

Evaluation of antidiabetic potential of *Plumbago indica* Roots: insights from *in vitro*, *in vivo* and *in silico* studies

Abdulkadir Abdu Mohmmmednor Ibrahim¹, Brahmjot Singh¹, Hossamaldeen Bakrey¹,
Hasandeep Singh², Atamjit Singh¹, Balbir Singh¹, Sarabjit Kaur¹

¹ Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, 143005, Punjab, India

² Department of Pharmaceutical Sciences, Amritsar Group of Colleges, Amritsar, 143005, Punjab, India

Corresponding author: Sarabjit Kaur (sarabjit.pharma@gndu.ac.in)

Received 14 July 2025 ♦ Accepted 24 September 2025 ♦ Published 19 December 2025

Citation: Ibrahim AAM, Singh B, Bakrey H, Singh H, Singh A, Singh B, Kaur S (2025) Evaluation of antidiabetic potential of *Plumbago indica* Roots: insights from *in vitro*, *in vivo* and *in silico* studies. Pharmacia 72: 1–17. <https://doi.org/10.3897/pharmacia.72.e165113>

Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose levels due to insufficient insulin production or decreased insulin sensitivity. Despite advances in antidiabetic pharmacotherapy, the limitations and adverse effects of current treatments highlight the need for natural products as alternative therapies. The present investigation was carried out to evaluate the anti-diabetic activity of the methanol extract (PIME) and ethyl acetate fraction (PIEAF) of roots of *Plumbago indica* using *in vitro*, *in vivo* and *in silico* models. The *in vitro* study was done by α -glucosidase and α -amylase inhibition assays. The effect of PIME and PIEAF on serum parameters and histopathological changes was assessed in streptozotocin/nicotinamide-induced type 2 diabetes mellitus in rats. The *in vitro* studies showed that PIEAF and PIME inhibited α -glucosidase and α -amylase enzymes in a dose dependent manner. *In vivo* studies indicated that administration of PIME and PIEAF led to the reduction in serum glucose, cholesterol, triglycerides, hepatic enzyme levels and significantly improved renal function and oxidative stress markers. Taking Plumbagin as the marker compound in *P. indica*, the HPLC analysis of PIME was carried out and it showed that the plumbagin content was 0.26%. *In silico* studies revealed that plumbagin inhibited α -glucosidase activity through optimal orientation and strong molecular affinity. *P. indica* roots showed strong promising potential as a treatment for diabetes, probably due to its antioxidant properties and higher content of plumbagin than other species of *Plumbago*.

Keywords

Plumbago indica, Antidiabetic, Streptozotocin- nicotinamide, Plumbagin, Molecular docking

Introduction

Diabetes mellitus is a metabolic disorder causing elevated blood sugar levels due to reduced insulin production and sensitivity (Mukhtar et al. 2020). Type 2 diabetes mellitus (T2DM) is the most predominant type, accounting for around 90% of diabetes cases worldwide

(Galicia-Garcia et al. 2020). T2DM is strongly linked to obesity rates, particularly among individuals aged 45 and older (Hillier and Pedula 2001). However, there is an alarming increase in T2DM cases among children and teenagers due to the rising prevalence of obesity and sedentary lifestyles (Amschler 2002). Diabetes mellitus has been considered as a silent killer disease since its evolu-

tion (Joseph and Vadasseril 2023). Pancreatic β -cells are responsible for glucose homeostasis and the cells can be degenerated in diabetic patients with a loss of the ability to control the high glucose level, resulting in diabetic complications (Khin et al. 2023). A decrease in secretion of insulin from the pancreas, an increase in blood glucose level and unutilized blood glucose by tissues leads to symptoms of diabetes, such as polyphagia, polyuria and polydipsia (Adu-Sarkodie 2017). Recent research has shown that the prevalence of diabetes is increasing rapidly in many developing and developed countries (Khan et al. 2019). Currently, DM is controlled either by administration of exogenous insulin, hypoglycemic agents or by insulin secretagogues (Satin et al. 2021). However, these approaches are associated with various side effects and toxicity. Some hypoglycemic synthetic agents are even known to produce life-threatening hypoglycemia (Mishra et al. 2021; Tasnim et al. 2024). The drawback of current medications arises from the lack of efficacy, high risk and high cost of the treatment. Due to adverse effects of various synthetic antidiabetic agents, there is an increasing interest in the prediction of antidiabetic potential of herbal plants. Therefore, the plant-based therapy for diabetes using traditional medicines is growing widely which are in high demand for safe, economical and readily available treatments (Ansari et al. 2023; Mishra et al. 2024).

P. indica Linn. is a medicinal plant belonging to Plumbaginaceae family, used in Thai traditional medicine for gastric stimulation, flatulence, hemorrhoids and improving appetite. Moreover, in eastern Africa and India, it is used as gastric stimulant, abortifacient and oral contraceptive (Ibrahim et al. 2018). The roots of this plant serve as a major source of plumbagin, a naturally occurring yellow pigment with a wide range of pharmacological properties (Lenora et al. 2012). There is substantial evidence indicating that Plumbagin have a significant role in the treatment of inflammation, breast cancer, brain tumors, lung cancer, hepatocellular carcinoma, cardiovascular diseases and other conditions (Sharma et al. 2024). As the principal and bioactive component present in the roots of this plant is plumbagin, therefore its initial extraction and subsequent testing by HPLC was done in this study to verify the presence of the intended bioactive compound.

P. indica is rich in naphthoquinones, flavonoids and terpenoids which have shown their potential in diabetes management (Priyanjani et al. 2021). It has been reported that this plant possesses antioxidant activity and reduces chronic inflammation which are linked to diabetes and its complications (Ittiyavirah et al. 2012; Eldhose et al. 2013). Moreover, other species of genus *Plumbago* eg. *P. zeylanica* and *P. auriculata* have been reported to exhibit antidiabetic activity (Zia et al. 2024).

As there is no scientific report available on hypoglycemic activity of *P. indica*, therefore, this study was carried out to evaluate the antidiabetic potential of this plant.

Materials and methods

Drugs and chemicals

Streptozotocin (STZ) at a purity of 98% and nicotinamide at a purity of 99.5% were procured from Sisco Research Laboratories Pvt. Ltd India. Nitro blue tetrazolium (NBT), reduced glutathione (GSH) and 5, 5'-dithio-bis [2-nitrobenzoic acid] (DTNB) were obtained from Loba Chemie, Mumbai, India. All remaining reagents utilized in the experiment were of analytical grade.

Plant material

Plumbago indica roots were procured from Jagdeesh Herbals, Kerala and its authentication was done by Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar and voucher specimen no. (3979) has been kept at the Department Herbarium.

Preparation of plant extracts and their active fractions

The plant material, after cleaning and authentication, was grounded into a coarse powder using a mechanical grinder. The dried plant material (1000 g) was first defatted with hexene and successively extracted with chloroform and methanol in a Soxhlet apparatus for 48 hours. Further fractions were obtained from the most potent extract using ethyl acetate and n-butanol. Each extract and fractions were concentrated using a rotary evaporator after filtration and their weight was measured to calculate the percentage yield from the air-dried plant material. The concentrated extracts/fractions were stored in a refrigerator to prevent microbial contamination (Rajput et al. 2023).

Preliminary phytochemical screening

Preliminary testing for the presence of secondary metabolites was performed for the most bioactive extract using standard protocols for various classes of phytoconstituents such as alkaloids, terpenoids, flavonoids, glycosides and tannins (Afzal et al. 2022).

In Vitro studies

Antioxidant assay

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) free radical scavenging assay

To assess the hydrogen-donating capacity of natural compounds or plant extracts, DPPH assay was used. The process of converting the DPPH radical to a stable molecule allows for the measurement of the capacity to scavenge free radicals. The IC₅₀ value for the DPPH scavenging activity was calculated. Different concentrations of extracts and

fractions (25, 50, 100, 200 and 400 µg/ml) were prepared in methanol, added to test tubes and mixed with methanolic DPPH solution. After incubation, absorbance was measured at 517 nm against a blank with lower absorbance indicating higher scavenging capacity (Rajput et al. 2023). The equation for calculating scavenging activity is as follows:

$$\text{Inhibition potential} = (A_c - A_s) / A_c \times 100,$$

where A_c is the absorbance of control and A_s is the absorbance of sample.

Ferric reducing antioxidant power assay (FRAP)

The FRAP assay was conducted using various concentrations of extracts and fractions ranging from 25–400 µg/ml. This assay assessed the ability of the extracts/fractions to convert potassium ferricyanide (Fe^{3+}) into potassium ferrocyanide (Fe^{2+}). This Fe^{2+} then reacts with ferric chloride, forming a ferric-ferrous complex measurable via spectrophotometry. Each concentration (1ml) was mixed with 2.5 ml of 1% potassium ferricyanide and 2.5 ml of phosphate buffer (200 mM, pH 6.6) and the mixture was incubated at 50 °C for 20 minutes. Eventually, 2.5ml of 10% TCA (trichloroacetic acid) was added and centrifuged at 3000 rpm for 10 minutes. Later, 2.5 ml of supernatant was mixed with 2.5ml of double distilled water and 0.5 ml of Ferric chloride (0.1%) was added and the absorbance was measured at 765 nm (Koffi et al. 2022). Ascorbic acid was used as the standard drug. Inhibition potential was calculated using the following formula:

$$\text{Ferricyanide reduction Potential} = \frac{\text{Absorbance of ascorbic acid} - \text{Absorbance of sample}}{\text{Absorbance of ascorbic acid}} \times 100.$$

Antidiabetic assay

Alpha-glucosidase inhibition assay

For the α -glucosidase inhibition assay, a microtiter 96-well plate was utilized. In this assay, wells of the plate were filled with a solution containing 50 µl of phosphate buffer (pH 6.8), 10 µl of α -glucosidase enzyme solution (1U/ml) and the extracts and fractions (25, 50, 100, 150, 200 and 250 µg/ml) were added and pre-incubated for 5 minutes at 37 °C (Wang and Zhao 2019). After the pre-incubation period, 20 µl of a 2 mmol/l solution of p-nitrophenol- α -D-glucopyranoside (pNPG) substrate from HiMedia was added. The mixture was then incubated at 37 °C for 30 minutes. Afterwards, 50 µl of sodium carbonate solution (100 mmol/l) was added to stop the reaction. Acarbose served as the reference compound. The conversion of pNPG into yellow-colored p-nitrophenol was determined by measuring the absorbance at 405nm using a spectrophotometer. The assay was conducted in triplicate along with appropriate blanks.

Alpha-amylase inhibition assay

The α -amylase enzyme inhibition assay was conducted using a microliter 96-well plate. A solution comprising 20% methanol, 25 µL of phosphate buffer (pH 6.9) and porcine α -amylase, was dispensed into each well of the plate, after which extracts and fractions (25–250 µg/ml) were added. The reaction mixture was incubated at 25 °C for 10 minutes. The enzymatic reaction was halted by adding 50 µL of 96 mM 3, 5-dinitrosalicylic acid (DNS) color reagent. The IC₅₀ values of the samples were determined and reported as the mean $\pm$ standard deviation of three independent experiments (Wang and Zhao 2019).

List of abbreviations

PIME: *Plumbago indica* Methanol Extract; **PICE:** *Plumbago indica* Chloroform Extract; **IEAF:** *Plumbago indica* Ethyl Acetate Fraction; **PIBF:** *Plumbago indica* n-Butanol Fraction; **STZ:** Streptozotocin; **NBT:** Nitro blue tetrazolium; **DTNB:** Dithio-bis 2-Nitrobenzoic acid; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **FRAP:** Ferric reducing antioxidant power assay; **DNS:** Dinitro salicylic acid; **IAEC:** Institutional Animal Ethics Committee; **CCSEA:** Committee for Control and Supervision of Experiments on Animals; **NA:** Nicotinamide; **CMC:** Carboxymethyl-cellulose; **SGOT:** Serum glutamic oxaloacetic transaminase; **SGPT:** Serum glutamate pyruvate transaminase; **ALP:** Alkaline phosphate; **HDL:** High-Density lipoproteins; **TBARS:** Thiobarbituric acid reactive substances; **SAG:** Superoxide anion generation; **GSH:** Glutathione.

In vivo study

Animals

Wistar rats (200-250 gram) were procured from Guru Nanak Dev University, Amritsar, Punjab, India. They were housed in the animal house with free access of water and laboratory pellet diet. The rats were exposed to normal cycle of light and dark. The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. The experimental protocol no. (226/CPCSEA/2023/22) of Guru Nanak Dev University, Amritsar was duly approved by the Institutional Animal Ethics Committee (IAEC) and animals were taken care of as per the guidelines of Committee for Control and Supervision of Experiments on Animals (CCSEA).

Acute toxicity study

The acute toxicity test for the most potent extract i.e methanol extract of *P. indica* (PIME) was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) 423 guidelines (OECD 2001). Before administering the dose, the animals were fasted overnight, with access to water only. The following morn-

ing, methanol extract was given orally at a dose of 2000 mg/kg. After dosing, the animals were allowed to resume normal access to food and water. Observations for signs of toxicity and behavioral changes were made every 24 hours over the course of 14 days to assess any potential mortality.

Induction of diabetes mellitus

Diabetes was induced in the rats through intraperitoneal injection of streptozotocin (STZ) and nicotinamide (NA) at doses of 50 and 110 mg/kg body weight respectively (Sadasivam and Manickam 1996). STZ was dissolved in freshly prepared citrate buffer at pH 4.5 and administered to overnight fasted rats, 15 minutes following intraperitoneal administration of nicotinamide at a dose of 110 mg/kg body weight. To prevent acute hypoglycemic conditions resulting from the release of a high concentration of insulin during beta islet destruction, rats were immediately provided with 10% dextrose in their drinking water for 24 hours post-STZ injection. Subsequently, rats were allowed access to regular food and drinking water. After one-week, fasting blood glucose levels were measured using a digital glucometer (BG-03 Gluco One Glucometer, Dr. Morepen) and animals showing blood glucose levels exceeding 200 mg/dl were selected for further experimentation.

The experimental animals received daily treatment with methanol extract of *P. indica* (PIME) at the doses of 100, 200 and 400mg/kg and ethyl acetate fraction of roots of *P. indica* (PIEAF) at the doses of 10, 20 and 40 mg/kg for 21 days, with 5mg/kg Gliclazide serving as the standard drug. After that blood samples were collected from fasted and anesthetized rats via retro-orbital sinus vein puncture and serum was obtained through centrifugation. Subsequently, all rats were euthanized and their kidneys and pancreas were harvested for analysis of biochemical, hematological and histopathological parameters.

Experimental design

Animals were divided into 9 groups (with n=6 in each group). Animals were treated with streptozotocin (STZ) and nicotinamide (NA) to induce diabetes. Diabetic rats were treated with Methanol extract of *P. indica* and its ethyl acetate fraction (PIME and PIEAF) in different doses orally which were prepared in 0.5% carboxymethylcellulose (CMC). STZ was dissolved in freshly prepared citrate buffer solution (pH=4.5) and gliclazide was used as standard drug. The experimental groups for animal studies were as follows:

- Normal control (n=6): rats received 0.5 ml of 0.5% CMC daily for 3 weeks.
- Diabetic control (n=6): rats received STZ (50 mg/kg) and NA (110 mg/kg) once and after the induction of diabetes, vehicle was administered daily for 3 weeks.
- Standard drug (n=6): diabetic rats of this group received gliclazide (5 mg/kg/day) for 3 weeks. Diabetic rats were administered daily with PIME suspended in CMC for 3 weeks at doses of 100, 200 and 400 mg/kg and PIEAF at doses of 10, 20 and 40 mg/kg.

After completion of the treatment period, rats were placed in an individual metabolic cage for 24h and kept fasting for 12h. The rats were anesthetized with sodium pentobarbital and blood was collected using retro-orbital puncture and then they were sacrificed. Both the kidneys and pancreas were harvested immediately and rinsed with KCl buffer solution (pH- 7.4). Serum was isolated from the collected blood by centrifugation at 5000×g using REMI C- 24 PLUS centrifuge for biochemical estimations. The harvested kidney and small part of pancreas were preserved in 10% formalin solution for histopathological investigations. Rest part of pancreas was homogenized (10% w/v) in potassium chloride solution with the help of a Teflon homogenizer, then centrifuged at 5000×g for 20 minutes to isolate the supernatant for estimation of oxidative stress biomarkers (Singh et al. 2020).

Evaluation of serum glucose, liver enzymes and lipid profiles

Glucose, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP), cholesterol, triglycerides, low density lipoproteins (LDL) and high-density lipoproteins (HDL) in serum were assessed using commercially available kits (Erba Diagnostics India) (Singh et al. 2022).

Renal functions parameters

The estimation of creatinine, urea and uric acid in serum was carried out using commercially available estimation kits (Erba Diagnostics India).

Estimation of oxidative stress parameters in pancreatic tissue

Oxidative stress markers were assessed in tissue homogenates of pancreas from each experimental group. The level of lipid peroxidation, a crucial indicator of oxidative damage, was quantified by measuring thiobarbituric acid reactive substances (TBARS). Superoxide anion generation (SAG) in the tissues was evaluated by assessing the reduction in nitro blue tetrazolium. Additionally, the concentration of reduced glutathione (GSH) in the tissue was determined spectrophotometrically (Ahmed et al. 2015). These analyses provided insights into the oxidative stress status and antioxidant capacity of the pancreatic tissues in each experimental group.

Hematoxylin and Eosin Staining

Preserved kidneys and pancreas were initially submerged in a 10% formalin solution before undergoing a series of preparatory steps. These included dehydration through successive ethanol concentration, followed by immersion in xylene and subsequent fixation in paraffin wax. The resulting tissue sections, with a thickness of four micrometers, were then mounted onto glass slides using commercial Bakers mounting fluid. To ensure proper adhesion, the slides were gently heated to melt the paraffin wax and then rinsed with xylene. Subsequent washing steps in-

volved washing with absolute alcohol and hydration with water. Finally, the sections were stained with hematoxylin and eosin (Rowley et al. 2019). The prepared slides were examined under a microscope for any gross histopathological changes.

High performance liquid chromatography (HPLC) of PIME

The methanol extract was analyzed on Agilent technology HPLC system, provided with a photodiode array (PDA) detector using C-18 (150 mm × 4.6 mm, i.d. 5 µm) column. The mobile phase consisting of methanol and water in the proportion of 3:1 v/v was used. The column was equilibrated with mobile phase, pumped at the flow rate of 0.8ml/min and the injection volume was 5 mL. For calibration curve, plumbagin was prepared as standard at different concentrations 1–20 µg/ml in ethanol as solvent. Both the standard and extract were performed in triplicate and the detection was monitored at the wavelength of 265 nm. Identification and quantification of plumbagin were performed based on retention time and peak area was analyzed using EZChrome Elite chromatography data system software (CDS) (Tiwari et al. 2021).

Molecular docking studies

Previously developed and validated homology model of α-glucosidase on isomaltase (*Saccharomyces cerevisiae*) template (PDB: 3A4A) was utilized to analyze plumbagin interactions within the most favorable binding site in α-glucosidase (Singh et al. 2024). Structure of plumbagin was drawn on ChemDraw Ultra (2013) and its energy was minimized by using MM2 (molecular mechanics) force field in Chem3D Ultra software. The prepared structure of plumbagin was protonated in aqueous solution using ligand preparation module in LeadIt and docked into defined binding site using FlexX docking module in LeadIt (Singh et al. 2022). All FlexX solutions yielded were scored using Consensus scoring function (CScore) and ranked accordingly. The top best pose with the highest score was selected for investigation of interactions, HYDE assessment and calculation of free energy of binding (ΔG). 2D and 3D enzyme-hybrid interactions were visualized using Discovery Studio Visualizer (Singh et al. 2023). Briefly, the prepared homology model on the isomaltase (*Saccharomyces cerevisiae*) template using SWISS-MODEL pipeline was verified by overlapping with the template crystallographic structure and visualizing binding site residues (including His111, Asp214, Glu276, His348, and Asp349) of the co-crystallized ligand alpha-D-glucopyranose. Binding site residues were devoid of any deviations. The model was further validated using PROCHECK, which showed 89.5% residues in most favorable regions in the generated Ramachandran plot (Suppl. material 1). The accuracy of the docking protocol was established by docking the co-crystallized parent template protein ligand isomaltose in the bind-

ing site, which generated ligand confirmation with a root mean square deviation (RMSD) value of 0.5614, indicating the reliability of the docking protocol.

Statistical analysis

All the results were calculated as mean ± S.E.M. Statistical evaluation was performed using Graph Pad Prism software version 9.5.1 by using one-way ANOVA. $P < 0.05$ was considered as significant.

Results

Preliminary phytochemical screening

The extracts prepared from the roots of *P. indica* were subjected to preliminary phytochemical screening in order to check the presence of particular class of components present. Methanol extract showed the presence of alkaloids, flavonoids, tannins and cardiac glycosides.

In vitro antioxidant studies

The antioxidant ability of methanol, chloroform extracts and the separated fractions ethyl acetate fraction (PIEAF) and n-butanol fraction (PIBF) of active methanol extract of *P. indica* roots were assessed using *in vitro* assays such as DPPH and ferricyanide ion reduction assay.

DPPH method

The inhibitory potential of the extracts and fractions from *P. indica* root was evaluated in DPPH assay for its radical scavenging activity was compared to ascorbic acid as standard. Ethyl acetate fraction of *P. indica* (PIEAF) showed the highest antioxidant potential with an IC₅₀ value of 121.86 µg/mL, followed by the methanol extract of *P. indica* (PIME) with an IC₅₀ of 148.25 µg/mL compared to ascorbic acid (IC₅₀ 53.10 µg/mL) as shown in Fig. 1. The results revealed that free radical scavenging activity of the PIME and PIEAF increased with an increase in the concentration.

Ferric-reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) assay was carried out at different concentrations of all extracts and fractions of *P. indica*. PIEAF and PIME with IC₅₀ value of 122.75 µg/mL and 134.56 µg/mL respectively, exhibited the highest reducing power, while PICE (IC₅₀ value 302.24 µg/mL) and PIBF (IC₅₀ 241.01 µg/mL) showed relatively weaker activities as shown in Fig. 2.

From these observations, it was concluded that among all the extracts and fractions, methanol extract and its fraction showed maximum antioxidant activity in DPPH and FRAP assays. Pharmacological reports have shown that many plants with antioxidant activity may help manage diabetes by mitigating oxidative stress, a key factor in the disease's progression, therefore further invitro antidia-

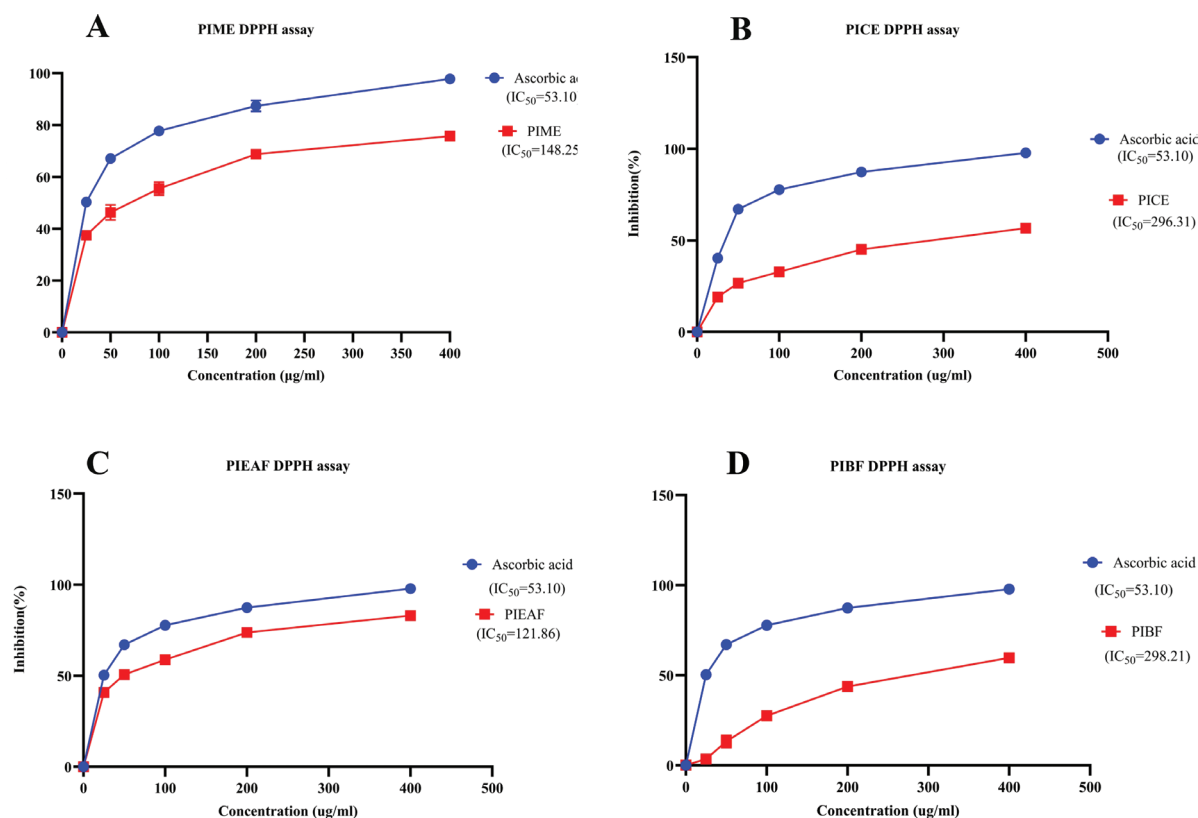


Figure 1. Percentage inhibition of DPPH free radical activity of extracts and fractions of *P.indica*: **A.** PIME (Methanol extract of *P. indica*); **B.** PICE (Chloroform extract of *P. indica*); **C.** PIEAF(ethyl acetate fraction of *P. indica*); **D.** PIBF (butanol fraction of *P. indica*) at different concentrations (25–400 µg/ml), compared with standard antioxidant ascorbic acid.

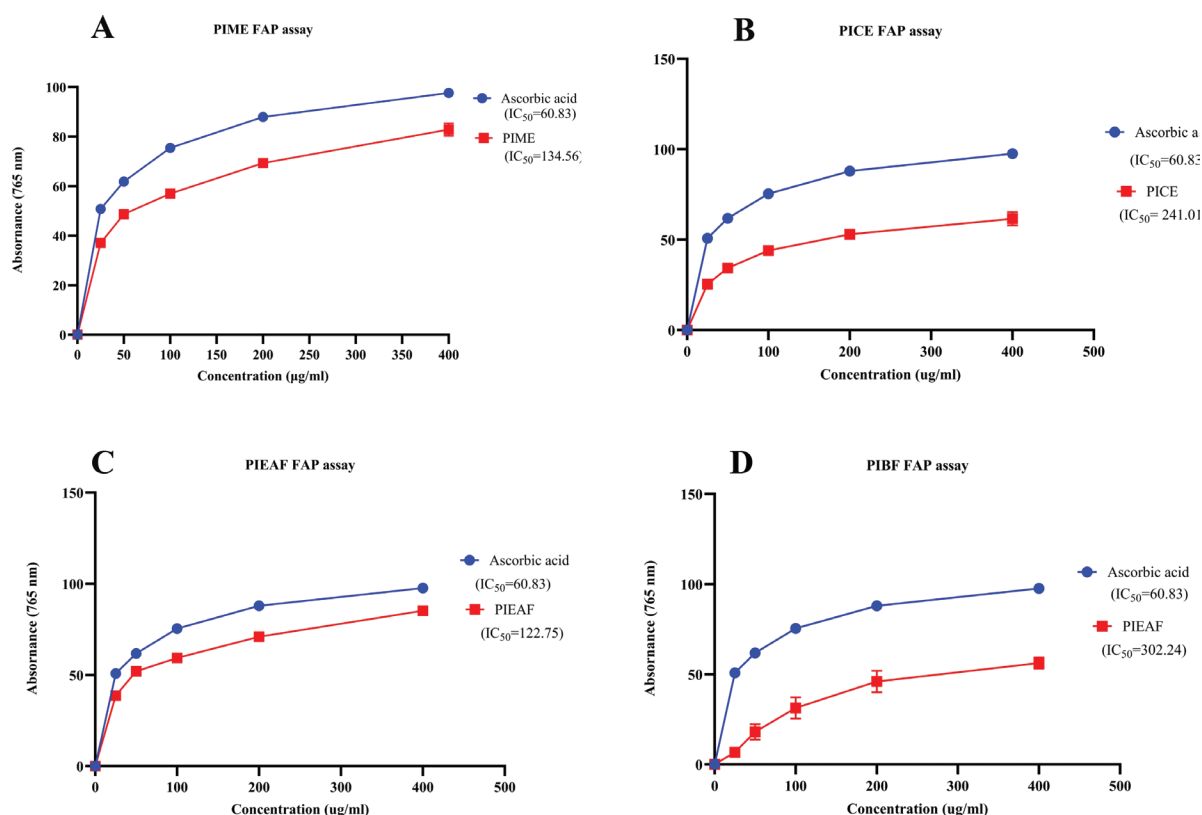


Figure 2. Percentage inhibition of hydroxyl radical scavenging activity of extracts and fractions of *P.indica*: **A.** PIME (Methanol extract of *P. indica*); **B.** PICE (Chloroform extract of *P. indica*); **C.** PIEAF(ethyl acetate fraction of *P. indica*); **D.** PIBF(butanol fraction of *P. indica*) at different concentrations (25–400 µg/ml), compared with ascorbic acid as antioxidant standard.

betic assays were done on extracts and fractions prepared from this plant to test its antihyperglycemic effect.

In vitro anti-diabetic studies

Alpha-glucosidase inhibition assay

P. indica extracts and fractions were tested for α -glucosidase inhibitory activity at doses ranging from 25 to 250 $\mu\text{g}/\text{mL}$. The standard drug used was acarbose ($\text{IC}_{50} = 70.92 \mu\text{g}/\text{mL}$). The results showed that the PIEAF exhibited the highest inhibitory effect ($\text{IC}_{50}=99.89 \mu\text{g}/\text{mL}$), followed by the PIME ($\text{IC}_{50}=109.82 \mu\text{g}/\text{mL}$). The butanol fraction (PIBF) and chloroform extract (PICE) showed minimum inhibition with $\text{IC}_{50} = 170.34 \mu\text{g}/\text{mL}$ and $\text{IC}_{50} = 214.71 \mu\text{g}/\text{mL}$ as represented in Fig. 3. PIEAF and PIME showed better α -glucosidase enzyme inhibition than PICE and PIBF.

Alpha-amylase inhibition assay

Alpha-amylase inhibitory activity of *P. indica* extracts and fractions was done at doses ranging from 25 to 250 $\mu\text{g}/\text{mL}$, using acarbose as the standard reference ($\text{IC}_{50} = 75.29 \mu\text{g}/\text{mL}$). The PIEAF showed the highest inhibition ($\text{IC}_{50} = 106.09 \mu\text{g}/\text{mL}$), followed by the PIME ($\text{IC}_{50} = 112.85 \mu\text{g}/\text{mL}$). The PICE and PIBF exhibited moderate inhibitory activity ($\text{IC}_{50} = 197.59 \mu\text{g}/\text{mL}$ and $213.56 \mu\text{g}/\text{mL}$) as shown in Fig. 4.

The results from the above assays indicated that the methanol extract of roots of this plant and its ethyl acetate fraction

possess notable hypoglycemic activity whereas its chloroform extract and butanol fraction was found to be less effective. Based on these aforementioned results, the bioactive extract and its fraction namely PIME and PIEAF were further subjected to in vivo testing using streptozotocin (STZ) and nicotinamide (NA) model of Type 2 diabetes in rats.

Acute toxicity studies

In acute toxicity studies, rats showed no signs of toxicity in the methanol extract of *P. indica* at a dose of 2000 mg/kg within the first 24 hours. Based on the OECD guidelines 423, a dose equal to one-tenth (200mg/kg) of this safe amount was chosen for experimental use, while lower (half, 100mg/kg) and higher (double, 400mg/kg) doses were used to evaluate any dose- dependent effects.

In-vivo evaluation of antidiabetic activity

The antidiabetic activity of methanol extract and its ethyl acetate fraction was investigated using STZ/NA induced diabetes in rats and various renal, hepatic and oxidative stress parameters were assessed in rats.

Serum glucose, liver enzymes and lipid profiles

The effects of PIME and PIEAF treatment on various biochemical parameters was assessed in STZ/NA induced diabetic rats. The diabetic control group had significantly higher levels of glucose, triglycerides, cholesterol, LDL, SGOT,

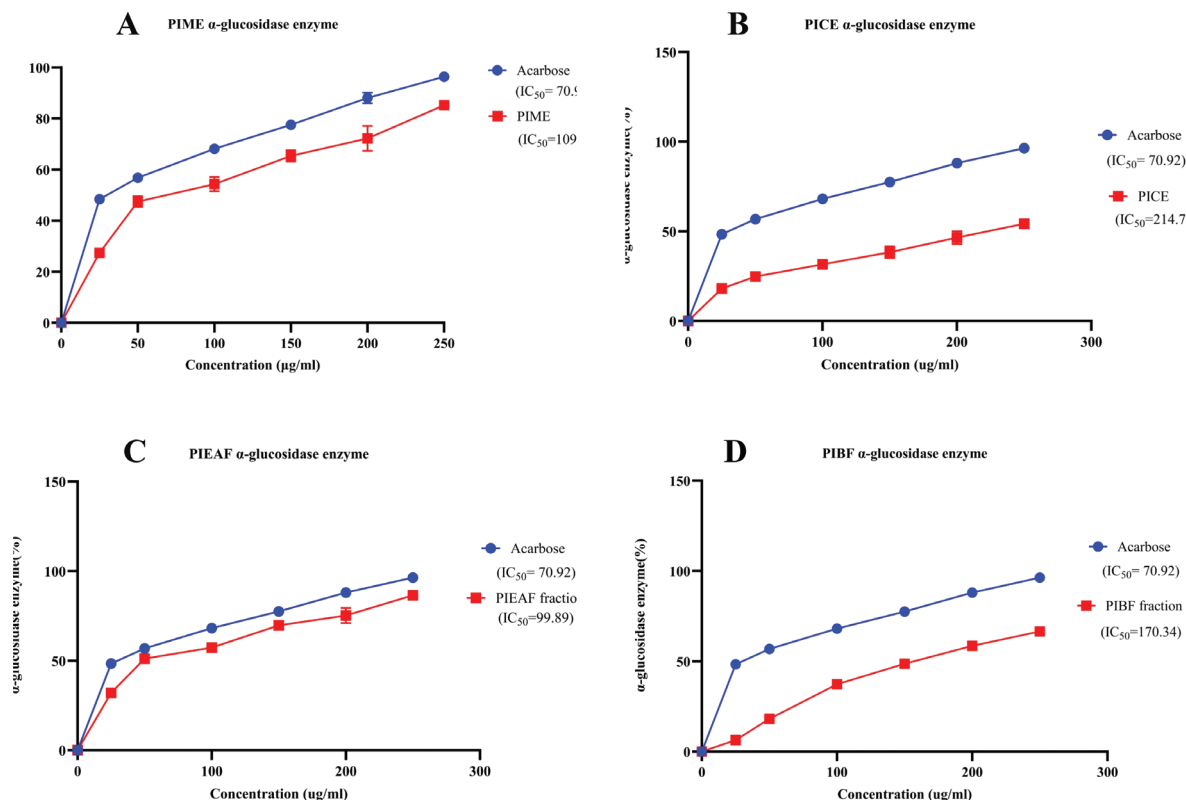


Figure 3. α -Glucosidase enzyme inhibition potential of extracts and fractions of *P. indica*: A. PIME (Methanol extract of *P. indica*); B. PICE (Chloroform extract of *P. indica*); C. PIEAF (ethyl acetate fraction of *P. indica*); D. PIBF (butanol fraction of *P. indica*) at different concentrations (25–250 $\mu\text{g}/\text{mL}$), compared with acarbose as standard.

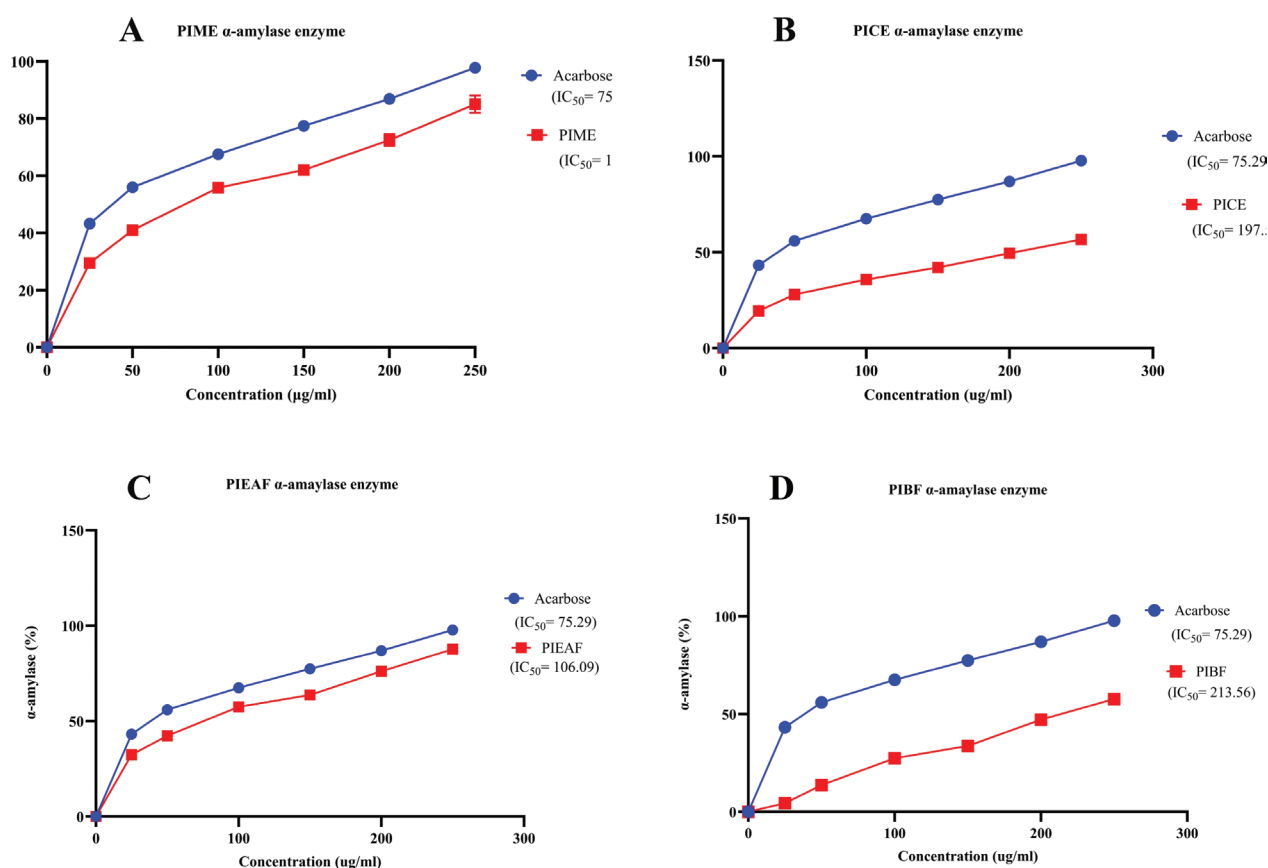


Figure 4. α- Amylase enzyme inhibition potential of extracts and fractions of *P. indica*: A. PIME; B. PICE; C. PIEAF; D. PIBF at different concentrations (25–250 μg/ml), compared with acarbose as standard.

SGPT and ALP, while also having lower HDL levels, compared to the normal control group. The treatment with PIME (100, 200 and 400 mg/kg) and PIEAF (10, 20 and 40 mg/kg) resulted in a significant reduction in glucose, triglycerides, cholesterol, LDL, SGOT, SGPT and ALP levels, in addition to a significant improvement in HDL levels, when compared to the diabetic control group as shown in Fig. 5A–H.

Among the doses evaluated, the higher dose of PIEAF (40 mg/kg) had the maximum effect on returning biochemical parameters to normal levels, suggesting its significant antihyperglycemic activity.

Renal functions and oxidative stress parameters

The effects of PIME and PIEAF on renal function and oxidative stress indicators in STZ/NA- induced diabetic rats was observed in this study. The diabetic control group had considerably higher levels of creatinine, urea, uric acid, TBARS (Thio barbituric Acid Reactive Substances) and SAG (Superoxide Anion Generation) as well as a significant decrease in GSH (Glutathione) as compared to the normal control group. When compared to the diabetic control, PIME (200 and 400 mg/kg) and PIEAF (20 and 40 mg/kg) significantly reduced the high levels of creatinine, urea, uric acid, TBARS and SAG while raising GSH levels in a dose-dependent manner Fig. 6A–G. Among the groups, the higher dose of PIEAF (40 mg/kg) exhibited significant improvement, demonstrating nephroprotective and antioxidant properties in diabetic rats.

Histopathological evaluation of tissues

Histopathological study of pancreatic tissue on STZ/NA-induced diabetic rats showed that the effects of PIME and PIEAF on islet cell morphology were dose-dependent. The control group exhibited pancreatic tissue with normal-situated islets of Langerhans (Fig. 7A). The diabetic control group showed extensive degeneration and distortion of islet cells, indicating serious pancreatic damage (Fig. 7B). The standard Gliclazide 5mg/kg drug treatment showed protection of islet cells (Fig. 7C). The groups treated with methanol extract of *P. indica* at 100 and 200mg/kg showed mild protective effects, but significant improvement was observed at the dose of 400 mg/kg, restoring islet structure (Fig. 7D–F). PIEAF-treated groups at lower dose showed minimal protection, but at the dose of 40 mg/kg significant improvement in islet cell structure was observed Fig. 7G–I.

These findings suggest that PIME and PIEAF may have dose-dependent protective effects in islets of Langerhans of pancreatic tissue which has been degenerated and distorted by diabetes and the maximum effect was observed at 40mg/kg dose of ethyl acetate fraction of *P.indica*.

After assessing the effect of different groups on pancreatic tissue, significant morphological differences were also observed in renal tissue of various groups. The normal control group had well-preserved glomeruli and intact tubules, indicating normal renal anatomy (Fig. 8A). The diabetic control group showed severe kidney injury, while the standard drug-treated group had typical glomerular

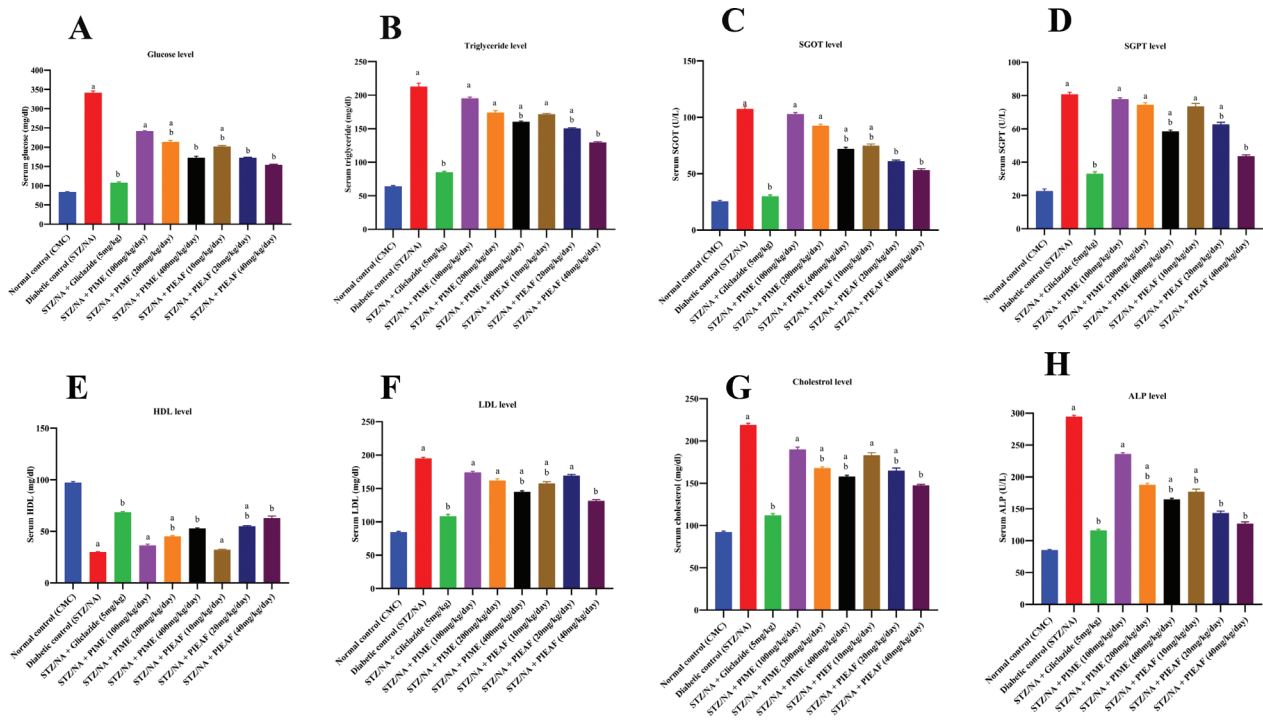


Figure 5. Effect of PIME and PIEAF on **A.** Glucose; **B.** Triglyceride; **C.** Cholesterol; **D.** HDL; **E.** LDL; **F.** SGOT; **G.** SGPT; **H.** ALP of diabetic rats. Data are expressed as mean $\pm$ SEM [n = 6 in each group], 'a' shows the mean significant difference when compared to the normal control group ($P < 0.05$). 'b' shows the mean significant difference when compared to the diabetic control group ($P < 0.05$).

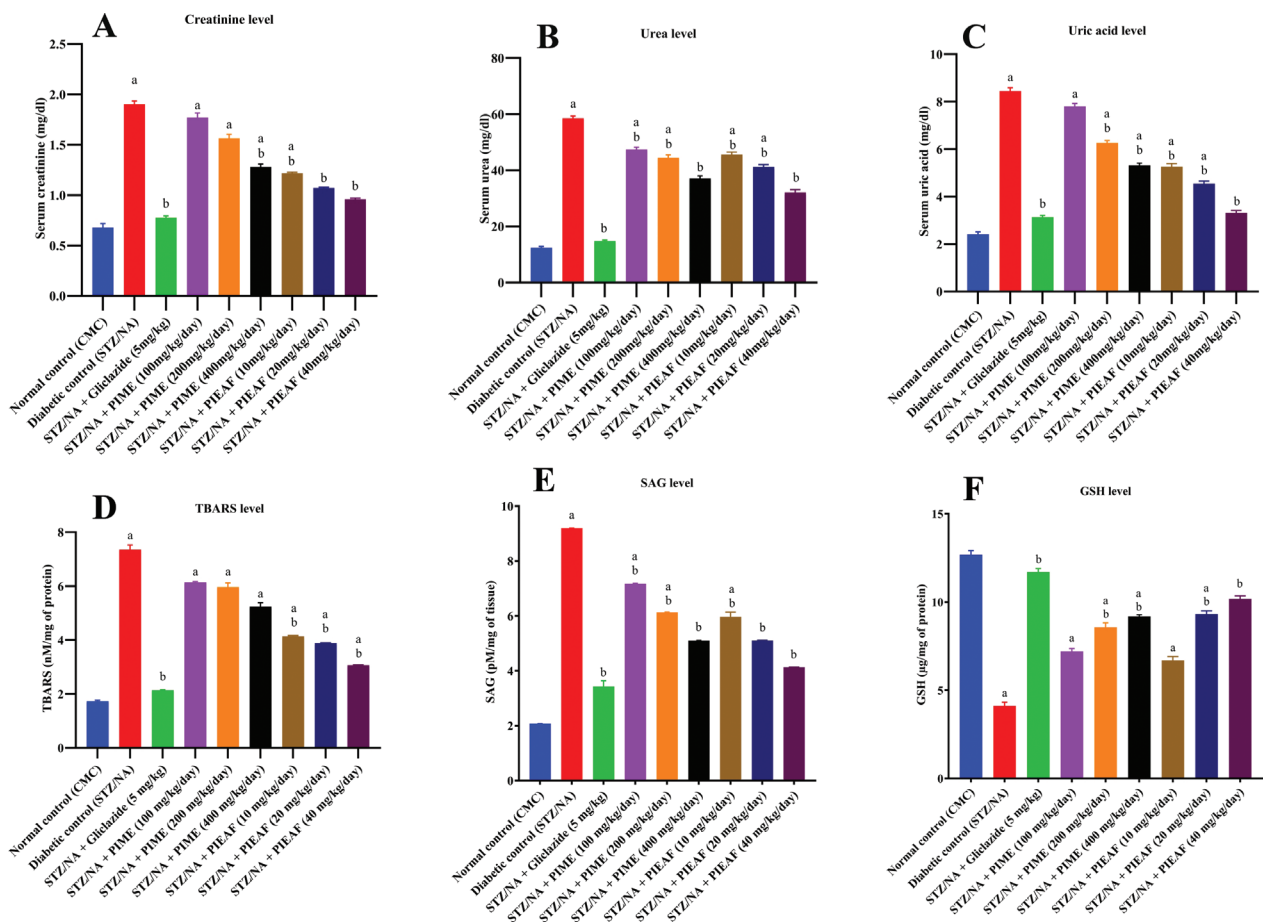


Figure 6. Effect of PIME and PIEAF on **A.** Creatinine; **B.** Urea; **C.** Uric acid; **D.** Bilirubin; **E.** TBARS (Thio barbituric acid reactive substances); **F.** SAG (Superoxide anion Generation); **G.** GSH (glutathione) in diabetic rats. Data are expressed as mean $\pm$ SEM [n = 6 in each group], 'a' shows the mean significant difference when compared to the normal control group ($P < 0.05$). 'b' shows the mean significant difference when compared to the diabetic control group ($P < 0.05$).

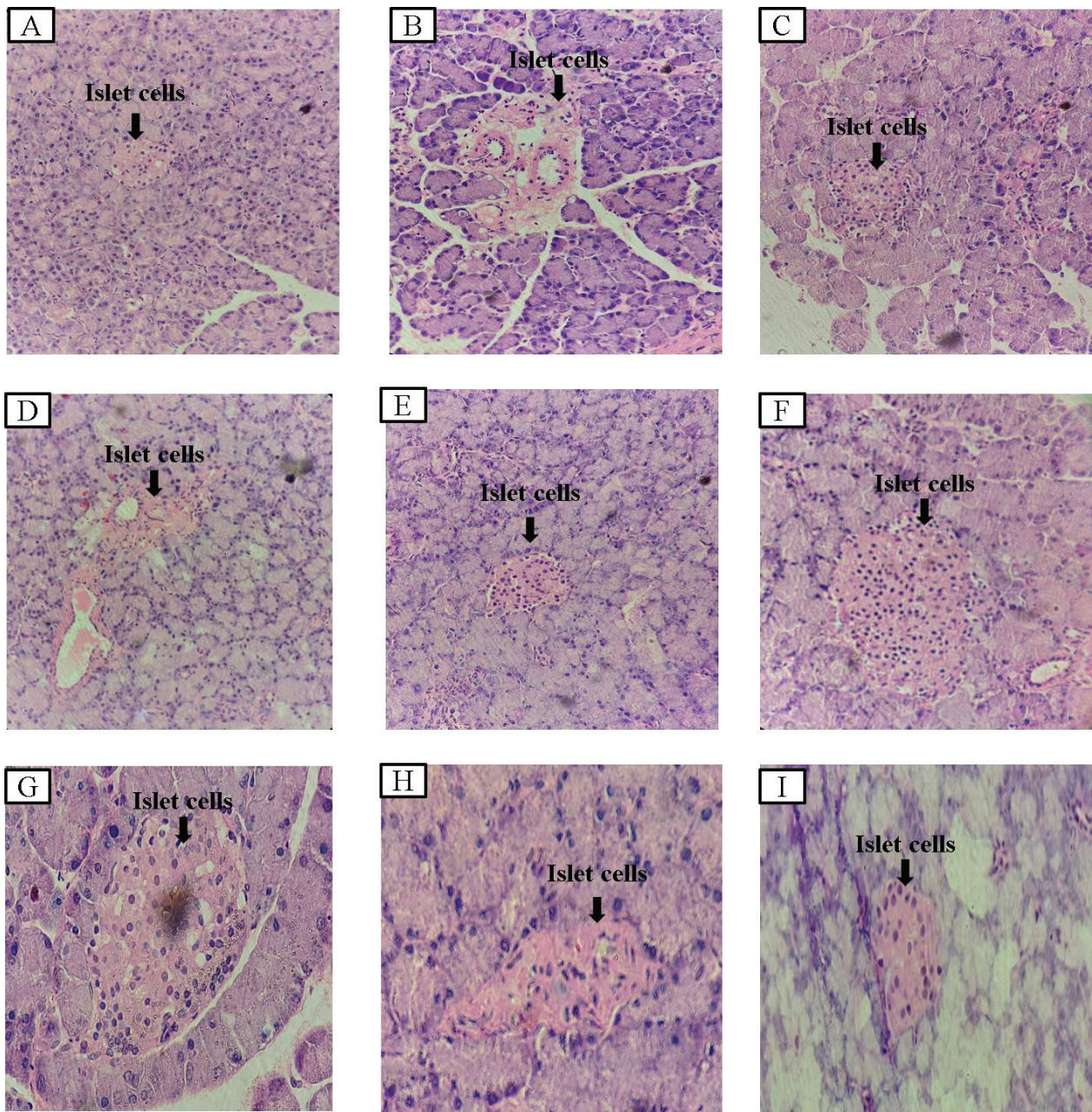


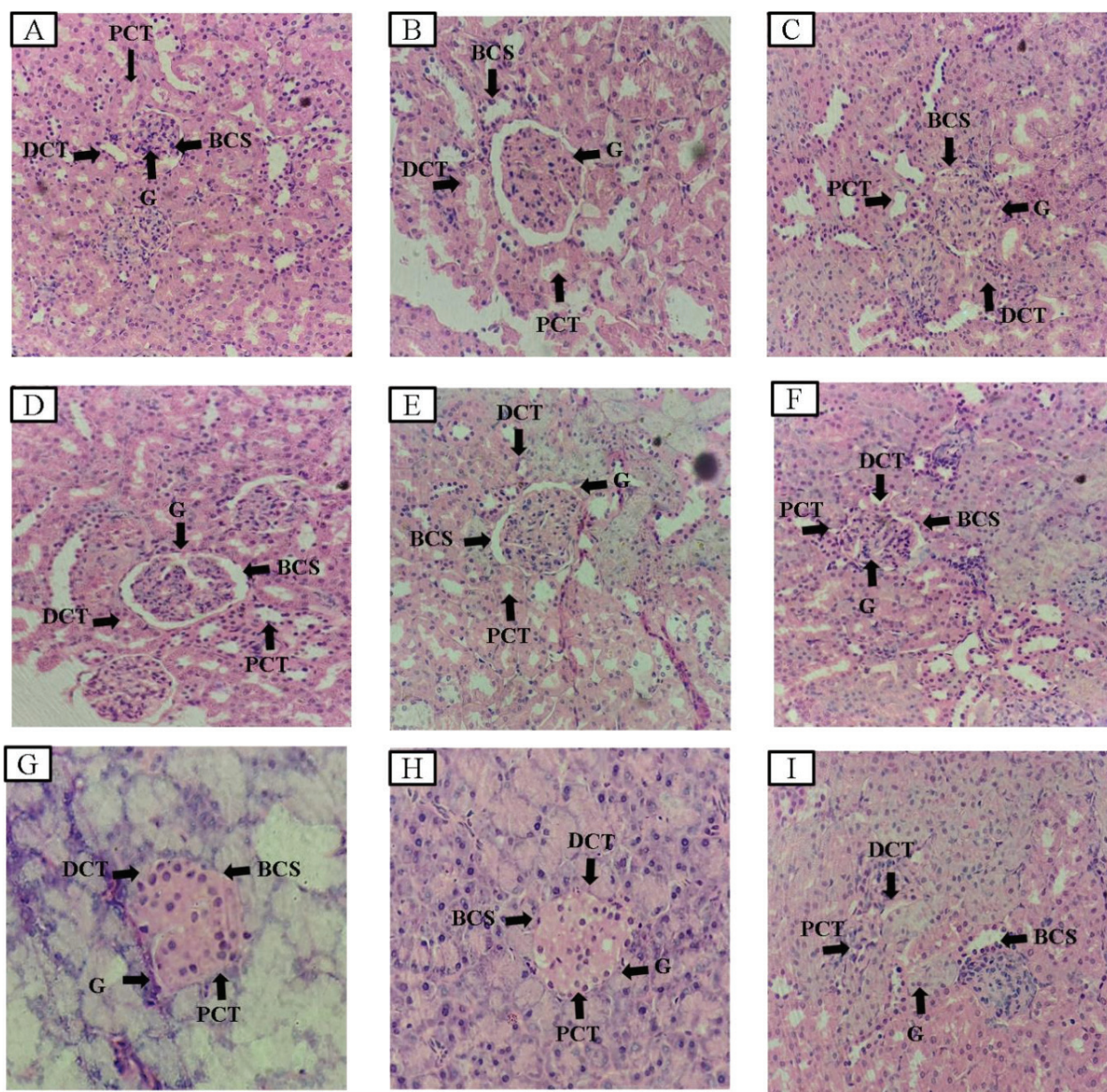
Figure 7. PIME and PIEAF **A.** Section showing pancreatic tissue with normally situated islets of Langerhans in normal group (H&E; 100×); **B.** Section showing degenerated islets of Langerhans in diabetic control group (H&E; 100×); **C.** Photomicrograph showing protection of islets of Langerhans in standard group; **D, E.** Section showing initiation of mild protection of islets cells in PIME treated (100 and 200 mg/kg) group (H&E; 100×); **F.** Photomicrograph showing initiation of moderate protection of islets cells in PIME (400 mg/kg) group; **G, H.** Sections showing partly protected islets cells in PIEAF treated (10, 20 mg/kg) group; **I.** Section exhibited significant protection of islets cells (PIEAF 40 mg/kg) (H&E; 100×).

structure and tubular morphology (Fig. 8B, C). PIME treatment at the doses of 100, 200 and 400mg/kg resulted in mild to moderate glomerular and tubular restoration (Fig. 8D, E, F), while PIEAF treatment at 10 and 20 mg/kg showed partial recovery of both glomeruli and tubules (Fig. 8G, H). The PIEAF treatment at the dose of 40 mg/kg significantly improved renal structure, demonstrating a dose-dependent nephroprotective effect (Fig. 8I).

The histopathological studies on pancreatic and renal tissues of rats subjected to various test groups revealed that the PIME and PIEAF possess antidiabetic and ne-

phroprotective potential as both the methanol extract and its fraction have shown protective effect in kidney and pancreatic tissues.

After observing the hypoglycemic effect methanol extract of the plant *P. indica*, further HPLC analysis of bioactive extract PIME was carried out to determine the content of major component Plumbagin in the bioactive extract. Plumbagin is the major bioactive compound found in the roots of the plant *Plumbago indica* and this natural naphthoquinone is responsible for many of the plant's reported pharmacological activities.



G-Glomerulus, BCS-Bowman Capsule Space, PCT-Proximal Convoluted Tubules, DC-Distal Convoluted Tubules

Figure 8. Histopathology of kidney tissue from different experimental groups A. Section showing normal glomerulus tubules in normal control group (H&E; 100×); B. Section showing degeneration of glomerulus and tubular necrosis in diabetic control group (H&E; 100×); C. Section showing the normal glomerulus in positive control (H&E; 100×) D, E. Sections showing mild glomerular and tubular changes in PIME treated (100, 200 mg/kg) group (H&E; 100×). F. Sections showing moderate protection in PIME treated (400 mg/kg) group; G, H. Sections showing partly protected glomerular and tubular tissue in PIEAF treated (100, 200 mg/kg) group; I. Section exhibited significant recovery of glomerular and tubular tissue (PIEAF 40 mg/kg) (H&E; 100×).

HPLC analysis of PIME

Methanol extract of *P. indica* roots was quantitatively determined for plumbagin content by HPLC method and the content of plumbagin in the extract was found to be 0.26%. The chromatograms of PIME and standard plumbagin are shown in Fig. 9. As plumbagin was found in high concentration in PIME, it can be considered responsible for the antidiabetic potential of plant *P. indica*.

After confirmation of antidiabetic effect of *P. indica* roots in in vitro and in vivo studies, further in silico studies were

done on bioactive component of the plant i.e. Plumbagin. In silico studies were designed to explore the binding affinity of the molecule with target protein which will help in virtual screening of biological activity and understanding of molecular mechanisms of the target molecule, plumbagin.

Molecular docking studies

Molecular docking studies were performed to get insight into various molecular interactions of plumbagin with α -glucosidase responsible for its inhibition. A previously

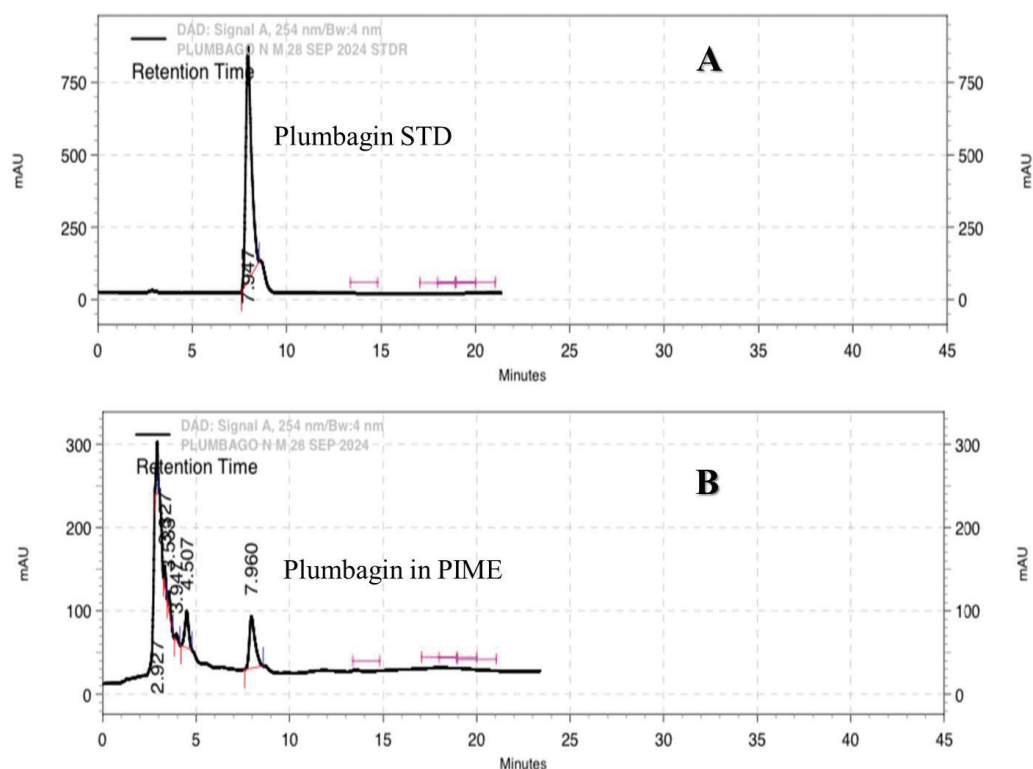


Figure 9. Representative HPLC chromatogram **A.** Plumbagin as standard; **B.** *Plumbago indica* methanol extract.

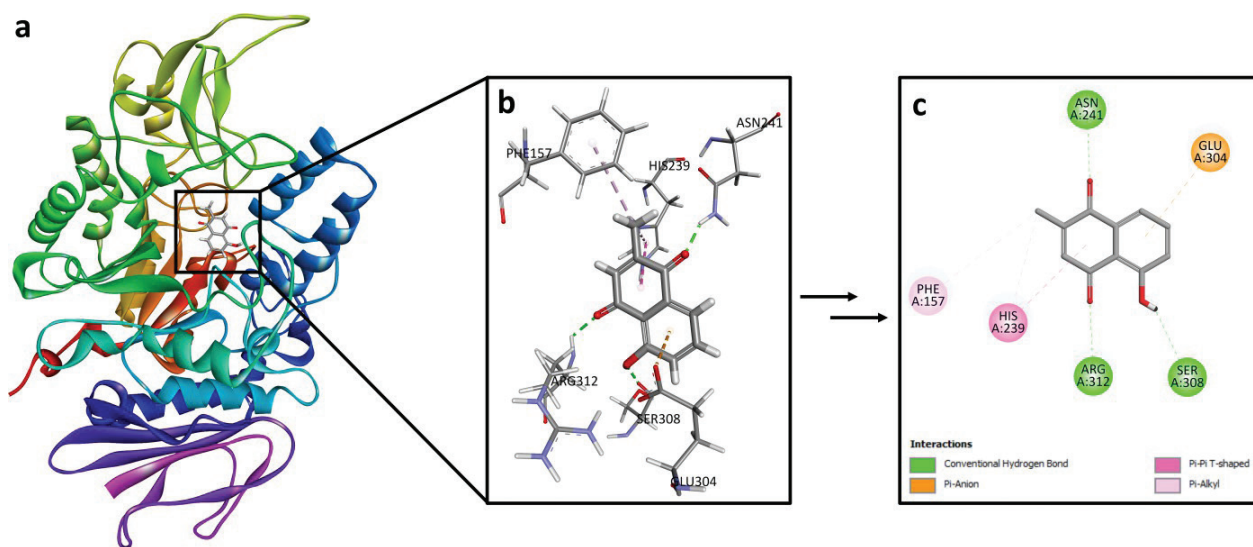


Figure 10. **A.** Plumbagin complex with α -glucosidase (PDB: 3A4A); **B.** 3D view of interactions of plumbagin with residues of the binding site of α -glucosidase; **C.** 2D view of interactions of plumbagin with the residues of the binding site of α -glucosidase.

developed and validated model was utilized in the analysis (Singh et al. 2024). The prepared and energy-minimized structure of plumbagin was docked into the defined binding site of α -glucosidase and the best pose with a docking score of -15.8913 was selected for discussion. Plumbagin docked in the most favorable binding site of the homology model of α -glucosidase from *Saccharomyces cerevisiae* based on the template of isomaltase from *Saccharomyces cerevisiae* (PDB: 3A4A) as shown in Fig. 10.

The overall binding pattern of plumbagin with residues of the binding site suggests that the hybrid molecule is well positioned in the cavity and properly stabilized by various favorable electrostatic interactions. Major interactions

with α -glucosidase include π -alkyl interaction, π -anion interaction, π - π T-shaped interaction and conventional hydrogen bond interaction. Plumbagin was found to be sandwiched between the Phe157, His239 and Asn241 on one side and Glu304, Ser308 and Arg312 on the other side. Both Phe157 and His239 showed π -alkyl interactions with the methyl substitution in plumbagin. The core nucleus of plumbagin showed π - π T-shaped interaction with His239 and π -anion interaction with Glu304. Both ketone groups in the structure made conventional hydrogen bond interactions with Asn241 (H-bond acceptor; with amine group: $d = 2.03$) and Arg312 (H-bond acceptor; with amine group: $d = 2.19$). Another conventional hydrogen

bond interaction of the hydroxyl group was observed with Ser308 (H-bond acceptor; with amine group: $d = 2.07$), all three presenting strongholds of plumbagin in the binding site. Thus, the overall study suggests that plumbagin is well equipped with small, rigid and planar groups making it the most eligible scaffold that seems to be able to satisfy the necessary pharmacophoric requirements of α -glucosidase inhibition hence making it a promising molecule in management of diabetes.

Discussion

The current study investigated the antidiabetic potential of *Plumbago indica* root extracts and its fractions using *in vitro*, *in vivo* and *in silico* assays. Initially, methanol and chloroform extract were prepared from *P. indica* roots. Then the methanol extract was fractionated into n-butanol (PIBF) and ethyl acetate (PIEAF) fractions.

Various extracts and fractions prepared from the roots of *P. indica* were then investigated for antioxidant activity using *in vitro* assays. The reducing power and free radical scavenging ability of the extracts and fractions were evaluated using the ferric-reducing antioxidant power (FRAP) assay and the DPPH free radical scavenging method (Andreadi et al. 2022; Melk et al. 2024). The results showed that IC₅₀ values of PIEAF were found to be 121.86 $\mu\text{g/mL}$ and 122.75 $\mu\text{g/mL}$ in the DPPH and FRAP assays, respectively, therefore PIEAF (ethyl acetate fraction of *P. indica*) demonstrated the highest level of antioxidant activity. However, among the extracts, PIME (methanol extract of *P. indica*) showed remarkable activity.

The secondary metabolites such as alkaloids, flavonoids, tannins and glycosides were found to be present in methanol extract after preliminary phytochemical screening. The antioxidant and antidiabetic potential of these secondary metabolites have been well established in various studies (Yaribeygi et al. 2020; Charlton et al. 2020). Recent studies have also shown that natural phenolic and flavonoid-rich extracts possess strong antioxidant capacity, which plays a key role in attenuating oxidative stress, a major factor contributing to β -cell dysfunction and insulin resistance in diabetes mellitus (Bungau et al. 2023; Bouslamti et al. 2023). Based on these reports, antioxidant potential of PIME and PIEAF can be attributed to the presence of phenolic compounds and flavonoids in them.

The *in vitro* α -glucosidase and α -amylase inhibition assays further demonstrated the antidiabetic potential of PIEAF and PIME. PIEAF showed IC₅₀ values of 99.89 $\mu\text{g/mL}$ and 106.09 $\mu\text{g/mL}$ against α -glucosidase and α -amylase, respectively. These enzyme inhibitory activities are important because they delay carbohydrate digestion and glucose absorption, thereby reducing postprandial hyperglycemia. Similar inhibitory activities have been reported for other medicinal plant extracts such as *Hawthorn fruit* and *Gymnema sylvestre*, which protect pancreatic β -cells while reducing carbohydrate breakdown. Hawthorn extract has been shown to inhibit pancreatic lipase, an enzyme involved in fat digestion, while *Gymnema syl-*

vestre can inhibit carbohydrate breakdown by inhibiting enzymes like α -amylase and α -glucosidase. Both plant extracts also possess antioxidant properties and help protect pancreatic β -cells from damage, although by different mechanisms, contributing to a potential antidiabetic effect (Ibrahim et al. 2017; Lien et al. 2023). Similar dual antioxidant and enzyme-inhibitory mechanism was observed in our study which suggests that PIEAF could provide superior glycemic control through its α -amylase and α -glucosidase inhibition compared to synthetic α -glucosidase inhibitors, which often cause gastrointestinal side effects.

Based on these *in vitro* assays, most bioactive extract and its fraction (PIME and PIEAF) were selected for *in vivo* studies using streptozotocin (STZ)/nicotinamide (NA)-induced diabetic rat model. Acute toxicity studies showed no adverse effects and mortality up to 2000 mg/kg of PIME, indicating a broad safety and confirming its appropriateness for long-term use in rats. In STZ-NA-induced diabetic rats, the biochemical parameters of type 2 diabetes were observed, which showed marked hyperglycemia, dyslipidemia, elevated liver enzymes and impaired renal function. After administration of PIME and PIEAF for 21 days, significant improvements were observed in fasting blood glucose levels, lipid profiles such as (triglycerides, cholesterol, LDL and HDL) and liver enzymes (SGOT, SGPT and ALP). PIEAF at 40 mg/kg showed significant amelioration of type 2 diabetes compared to all the tested doses, bringing a result of parameters near normal and outperforming the common antidiabetic medication gliclazide in certain tests. Such findings align with reports on other natural extracts, such as *Allium consanguineum* and *Rumex crispus*, which exhibited potent enzyme inhibition and glycemic regulation in diabetic models (Minh et al. 2019; Mahnashi et al. 2022). Compounds isolated from *A. consanguineum* showed potent inhibition of enzymes involved in carbohydrate metabolism and significantly reduced blood glucose levels in experimental models of diabetes. In a similar study, ethanolic extract of *Solanum torvum* fruits showed hypoglycemic, hypolipidemic and hepatoprotective activity in streptozotocin induced diabetic rats (Satyanarayana et al. 2022). The aforementioned studies also reported that the phytochemicals present in plants such as flavonoids, tannins can promote the regeneration of damaged pancreatic β -cells and normalize lipid profile and liver enzymes.

The protective effects of PIME and PIEAF on kidney function tests were also carried out in this study (Alanazi et al. 2020). Serum creatinine, urea and uric acid levels in diabetic control rats were markedly elevated, indicating renal impairment. These levels were markedly and dose-dependently decreased by PIME and PIEAF treatment at the dose of 400 and 40mg/kg respectively. Moreover, the diabetic control rats had significantly lower levels of reduced glutathione (GSH), a crucial antioxidant and higher levels of oxidative stress markers in pancreatic tissue, such as TBARS and superoxide anion generation (SAG). These patterns were reversed by treatment with the PIEAF, especially at higher dose (40mg/kg), indicating

a potent antioxidative effect *in vivo*. The antioxidant activity may decrease the oxidative stress in diabetic tissues and maintain pancreatic β -cell function. Similar nephro-protective and antioxidant activities have been reported for *Cenchrus purpureus* and mushroom polysaccharides, which mitigate oxidative stress and preserve renal functions (Arunachalam et al. 2022; Ojo et al. 2022). The phytochemicals present in these plants can increase the activity of antioxidant enzymes like SOD and CAT which results in reducing oxidative stress. This will further prevent lipid peroxidation; help prevent damage to kidney cells and preserve overall renal function.

Additionally, the confirmation of the protective effects of PIME and PIEAF has been seen biochemically by histopathological analysis. Significant islet cell damage, including degeneration and distortion, was seen in the pancreatic tissue of diabetic rats. Both PIME and PIEAF treatment especially at higher doses 400mg/kg and 40mg/kg, demonstrated islet structure preservation and regeneration, suggesting protective effects on β -cells. Similarly, glomerular and tubular damage was observed in diabetic kidney sections in diabetic control group, however, PIEAF treatment (40 mg/kg) led to restoration of normal renal structure. These findings corroborate the biochemical information and the nephroprotective as well as antidiabetic activity of methanol extract and its ethyl acetate fraction of *P. indica* roots. Comparable β -cell protective and nephroprotective effects have been demonstrated by extracts of plants *Crass-occephalum crepidioides* and *Ephedra alata*, highlighting the relevance of natural antioxidants in preventing structural damage of pancreas and kidneys caused by diabetes (Bahar et al. 2017; Tiss et al. 2022). Therefore, it can be inferred that the antioxidant property of PIME and PIEAF played an important role in restoring the histological architecture of the pancreas and kidneys in diabetic rats in this study.

Oral hypoglycemic agents are widely used in the treatment of T2DM but they can cause hypoglycemia, weight gain, fluid retention, heart failure, joint pain and pancreatitis. α -glucosidase inhibitors like acarbose may lead to abdominal pain, diarrhea, flatulence but *P. indica* can act as natural antioxidant and therefore be protective against oxidative damage caused by hyperglycemia and prevent the progression and development of diabetic complications (Dilworth et al. 2024).

After establishing the antidiabetic activity of *P. indica* roots, the content of the principal and bioactive component of this plant, plumbagin in PIME was determined by HPLC. Plumbagin, which is a bioactive naphthoquinone was found to be present in PIME, at a concentration of 0.26%. In a recent study, antioxidant and anti-inflammatory properties were exhibited by plumbagin. Since, increased oxidative stress and neuroinflammation are primarily associated with the onset of disorders including cancer and diabetes, plumbagin appears to be a viable therapeutic agent for the treatment or control of such chronic diseases due to its valuable inhibitory actions on oxidative stress and inflammation (Verma and Goyal 2024). Therefore, in our study, Plumbagin can be taken as

a probable contributor to the observed antidiabetic activity as established by its identification and quantification.

After the determination of marker compound in this plant, molecular docking studies were carried out to understand the mechanism of action of plumbagin in which a homology model of α -glucosidase was used. Plumbagin demonstrated a favorable docking score of -15.89 when it was docked into the active site of the enzyme. Phe157, His239, Asn241, Glu304, Ser308 and Arg312 were among the essential amino acid residues with which the molecule formed hydrogen bonds, π - π stacking, π -alkyl and π -anion interactions. The results of the docking study revealed that plumbagin was stabilized within the enzyme binding site by these interactions, indicating that it inhibited α -glucosidase activity through optimal orientation and strong molecular affinity.

Overall, the results of this investigation showed that *P. indica* roots, especially its ethyl fraction, have strong antioxidant and antidiabetic properties. These activities are probably mediated by reducing oxidative stress, protecting pancreatic and renal tissues and inhibiting enzymes that break down carbohydrates. HPLC and molecular docking studies have also confirmed that plumbagin plays a major role in these positive effects. The findings provide the scientific support for the traditional use of *P. indica* in the treatment of diabetes. To fully explore its therapeutic potential, more research is necessary, including formulation development and clinical evaluation.

Conclusion

In conclusion the present study highlights *Plumbago indica* roots promising antidiabetic potential, which is validated by *in vitro*, *in vivo* and *in silico* results. Key enzymes involved in the digestion of carbohydrates and the regulation of blood glucose, α -glucosidase and α -amylase, were significantly inhibited by the extract/fraction, especially PIEAF. The ability of the extract and fraction to scavenge free radicals was further demonstrated by the antioxidant assays, indicating their potential to reduce oxidative stress linked to diabetes. When *P. indica* extracts, particularly PIEAF 40mg/kg, were administered to streptozotocin/nicotinamide-induced diabetic rats *in vivo*, there was a significant decrease in blood glucose, cholesterol, triglycerides, LDL and hepatic enzymes, as well as an increase in HDL levels. Also, regenerative effects on renal and pancreatic tissues were demonstrated by histopathological analysis, suggesting tissue recovery and regeneration. Further, Plumbagin, a known bioactive compound, was found in the extracts, according to quantitative analysis. The molecular docking studies indicated that plumbagin interacts favorably with α -glucosidase, suggesting a potential mechanism of action. Overall, the potential of *P. indica* roots as natural antidiabetic agents is highlighted by their notable pharmacological activities, which support its traditional use. Further studies should be carried out on bioactive compound to determine its therapeutic efficacy and safety.

Acknowledgement

Authors are thankful to Department of Pharmaceutical Sciences, Guru Nanak Dev University, Amritsar, Punjab, India for providing the required facilities and assistance. Mr. Abdulkadir Abdu Mohammednor Ibrahim is thankful to ICCR (Indian Council for Cultural Relations) for providing scholarship.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: 226/CPCSEA/2023/22.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

Funding

No funding was reported.

Author contributions

A. A. M. I. performed the experimental design, data analysis and the first draft of the manuscript. B.S. contributed to the initial idea conception, manuscript preparation figure creation and critical review. H. B. helped with experimental study and data analysis. H.S. assisted with in vitro and in vivo studies. A.S. performed the molecular docking studies. B.S. reviewed and edited the manuscript for scientific accuracy and coherence. S. K. conceptualized and supervised the work. All authors reviewed the draft and approved the final version of the manuscript submitted.

Author ORCIDs

Abdulkadir Abdu Mohammednor Ibrahim  <https://orcid.org/0009-0003-7617-7574>

Hossamaldeen Bakrey  <https://orcid.org/0009-0002-6298-4992>

Hasandeep Singh  <https://orcid.org/0000-0001-9204-0883>

Atamjit Singh  <https://orcid.org/0000-0001-5439-8032>

Balbir Singh  <https://orcid.org/0000-0002-8174-062X>

Sarabjit Kaur  <https://orcid.org/0000-0002-7748-4336>

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

- Adu-Sarkodie NY (2017) Clinical management of diabetes mellitus in the older adult patient. *Current Diabetes Reviews* 13(3): 225–238. <https://doi.org/10.2174/1573399812666161206151706>
- Afzal T, Bibi Y, Ishaque M, Masood S, Qayyum A, Nisa S, Shah ZH, Alsamady H, Chung G (2022) Pharmacological properties and preliminary phytochemical analysis of *Pseudocaryopteris foetida* (D. Don) P.D. Cantino leaves. *Saudi Journal of Biological Sciences* 29(2): 1185–1190. <https://doi.org/10.1016/j.sjbs.2021.09.048>
- Ahmed D, Kumar V, Verma A, Shukla GS, Sharma M (2015) Antidiabetic, antioxidant and antihyperlipidemic effect of extract of *Euryale ferox* Salisb. with enhanced histopathology of pancreas, liver and kidney in streptozotocin-induced diabetic rats. *Springer Plus* 4: 1–10. <https://doi.org/10.1186/s40064-015-1059-7>
- Alanazi AZ, Alqahtani F, Mothana RAA, Mohany M, Abuhashish HM, Ahmed MM, Al-Rejaie SS (2020) Protective role of *Loranthus regularis* against liver dysfunction, inflammation and oxidative stress in streptozotocin diabetic rat model. *Journal of Evidence-Based Complementary and Alternative Medicine* 2020: e5027986. <https://doi.org/10.1155/2020/5027986>
- Amschler DH (2002) The alarming increase of type 2 diabetes in children. *Journal of School Health* 72(1): 39–41. <https://doi.org/10.1111/j.1746-1561.2002.tb06510.x>
- Andreadi A, Bellia A, Di Daniele N, Meloni M, Lauro R, Della-Morte D, Lauro D (2022) The molecular link between oxidative stress, insulin resistance and type 2 diabetes: a target for new therapies against cardiovascular diseases. *Current Opinion in Pharmacology* 62: 85–96. <https://doi.org/10.1016/j.coph.2021.11.010>
- Ansari P, Samia JF, Khan JT, Rafi MR, Rahman MS, Rahman AB, Abdel-Wahab YHA, Seidel V (2023) Protective effects of medicinal plant-based foods against diabetes: a review on pharmacology, phytochemistry and molecular mechanisms. *Nutrients* 15(14): e3266. <https://doi.org/10.3390/nu15143266>
- Arunachalam K, Sreeja PS, Yang X (2022) The antioxidant properties of mushroom polysaccharides can potentially mitigate oxidative stress, beta-cell dysfunction and insulin resistance. *Frontiers in Pharmacology* 13: e874474. <https://doi.org/10.3389/fphar.2022.874474>
- Bahar E, Akter K-M, Lee G-H, Lee H-Y, Rashid H-O, Choi M-K, Bhattarai KR, Hossain MMM, Ara J, Mazumder K, Raihan O, Chae H-J, Yoon H (2017) β -Cell protection and antidiabetic activities of *Crassocephalum crepidioides* (Asteraceae) Benth. S. Moore extract against alloxan-induced oxidative stress via regulation of apoptosis and reactive oxygen species (ROS). *BMC Complementary and Alternative Medicine* 17(1): e179. <https://doi.org/10.1186/s12906-017-1697-0>
- Bungau SG, Vesa CM, Bustea C, Purza AL, Tit DM, Brisc MC, Radu AF (2023) Antioxidant and hypoglycemic potential of essential oils in diabetes mellitus and its complications. *International Journal of Molecular Sciences* 24(22): e16501. <https://doi.org/10.3390/ijms242216501>
- Bouslamti M, Loukili EH, Elrherabi A, El Moussaoui A, Chebaibi M, Bencheikh N, Benjelloun AS (2023) Phenolic profile, inhibition of α -amylase and α -glucosidase enzymes, and antioxidant properties of

- Solanum elaeagnifolium* Cav. (Solanaceae): In vitro and in silico investigations. *Processes* 11(5): e1384. <https://doi.org/10.3390/pr11051384>
- Charlton A, Garzarella J, Jandeleit-Dahm KAM, Jha JC (2020) Oxidative stress and inflammation in renal and cardiovascular complications of diabetes. *Biology* 10(1): 1–18. <https://doi.org/10.3390/biology10010018>
- Dilworth L, Stennett D, Facey A, Omoruyi F, Mohansingh S, Omoruyi F (2024) Diabetes and the associated complications: The role of antioxidants in diabetes therapy and care. *Biomedicine & Pharmacotherapy* 181: e117641. <https://doi.org/10.1016/j.biopha.2024.117641>
- Eldhose B, Notario V, Latha (2013) Evaluation of phytochemical constituents and in vitro antioxidant activities of *Plumbago indica* root extracts. *Journal of Pharmacognosy and Phytochemistry* 2: 157–161.
- Galicía-García U, Benito-Vicente A, Jebari S, Larrea-Sebal A, Siddiqi H, Uribe KB, Ostolaza H, Martín C (2020) Pathophysiology of type 2 diabetes mellitus. *International Journal of Molecular Sciences* 21(17): e6275. <https://doi.org/10.3390/ijms21176275>
- Hillier TA, Pedula KL (2001) Characteristics of an adult population with newly diagnosed type 2 diabetes. *Diabetes Care* 24(9): 1522–1527. <https://doi.org/10.2337/diacare.24.9.1522>
- Ibrahim A, Babandi A, Sani AH, Wudil AM, Murtala Y, Umar IA (2017) HPLC profile, in vitro alpha-amylase, alpha-glucosidase inhibitory and antioxidant activities of *Gymnema sylvestre* ethyl acetate leaf extract. *Bayero Journal of Pure and Applied Sciences* 10(1): 72–80. <https://doi.org/10.4314/bajopas.v10i1.11>
- Ittiyavirah SP, Jobin KV, Jissa MS, Jomy M, Josmi TJ, Littin B (2012) Anti-inflammatory and analgesic activities of *Plumbago capensis* and *Plumbago indica*. *Advances in Pharmacology and Toxicology* 13(1): 1–47.
- Joseph T, Vadasseri JC (2023) Diabetes – a silent killer: a threat for cardiorespiratory fitness. *IntechOpen*. <https://doi.org/10.5772/intechopen.108164>
- Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Kaabi JA (2019) Epidemiology of type 2 diabetes – global burden of disease and forecasted trends. *Journal of Epidemiology and Global Health* 10(1): e107. <https://doi.org/10.2991/jegh.k.191028.001>
- Khin PP, Lee JH, Jun HS (2023) Pancreatic beta-cell dysfunction in type 2 diabetes. *European Journal of Inflammation* 21: e1721727X2311541. <https://doi.org/10.1177/1721727X231154152>
- Koffi N, Ernest AK, Benedicta DA, George NE, Jules B, Rachel E, Justin KK (2022) Analysis of the phenolic compounds and antioxidant activities of extracts of *Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). *Medical Sciences and Pharmacy* 1(2): 157–168. <https://doi.org/10.1016/j.msphr.2022.04.007>
- Lien HM, Lin HT, Huang SH, Chen YR, Huang CL, Chen CC, Chyau CC (2023) Protective effect of hawthorn fruit extract against high fructose-induced oxidative stress and endoplasmic reticulum stress in pancreatic β -cells. *Foods* 12(6): e1130. <https://doi.org/10.3390/foods12061130>
- Lenora RDK, Dharmadasa RM, Abeysinghe DC, Arawawala LDAM (2012) Investigation of plumbagin content in *Plumbago indica* Linn. grown under different growing systems. *Pharmacologia* 3(2): 57–60. <https://doi.org/10.5567/pharmacologia.2012.57.60>
- Melk MM, Melek FR, El-Sayed AF (2024) Enzymes inhibitory capabilities of phenolics from *Plumbago indica* L. and *Plumbago auriculata* Lam.: in vitro studies and molecular docking. *Process Biochemistry* 136: 1–13. <https://doi.org/10.1016/j.procbio.2023.11.011>
- Mishra AK, Pandey M, Pannu A, Dewangan HK, Sahoo PK (2024) Review on diabetes mellitus: an insight into the current scenarios, the challenges of therapy and application of traditional drugs. *Current Traditional Medicine* 10(3): e0666230501212125. <https://doi.org/10.2174/2215083810666230501212125>
- Mishra V, Nayak P, Sharma M, Albutti A, Alwashmi AS, Aljasir MA, Alsowayeh N, Tambuwala MM (2021) Emerging treatment strategies for diabetes mellitus and associated complications: an update. *Pharmaceutics* 13(10): e1568. <https://doi.org/10.3390/pharmaceutics13101568>
- Mahnashi MH, Alqahtani YS, Alqarni AO, Alyami BA, Alqahtani OS, Jan MS, Hussain F, Islam ZU, Ullah F, Ayaz M, Abbas M, Rashid U, Sadiq A (2022) Phytochemistry, anti-diabetic and antioxidant potentials of *Allium consanguineum* Kunth. *BMC Complementary Medicine and Therapies* 22(1): e154. <https://doi.org/10.1186/s12906-022-03639-5>
- Minh TN, Van TM, Andriana Y, Vinh LT, Hau DV, Duyen DH, Guzman-Gelani CD (2019) Antioxidant, xanthine oxidase, α -amylase and α -glucosidase inhibitory activities of bioactive compounds from *Rumex crispus* L. root. *Molecules* 24(21): e3899. <https://doi.org/10.3390/molecules24213899>
- Mukhtar Y, Galalain A, Yunusa U (2020) A modern overview on diabetes mellitus: a chronic endocrine disorder. *European Journal of Biology* 5(2): 1–14. <https://doi.org/10.47672/ejb.409>
- Ojo OA, Oni AI, Grant S, Amanze J, Ojo AB, Taiwo OA, Maimako RF, Evbuomwan IO, Iyobhebhe MCO, Osemwegie O, Agboola AO, Akintayo C, Asogwa NT, Aljarba NH, Alkahtani S, Mostafa-Hedeab G, Batiha GE-S, Adeyemi OS (2022) Antidiabetic activity of elephant grass (*Cenchrus purpureus* (Schumach.) Morrone) via activation of PI3K/Akt signaling pathway, oxidative stress inhibition, and apoptosis in Wistar rats. *Frontiers in Pharmacology* 13: e845196. <https://doi.org/10.3389/fphar.2022.845196>
- Priyanjani HSA, Senarath RMUS, Senarath WTPSK, Munasinghe MLMS (2021) Propagation, phytochemistry and pharmacology of *Plumbago indica* – a review. *Journal of Pharmaceutical Research International* 33(42B): 188–202. <https://doi.org/10.9734/jpri/2021/v33i42B32439>
- Rajput A, Sharma P, Kumar N, Kaur S, Arora S (2023) Neuroprotective activity of novel phenanthrene derivative from *Grewia tiliifolia* by in vitro and in silico studies. *Scientific Reports* 13: 1–12. <https://doi.org/10.1038/s41598-023-29446-7>
- Rajput A, Sharma P, Kumar N, Singh H, Singh T, Singh S, Bedi PMS, Singh B, Arora S, Kaur S (2023) Anticonvulsant potential of *Grewia tiliifolia* in pentylenetetrazole-induced epilepsy: insights from in vivo and in silico studies. *Metabolic Brain Disease* 38: 2355–2367. <https://doi.org/10.1007/s11011-023-01252-0>
- Sadasivam S, Manickam A (1996) *Biochemical methods* (2nd Edn.). New Age International (P) Ltd., India, 184–185.
- Satin LS, Soleimanpour SA, Walker EM (2021) New aspects of diabetes research and therapeutic development. *Pharmacological Reviews* 73(3): 1001–1015. <https://doi.org/10.1124/pharmrev.120.000160>
- Satyanarayana N, Chinni SV, Gobinath R, Sunitha P, Sankar AU, Muthuvenkatachalam BS (2022). Antidiabetic activity of *Solanum torvum* fruit extract in streptozotocin-induced diabetic rats. *Frontiers in Nutrition* 9: 1–11. <https://doi.org/10.3389/fnut.2022.987552>
- Sharma B, Dhiman C, Hasan GM, Shamsi A, Hassan MI (2024) Pharmacological features and therapeutic implications of plumbagin in cancer and metabolic disorders: A narrative review. *Nutrients* 16: e3033. <https://doi.org/10.3390/nu16173033>
- Silva NR, Gonçalves CET, Gonçalves DLN, Cotta RMM, Da Silva LS (2021) Association of uric acid and uric acid to creatinine ratio with chronic kidney disease in hypertensive patients. *BMC Nephrology* 22: e2521. <https://doi.org/10.1186/s12882-021-02521-9>

- Singh A, Bhagat M, Kaur S, Arora D, Walia R, Singh B (2023) Ameliorative effects of *Grewia asiatica* leaves in animal models of pain and inflammation. *Journal of Herbs, Spices and Medicinal Plants* 29(1): 63–72. <https://doi.org/10.1080/10496475.2022.2089429>
- Singh A, Kumar S, Bhagat M, Kaur S, Arora D, Walia R, Singh B (2022) Design, synthesis and biological evaluation of isatin indole-3-carboxaldehyde hybrids as a new class of xanthine oxidase inhibitors. *Archives of Pharmacy* 355(6): e2200033. <https://doi.org/10.1002/ardp.202200033>
- Singh A, Kumar S, Kumar D, Walia R, Arora D, Bhagat M, Kaur S, Singh B (2024) Discovery of triazole tethered thymol/carvacrol-coumarin hybrids as a new class of α -glucosidase inhibitors with potent in vivo antihyperglycemic activities. *European Journal of Medicinal Chemistry* 263: e115948. <https://doi.org/10.1016/j.ejmech.2023.115948>
- Singh B, Kumar A, Singh H, Kaur S, Arora S, Singh B (2022) Protective effect of vanillic acid against diabetes and diabetic nephropathy by attenuating oxidative stress and upregulation of NF- κ B, TNF- α and COX-2 proteins in rats. *Phytotherapy Research* 36: 1338–1352. <https://doi.org/10.1002/ptr.7392>
- Singh H, Singh R, Kaur S, Arora R, Mannan R, Buttar HS, Arora S, Singh B (2020) Protective role of *Phyllanthus fraternus* in alloxan-induced diabetes in rats. *Journal of Ayurveda and Integrative Medicine* 11: 391–398. <https://doi.org/10.1016/j.jaim.2019.09.008>
- Singh H, Singh R, Singh A, Singh H, Singh G, Kaur S, Singh B (2023) Role of oxidative stress in diabetes-induced complications and their management with antioxidants. *Archives of Physiology* 1: 1–26. <https://doi.org/10.1080/13813455.2023.2243651>
- Tasnim J, Hashim NM, Han HC (2024) A comprehensive review on potential drug–drug interactions of proton pump inhibitors with anti-diabetic drugs metformin and DPP-4 inhibitors. *Cell Biochemistry and Function* 42(2): e3967. <https://doi.org/10.1002/cbf.3967>
- Tiwari R, Kumar A, Solanki P, Dhobi M, Sundaresan V, Kalaiselvan V, Raghuvanshi RS (2021) Analytical quality-by-design (AQbD) guided development of a robust HPLC method for the quantification of plumbagin from *Plumbago* species. *Journal of Liquid Chromatography & Related Technologies* 44(11–12): 529–537. <https://doi.org/10.1080/10826076.2021.1973027>
- Tiss M, Souiy Z, Achour L, Hamden K (202) *Ephedra alata* extracts exert anti-obesity, anti-hyperglycemia, anti-pyretic and analgesic effects. *Nutrition and Food Science* 52(1): 119–128. <https://doi.org/10.1108/NFS-03-2021-0086>
- Wang KJ, Zhao JL (2019) Corn silk (*Zea mays* L.), a source of natural antioxidants with α -amylase, α -glucosidase, advanced glycation and diabetic nephropathy inhibitory activities. *Biomedicine & Pharmacotherapy* 110: 510–517. <https://doi.org/10.1016/j.biopha.2018.11.126>
- Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A (2020) Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative Medicine and Cellular Longevity* 9: e8609213. <https://doi.org/10.1155/2020/8609213>
- Verma A, Goyal A (2024) Unravelling the potent anti-oxidant and anti-inflammatory actions of plumbagin: A review of preclinical discoveries. *Pharmacological Research – Modern Chinese Medicine* 10: 1–12. <https://doi.org/10.1016/j.prmcm.2023.100351>

Supplementary material 1

Supplementary figure

Authors: Abdulkadir Abdu Mohammednor Ibrahim, Brahmajot Singh, Hossamaldeen Bakrey, Hasandeep Singh, Atamjit Singh, Balbir Singh, Sarabjit Kaur

Data type: png

Explanation note: Ramachandran Plot.

Copyright notice: This dataset is made available under the Open Database License (<http://opendatacommons.org/licenses/odbl/1.0>). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: <https://doi.org/10.3897/pharmacia.72.e165113.suppl1>