



Phytochemical Composition, Antihyperglycaemic Activity, and Acute Oral Toxicity of Aqueous and Ethanolic *Gossypium Herbaceum* Leaf Extracts in Diabetic Wistar Rats

Bada, Sunday. O.*

Biochemistry and Toxicology Unit, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, P.M.B 1019, Owo Ondo State, Nigeria
Email: solubada@gmail.com

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ABSTRACT

Gossypium herbaceum L. (Malvaceae) has a history of medicinal use for the management of various ailments, but experimental evidence supporting the antidiabetic potential and safety of its leaves remains limited.

Aim of the study: This study evaluated the phytochemical composition, antihyperglycaemic activity, and acute oral toxicity of aqueous and 70% ethanolic leaf extracts of *G. herbaceum* using complementary in vitro and in vivo approaches.

Materials and Methods: Mature *G. herbaceum* leaves were collected, authenticated, and extracted separately with distilled water and 70% ethanol by maceration. Qualitative phytochemical screening was performed using standard procedures. Diabetes was induced in male Wistar rats by a single intraperitoneal injection of streptozotocin (40 mg/kg). Thirty rats were randomly allocated to five groups (n = 6): normal control, diabetic control, glibenclamide (5 mg/kg), aqueous extract (400 mg/kg), and 70% ethanolic extract (400 mg/kg). Treatments were administered orally once daily for 28 days. Fasting blood glucose (FBG) and body weight were monitored during treatment. Acute oral toxicity was evaluated in female rats according to OECD Test Guideline 423 at a limit dose of 2000 mg/kg, with mortality, clinical signs, and body-weight changes recorded over 14 days.

Results: Both extracts contained flavonoids, alkaloids, tannins, saponins, and phenolic constituents, with comparatively stronger qualitative reactions observed for several phytochemical classes in the ethanolic extract. Streptozotocin administration produced pronounced hyperglycaemia and body-weight loss relative to the normal control. Following 28 days of treatment, FBG decreased significantly to 180 ± 15 mg/dL and 140 ± 12 mg/dL in the aqueous and ethanolic extract groups, respectively, from 350 ± 25 mg/dL in diabetic controls ($p < 0.05$), representing reductions of 48.6% and 60.0%. Glibenclamide reduced FBG to 101 ± 14 mg/dL, corresponding to a 71.1% reduction. Both extracts significantly attenuated diabetes-associated weight loss, with final body weights of 214 ± 16 g and 220 ± 18 g for the aqueous and ethanolic extracts, respectively, compared with 200 ± 15 g in diabetic controls ($p < 0.05$). At the acute toxicity limit dose of 2000 mg/kg, neither extract caused mortality nor overt treatment-related clinical abnormalities, and the body-weight progression remained normal throughout the 14-day observation period. The acute LD₅₀ was therefore considered >2000 mg/kg under the experimental conditions.

Conclusion: Aqueous and 70% ethanolic *G. herbaceum* leaf extracts exhibited significant antihyperglycaemic activity in streptozotocin-induced diabetic rats, with the ethanolic extract showing greater efficacy. The enhanced activity of the ethanolic preparation may reflect more efficient extraction of pharmacologically relevant constituents, although quantitative phytochemical profiling and mechanistic studies are needed to confirm this hypothesis. Both extracts demonstrated a favourable acute oral safety profile at 2000 mg/kg. These findings support further investigation of *G. herbaceum* leaves as a potential source of antidiabetic bioactive compounds, particularly through bioactivity-guided fractionation, compound identification, mechanistic validation, and longer-term toxicological studies.

Keywords: *Gossypium herbaceum*; antihyperglycaemic activity; antidiabetic activity; streptozotocin; phytochemical composition; ethanolic extract; aqueous extract; acute oral toxicity; Wistar rats.

1. Introduction

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most important and rapidly expanding non-communicable diseases worldwide, with substantial consequences for morbidity, mortality, and healthcare expenditure. The International Diabetes Federation (IDF) estimated that 536.6 million adults aged 20–79 years were living with diabetes in 2021, corresponding to approximately 10.5% of the global adult population, and projected that the number could increase to 783.2 million by 2045 (International Diabetes Federation [IDF], 2021; Sun et al., 2022). Type 2 diabetes mellitus (T2DM) constitutes approximately

90–95% of all diabetes cases and is primarily characterized by insulin resistance accompanied by progressive deterioration of pancreatic β -cell function (American Diabetes Association Professional Practice Committee, 2024). Persistent hyperglycemia contributes to vascular and metabolic injury and increases the risk of cardiovascular, renal, neurological, and ocular complications, thereby imposing a considerable clinical and socioeconomic burden (American Diabetes Association Professional Practice Committee, 2024).

Current diabetes management is centered on lifestyle modification and pharmacological treatment aimed at achieving sustained glycemic control and reducing diabetes-associated complications. Available pharmacological options include insulin, metformin, sulfonylureas, α -glucosidase inhibitors, sodium–glucose cotransporter-2 inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase-4 inhibitors, and thiazolidinediones. Although these therapeutic classes have significantly improved diabetes care, their clinical use may be constrained by adverse effects, treatment cost, accessibility, adherence, and variable therapeutic responses (Chaudhury et al., 2017). Sulfonylureas, for example, promote insulin secretion from pancreatic β -cells but may cause hypoglycemia and weight gain. These therapeutic limitations, coupled with the increasing global prevalence of diabetes, have spurred ongoing investigation into complementary therapeutic strategies and novel bioactive molecules, including those derived from medicinal plants (Rios et al., 2015).

Medicinal plants constitute an important reservoir of chemically diverse compounds with potential pharmacological activity. Their long-standing use in traditional healthcare systems has provided an important starting point for the discovery of pharmacologically active natural products. African medicinal flora, in particular, contains numerous species traditionally used for metabolic disorders and other chronic diseases, although the pharmacological efficacy, chemical composition, pharmacokinetics, and toxicological characteristics of many of these plants remain insufficiently characterised (Mahomoodally, 2013). Consequently, scientific validation of medicinal plants requires an integrated approach incorporating phytochemical characterisation, in vitro biological screening, in vivo pharmacological evaluation, and systematic safety assessment. Such an approach is essential for distinguishing promising therapeutic candidates from preparations whose traditional use is not necessarily supported by adequate evidence of efficacy or safety (Ekor, 2014).

Gossypium herbaceum L. (Malvaceae), commonly referred to as Levant cotton, is an economically important member of the genus *Gossypium*. Although the species is principally recognised for its contribution to cotton fibre production, different organs of *Gossypium* plants contain a wide range of phytochemicals and have attracted increasing scientific interest for potential nutritional, pharmaceutical, and industrial applications. Investigations of cotton tissues have identified diverse classes of secondary metabolites and other constituents, including phenolics, flavonoids, terpenoids, fatty acids, and related compounds, although their distribution varies considerably with plant organ and species (Egbuta et al., 2017). More specifically, experimental analysis of *G. herbaceum* leaves has demonstrated the presence of several phytochemical classes and reported antioxidant and other biological activities, supporting further investigation of the leaves as a source of potentially bioactive constituents (Larayetan et al., 2021). These findings provide a scientific basis for evaluating the pharmacological relevance of *G. herbaceum* leaf preparations beyond their conventional agricultural importance.

The biological activity of plant extracts is closely related to their chemical composition, which can be markedly influenced by the extraction procedure and solvent system. Solvent polarity, extraction time, temperature, plant-to-solvent ratio, and matrix characteristics all influence the selective recovery of phytochemicals and, consequently, the biological properties of the resulting extract (Chemat et al., 2019). Water is particularly relevant because it is the principal solvent in many traditional herbal preparations, whereas hydroethanolic systems can extract a broader range of polar and moderately polar compounds. Comparative evaluation of aqueous and hydroethanolic extracts may therefore reveal differences in phytochemical recovery and biological potency that are not apparent when a single extraction system is employed. Such comparisons are especially relevant for *G. herbaceum*, for which the relationship between extraction solvent, phytochemical composition, antidiabetic activity, and toxicological profile remains inadequately characterised.

Streptozotocin (STZ)-induced diabetes is a widely used experimental approach for evaluating candidate antidiabetic interventions in rodents. STZ is preferentially taken up by pancreatic β -cells and produces cellular injury that leads to impaired insulin secretion and hyperglycaemia. Depending on the dose, administration regimen, and incorporation of dietary or metabolic manipulations, STZ can generate models representing severe insulin deficiency or selected features of T2DM (Furman, 2021). Consequently, STZ-induced diabetic rodents remain extensively used for the preclinical assessment of antihyperglycaemic compounds and medicinal plant preparations. Monitoring fasting blood glucose together with body-weight changes provides useful measures of treatment-associated improvement or deterioration in metabolic status.

Demonstration of antidiabetic activity must, however, be accompanied by rigorous evaluation of safety. The assumption that natural products are inherently safe is not scientifically justified because plant-derived preparations may contain pharmacologically active or potentially toxic constituents whose concentrations depend on botanical identity, plant part, geographical origin, processing, extraction conditions, and dose. Adverse reactions, herb–drug interactions, and organ-specific toxicities have been documented for medicinal herbal preparations, emphasising the need for systematic toxicological assessment before therapeutic translation (Ekor, 2014). The OECD Test Guideline 423 provides a standardised acute oral toxicity protocol based on the acute toxic class method for estimating the toxicity category and relevant dose range while limiting animal use (Organisation for Economic Co-operation and Development [OECD], 2002).

Despite increasing evidence of biological activity in *Gossypium* species, the pharmacological and toxicological characterisation of *G. herbaceum* leaf extracts remains comparatively limited. Existing research has provided evidence for phytochemical diversity and antioxidant, antimicrobial, cytotoxic, and antimalarial activities of *G. herbaceum* leaf extracts (Larayetan et al., 2021), while broader reviews have described the chemical constituents and biological importance of cotton plant tissues (Egbuta et al., 2017). However, comparatively little integrated evidence is available on the influence of extraction solvent on the antidiabetic efficacy and safety of *G. herbaceum* leaves. In particular, comparative evaluation of aqueous and 70% ethanolic

extracts incorporating phytochemical screening, and acute toxicological endpoints could provide a more comprehensive assessment of the therapeutic promise of this plant.

The present study therefore investigated the phytochemical composition, antidiabetic activity, and safety profile of aqueous and 70% ethanolic leaf extracts of *Gossypium herbaceum*. By integrating phytochemical, in vivo antidiabetic, and toxicological investigations, this study provides a comprehensive preclinical evaluation of *G. herbaceum* leaf extracts and establishes a basis for further bioactivity-guided investigations.

2. Materials and Methods

Fresh mature leaves of *Gossypium herbaceum* L. were collected from Amurin farmland, Owo, Ondo State, southwestern Nigeria (approximately 7°11' 46''N, 5°35' 11''E) during the early morning hours to minimise potential deterioration of thermolabile and photosensitive phytoconstituents. The collected plant material was placed in clean, labelled polyethylene bags and transported to the laboratory for processing.

The plant material was taxonomically authenticated by a qualified taxonomist at the Herbarium of the Environmental Biology Unit, Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Nigeria. A voucher specimen was prepared, assigned an appropriate voucher number, and deposited in the institutional herbarium for future reference and verification.

2.1 Preparation of aqueous and ethanolic leaf extracts

The collected leaves were carefully inspected and manually sorted to remove damaged material, extraneous matter, and other plant materials. The leaves were washed gently under running potable water to remove adhering soil and particulate contaminants and subsequently rinsed with distilled water. The cleaned leaves were spread in a single layer and air-dried at room temperature (25–30 °C) in a shaded, well-ventilated environment for 14 days. Direct exposure to sunlight was avoided to minimise degradation of light-sensitive phytochemicals.

The dried leaves were pulverised into a fine, homogeneous powder using a mechanical grinder. The powdered material was stored in clean, airtight containers until extraction. For extraction, 200 g of the powdered leaf material was separately macerated in 2.0 L of distilled water and 2.0 L of 70% (v/v) ethanol, giving a plant material-to-solvent ratio of 1:10 (w/v). Each extraction mixture was maintained at room temperature for 48 h with intermittent agitation to enhance solvent penetration and mass transfer.

Following extraction, the mixtures were filtered using muslin cloth. The ethanolic filtrate was concentrated under reduced pressure using a rotary evaporator maintained at approximately 50 °C, whereas the aqueous filtrate was concentrated by lyophilisation (freeze-drying). The dried extracts were weighed to determine extraction yield and transferred into sterile, airtight amber containers and stored at 4 °C until phytochemical, in vivo antidiabetic and toxicological, analyses. Before administration, the dried extracts were freshly reconstituted in an appropriate vehicle at the required concentrations.

2.2 Qualitative phytochemical screening

Qualitative phytochemical screening was performed separately on the aqueous and 70% ethanolic leaf extracts using established pharmacognostic procedures. The extracts were screened for major classes of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, phenolic compounds, cardiac glycosides, steroids, and terpenoids, following standard procedures described by Harborne (1998) and Trease and Evans (2009).

2.3 Experimental animals and ethical considerations

Healthy male Wistar rats weighing 120–150 g (6–8 weeks old) were obtained from the laboratory animal facility of Federal Medical Centre, Owo, Nigeria. Upon arrival and throughout the experimental period, animals were examined and monitored for general health, behavior, food and water consumption, body weight changes, and clinical signs of toxicity. Animals were housed in clean, well-ventilated polypropylene cages under controlled environmental conditions of approximately 25 ± 2 °C, a 12-h light/12-h dark cycle, and relative humidity of 50–60%. Animals were provided unrestricted access to standard commercial rat chow and clean drinking water throughout the acclimation period (14 days) and the experimental period, except during specified fasting periods. Experimental procedures were conducted in accordance with institutional animal care requirements and internationally accepted principles for the humane use of laboratory animals.

2.4 In vivo antidiabetic study

2.4.1 Experimental design and allocation

Thirty male Wistar rats were randomly allocated into five experimental groups (n = 6 per group). Table 1 presents the experimental groups and treatment regimens. Treatments were administered orally once daily for 28 consecutive days. The selected extract dose of 400 mg/kg body weight was based on available preliminary toxicity information. Glibenclamide (5 mg/kg body weight) was used as the reference antidiabetic drug.

Table 1. Experimental groups and treatment protocol

S/N	Group	Treatment	Dose (mg/kg body weight)
I	Normal control	Vehicle/Distilled water	-
II	Diabetic control	Vehicle/Distilled water	-
III	Standard drug	Glibenclamide	5.0
IV	Diabetic + aqueous extract	<i>G. herbaceum</i> aqueous extract	400.0
V	Diabetic + ethanolic extract	<i>G. herbaceum</i> ethanolic extract	400.0

Fasting blood glucose concentration and body weight were monitored at baseline and subsequently at predetermined weekly intervals throughout the 28-day treatment period. All measurements were performed using standardised procedures at approximately the same time of day to minimise diurnal variation.

2.4.2 Induction of experimental diabetes

Experimental diabetes was induced using streptozotocin (STZ), a pancreatic β -cell cytotoxic agent commonly employed to produce experimental models of diabetes in rodents (Szkudelski, 2001). Following an overnight fast, rats designated for diabetes induction received a single intraperitoneal injection of freshly prepared STZ at a dose of 40 mg/kg body weight. STZ was dissolved immediately before administration in ice-cold 0.1 M citrate buffer adjusted to pH 4.5.

Following STZ administration, and because STZ-induced diabetes may be accompanied by transient metabolic disturbances, animals were monitored closely for signs of distress and excessive hyperglycaemia during the post-induction period. Seventy-two hours after STZ administration, fasting blood glucose (FBG) was determined using a calibrated glucometer. Rats exhibiting FBG concentrations greater than 250 mg/dL were classified as diabetic and used in the treatment phase.

2.5 Toxicological Evaluation Protocol

2.5.1. Test Substance and Dose Preparation

The leaf extracts (aqueous and ethanolic) were dissolved or suspended in 0.5% (w/v) sodium carboxymethylcellulose in distilled water immediately before administration. Fresh preparations are made daily to minimise degradation, precipitation, or alteration of the test material during storage. The test substance is administered by oral gavage using a suitable stainless-steel or flexible cannula at a constant volume, typically 10 mL/kg body weight. The dose administered to each animal was calculated from its most recent body weight and expressed as mg/kg body weight. All animals within a treatment group received the same nominal dose on a body-weight basis.

2.5.2 Acute Oral Toxicity Assessment

The acute oral toxicity of the aqueous and ethanolic *G. herbaceum* leaf extracts was evaluated according to OECD Test Guideline 423 (OECD, 2002) using the acute toxic class method.

A limit test was performed at a single dose of 2000 mg/kg body weight. Three female rats, fasted overnight (with water available), were administered the extract by oral gavage. Animals were observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention during the first 4 hours, and daily thereafter for 14 days.

2.6 Statistical analysis

All quantitative data were expressed as mean $\pm$ standard deviation (SD). Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Statistical significance was set at $p < .05$. All statistical analyses were conducted using the individual animal as the experimental unit.

3. Results and Discussion

3.1 Qualitative Phytochemical Analysis

Qualitative phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, saponins, and other phenolic constituents in both aqueous and 70% ethanolic extracts of *Gossypium herbaceum* leaves (Table 2). However, the intensity of the reactions varied according to the extraction solvent, with the ethanolic extract generally exhibiting stronger reactions for flavonoids, phenolic compounds, alkaloids, and tannins. This solvent-dependent

pattern is consistent with the established influence of solvent polarity, solvent–solute interactions, and plant-matrix characteristics on the recovery of secondary metabolites during extraction (Chemat et al., 2019; Zhang et al., 2018).

Flavonoids and phenolic compounds exhibited the strongest reactions in both extracts, with the ethanolic extract recording a higher intensity (++++), compared with the aqueous extract (+++). The comparatively greater detection intensity in the 70% ethanolic extract may be attributable to the ability of aqueous ethanol to solubilise phenolic constituents with a broad range of polarity. Phenolic compounds occur in plants as structurally diverse molecules, including phenolic acids, flavonoids, tannins, and related conjugated forms, and their extractability is strongly influenced by solvent composition and extraction conditions (Lefebvre et al., 2021; Sridhar et al., 2021). Hydroethanolic systems can provide a favourable balance between water-mediated extraction of polar constituents and ethanol-mediated solubilisation of moderately polar compounds, thereby facilitating broader recovery of plant phenolics than either solvent used alone (Chemat et al., 2019; Zhang et al., 2018). The occurrence of phenolic and flavonoid constituents is also consistent with the recognised phytochemical complexity of *Gossypium* species, which contain a range of phenolic and other specialised metabolites of potential biological significance (Egbuta et al., 2017). Phenolic and flavonoid compounds are recognised for their ability to participate in redox reactions and, depending on their molecular structure and concentration, may contribute to antioxidant and other pharmacological activities. Given that phenolic and flavonoid compounds are strongly associated with antioxidant, anti-inflammatory, and anticancer activities, the ethanolic extract of *G. herbaceum* leaves may hold greater potential for oxidative stress-related therapeutic applications (Zhang et al., 2018; Bitwell et al., 2023). The relatively stronger detection of flavonoids and phenolics in the ethanolic extract may have important implications for subsequent biological investigations.

Alkaloids were detected at moderate intensity (++) in the aqueous extract and at a higher intensity (+++) in the ethanolic extract. This difference may reflect the chemical diversity of alkaloids, whose extraction behaviour depends on molecular structure, ionisation state, solvent composition, and pH. Although many protonated alkaloids exhibit appreciable aqueous solubility, their neutral or free-base forms can show greater affinity for organic or hydro-organic solvents. Consequently, the stronger reaction observed in the ethanolic extract may indicate more effective recovery of alkaloidal constituents under the extraction conditions employed (Zhang et al., 2018; Bitwell et al., 2023). Cotton plants are known to contain a chemically diverse array of secondary metabolites, including terpenoid aldehydes and phenolic constituents, further supporting the potential phytochemical complexity of *Gossypium* leaf extracts (Egbuta et al., 2017).

Tannins were detected at low intensity (+) in the aqueous extract and moderate intensity (++) in the ethanolic extract. The greater reaction intensity in the hydroethanolic extract may be related to the structural heterogeneity of tannins and the influence of solvent composition on their solubility and diffusion from the plant matrix. Tannins comprise diverse high-molecular-weight polyphenolic structures, and their extraction can be affected by solvent polarity, temperature, particle size, extraction time, and the physicochemical characteristics of the plant material (Sridhar et al., 2021). The present observation therefore suggests that 70% ethanol may have provided more favourable conditions for recovering detectable tannin-like constituents from *G. herbaceum* leaves.

Saponins were detected at moderate intensity (++) in both aqueous and ethanolic extracts. The relatively similar response between the two extraction systems may be explained by the amphiphilic nature of saponins, which contain hydrophilic glycosidic components linked to relatively hydrophobic aglycone moieties. This structural characteristic enables different saponin molecules to exhibit appreciable affinity for both aqueous and hydro-organic extraction media. The detection of saponins in both extracts consequently suggests that these constituents are not exclusively associated with either highly polar or moderately polar solvent fractions (Chemat et al., 2019; Lefebvre et al., 2021). Their presence is noteworthy because saponins have been investigated for a range of biological properties; however, biological activity cannot be inferred from their qualitative detection alone and depends on the identity, concentration, structure, and bioavailability of the individual saponins.

Taken together, the qualitative phytochemical profile demonstrates that both water and 70% ethanol were capable of extracting several major classes of secondary metabolites from *G. herbaceum* leaves, although the ethanolic extract generally produced stronger qualitative responses. The broader and more intense reactions observed with 70% ethanol are consistent with the capacity of hydroethanolic solvents to extract chemically diverse metabolites through favourable solvent–solute interactions and enhanced penetration of the plant matrix (Chemat et al., 2019; Lefebvre et al., 2021). Accordingly, these findings provide a preliminary phytochemical basis for prioritising the 70% ethanolic extract for subsequent quantitative and bioactivity-guided investigations.

Table 2: Qualitative Phytochemical Composition of *Gossypium herbaceum* Leaf Extracts

Compound	Aqueous Extract	Ethanol Extract
Flavonoids	+++	++++
Alkanoids	++	+++
Tannis	+	++
Saponins	++	++
Phenolic Compounds	+++	++++

Legend: + = Slight; ++ = Moderate; +++ = Abundant

3.2 In Vivo Antidiabetic Activity

The in vivo studies validated the antidiabetic effects, as evidenced by significant reductions in fasting blood glucose levels and improved body weight in diabetic rats.

3.2.1 Blood Glucose Levels

The effect of *Gossypium herbaceum* leaf extracts on fasting blood glucose (FBG) in streptozotocin (STZ)-induced diabetic rats is presented in Table 3. The normal control animals exhibited an FBG concentration of 90 ± 10 mg/dL, whereas the diabetic control group showed a marked elevation to 350 ± 25 mg/dL. This pronounced hyperglycaemia confirms the establishment of the diabetic state and demonstrates the substantial metabolic disturbance produced by STZ. STZ-induced experimental diabetes is widely used to evaluate antihyperglycaemic agents because STZ induces pancreatic β -cell injury and consequently compromises insulin production and glucose homeostasis. Accordingly, persistent elevation of blood glucose in untreated STZ-diabetic animals provides an appropriate basis for evaluating the glucose-lowering effects of candidate therapeutic agents (Omale et al., 2023; Furman, 2021).

Treatment with *G. herbaceum* extracts substantially attenuated the STZ-associated hyperglycaemia. The aqueous extract reduced FBG from 350 ± 25 mg/dL in the diabetic control to 180 ± 15 mg/dL, representing an approximate 48.6% reduction relative to the diabetic control. More pronounced activity was observed with the ethanolic extract, which reduced FBG to 140 ± 12 mg/dL, corresponding to an approximate 60.0% reduction. Thus, the ethanolic extract produced a greater absolute glucose-lowering effect than the aqueous extract, reducing FBG by approximately 40 mg/dL more than the aqueous preparation. This solvent-dependent difference is consistent with the broader observation that extraction solvent can influence the recovery and relative abundance of bioactive phytochemicals and, consequently, the biological activity of plant extracts. Recent reviews and experimental studies indicate that plant-derived antidiabetic effects may involve complementary actions on insulin secretion, insulin sensitivity, glucose transport, hepatic glucose metabolism, and intestinal carbohydrate digestion (Ahdaa et al., 2023; Omale et al., 2023).

The stronger activity of the ethanolic extract may be related to its ability to extract a broader or greater quantity of moderately polar phytoconstituents from the leaves. This interpretation is particularly relevant to the phytochemical findings obtained in the present study, in which the ethanolic extract demonstrated stronger qualitative reactions for flavonoids and phenolic compounds than the aqueous extract. Flavonoids and other polyphenolic constituents have been extensively investigated for antihyperglycaemic activity and may influence glucose homeostasis through multiple molecular pathways, including modulation of α -amylase and α -glucosidase activity, improvement of peripheral glucose uptake, attenuation of oxidative stress, and regulation of insulin-signalling pathways (Proença et al., 2022a; Dirir et al., 2022).

The reduction in FBG observed with the aqueous extract is also biologically relevant. Its ability to lower glucose from 350 to 180 mg/dL indicates that water-soluble constituents in *G. herbaceum* leaves may possess appreciable antihyperglycaemic activity. Similar glucose-lowering effects of aqueous and hydroalcoholic plant extracts have been reported in STZ-induced diabetic animal models, supporting the concept that both highly polar and moderately polar phytochemicals can contribute to glucose regulation (Omale et al., 2023). A recent study using an aqueous leaf extract in STZ-induced diabetic rats likewise reported significant reductions in blood glucose following repeated administration, reinforcing the utility of aqueous botanical preparations in experimental antidiabetic investigations.

Glibenclamide produced the lowest reported FBG among the treatment groups, reducing blood glucose to 101 ± 14 mg/dL. This strong response is consistent with the established pharmacological action of sulfonylureas, which stimulate pancreatic β -cells to increase insulin secretion. The response to glibenclamide therefore provides an appropriate pharmacological benchmark against which to evaluate the activity of the plant extracts. Importantly, the ethanolic extract lowered FBG to 140 ± 12 mg/dL, indicating substantial antihyperglycaemic activity but a less pronounced effect than glibenclamide under the conditions of the present experiment. Comparable experimental studies commonly use glibenclamide as a positive control when assessing plant extracts in STZ-induced diabetic rodents, although the magnitude of response depends strongly on STZ dose, duration of diabetes, treatment dose, treatment duration, and the residual functional capacity of pancreatic β -cells (Ahdaa et al., 2023).

The pattern observed in Table 3 therefore suggests a solvent-dependent antihyperglycaemic effect, with the ethanolic extract showing greater efficacy than the aqueous extract and both extracts demonstrating activity relative to the untreated diabetic control. This finding is consistent with contemporary evidence that medicinal plants may exert antidiabetic actions through several interacting mechanisms rather than through a single pharmacological target (Faloye et al., 2024). These may include enhanced insulin secretion or signalling, increased cellular glucose uptake, suppression of excessive hepatic glucose production, inhibition of carbohydrate-digesting enzymes, and reduced oxidative and inflammatory stress (Omale et al., 2023; Ahdaa et al., 2023).

Overall, the results provide preliminary in vivo evidence that *G. herbaceum* leaf extracts possess antihyperglycaemic activity in STZ-induced diabetic rats, with the ethanolic extract demonstrating greater efficacy than the aqueous extract. The findings are particularly significant because the ethanolic extract also showed stronger phytochemical responses in the preceding qualitative screening, suggesting that solvent-dependent enrichment of bioactive constituents may contribute to the observed difference in activity.

Table 3: Effect of *Gossypium herbaceum* Leaf Extracts on Fasting Blood Glucose Levels in Streptozotocin-Induced Diabetic Rats

Treatment Group	Fasting Blood Glucose (mg/dL)	Percentage Reduction (%)
Distilled Water	90 ± 10	-
Distilled water (diabetic control)	350 ± 25	-
Glibenclamide	101 ± 14	71.1
Aqueous extract of <i>G. herbaceum</i>	180 ± 15	48.6
Ethanol extract of <i>G. herbaceum</i>	140 ± 12	60.0

3.2.2 Body Weight Changes

Body-weight changes provide an important complementary indicator of the metabolic consequences of experimental diabetes and the overall response to treatment. The effect of *Gossypium herbaceum* leaf extracts on body weight in the experimental groups is presented in Table 4. The normal control animals recorded the highest mean body weight (250 ± 20 g), whereas the untreated diabetic group had a substantially lower body weight of 200 ± 15 g. Relative to the normal control, this represents an approximately 20% reduction in body mass. In contrast, treatment with glibenclamide, the aqueous extract, and the ethanolic extract resulted in higher mean body weights of 225 ± 17, 214 ± 16, and 220 ± 18 g, respectively. The reported statistically significant improvement in body weight in the extract-treated diabetic animals compared with the untreated diabetic control ($p < 0.05$) indicates attenuation of the weight loss associated with experimental diabetes. This pattern is consistent with observations from experimental studies in which effective antidiabetic interventions ameliorated diabetes-associated reductions in body mass (Furman, 2021; Omale et al., 2023).

The reduced body weight observed in the diabetic control group is consistent with the metabolic consequences of STZ-induced diabetes. STZ produces pancreatic β -cell injury and, depending on the dose and experimental protocol, can generate pronounced insulin deficiency and sustained hyperglycaemia. In insulin-deficient states, impaired glucose utilization by peripheral tissues promotes a catabolic shift in energy metabolism, with increased mobilization of adipose lipid and muscle protein to meet energy requirements. Persistent hyperglycaemia may also result in glucosuria, osmotic diuresis, and loss of water and energy substrates, thereby contributing further to body-weight reduction (Furman, 2021).

The partial restoration of body weight following treatment with *G. herbaceum* extracts suggests an overall improvement in the metabolic disturbances associated with diabetes. The ethanolic extract produced a mean body weight of 220 ± 18 g, compared with 214 ± 16 g for the aqueous extract, while glibenclamide produced 225 ± 17 g. Thus, both extracts attenuated the reduction in body mass relative to the untreated diabetic group, with the ethanolic preparation showing a slightly greater effect than the aqueous preparation. This finding is particularly relevant when considered alongside the glucose-lowering effects reported in Table 3, where the ethanolic extract also produced the greater reduction in fasting blood glucose. Similar associations between improved glycaemic control and attenuation of body-weight loss have been reported in experimental diabetic models treated with plant-derived preparations (Omale et al., 2023; Satyanarayana et al., 2022).

The greater body-weight preservation observed with the ethanolic extract may, at least in part, reflect differences in its phytochemical composition. The ethanolic extract showed stronger qualitative reactions for phenolic and flavonoid constituents than the aqueous extract in the present study. Plant polyphenols have been investigated extensively for their ability to modulate glucose metabolism, oxidative stress, inflammation, and other processes associated with diabetes (Proença et al., 2022b).

Oxidative stress provides another biologically plausible link between diabetes and alterations in body weight. Persistent hyperglycaemia promotes reactive oxygen species generation and disturbances in redox homeostasis, which can contribute to cellular dysfunction and progression of diabetic pathology (Yaribeygi et al., 2020). Polyphenolic constituents of medicinal plants may counter oxidative stress through direct radical-scavenging activity and modulation of endogenous antioxidant systems.

The response observed following glibenclamide treatment is also noteworthy. Glibenclamide-treated animals had a mean body weight of 225 ± 17 g, the highest among the diabetic treatment groups. As an insulin secretagogue, glibenclamide lowers blood glucose primarily by stimulating insulin secretion from pancreatic β -cells. The magnitude of its response in an STZ model depends on the extent of β -cell destruction and the amount of residual β -cell function (Furman, 2021). It is also important to recognize that the present table provides terminal body weight rather than serial body-weight measurements. Consequently, the results demonstrate differences in final body mass among the groups but do not, by themselves, characterize the temporal trajectory of weight loss or recovery. (Masky et al., 2024).

The present results are consistent with recent experimental evidence showing that treatment of STZ-induced diabetic animals with plant extracts can improve both glycaemic status and diabetes-associated alterations in body weight. For example, a recent study of *Solanum torvum* fruit extract reported significant increases in body weight alongside improved glycaemic parameters in STZ-induced diabetic rats, supporting the concept that attenuation of body-weight loss can accompany botanical antihyperglycaemic activity (Satyanarayana et al., 2022). Similar observations have been reported in other phytotherapeutic studies, although the magnitude and direction of body-weight responses vary by plant species, extraction method, dose, treatment duration, diabetic model, and experimental conditions.

Generally, the results indicate that STZ-induced diabetes was associated with a pronounced reduction in body weight. In contrast, administration of *G. herbaceum* leaf extracts significantly attenuated this loss relative to the untreated diabetic control. The ethanolic extract produced a slightly greater preservation of body weight than the aqueous extract. These findings provide additional preclinical evidence supporting the biological activity of *G. herbaceum* leaves and suggest that the extract may ameliorate some of the systemic metabolic consequences of experimental diabetes.

Table 4: Effect of *Gossypium herbaceum* leaf extracts on terminal body weight of streptozotocin-induced diabetic rats.

S/N	Group	Body weight (g)
I	Distilled water	250 ± 20
II	Diabetic	200 ± 15
III	Glibenclamide	225 ± 17
IV	Aqueous extract <i>G. herbaceum</i>	214 ± 16
V	Ethanol extract <i>G. herbaceum</i>	220 ± 18

Diabetic rats treated with the extracts showed a significant improvement in body weight compared to the untreated diabetic control group ($p < 0.05$).

3.3 Toxicological Study - Acute Oral Toxicity Study

3.3.1 Mortality and Clinical Observations

The acute oral toxicity profiles of the aqueous and ethanolic leaf extracts of *Gossypium herbaceum* (Table 5.1) were evaluated in female rats following a single oral administration of 2000 mg/kg body weight, using the acute toxic class approach described in OECD Test Guideline 423 (OECD, 2002). No mortality occurred in either extract-treated group during the 14-day observation period, with all three animals in each group surviving to study termination. Furthermore, no treatment-related clinical abnormalities were observed during the intensive post-administration observation period or during subsequent daily monitoring. Specifically, the animals did not exhibit overt alterations in locomotor activity, respiration, grooming behaviour, salivation, piloerection, convulsions, or other abnormal behavioural responses. The absence of mortality and overt clinical toxicity at the highest dose investigated provides evidence that neither extract produced evident acute oral toxicity at 2000 mg/kg under the conditions of the present study.

This finding is consistent with the principle underlying OECD TG 423, in which mortality and overt signs of toxicity following sequential administration of defined dose levels are used to establish an acute toxicity class or LD₅₀ cut-off range rather than to generate a precise point estimate of LD₅₀. When no mortality occurs at 2000 mg/kg, the result indicates that the acute oral LD₅₀ lies above the tested dose; accordingly, the present findings support an LD₅₀ above 2000 mg/kg for the tested extracts.

The absence of clinical signs is also toxicologically relevant because acute toxicity may manifest through neurological, autonomic, respiratory, gastrointestinal, or behavioural disturbances even in the absence of mortality. OECD guidance recommends systematic observation of parameters including changes in skin and fur, eyes and mucous membranes, respiratory and circulatory function, autonomic responses, motor activity, and behaviour during the observation period (OECD, 2002). The absence of such observable abnormalities in the present experiment therefore strengthens the evidence that administration of the extracts at 2000 mg/kg did not produce overt acute systemic toxicity.

The present findings are comparable with recent toxicological investigations of botanical preparations in which single oral administration of 2000 mg/kg resulted in no mortality or major clinical signs during a 14-day observation period. For example, an acute toxicity study of a polyherbal preparation conducted according to OECD TG 423 reported no mortality or signs of toxicity following administration of 2000 mg/kg, while a separate recent investigation of a standardized botanical preparation also reported absence of mortality and clinical toxicity at the same dose. Similarly, Ugwah-Oguejiofor et al. (2019) reported no lethal effects or behavioural signs of toxicity following acute administration of an aqueous plant extract in rodents, with the acute LD₅₀ cut-off exceeding 2000 mg/kg. These observations provide useful contextual support for the tolerability pattern observed for *G. herbaceum* leaf extracts in the present study.

The absence of acute toxicity is particularly relevant because plant-derived preparations may contain chemically diverse secondary metabolites whose toxicological properties can vary according to plant part, extraction solvent, dose, geographical origin, and processing conditions. Consequently, safety cannot be inferred solely from the traditional use of a plant or from its botanical identity. Rather, standardized experimental toxicology is required to establish dose-related tolerability. Contemporary toxicology literature emphasizes that acute toxicity testing provides an essential initial safety screen, while recognizing that a negative acute-toxicity finding does not exclude delayed, cumulative, organ-specific, reproductive, genotoxic, or other chronic toxic effects (Erhirhie et al., 2018). The present findings indicate that single oral exposure to 2000 mg/kg of either aqueous or ethanolic *G. herbaceum* leaf extract was not associated with mortality or overt clinical toxicity during the 14-day observation period.

3.3.2 Body Weight Changes

Changes in body weight during the 14-day observation period are presented in Table 5.2. Both treatment groups exhibited progressive increases in body weight following administration of the extracts. Rats receiving the aqueous extract increased from 182 ± 9 g at baseline to 201 ± 10 g on day 7 and 219

± 12 g on day 14, corresponding to a mean increase of 37 ± 5 g. Similarly, animals treated with the ethanolic extract increased from 180 ± 8 g initially to 199 ± 10 g on day 7 and 217 ± 11 g on day 14, resulting in a mean gain of 37 ± 4 g. The reported absence of a statistically significant difference in body-weight gain between the two extract groups ($p > .05$) indicates that the two preparations produced broadly comparable effects on this general toxicological endpoint.

The progressive gain in body weight observed in both groups provides supportive evidence of good general tolerance during the observation period. Body weight is routinely monitored in toxicity studies because marked weight loss, failure to gain expected weight, or persistent deterioration in weight trajectory may signal systemic toxicity, reduced food consumption, gastrointestinal dysfunction, altered metabolism, dehydration, or other adverse effects. OECD acute oral toxicity protocols incorporate serial body-weight assessment as part of the overall clinical evaluation, thereby allowing potential treatment-related changes to be identified alongside mortality and clinical observations (OECD, 2002).

The approximately 20% increase in body weight recorded in both treatment groups over 14 days is therefore more consistent with continued growth and maintenance of general physiological status than with an adverse effect of the extracts. Similar findings have been reported in recent acute toxicity studies of botanical preparations, in which animals receiving a single 2000 mg/kg oral dose demonstrated normal body-weight trajectories over the subsequent observation period and showed no significant differences from corresponding controls. These observations reinforce the usefulness of body-weight progression as a complementary indicator of short-term tolerability, although it should not be interpreted independently of other toxicological endpoints.

The similar body-weight trajectories of the aqueous and ethanolic extract groups are noteworthy because the two extracts differed in their phytochemical profiles and extraction yields in the present study. Despite the stronger qualitative phytochemical reactions observed for the ethanolic extract, its administration did not result in evidence of growth retardation or acute deterioration relative to the aqueous preparation. This observation suggests that the greater phytochemical extraction achieved with the ethanolic solvent was not accompanied by an overt increase in acute toxicity at the tested dose.

The body-weight results also complement the mortality and clinical-observation findings in Table 5.1. Taken together, the absence of deaths, lack of observable toxicity signs, and continued body-weight gain provide convergent evidence that a single oral administration of 2000 mg/kg of either *G. herbaceum* leaf extract was well tolerated during the 14-day observation period. Recent toxicological investigations similarly emphasize that interpretation of acute safety is strengthened when survival, clinical observations, and body-weight trajectories show concordant patterns rather than relying on mortality alone.

Overall, the present acute toxicity findings indicate that both aqueous and ethanolic *G. herbaceum* leaf extracts were tolerated following a single oral administration of 2000 mg/kg in female rats, with no mortality or overt clinical signs of toxicity and with continued body-weight gain throughout the 14-day observation period. The comparable body-weight responses between the extracts further support their short-term tolerability at the investigated dose. These findings provide a useful preliminary safety basis for subsequent pharmacological investigations.

Table 5.1: Mortality and clinical observations following single oral administration of *G. herbaceum* leaf extracts at 2000 mg/kg body weight

Extract	Dose (mg/kg)	number of rats	mortality (dead/total)	clinical signs (0- 4 h)	clinical signs (24h-14days)	outcome
Aqueous	2000	3 female	0/3	none observed	none observed	survived, normal
Ethanol	000	3 female	0/3	none observed	none observed	survived, normal

Table 5.2: Body weight changes of female rats after single oral administration of *G. herbaceum* leaf extracts at 2000 mg/kg body weight

Extract	Initial body weight (g)	Day 7 body weight (g)	Day 14 body weight (g)	Body weight gain (g)
Aqueous	182 ± 9	201 ± 10	219 ± 12	37 ± 5
Ethanol	180 ± 8	199 ± 10	217 ± 11	37 ± 4

Values are expressed as mean $\pm$ SD. No statistically significant differences in body weight gain were observed ($p > 0.05$).

4. Conclusion

The present study provides comprehensive preclinical evidence supporting the ethnopharmacological potential of *Gossypium herbaceum* L. leaf extracts as a promising candidate for antidiabetic therapy. Qualitative phytochemical screening confirmed the presence of medicinally important secondary metabolites—including flavonoids, alkaloids, tannins, saponins, and phenolic compounds—in both aqueous and ethanolic extracts, with the 70% ethanolic extract consistently demonstrating superior extraction efficiency for these bioactive constituents. This solvent-dependent enrichment of phytochemicals correlated well with the observed pharmacological outcomes.

The in vivo antidiabetic evaluation in streptozotocin-induced diabetic Wistar rats demonstrated that both aqueous and ethanolic leaf extracts produced significant reductions in fasting blood glucose levels, with the ethanolic extract exhibiting superior antihyperglycaemic activity (60.0% reduction) compared to the aqueous extract (48.6% reduction). Furthermore, both extracts significantly attenuated diabetes-associated body weight loss, with the ethanolic preparation showing slightly greater efficacy. Although the glucose-lowering effect of the extracts was less pronounced than that of

glibenclamide, the reference sulfonylurea drug, the observed activity nonetheless represents a substantial therapeutic effect that warrants further investigation.

The acute oral toxicity assessment conducted in accordance with OECD Test Guideline 423 established that both extracts were well tolerated at the limit dose of 2000 mg/kg body weight, with no mortality, no treatment-related clinical abnormalities, and normal body weight trajectories observed throughout the 14-day observation period. These findings indicate that the acute oral LD₅₀ of both extracts exceeds 2000 mg/kg, suggesting a favourable preliminary safety profile.

Collectively, these results provide a scientific basis for the traditional use of *G. herbaceum* leaves in managing diabetes mellitus and highlight the ethanolic extract as the more promising candidate for further development. The correlation between the phytochemical profile and the observed antidiabetic activity suggests that the phenolic and flavonoid constituents may be principal contributors to the therapeutic effects. However, further studies are warranted to quantitatively determine the bioactive constituents and establish their mechanisms of action at the molecular level, evaluate the chronic toxicity and organ-specific safety of the extracts, isolate and characterise the specific antidiabetic compounds through bioactivity-guided fractionation, and conduct clinical trials to validate the translational potential of these findings in human subjects. Such investigations would contribute significantly to developing novel, plant-derived therapeutic agents for managing diabetes mellitus and associated metabolic disorders.

REFERENCES

- American Diabetes Association Professional Practice Committee. (2024). 2. Diagnosis and classification of diabetes: Standards of Care in Diabetes 2024. *Diabetes Care*, 47(Supplement_1), S20–S42. <https://doi.org/10.2337/dc24-S002>
- Ahda, M., Jaswir, I., Khatib, A., Ahmed, Q. U., Mahfudh, N., & Ardini, Y. D. (2023). A review on selected herbal plants as alternative anti-diabetes drugs: Chemical compositions, mechanisms of action, and clinical study. *International Journal of Food Properties*, 26(1), 1414–1425. <https://doi.org/10.1080/10942912.2023.2215475>
- Bitwell, C., Indra, S. S., Chimuka, L., & Maseka, K. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, 19, e01585. <https://doi.org/10.1016/j.sciaf.2023.e01585>
- Chaudhury, A., Duvoor, C., Dendi, V. S. R., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N. S., Montales, M. T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C. K., Lohani, G. P., & Mirza, W. (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Frontiers in Endocrinology*, 8, 6. <https://doi.org/10.3389/fendo.2017.00006>
- Chemat, F., Vian, M. A., Ravi, H. K., Khadhraoui, B., Hilali, S., Perino, S., & Fabiano-Tixier, A.-S. (2019). Review of alternative solvents for green extraction of food and natural products: Panorama, principles, applications and prospects. *Molecules*, 24(16), 3007. <https://doi.org/10.3390/molecules24163007>
- Dirir, A. M., Daou, M., Yousef, A. F., & Yousef, L. F. (2022). A review of alpha-glucosidase inhibitors from plants as potential candidates for the treatment of type-2 diabetes. *Phytochemistry Reviews*, 21(4), 1049–1079. <https://doi.org/10.1007/s11101-021-09773-1>
- Egbuta, M. A., McIntosh, S., Waters, D. L. E., Vancov, T., & Liu, L. (2017). Biological importance of cotton by-products relative to chemical constituents of the cotton plant. *Molecules*, 22(1), 93. <https://doi.org/10.3390/molecules22010093>
- Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4, 177. <https://doi.org/10.3389/fphar.2013.00177>
- Erhirhie, E. O., Ihewereme, C. P., & Ilodigwe, E. E. (2018). Advances in acute toxicity testing: Strengths, weaknesses and regulatory acceptance. *Interdisciplinary Toxicology*, 11(1), 5–12. <https://doi.org/10.2478/intox-2018-0001>
- Faloye, K. O., Famuyiwa, F. G., Olanudun, E. A., Ayoola, M. D., Ayoade, P. T., Ugwo, J. P., Obaidullah, A. J., Alotaibi, J. M., Akinniyi, G., & Famuyiwa, S. O. (2024). Unraveling the antidiabetic effect of *Alternanthera brasiliana* (L.) Kuntze: A combined in vitro and in vivo approach. *Natural Product Communications*, 19(10). <https://doi.org/10.1177/1934578X241292182>
- Furman, B. L. (2021). Streptozotocin-induced diabetic models in mice and rats. *Current Protocols*, 1(4), e78. <https://doi.org/10.1002/cpz1.78>
- Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.
- International Diabetes Federation. (2021). IDF diabetes atlas** (10th ed.). International Diabetes Federation.
- Larayetan, R. A., Ayeni, G., Yahaya, A., Ajayi, A., Omale, S., Ishaq, U., Abiodun, D. J., Olisah, C., Aigbogun, J., & Enyioma-Alozie, S. (2021). Chemical composition of *Gossypium herbaceum* Linn and its antioxidant, antibacterial, cytotoxic and antimalarial activities. *Clinical Complementary Medicine and Pharmacology*, 1(1), 100008. <https://doi.org/10.1016/j.ccmp.2021.100008>
- Lefebvre, T., Destandau, E., & Lesellier, E. (2021). Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A*, 1635, 461770. <https://doi.org/10.1016/j.chroma.2020.461770>
- Mahomoodally, M. F. (2013). Traditional medicines in Africa: An appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine*, 2013, 617459. <https://doi.org/10.1155/2013/617459>

- Masky, B., Adjia, H., Miaffo, D., Aboubakar Oumarou, B. F., Foyet, H. S., Maguirgue, K., Talla, E. R., Bello, A. K., Bonabé, C., & Ntchapda, F. (2024). Antidiabetic activity of the aqueous extract of *Erigeron floribundus* leaves in streptozotocin-induced type 1 diabetes model in Wistar rats. *Metabolism Open*, 22, 100288. <https://doi.org/10.1016/j.metop.2024.100288>
- Organisation for Economic Co-operation and Development. (2002). *Test No. 423: Acute oral toxicity—Acute toxic class method*. OECD Publishing. <https://doi.org/10.1787/9789264071001-en>
- Omale, S., Amagon, K. I., Johnson, T. O., Bremner, S. K., & Gould, G. W. (2023). A systematic analysis of anti-diabetic medicinal plants from cells to clinical trials. *PeerJ*, 11, e14639. <https://doi.org/10.7717/peerj.14639>
- Proença, C., Ribeiro, D., Freitas, M., & Fernandes, E. (2022a). Flavonoids as potential agents in the management of type 2 diabetes through the modulation of α -amylase and α -glucosidase activity: A review. *Critical Reviews in Food Science and Nutrition*, 62(12), 3137–3207. <https://doi.org/10.1080/10408398.2020.1862755>
- Proença, C., Ribeiro, D., Freitas, M., Carvalho, F., & Fernandes, E. (2022b). A comprehensive review on the antidiabetic activity of flavonoids targeting PTP1B and DPP-4: A structure-activity relationship analysis. *Critical Reviews in Food Science and Nutrition*, 62(15), 4095–4151. <https://doi.org/10.1080/10408398.2021.1872483>
- Ríos, J. L., Francini, F., & Schinella, G. R. (2015). Natural products for the treatment of type 2 diabetes mellitus. *Planta Medica*, 81(12–13), 975–994. <https://doi.org/10.1055/s-0035-1546131>
- Satyanarayana, N., Chinni, S. V., Gobinath, R., Sunitha, P., Uma Sankar, A., & Muthuvenkatachalam, B. S. (2022). Antidiabetic activity of *Solanum torvum* fruit extract in streptozotocin-induced diabetic rats. *Frontiers in Nutrition*, 9, 987552. <https://doi.org/10.3389/fnut.2022.987552>
- Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D.-V. N., & Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: A review. *Environmental Chemistry Letters*, 19(4), 3409–3443. <https://doi.org/10.1007/s10311-021-01217-8>
- Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, 50(6), 537–546. <https://doi.org/10.33549/physiolres.930111>
- Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B. B., Stein, C., Basit, A., Chan, J. C. N., Mbanya, J. C., Pavkov, M. E., Ramachandaran, A., Wild, S. H., James, S., Herman, W. H., Zhang, P., Bommer, C., Kuo, S., Boyko, E. J., & Magliano, D. J. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Research and Clinical Practice*, 183, 109119. <https://doi.org/10.1016/j.diabres.2021.109119>
- Trease, G. E., & Evans, W. C. (2009). *Trease and Evans' pharmacognosy* (16th ed.). Saunders/Elsevier. <https://doi.org/10.1016/B978-0-7020-2933-2.X0001-9>
- Ugwah-Oguejiofor, C. J., Okoli, C. O., Ugwah, M. O., Umaru, M. L., Ogbulie, C. S., Mshelia, H. E., Umar, M., & Njan, A. A. (2019). Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon*, 5(1), e01179. <https://doi.org/10.1016/j.heliyon.2019.e01179>
- Yaribeygi, H., Sathyapalan, T., Atkin, S. L., & Sahebkar, A. (2020). Molecular mechanisms linking oxidative stress and diabetes mellitus. *Oxidative Medicine and Cellular Longevity*, 2020, 8609213. <https://doi.org/10.1155/2020/8609213>
- Zhang, H., Birch, J., Yang, H., Xie, C., Kong, L., Dias, G., & Bekhit, A.-E. (2018). Effect of solvents on polyphenol recovery and antioxidant activity of isolates of *Asparagus officinalis* roots from Chinese and New Zealand cultivars. *International Journal of Food Science & Technology*, 53(10), 2369–2377. <https://doi.org/10.1111/ijfs.13829>