepiride, individually and combined, on catalase (CAT) activity in STZ-induced diabetic rats. Data are presented as mean ± S.E.M. (n = 6); significance determined by One-way ANOVA with Dunnett's test.**

Treatment groups	CAT (U/min/mg of protein)	% Change in CAT level
Normal group	5.60 ± 0.67*c	60.78%
Diabetic control (STZ)	1.99 ±0.43	---
Glimepiride	4.67 ± 0.21^{c}	37.98%
Gymnema sylvestre extract (200 mg/kg)	3.76 ± 0.45	38.43%
Gymnema sylvestre extract (400 mg/kg)	3.98±1.34	21.47%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	3.23 ± 0.54	10.59%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	3.45 ± 0.45	28.30%

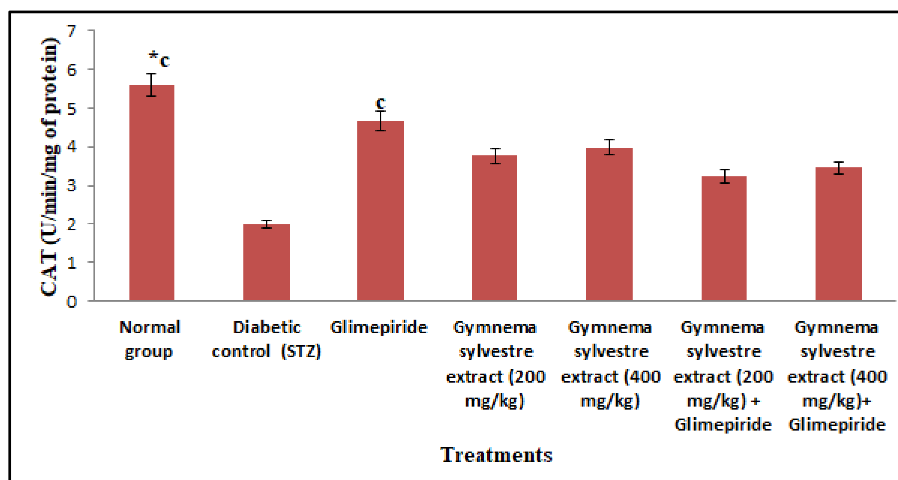

Figure 6: Gymnema sylvestre and glimepiride effects on CAT in diabetic rats (n = 6); mean ± S.E.M., significance: *P < 0.05 vs. DC, aP < 0.05 vs. glimepiride.

3.9 Effect of Gymnema sylvestre extract on Glutathione level in diabetic rats

STZ-induced diabetes significantly lowered glutathione (GSH) levels, indicating compromised antioxidant defense. Glimepiride restored GSH (+47%), while Gymnema sylvestre extract increased levels dose-dependently, with 400 mg/kg showing greater recovery. Combination therapy (400 mg/kg + glimepiride) nearly normalized GSH (+47%), suggesting synergistic enhancement of antioxidant protection in diabetes.

Table 9: **Gymnema sylvestre and glimepiride effects on GSH in diabetic rats (n = 6); mean ± S.E.M., analyzed by One-way ANOVA with Dunnett's test.**

Treatment groups	GSH (U/min/mg of protein)	% Change in GSH level
Normal group	27.56 ± 1.65*c	57.87%
Diabetic control (STZ)	12.45 ±0.76***c	---
Glimepiride	22.45± 0.64	46.92%
Gymnema sylvestre extract (200 mg/kg)	18.56 ± 0.81[b]	34.21%
Gymnema sylvestre extract (400 mg/kg)	21±0.38[c]	44.27%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	23.56 ± 1.78[b]	37.19%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	25.67 ± 1.26[c]	47.31%

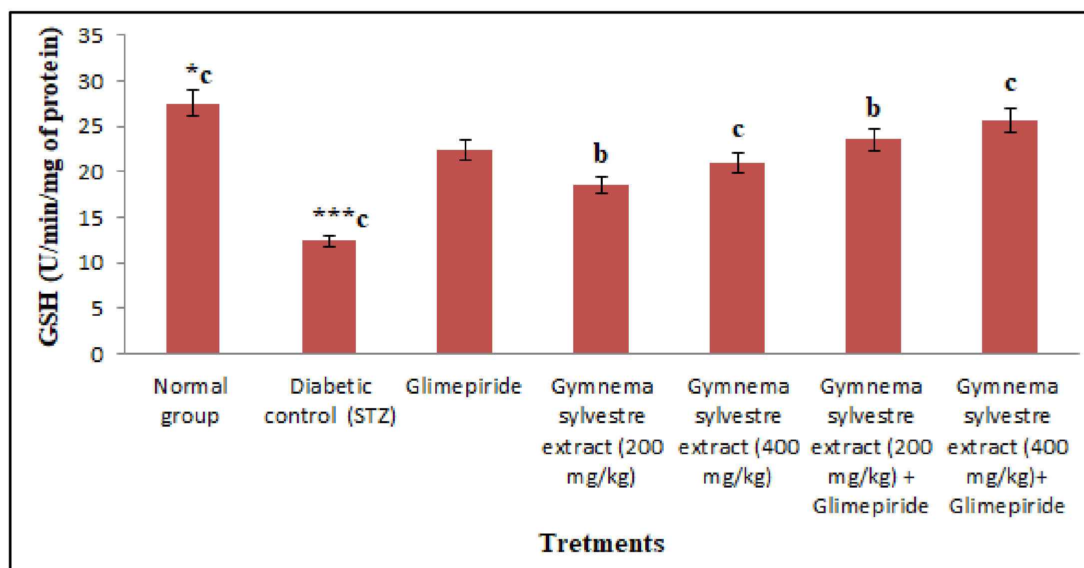

Figure 7: **Gymnema sylvestre and glimepiride effects on GSH in STZ-diabetic rats (n = 6); mean ± S.E.M. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001 vs. diabetic control; aP < 0.05, bP < 0.01, cP < 0.001 vs. glimepiride (One-way ANOVA, Dunnett's test).**

3.10 Effect of Gymnema sylvestre extract on Liped peroxidatse(LPO) level in diabetic rats

STZ-induced diabetes significantly increased lipid peroxidation (LPO), reflecting oxidative damage. Glimepiride reduced LPO by 58%, while Gymnema sylvestre extract lowered LPO dose-dependently, with 400 mg/kg being more effective. Combination therapy (400 mg/kg + glimepiride) further decreased LPO by ~73%, nearing normal levels, indicating a synergistic antioxidant effect that protects against diabetes-related oxidative stress.

Table 10: **Gymnema sylvestre and glimepiride effects on LPO in STZ-diabetic rats (n = 6); mean ± S.E.M. Statistical analysis: One-way ANOVA followed by Dunnett's test.**

Treatment groups	LPO (U/min/mg of protein)	% Change in LPO level
Normal group	1.23 ± 0.76c	82.27%
Diabetic control (STZ)	10.25 ±0.71***	---
Glimepiride	4.37± 0.37[c]	57.91%
Gymnema sylvestre extract (200 mg/kg)	4.67± 0.71[c]	69.23%
Gymnema sylvestre extract (400 mg/kg)	3.78±0.76[c]	73.79%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	3.89 ± 0.90[b]	71.14%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	3.34 ± 0.76*[c]	73.48%

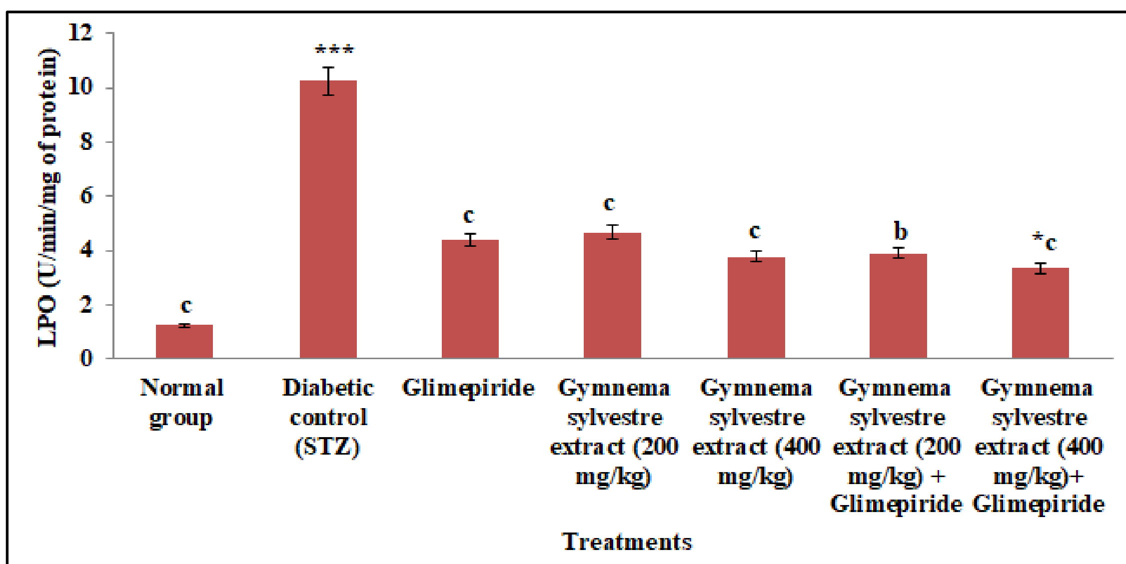

Figure 8: **Gymnema sylvestre and glimepiride effects on LPO in STZ-diabetic rats (n = 6); mean ± S.E.M. Significance: *P < 0.05, **P < 0.01, ***P < 0.001 vs. diabetes control; aP < 0.05, bP < 0.01, cP < 0.001 vs. glimepiride (One-way ANOVA, Dunnett's test).**

3.11 Effect of Gymnema sylvestre extract on ALP level in diabetic rats

STZ-induced diabetes caused a marked increase in lipid peroxidation (LPO), reflecting severe oxidative stress and membrane damage. Glimepiride reduced LPO by 41%, while Gymnema sylvestre extract decreased it dose-dependently, with the 400 mg/kg dose showing a 59% reduction. Combination therapy offered similar protection, indicating that high-dose extract alone is highly effective. These findings highlight the strong antioxidant potential of Gymnema sylvestre, capable of reducing

oxidative damage in diabetes either alone or alongside glimepiride.

Table 11: Glimepiride and Gymnema sylvestre effects on LPO in STZ-induced diabetic rats (n = 6); values as mean ± S.E.M. Statistical analysis by One-way ANOVA followed by Dunnett's test.

Treatment groups	LPO (U/min/mg of protein)	% Change in LPO level
Normal group	82.43 ± 1.26***c	76.13%
Diabetic control (STZ)	324.00 ± 2.05***	---
Glimepiride	197.01 ± 1.28[c]	41.08%
Gymnema sylvestre extract (200 mg/kg)	160.23 ± 2.21***[c]	52.13%
Gymnema sylvestre extract (400 mg/kg)	138.02 ±3.09***[c]	59.19%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	167.01 ± 2.50***[c]	50.18%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	138.25 ± 1.05***[c]	59.08%

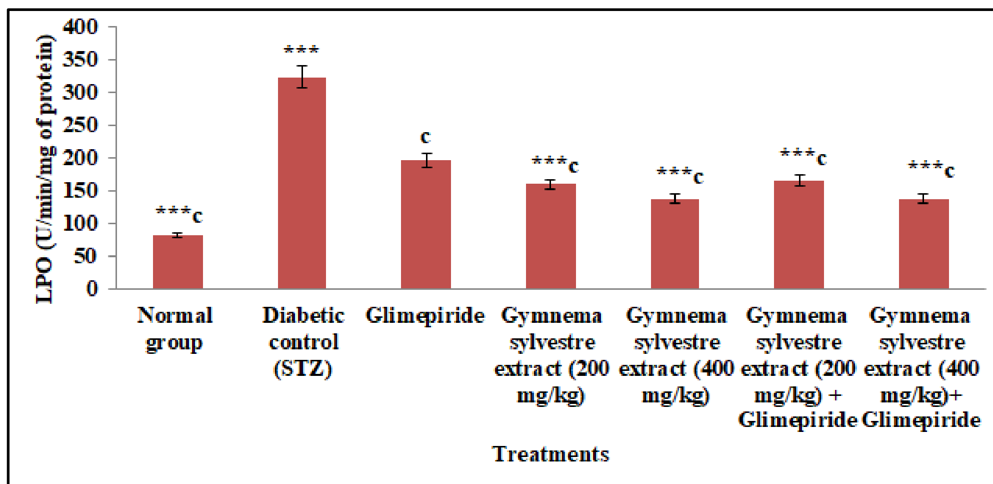

Figure 9: Effect of glimepiride and Gymnema sylvestre on LPO in STZ-induced diabetic rats (n = 6); values are mean ± S.E.M. Significance: *P < 0.05, **P < 0.01, ***P < 0.001 vs. diabetic control; aP < 0.05, bP < 0.01, cP < 0.001 vs. glimepiride (One-way ANOVA, Dunnett's test).

3.12 Effect of Gymnema sylvestre extract on AST level in diabetic rats

STZ-induced diabetes significantly elevated AST levels, indicating liver damage and oxidative stress. Glimepiride moderately reduced AST (21%), whereas Gymnema sylvestre extract restored liver enzyme activity more effectively, with the 400 mg/kg dose achieving an 81% reduction. Combination therapy (400 mg/kg + glimepiride) provided the greatest hepatoprotective effect (82%), suggesting synergism. These findings highlight the liver-protective potential of Gymnema sylvestre, alone and alongside glimepiride, in diabetes management.

Table 12: **Effect of Gymnema sylvestre and Glimepiride on AST in STZ-diabetic rats (U/min/mg protein, mean ± SEM); % change shows reduction vs. diabetic control. Significance: ***P < 0.001, bP < 0.05, c vs. normal.**

Treatment groups	AST (U/min/mg of protein)	% Change in AST level
Normal group	79 ± 0.26***c	66.33%
Diabetic control (STZ)	229.44 ± 5.35***	---
Glimepiride	182.11 ± 7.48^{b}	21.18%
Gymnema sylvestre extract (200 mg/kg)	81.22 ± 2.44***^{c}	65.43%
Gymnema sylvestre extract (400 mg/kg)	45.34 ± 1.39***^{c}	81.39%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	88.77 ± 1.64***^{c}	60.06%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	43.53 ± 0.78***^{c}	82.43%

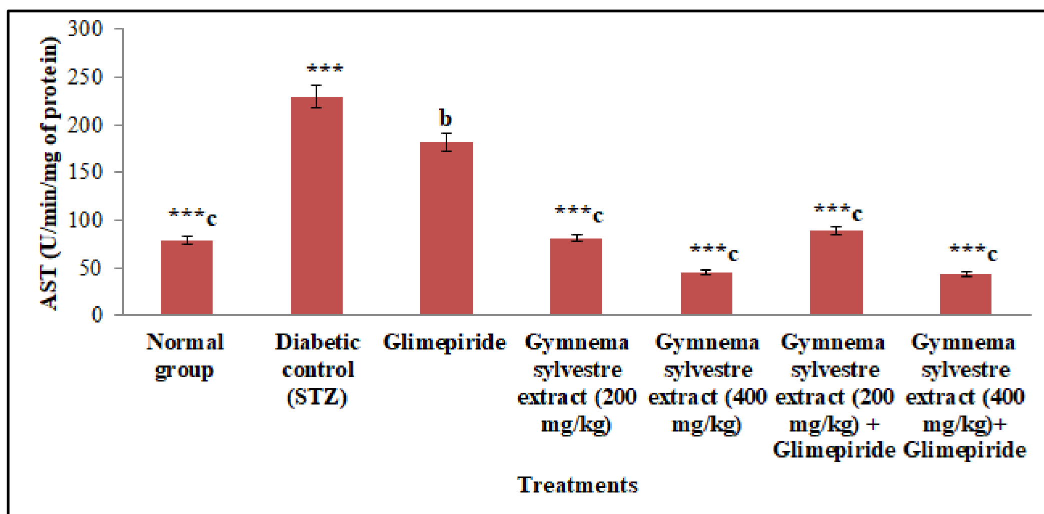

Figure 10: **Effect of Glimepiride and Gymnema sylvestre extract on AST activity in STZ-induced diabetic rats. Data are mean ± SEM; % change indicates reduction vs. diabetic control. Significance vs. normal group: ***P < 0.001, bP < 0.05.**

3.13 Result of Gymnema sylvestre extract on ALT level in diabetic rats

Table 13 presents the effects of Gymnema sylvestre extract and glimepiride on serum ALT in STZ-induced diabetic rats. The diabetic control group showed a marked ALT increase (115.55 ± 4.25 U/L), indicating liver injury. Glimepiride reduced ALT to 86.01 ± 1.56 U/L (−26.13%), while G. sylvestre extract lowered ALT dose-dependently to 78.02 ± 2.77 U/L (200 mg/kg, −33.42%) and 61.67 ± 1.67 U/L (400 mg/kg, −48.29%). Combination therapy produced similar or slightly better reductions, highlighting the extract's hepatoprotective effect and potential synergism with glimepiride.

Table 13: Effect of Gymnema sylvestre extract and Glimepiride on serum ALT levels in STZ-induced diabetic rats. Data are mean ± SEM (U/L); % change represents reduction vs. diabetic control. Statistical significance vs. normal group: ***P < 0.001, **P < 0.01, bP < 0.05.

Treatment groups	ALT U/L	% Change in ALT level
Normal group	37.76 ± 1.36***	69.23%
Diabetic control (STZ)	115.55 ± 4.25***	---
Glimepiride	86.01 ±1.558^{b}	26.13%
Gymnema sylvestre extract (200 mg/kg)	78.02 ± 2.77**^{c}	33.42%
Gymnema sylvestre extract (400 mg/kg)	61.67 ± 1.67***^{c}	48.29%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	77.01 ± 1.09**^{c}	34.65%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	65.52 ± 1.765***^{c}	44.48%

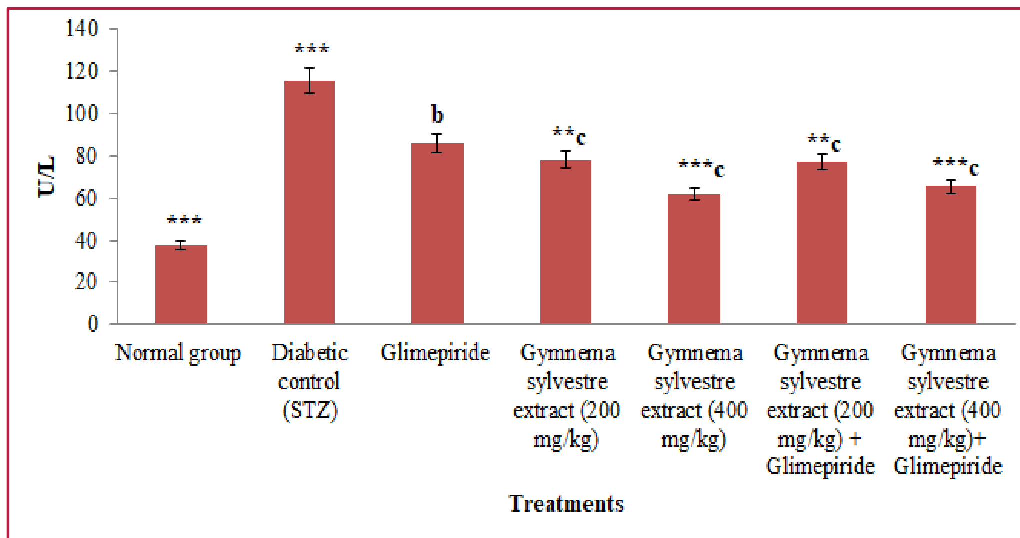

Figure 11: Gymnema sylvestre extract significantly improved oral glucose tolerance in STZ-induced diabetic rats, producing dose- and time-dependent reductions in blood glucose, with the 400 mg/kg dose showing the greatest effect. Co-administration with glimepiride further enhanced glycaemic control, indicating additive or synergistic antihyperglycemic activity.

Table 14: Effect of Gymnema sylvestre extract on OGT level in diabetic rats

Treatment groups	30 min	% change	60 min	% change	120 min	% change
Normal group	218.9± 3.7***	18.97%	163.9± 1.9***c	54.89%	156.7± 1.3***	50.85%
Diabetic control (STZ)	266.6 ± 1.7	----	257.3 ± 1.6	----	308.4 ± 2.48	----
Glimepiride	250.4 ± 1.14[c]	7.15%	282.68 ± 3.4[c]	20.54%	225.7 ± 4.5[c]	28.34%
Gymnema sylvestre extract (200 mg/kg)	251..0 ± 2.02	7.58%	312.1 ± 1.5***	12.32%	229.0 ± 1.8***	36.12%
Gymnema sylvestre extract (400 mg/kg)	258.3 ± 2.5[a]	2.03%	296.3± 2.7[c]	17.15%	183.4± 2.5***	41.37%
Gymnema sylvestre extract (200 mg/kg) + Glimepiride	256.2 ± 2.7**	4.61%	283.3. ± 1.2[c]	21.07%	196.9 ± 1.4***	37.18%
Gymnema sylvestre extract (400 mg/kg)+ Glimepiride	258.6± 2.2*	3.02%	285,0± 168[c]	20.44%	188.6± 1.6***	39.31%

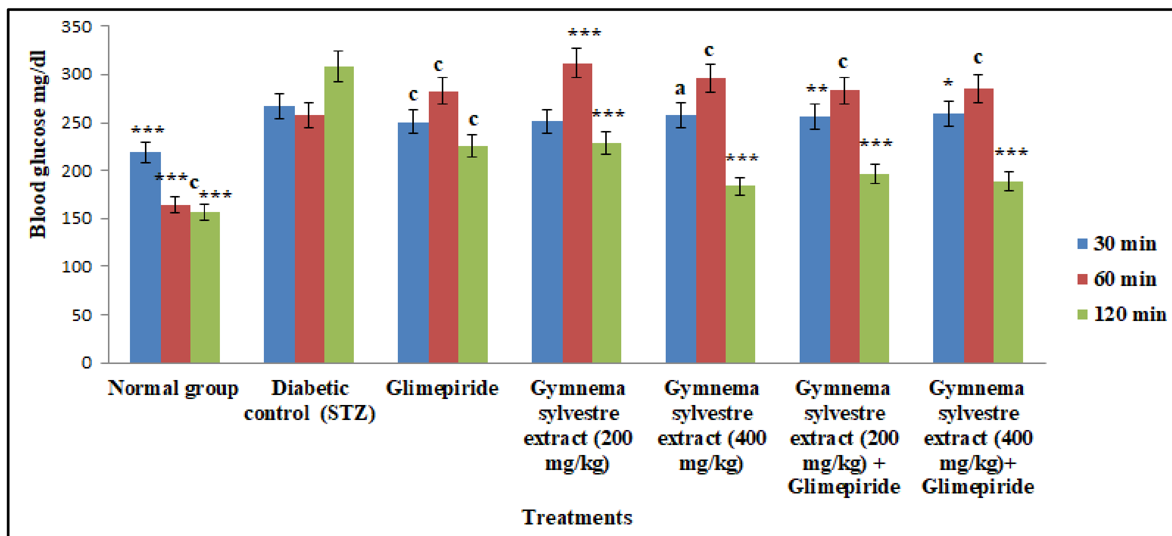

Figure 12: **Effect of Gymnema sylvestre extract and glimepiride on time-dependent blood glucose levels in STZ-induced diabetic rats. Data are presented as mean ± SEM; % change indicates reduction vs. diabetic control. Statistical significance: *P < 0.05, **P < 0.01, ***P < 0.001; a/b/c indicate comparison with control or normal group.**

3.14 Pharmacokinetic intraction of Gymnema sylvestre extract and Glimepiride

Table 15 presents the pharmacokinetic profile of glimepiride alone and in combination with Gymnema sylvestre extract. Co-administration of the extract altered several parameters in a dose-dependent manner. The peak plasma concentration (Cmax) slightly decreased from 78.64 ± 4.24 mg/ml to 73.27 ± 5.38 mg/ml and 70.14 ± 4.33 mg/ml with 200 and 400 mg/kg extract, respectively, while the area under the curve (AUC0t) declined from 572.33 ± 10.22 to 534.7 ± 19.27 and 414.30 ± 11.07 mg·h/ml, suggesting reduced systemic exposure. The half-life (T1/2) shortened significantly (3.55 ± 0.21, 1.55 ± 0.07, 1.16 ± 0.04 h, ***p < 0.001), indicating faster elimination, and Tmax increased from 5.1 to 8.1 h, reflecting delayed peak concentration. Mean residence time (MRT) remained largely unchanged. These findings highlight that higher doses of G. sylvestre extract can influence glimepiride's pharmacokinetics, emphasizing the need to assess potential herb drug interactions.

Table 15: Pharmacokinetic profile of Glimepiride alone and with Gymnema sylvestre extract in rats. Data are mean ± SEM; statistical significance: ***P < 0.001 vs. Glimepiride alone.

Parameters	Glimepiride	Gymnema sylvestre extract (200 mg/kg) + Glimepirid	Gymnema sylvestre extract (400 mg/kg)+ Glimepiride
C_{max} (mg/ml)	78.64±4.24	73.27±5.38	70.14±4.33***
AUC_{0-t} (mg/ml* h)	572.33±10.22	534.7±19.27	414.30±11.07
$T_{1/2}$ (h)	3.55±0.21	1.55±0.07***	1.16±0.04***
T_{max} (h)	5.1±0	8.1±0	8.1±0
MRT (h)	7.36±0.04	6.40±0.13	7.02±0.22

DISCUSSION:

The present study highlights the therapeutic potential of Gymnema sylvestre aqueous extract in the management of STZ-induced diabetes, both alone and in combination with glimepiride. Phytochemical and HPTLC analyses confirmed the presence of bioactive constituents, particularly flavonoids such as quercetin, which are known to contribute to antihyperglycemic, antioxidant, and hepatoprotective effects. Pharmacodynamically, the extract produced dose-dependent reductions in blood glucose levels and significantly improved oral glucose tolerance, indicating enhanced insulin sensitivity and glucose utilization. The combination of G. sylvestre with glimepiride resulted in superior glycemic control, suggesting additive or synergistic mechanisms. Additionally, the extract effectively restored antioxidant enzyme levels (SOD, CAT, and GSH) and reduced lipid peroxidation, thereby attenuating oxidative stress associated with diabetes. Hepatic protection was evidenced by the normalization of AST and ALT levels, with the higher extract dose and combination therapy showing greater efficacy than glimepiride alone. Pharmacokinetic and in vitro CYP3A studies revealed that G. sylvestre can modulate glimepiride metabolism, leading to altered drug exposure. These findings underscore the clinical relevance of potential herb–drug interactions and support the use of G. sylvestre as a beneficial adjunct in diabetes therapy, with appropriate monitoring and dose adjustment.

CONCLUSION

The present study demonstrates that *Gymnema sylvestre* aqueous extract possesses significant antihyperglycemic, antioxidant, and hepatoprotective activities in STZ-induced diabetic rats. The extract improved glucose tolerance, restored endogenous antioxidant defenses, reduced lipid peroxidation, and normalized hepatic enzyme levels in a dose-dependent manner. When co-administered with glimepiride, *G. sylvestre* enhanced glycemic control and protective effects, indicating additive or synergistic therapeutic benefits. However, modulation of glimepiride pharmacokinetics and CYP3A activity highlights the potential for herb–drug interactions, emphasizing the need for careful dose optimization and monitoring. Overall, *Gymnema sylvestre* shows promise as an effective adjunct in diabetes management, warranting further clinical investigation..

REFERENCES

1. Sapra A, Bhandari P. Diabetes Mellitus - StatPearls - NCBI Bookshelf. StarPearls Publication. 2021.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;

3. Ogurtsova K, Guariguata L, Barengo NC, Ruiz PLD, Sacre JW, Karuranga S, et al. IDF diabetes Atlas: Global estimates of undiagnosed diabetes in adults for 2021. Diabetes Res Clin Pract. 2022;

4. Egan AM, Dinneen SF. What is diabetes? Medicine (United Kingdom). 2022.

5. Antony A, Farid M. Effect of Temperatures on Polyphenols during Extraction. Applied Sciences (Switzerland). 2022.

6. Abubakar AR, Haque M. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. Journal of Pharmacy and Bioallied Sciences. 2020.

7. Ahmed AH, Musa KY, Ahmed A, Mahmud AF. Microscopic, Physicochemical and Phytochemical Evaluation of Stem bark of Aubrevillea kerstingii (Harms) Pellegr. (Mimosaceae). Dutse J Pure Appl Sci. 2022;

8. Ummu Tukur, AI Umar, Sarkingobir Y, Ahmad Zayyanu. Assessment of Proximate and Phytochemical contents of Some Herbal Snuffs Sold in Sokoto Metropolis, Nigeria. Indones Chim Lett. 2023;

9. Fitrasyah SI, Ariani A, Rahman N, Nurulfuadi N, Aiman U, Nadila D, et al. Analysis of chemical properties and antioxidant activity of sambiloto (Andrographis paniculata nees.) leaf tea formula as a functional drink in preventing coronavirus diseases and degenerative diseases. Open Access Maced J Med Sci. 2021;

10. Ahmed Z, Aziz S, Hanif M, Mohiuddin SG, Khan SHA, Ahmed R, et al. Phytochemical screening and enzymatic and antioxidant activities of Erythrina suberosa (Roxb) bark. J Pharm Bioallied Sci. 2020;

11. RAIHANI SAIRUN MH, YUSUF N, ABDUL WAHAB NH. PHYTOCHEMICAL SCREENING AND EVALUATION OF THE ENZYMATIC ANTIOXIDANT ACTIVITIES OF SELECTED Mangifera spp. LEAVES (ANACARDIACEAE). Univ Malaysia Teren J Undergrad Res. 2020;

12. Mansoori A, Singh N, Dubey SK, Thakur TK, Alkan N, Das SN, et al. Phytochemical Characterization and Assessment of Crude Extracts From Lantana camara L. for Antioxidant and Antimicrobial Activity. Front Agron. 2020;

13. Praveena B, Murthy SDS. CHARACTERISATION OF POSSIBLE ENZYMATIC AND NON-ENZYMATIC ADAPTATIONS AGAINST UV-B STRESS IN THE CYANOBACTERIUM, SPIRULINA PLATENSIS. INDO Am J Pharm Sci. 2019;

14. Yusuf N, Masor NF, Zolkeflee P, Zakaria NS, Abdul Wahab NH, Asari A, et al. Qualitative phytochemical analysis, enzymatic and non-enzymatic antioxidant activities in stems and leaves of Vanilla planifolia (Orchidaceae). Food Res. 2023;

15. Suriyakalaa U, Ananth DA, Ramachandran R, Sankarganesh D, Angayarkanni J, Tietel Z, et al. Analysis of phytochemical composition of a leaf extract of sacred fig (Ficus religiosa L.) by UPLC-QqQ-MS and assessment of its hepatocurative potential in mouse model. South African J Bot. 2022;

16. Chigurupati S, Abdul Rahman Alharbi N, Sharma AK, Alhowail A, Vardharajula VR, Vijayabalan S, et al. Pharmacological and pharmacognostical valuation of Canna indica leaves extract by quantifying safety profile and neuroprotective potential. Saudi J Biol Sci. 2021;

17. Sadeer NB, Sinan KI, Cziáky Z, Jekő J, Zengin G, Jeewon R, et al. Assessment of the pharmacological properties and phytochemical profile of bruguiera gymnorhiza (L.) lam using in vitro studies, in silico docking, and multivariate analysis. Biomolecules. 2020;

18. Dias C, Fonseca AMA, Amaro AL, Vilas-Boas AA, Oliveira A, Santos SAO, et al. Natural-based antioxidant extracts as potential mitigators of fruit browning. Antioxidants. 2020;

19. Anarado CE, Chukwubueze FM, Anarado CJO, Umedum NL, Nwanya CB. Comparative Phytochemical and in vitro Antioxidant Screening of the Root and Stem Bark of Annona muricata Linn. Int Res J Pure Appl Chem. 2020;

20. Gomes AB, Corte VB, de Farias CC, Filho JR, Perini FM, Mariquito A, et al. Allelopathic potential of ethanolic extract and its fractions from leaves of Geonoma schottiana Mart. Comun Sci. 2024;

21. Gheraissa N, Chemsa AE, Cherrada N, Ebru E, Elsharkawy ER. Anabasis oropediorum Maire. as a health-promoting source: Phytochemical content, in vitro antioxidant, antidiabetic, antibacterial, and anti-inflammatory potential. J Res Pharm. 2023;

22. Aljawdah HMA, Abdel-Gaber R, Al-Shaebi EM, Thagfan FA, Al-Quraishy S, Qasem MAA, et al. Hepatoprotective activity of Eucalyptus camaldulensis extract in murine malaria mediated by suppression of oxidative and inflammatory processes. Front Cell Infect Microbiol. 2022;

23. Ezeako EC, Nworah FN, Osuji DO. Phytocompounds, antioxidant potential, and inhibitory actions of ethanolic leaf fraction of Sida linifolia Linn. (Malvaceae) on enzymes linked to inflammation, diabetes, and neurological disorders. Futur J Pharm Sci. 2023;

24. Sumithira G, Kumar GPS, Sharma M, Krisnamoorthy B, Suresh R, Balaraman AK. Antioxidant and antihyperlipidemic activity of methanolic fraction of maytenus heyneana root on stz induced diabetic wistar rats. Curr Trends Biotechnol Pharm. 2020;

25. Kapepula PM, Wamba HL, Lukusa DM, Franck T, Bahati PL, Fundu TM, et al. Congolese edible caterpillars, valuable sources of bioactive compounds with human health benefits. J Insects as Food Feed. 2023;

26. Nwaogu J, Abubakar B, Aliyu MS. Phytochemical Analysis and Antioxidants effects of Methanol Stem Bark Extract of Eucalyptus Camaldulensis in Wister Albino Rats. J Complement Altern Med Res. 2021;

27. Lawag IL, Nolden ES, Schaper AAM, Lim LY, Locher C. A Modified Folin-Ciocalteu Assay for the Determination of Total Phenolics Content in Honey. Appl Sci. 2023;

28. Meyer D, Morlock G. High-performance thin-layer chromatography analysis of industrial bamboo tableware for genotoxins, melamine and formaldehyde. J Planar Chromatogr - Mod TLC. 2023;

29. Bowonsomsarit W, Anegkamol W, Chuaypen N, Kaewwongse M, Tosukhowong P, Prapunwattana P, et al. Four-week induction of type 2 diabetes mellitus in rats by streptozotocin and high-fat diet. Chulalongkorn Med J. 2021;

30. AlAmri OD, Albeltagy RS, M. A. Akabawy A, Mahgoub S, Abdel-Mohsen DM, Abdel Moneim AE, et al. Investigation of antioxidant and anti-inflammatory activities as well as the renal protective potential of green coffee extract in high fat-diet/streptozotocin-induced diabetes in male albino rats. J Funct Foods. 2020;

31. Nahar N, Nazmul Hasan Zilani M, Biswas P, Morsaline Billah M, Bibi S, Albekairi NA, et al. Profiling of secondary metabolite and evaluation of anti-diabetic potency of Crotalaria quinquefolia (L): In-vitro, in-vivo, and in-silico approaches. Saudi Pharm J. 2024;

32. Sulaiman CT, Deepak M, Praveen TK, Lijini KR, Salman M, Sadheeshnakumari S, et al. Metabolite profiling and anti-cancer activity of two medicinally important Euphorbia species. Med Omi. 2023;

33. Arya A, Chahal R, Almutairi MH, Kaushik D, Aleya L, Kamel M, et al. Green approach for the recovery of secondary metabolites from the roots of Nardostachys Jatamansi (D. Don) DC using microwave radiations: Process optimization and anti-alzheimer evaluation. Front Plant Sci. 2022;

34. Mohammed HA, Khan RA, Abdel-Hafez AA, Abdel-Aziz M, Ahmed E, Enany S, et al. Phytochemical profiling, in vitro and in silico anti-microbial and anti-cancer activity evaluations and staph gyraseb and h-top-iiβ receptor-docking studies of major constituents of zygophyllum coccineum l. Aqueous-ethanolic extract and its subsequent fra. Molecules. 2021;

35. Adel A, Elnaggar MS, Albohy A, Elrashedy AA, Mostafa A, Kutkat O, et al. Evaluation of antiviral activity of Carica papaya leaves against SARS-CoV-2 assisted by metabolomic profiling. RSC Adv. 2022;

36. Youssef FS, Ovidi E, Al Musayeib NM, Ashour ML. Morphology, anatomy and secondary metabolites investigations of premna odorata blanco and evaluation of its anti-tuberculosis activity using in vitro and in silico studies. Plants. 2021;

37. Karakoyun, Bozkurt B, Çoban G, Masi M, Cimmino A, Evidente A, et al. A comprehensive study on narcissus tazetta subsp. tazetta L.: Chemo-profiling, isolation, anticholinesterase activity and molecular docking of amaryllidaceae alkaloids. South African J Bot. 2020;

38. Anyanwu, R. O., Onochie, A. U., Idama, F. O. Assessment of Hepatoprotective Potential of Ethanolic Extract of Allium sativum (Garlic) in Diabetes Induced Male Wistar Albino Rats. J Adv Med Pharm Sci. 2023;

39. Saxena PK, Nanda D, Gupta R. Assessment of Hepatoprotective Potential of Manilkara hexandra STEM Bark: An In-vitro Analysis. J Pharm Res Int. 2021;

40. Jaffar HM, Al-Asmari F, Khan FA, Rahim MA, Zongo E. Silymarin: Unveiling its pharmacological spectrum and therapeutic potential in liver diseases—A comprehensive narrative review. Food Science and Nutrition. 2024.

41. Godugu C, Khurana A, Saifi MA. Rare earth cerium oxide nanoparticles attenuated liver fibrosis in bile duct ligation mice model. J Trace Elem Med Biol. 2023.