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Peer Review Report for "Phytochemical Characterization and Antidiabetic Evaluation of *Saurauia bracteosa* Leaf Extract in a Type 2 Diabetes Mellitus Model"

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Review Timeline:

Submit Date:	21 Jul 2025
Revised Date:	13 Dec 2025
Accepted Date:	13 Dec 2025

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFeree : Reviewer

25 Jul 2025

I have reviewed the statistical components of this manuscript. The statistical methods are generally well-chosen and applied correctly. The reporting of results is clear. I have a few minor suggestions for clarity,

It is best to report numbers to two decimal places to maintain consistency throughout the paper.

In the Results section, lines 270-272, the results of another study are mentioned, so it is better to present only the results of your study in this section.

Esmail Mohammadian : Reviewer

25 Jul 2025

Dear Editor

Have a nice day.

The author investigated Phytochemical Characterization and Antidiabetic Evaluation of *Saurauia bracteosa* Leaf Extract in a Type 2 Diabetes Mellitus Model.

I would suggest reconsidering the manuscript for publication only after the following major revision.

1. Provide an LC-HRMS chromatogram.
2. It is necessary to rewrite the conclusion section more completely.
3. In the section on cellular mechanisms, more up-to-date and accurate references should be provided.
4. What is the effective role of Juglalin and Quercitrin?
5. How do you explain the effect of the solvent (ethanol)?
6. For Figures 3 and 4, numerical data (with tables) should also be provided.

REFEREE : Reviewer

12 Aug 2025

1. The T2DM model was induced using a high-fat/fructose diet combined with a low-dose streptozotocin (STZ) injection. While this model is commonly used, STZ has beta-cell cytotoxic effects that may mimic aspects of type 1 diabetes. How do the authors justify that this model accurately represents the pathophysiology of type 2 diabetes, particularly insulin resistance with preserved beta-cell mass initially, rather than a hybrid or type 1-like model?
2. The doses of *S. bracteosa* extract (100, 200, 400 mg/kg) were chosen, but the manuscript does not explain the basis for these specific values. Were they derived from preliminary toxicity studies, traditional usage data, or previous literature? Was a dose-response relationship truly observed, especially since blood glucose reduction was similar across all doses after three weeks?
3. While the study evaluates efficacy, there is no mention of acute or subacute toxicity testing of the extract. Given the potential for future therapeutic development, were any liver or kidney function markers (e.g., AST, ALT, creatinine) assessed to rule out organ toxicity?
4. The immunohistochemistry shows increased GLUT4 expression in the cytoplasm, but the text suggests enhanced translocation. However, effective glucose uptake requires GLUT4 translocation to the plasma membrane. Since the staining appears diffuse and not membrane-localized, how do the authors interpret this pattern? Does this indicate increased synthesis rather than translocation? Was membrane fraction analysis or confocal microscopy considered to better assess translocation?
5. Total cholesterol was significantly reduced even at the lowest dose (100 mg/kg), but triglycerides were only lowered at the highest dose (400 mg/kg). What phytochemical components or mechanisms might explain this differential dose-response? Could this suggest different pathways regulating cholesterol vs. triglyceride metabolism?
6. The reported HbA1c level in normal control rats (~122 µg/mL) appears unusually high when compared to typical values in healthy rodents (often <6%). The cited

reference (Jin et al., 2019) reports ~100 µg/mL as normal—was this unit conversion (e.g., from % to µg/mL) accurately applied? Could there be an error in assay calibration or unit reporting?

7. The LC-HRMS identified 22 compounds, including flavonoids (e.g., rutin, quercitrin) and triterpenoids (e.g., oleanolic acid). However, no quantitative analysis (e.g., % composition or concentration) was provided. Without knowing the relative abundance or pharmacokinetics, how can the authors attribute the observed effects to specific compounds? Was any bioactivity-guided fractionation performed to identify the most active constituents?

8. The discussion suggests activation of AMPK and Akt pathways based on literature about individual compounds. However, no direct measurements of p-Akt, p-AMPK, or related signaling molecules were made in this study. Are the proposed mechanisms speculative, or are there plans for future studies to validate these pathways?

9. The Federer's formula was used to determine sample size, resulting in 4 rats per group. Given the high variability observed in some parameters (e.g., HbA1c SEM = 106 in negative control), was a formal power analysis conducted to ensure adequate statistical power? Could the small sample size have masked significant differences between dose groups?

10. The treatment duration was four weeks. Do the authors have any data on whether the improvements in islet morphology and metabolic parameters are sustained after treatment cessation? Could this extract potentially reverse established diabetic complications?

11. The introduction notes that *S. vulcani* has been more extensively studied. Given the similarity between *S. bracteosa* and *S. vulcani*, are there plans for a comparative study to evaluate which species has superior antidiabetic activity or a more favorable safety profile?

12. The manuscript is generally clear and understandable, with a logical structure and sound scientific content. However, the English writing contains several grammatical errors, awkward phrasings, inconsistent terminology, and stylistic issues that affect readability and professionalism. These issues are common in non-native English writing and can be improved with careful editing.

Examples and Evaluation

1. Subject-Verb Agreement and Article Usage

Example from Text (Abstract):

"*S. bracteosa* extract doses of 200 and 400 reduced hemoglobin A1c (HbA1c) levels..."

Improved Version:

"Treatment with *S. bracteosa* extract at doses of 200 and 400 mg/kg significantly reduced hemoglobin A1c (HbA1c) levels..."

Comment: Adding "mg/kg" clarifies the unit, and "treatment with" improves flow. The verb agrees with the plural subject.

2. Awkward or Unnatural Phrasing

Example from Text (Background):

"*Saurauia bracteosa* an endemic plant in North Sumatra, Indonesia, has been

traditionally used..."

Corrected:

"*Saurauia bracteosa* is an endemic plant in North Sumatra, Indonesia, and has been traditionally used..."

Example from Text (Methods):

"The Federer's formula, used to determine the minimum number of rats: $(T-1)(N-1) \geq 15$..."

Improved:

"The minimum number of rats was determined using Federer's formula: $(T-1)(N-1) \geq 15$, where T is the number of groups and N is the number of animals per group."

3. Inconsistent or Incorrect Terminology

Example from Text (Results):

"The in vivo test results showed that *S. bracteosa* extract regulated the blood glucose levels in T2DM animal model..."

Improved:

"The in vivo results showed that *S. bracteosa* extract significantly lowered blood glucose levels in a T2DM rat model."

4. Redundancy and Wordiness

Example from Text (Discussion):

"The marked decrease in blood glucose and HbA1c levels following extract administration suggests enhanced long-term glycemic control."

Issue: "marked decrease" and "following extract administration" are acceptable but could be tightened.

More Concise:

"The significant reduction in blood glucose and HbA1c levels indicates improved long-term glycemic control."

5. Incorrect Preposition or Word Choice

Example from Text (Methods):

"Pancreatic and gastrocnemius muscle tissues were excised from the rats."

Better:

"Pancreatic and gastrocnemius muscle tissues were harvested for histological and immunohistochemical analysis."

6. Incomplete Sentences

Example from Text (Results):

"In the group receiving a 400 mg/kg dose of *S. bracteosa* extract."

Correction:

"The greatest reduction in HbA1c was observed in the group receiving a 400 mg/kg dose of *S. bracteosa* extract."

7. Inconsistent Verb Tense

Example from Text (Discussion):

"Diabetic animal models exhibit characteristic histopathological changes... The

preservation of pancreatic islet morphology and increased islet diameter suggest that the extract may have a protective effect..."

Comment: Mixed present ("exhibit", "suggest") and modal ("may have") is acceptable, but consistency in tense is important. Since the discussion interprets current results, present tense is appropriate.

8. Awkward Passive Voice

Example from Text (Methods):

"The tissue was incubated with GLUT4 monoclonal antibody for 30 min."

Comment: Passive voice is standard in methods sections, but clarity matters.

□ This is acceptable in scientific writing, though adding the agent can help:

"Sections were incubated with a GLUT4 monoclonal antibody (Bioworld, China) for 30 min at room temperature."

9. Punctuation and Spacing Issues

Example from Text (Figure Legend):

"ns: not significant; *p < 0.05; ** p < 0.01"

Correction:

"ns: not significant; *p < 0.05; **p < 0.01"

10. Overuse of Repetition

Example from Text (Conclusion):

"Saurauia bracteosa leaf extract demonstrated antidiabetic effects... by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, and preserving islet morphology. The extract also improved lipid profiles..."

Improved:

"Saurauia bracteosa leaf extract demonstrated antidiabetic effects by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, preserving islet morphology, and improving lipid profiles."

REFeree : Associate Editor

13 Aug 2025

The Ms needs some major revisions before suitable for publication in JJNPP.

REFeree : EIC

13 Aug 2025

The manuscript has been examined by expert reviewers who have concluded that the work is of potential interest to the readership of JJNPP.

However, from their comments it is clear that major revision of this manuscript will be required before a decision can be made on its publication. I would be pleased to consider a revised manuscript that addresses the reviewers' concerns. Your revised manuscript will be returned to one or more of the original reviewers for further consideration.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reply to Reviewer

Reviewer 3

Review:

I have reviewed the statistical components of this manuscript. The statistical methods are generally well-chosen and applied correctly. The reporting of results is clear. I have a few minor suggestions for clarity,

It is best to report numbers to two decimal places to maintain consistency throughout the paper.

In the Results section, lines 270-272, the results of another study are mentioned, so it is better to present only the results of your study in this section.

Answer: Thank you very much for your comment on the statistical method. I have revised the report numbers to two decimal places to maintain consistency throughout the paper.

Revision:

Page 5, lines 187-188: Table 1, Retention time (minute) and mzCloud match (%)

Page 6, lines 196: 14.70 minutes

In the Results section, Page 6, lines 213-218: I have removed another study.

Reviewer 5

1. Review

The T2DM model was induced using a high-fat/fructose diet combined with a low-dose streptozotocin (STZ) injection. While this model is commonly used, STZ has beta-cell cytotoxic effects that may mimic aspects of type 1 diabetes. How do the authors justify that this model accurately represents the pathophysiology of type 2 diabetes, particularly insulin resistance with preserved beta-cell mass initially, rather than a hybrid or type 1-like model?

Answer:

Thank you for this insightful comment. The definition of diabetes mellitus is a group of symptoms resulting from disturbances in the metabolism of fats, carbohydrates, and proteins caused by insufficient insulin secretion, inadequate insulin activity (insulin resistance), or both. We established an animal model exhibiting insulin resistance and reduced insulin secretion. In our experimental animals, insulin resistance was induced by administering a high-fat and fructose diet (Skovsø, 2014; Srinivasan et al., 2005). This approach was based on the theory that such a diet can lead to obesity, accompanied by elevated free fatty acids, glycerol, leptin, and pro-inflammatory cytokines, while decreasing adiponectin, which collectively result in insulin resistance. A single injection of streptozotocin was administered at a dose of 25 mg/kg body weight to induce insulin deficiency (Gheibi et al., 2017; Srinivasan et al., 2005). The regimen was designed to prevent the destruction of pancreatic β -cells, causing only partial damage. This was evidenced by the fact that the animals remained alive throughout the study without exogenous insulin administration. These findings justify that the animals developed type 2 diabetes mellitus, as indicated by elevated blood glucose levels following induction, which were significantly different from the normal control group (Figure 2).

Reference:

Skovsø, S. (2014). Modeling type 2 diabetes in rats using high fat diet and streptozotocin. *Journal of diabetes investigation*, 5(4), 349-358.

Srinivasan, K., Viswanad, B., Asrat, L., Kaul, C. L., & Ramarao, P. J. P. R. (2005). Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. *Pharmacological research*, 52(4), 313-320.

Gheibi, S., Kashfi, K., & Ghasemi, A. (2017). A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomedicine & pharmacotherapy*, 95, 605-613.

2. Review

The doses of S. bracteosa extract (100, 200, 400 mg/kg) were chosen, but the manuscript does not explain the basis for these specific values. Were they derived from preliminary toxicity studies, traditional usage data, or previous literature? Was a dose-response relationship truly observed, especially since blood glucose reduction was similar across all doses after three weeks?

Answer:

Thank you for your review. The doses of 100, 200, and 400 mg/kg were determined based on several considerations:

- Traditional use and human-to-animal dose conversion. The dose selection was guided by the estimated human equivalent intake of approximately 3 g of dried *Saurauia bracteosa* leaves per day in a 60-kg adult, corresponding to 50 mg/kg. Using body surface area (BSA) scaling (Km human = 37; Km rat = 6), this is equivalent to ~308 mg/kg in rats (U.S. FDA, 2005; Reagan-Shaw, Nihal, & Ahmad, 2008). Considering both the calculated equivalent dose and the need to explore a wider dose-response range within the safe limit (<2000 mg/kg according to OECD guidelines), three pragmatic dose levels were specified: 100 mg/kg (low), 200 mg/kg (medium), and 400 mg/kg (high). These doses were chosen to evaluate submaximal, moderate, and near-equivalent exposures while maintaining a safety margin for repeated administration.

Reference:

U.S. Food and Drug Administration (FDA). (2005). Guidance for industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Rockville, MD: Center for Drug Evaluation and Research (CDER).

Reagan-Shaw, S., Nihal, M., & Ahmad, N. (2008). Dose translation from animal to human studies revisited. *The FASEB Journal*, 22(3), 659–661. <https://doi.org/10.1096/fj.07-9574LSF>

- Evidence from prior studies on related plants. A previous in vivo antidiabetic study by Surbakti et al. (2019) employed a 100 mg/kg dose of ethanol extract from *Saurauia vulcani* leaves and demonstrated significant glucose-lowering effects as well as improvements in other parameters. Given the close taxonomic relationship between *Saurauia vulcani* and *Saurauia bracteosa*, this provided a rationale for selecting comparable doses. Accordingly, we chose 100, 200, and 400 mg/kg to evaluate dose-response relationships.

Reference:

Surbakti, C., Sitorus, P., & Satria, D. (2019). Effect of *Saurauia vulcani* Korth. leaves on superoxide dismutase, HbA1c levels and insulin expression in hyperglycemic rats. *Open Access Macedonian Journal of Medical Sciences*, 7(22), 3741.

Revision:**Page 3, lines 109-115**

Diabetic rats were randomly assigned to five groups (n = 4 per group): (I) negative control (CMC-Na vehicle), (II–IV) treatment groups receiving the extract at 100, 200, and 400 mg/kg, respectively, (V) positive control (metformin 40 mg/kg), and one normal control group. Treatments were administered once daily for four weeks, and blood glucose levels were measured weekly. Dose selection was guided by traditional use, human-to-animal dose conversion, and previous research with modification (Reagan-Shaw et al., 2008; Surbakti et al., 2019).

- We analyzed the dose–response relationship in blood glucose reduction between doses; however, in weeks 3 and 4 indicated that further dose escalation no longer enhanced the therapeutic effect. This phenomenon may occur for several reasons. In pharmacodynamics, the ceiling effect (Emax) refers to the maximal response a drug can elicit. Once this plateau is reached, additional dose increases will not confer further benefit but may instead elevate the risk of adverse effects. This principle underlies the dosing limitations commonly observed with many antidiabetic agents.

Reference:

Dowd, F. J., & Abel, P. W. (2017). *Concentration–response relationships*. In F. J. Dowd, B. T. Johnson, & A. Mariotti (Eds.), *Pharmacology and therapeutics for dentistry* (7th ed., pp. 35–48). St. Louis, MO: Elsevier.

Revision:**Page 9, lines: 306-312**

The dose–response relationship in blood glucose reduction observed in weeks 3 and 4 indicated that further dose escalation no longer enhanced the therapeutic effect. This phenomenon may occur for several reasons. In pharmacodynamics, the ceiling effect (Emax) refers to the maximal response a drug can elicit. Once this plateau is reached, additional dose increases will not confer further benefit but may instead elevate the risk of adverse effects. This principle underlies the dosing limitations commonly observed with many antidiabetic agents (Dowd and Abel, 2017).

3. Review

While the study evaluates efficacy, there is no mention of acute or subacute toxicity testing of the extract. Given the potential for future therapeutic development, were any liver or kidney function markers (e.g., AST, ALT, creatinine) assessed to rule out organ toxicity?

Answer:

We thank the reviewer for this important comment. Although *Saurauia bracteosa* leaves have been traditionally used by the Batak Toba and Karo communities, and previous studies by Widjaja et al. (2021) have demonstrated the safety of *Saurauia vulcani*, we acknowledge that specific acute or subacute toxicity studies of *S. bracteosa* have not yet been performed. In the present study, we did not measure liver or kidney function markers such as AST, ALT, or creatinine. This limitation has been clearly stated in the limitation section of the manuscript,

and we recognize that future studies should include comprehensive toxicity evaluations to confirm the safety profile of *S. bracteosa* for potential therapeutic development.

Reference:

Widjaja, S. S., Pertiwi, D., Simanjuntak, N. J. P., & Satria, D. (2021). Phytochemical Constituent and Subchronic Toxicity Evaluation of Ethanol Extract of *Saurauia vulcani* Korth Lour. Leaves In Wistar Rats. *Rasayan Journal of Chemistry*, 14(3).

Revision:

Page: 10, lines: 355-362

This study has several limitations. First, LC-HRMS profiling was conducted qualitatively without quantification, so the exact levels of the identified metabolites remain undetermined. Second, immunohistochemistry showed increased GLUT4 expression but did not confirm membrane translocation; more specific approaches (e.g., membrane fractionation or confocal imaging) are needed. Finally, safety assessments were not performed in this study. Future work should include quantitative phytochemical analysis, molecular pathway validation (e.g., Western blot), and comprehensive safety evaluation.

Review

4. *The immunohistochemistry shows increased GLUT4 expression in the cytoplasm, but the text suggests enhanced translocation. However, effective glucose uptake requires GLUT4 translocation to the plasma membrane. Since the staining appears diffuse and not membrane-localized, how do the authors interpret this pattern? Does this indicate increased synthesis rather than translocation? Was membrane fraction analysis or confocal microscopy considered to better assess translocation?*

Answer:

Thank you for this insightful observation. We agree that effective glucose uptake requires GLUT4 translocation to the plasma membrane, and that diffuse cytoplasmic staining in immunohistochemistry primarily reflects overall protein expression rather than definitive membrane localization. In our study, the IHC results demonstrated an increase in GLUT4 immunoreactivity in the cytoplasm of muscle fibers, which we interpreted as upregulation of GLUT4 synthesis.

We acknowledge that this does not directly confirm translocation to the plasma membrane. Techniques such as membrane fractionation or confocal microscopy would indeed provide more precise evidence of GLUT4 translocation. These approaches were beyond the scope of the present study but will be considered in future work to strengthen the mechanistic insight.

To avoid overinterpretation, we have revised the text to clarify that our findings indicate an increase in GLUT4 expression, which suggests a potential to enhance glucose utilization, but do not directly demonstrate translocation to the membrane.

Revision:

Page: 7 lines: 239-249

As shown in **Figure 5 and Table 3**, immunohistochemical analysis revealed minimal GLUT4 expression in the skeletal muscle tissue of the negative control group. In contrast, rats treated with *S. bracteosa* extract at doses of 100, 200, and 400 mg/kg as well as metformin (45 mg/kg) and the normal control group, showed increased GLUT4 expression compared with the negative control. The brown immunostaining, indicative of GLUT4 presence, appeared more intense in treated groups. However, the distribution was diffuse throughout the cytoplasm of

muscle fibers and not strictly confined to the plasma membrane. These findings suggest an upregulation of GLUT4 protein abundance, which may contribute to improved glucose utilization, although definitive evidence of membrane translocation was not obtained in this study.

Review

5. Total cholesterol was significantly reduced even at the lowest dose (100 mg/kg), but triglycerides were only lowered at the highest dose (400 mg/kg). What phytochemical components or mechanisms might explain this differential dose-response? Could this suggest different pathways regulating cholesterol vs. triglyceride metabolism?

Answer:

Thank you for this important question. The differential dose-response observed between cholesterol and triglyceride reduction may indeed reflect distinct regulatory pathways governing lipid metabolism.

The extract of *Saurauia bracteosa* contains flavonoids (e.g., quercetin) and triterpenoids (e.g., ursolic acid), which are known to modulate lipid homeostasis through distinct mechanisms. Flavonoids such as quercetin have been investigated as potential HMG-CoA reductase inhibitors (Sung et al., 2004; Cuccioloni et al., 2016). Besides that, quercetin also promotes cholesterol-to-bile acid conversion and cholesterol efflux. Meanwhile, triterpenoids such as ursolic acid have been identified to primarily target hydroxy-3-methylglutaryl coenzyme A synthase 1 (HMGCS1), a key upstream enzyme of HMG-CoA reductase in lipid metabolism (Ma et al., 2022). By acting on sequential steps of the mevalonate pathway, these compounds may exert synergistic effects in suppressing cholesterol biosynthesis. Besides, quercetin is associated with the Akt signaling pathway and regulates the expression of sterol regulatory element-binding proteins (SREBPs), which in turn control HMG-CoA reductase and low-density lipoprotein (LDL) receptor expression, both of which are critical for cholesterol homeostasis (Peng et al., 2017). Other secondary metabolites also exhibit lipid-modulating effects; for example, glycitein has been shown to decrease total cholesterol *in vivo*, while rutin reduces total cholesterol (Lee et al., 2007; Bayzar et al., 2023).

The effect on triglycerides can be attributed to rutin, which has been demonstrated to reduce triglyceride levels (Bayzar et al., 2023). On the other hand, quercetin ameliorates hypertriglyceridemia by suppressing lipogenic proteins acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and sterol regulatory element-binding protein-1c (SREBP-1c) while upregulating β -oxidation proteins such as peroxisome proliferator-activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1A), thereby enhancing fatty acid catabolism and reducing triglyceride accumulation (Jiang et al., 2025). According to available evidence, fewer chemical constituents from *Saurauia bracteosa* extract are known to reduce triglyceride levels compared to total cholesterol, suggesting that triglyceride modulation may only become significant at higher extract concentrations.

The more pronounced cholesterol-lowering effect at lower doses compared with triglyceride reduction may reflect the greater sensitivity of cholesterol biosynthesis and clearance pathways to secondary metabolites such as glycitein, rutin, quercetin, as well as ursolic acid, whereas triglyceride reduction often requires stronger modulation of lipid mobilization and utilization.

References:

Sung, J. H., Lee, S. J., Park, K. H., & Moon, T. W. (2004). Isoflavones inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase in vitro. *Bioscience, biotechnology, and biochemistry*, 68(2), 428-432.

Cuccioloni, M., Bonfili, L., Mozzicafreddo, M., Cecarini, V., Pettinari, R., Condello, F., ... & Eleuteri, A. M. (2016). A ruthenium derivative of quercetin with enhanced cholesterol-lowering activity. *RSC Advances*, 6(46), 39636-39641.

Ma, X., Bai, Y., Liu, K., Han, Y., Zhang, J., Liu, Y., ... & Bai, G. (2022). Ursolic acid inhibits the cholesterol biosynthesis and alleviates high fat diet-induced hypercholesterolemia via irreversible inhibition of HMGCS1 in vivo. *Phytomedicine*, 103, 154233.

Peng J, Li Q, Li K, Zhu L, Lin X, Lin X, et al. Quercetin Improves Glucose and Lipid Metabolism of Diabetic Rats: Involvement of Akt Signaling and SIRT1. *J Diabetes Res*. 2017;3417306:1–10.

Lee SO, Renouf M, Ye Z, Murphy PA, Hendrich S. Isoflavone glycitein diminished plasma cholesterol in female golden Syrian hamsters. *J Agric Food Chem*. 2007;55(26):11063–7.

Bazyar H, Moradi L, Zaman F, Zare Javid A. The effects of rutin flavonoid supplement on glycemic status, lipid profile, atherogenic index of plasma, brain-derived neurotrophic factor (BDNF), some serum inflammatory, and oxidative stress factors in patients with type 2 diabetes mellitus: a double-blind, placebo-controlled trial. *Phytother Res*. 2023;37(1):271–84.

Jiang L, Yi R, Chen H, Wu S. Quercetin alleviates metabolic-associated fatty liver disease by tuning hepatic lipid metabolism, oxidative stress and inflammation. *Anim Biotechnol*. 2025;36(1):2442351.

Revision: Page: 9 lines: 318-339

With respect to lipid regulation, flavonoids such as quercetin have been investigated as potential inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) (30). Additionally, quercetin promotes cholesterol-to-bile acid conversion and cholesterol efflux. Meanwhile, triterpenoids such as ursolic acid have been identified to primarily target hydroxy-3-methylglutaryl coenzyme A synthase 1 (HMGCS1), a key upstream enzyme of HMG-CoA reductase in lipid metabolism (31). By acting on sequential steps of the mevalonate pathway, these compounds may exert synergistic effects in suppressing cholesterol biosynthesis. Additionally, quercetin is linked to the Akt signalling pathway. It regulates the expression of sterol regulatory element-binding proteins (SREBPs), which in turn control the expression of HMG-CoA reductase and the LDL receptor, both of which are crucial for maintaining cholesterol homeostasis (32). On the other hand, quercetin ameliorates hypertriglyceridemia by suppressing lipogenic proteins acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and sterol regulatory element-binding protein-1c (SREBP-1c) while upregulating β -oxidation proteins such as peroxisome proliferator-activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1A), thereby enhancing fatty acid catabolism and reducing triglyceride accumulation (33). According to available evidence, fewer chemical constituents from *S. bracteosa* extract are known to reduce triglyceride levels compared to total cholesterol, suggesting that triglyceride modulation may only become significant at higher concentrations of the extract.

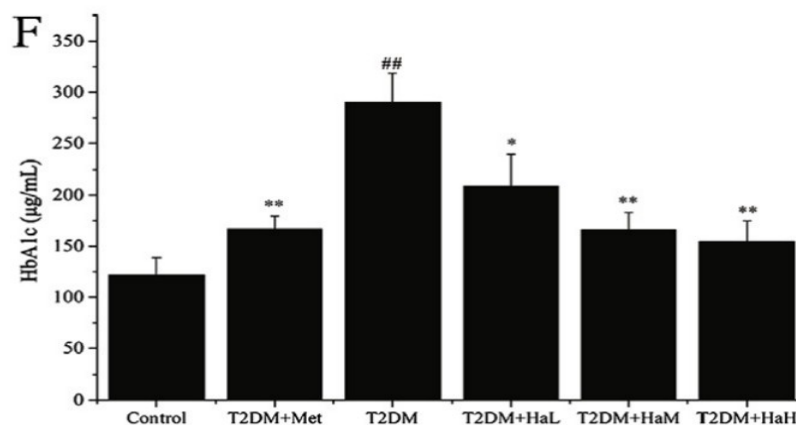
Review

6. The reported HbA1c level in normal control rats (~122 µg/mL) appears unusually high when compared to typical values in healthy rodents (often <6%). The cited reference (Jin et al., 2019) reports ~100 µg/mL as normal—was this unit conversion (e.g., from % to µg/mL) accurately applied? Could there be an error in assay calibration or unit reporting?

Answer:

We thank the reviewer for this important comment. In our study, HbA1c was quantified using an ELISA kit that reports results in absolute concentration (µg/mL) rather than as a percentage of total hemoglobin. This explains why the reported values (e.g., ~122 µg/mL in normal controls) appear numerically higher compared to typical clinical values (<6%). A similar reporting format was also adopted by Jin et al. (2019), who observed ~100–150 µg/mL in normal rats. Thus, our data are consistent with previous studies that employed the same assay principle.

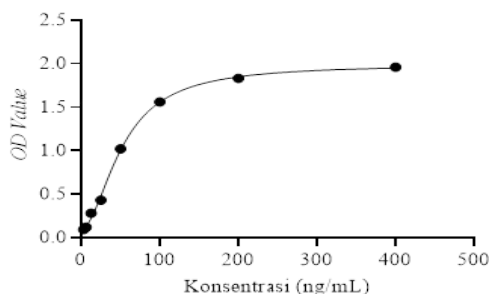
In the present study, the serum HbA1c level in the normal control group was 121.91 ± 4.26 µg/mL, whereas the negative control group exhibited markedly higher levels, reaching 458.00 ± 106.71 µg/mL. These findings are in line with the report of Jin et al. (2019), who observed HbA1c levels in normal rats at approximately 100–150 µg/mL and in negative control (T2DM) rats at approximately 250–325 µg/mL.



The figure above is the data from Jin et al (2019).

Reference:

Jin, D. X., He, J. F., Luo, X. G., & Zhang, T. C. (2019). Hypoglycemic effect of Hypericum attenuatum Choisy extracts on type 2 diabetes by regulating glucolipid metabolism and modulating gut microbiota. *Journal of Functional Foods*, 52, 479-491.



Based on the ELISA measurements, the HbA1c standard curve was fitted using a four-parameter logistic (4PL) regression model with the following equation:

$$y = d + \frac{(a - d)}{(1 + (\frac{x}{c})^b)}$$

The values of each parameter were calculated, and the results are presented in the following table.

Parameters of the HbA1c standard curve:

- **a:** the minimum asymptote (response at zero concentration).
- **d:** the maximum asymptote (response at infinite concentration).
- **c:** the inflection point (EC50), representing the concentration corresponding to the midpoint between the minimum and maximum response.
- **b:** the slope (Hill's slope), indicating the steepness of the curve at the inflection point.

Parameter	Value
a	0.098947
b	1.878019
c	52.40886
d	1.98942
R ²	0.9967

The obtained parameters were then substituted into the regression equation, allowing the concentration value (x) to be calculated from the optical density (OD) measured in the samples.

Review

7. The LC-HRMS identified 22 compounds, including flavonoids (e.g., rutin, quercitrin) and triterpenoids (e.g., oleanolic acid). However, no quantitative analysis (e.g., % composition or concentration) was provided. Without knowing the relative abundance or pharmacokinetics, how can the authors attribute the observed effects to specific compounds? Was any bioactivity-guided fractionation performed to identify the most active constituents?

Answer:

Thank you for this question. In the present study, chemical identification was carried out only qualitatively. However, we presented the peak area (a.u.) as an indicator of the relative abundance of each compound compared with others. Quantitative analysis using authentic standards was not performed in this study. The correlation between the identified compounds and the observed antidiabetic effects was established based on the literature, as described in the Discussion section. We acknowledge this as a limitation of our work, and it will be addressed in future studies to provide a stronger justification of the antidiabetic activity.

Revision:

Page: 10 lines: 341-348

This study has several limitations. First, LC-HRMS profiling was conducted qualitatively without quantification, so the exact levels of the identified metabolites remain undetermined. Second, immunohistochemistry showed increased GLUT4 expression but did not confirm membrane translocation; more specific approaches (e.g., membrane fractionation or confocal imaging) are needed. Finally, safety assessments were not performed in this study. Future work should include quantitative phytochemical analysis, molecular pathway validation (e.g., Western blot), and comprehensive safety evaluation.

Review

8. The discussion suggests activation of AMPK and Akt pathways based on literature about individual compounds. However, no direct measurements of p-Akt, p-AMPK, or related signaling molecules were made in this study. Are the proposed mechanisms speculative, or are there plans for future studies to validate these pathways?

Answer:

We appreciate the reviewer's insightful comment. Indeed, the discussion regarding potential activation of AMPK and Akt pathways was speculative and based on published literature concerning the identified compounds, as no direct measurements of p-AMPK, p-Akt, or other signaling proteins were performed in the present study. In our future work, we plan to validate these proposed mechanisms by conducting molecular assays, including Western blot analyses to assess p-AMPK and p-Akt expression levels.

Review:

9. The Federer's formula was used to determine sample size, resulting in 4 rats per group. Given the high variability observed in some parameters (e.g., HbA1c SEM = 106 in negative control), was a formal power analysis conducted to ensure adequate statistical power? Could the small sample size have masked significant differences between dose groups?

Answer:

Thank you for this valuable feedback. We acknowledge that a formal statistical power analysis was not performed prior to the experiment. It is true that the small sample size may have masked potential significant differences among groups due to the presence of outliers. Nevertheless, the current data still demonstrate consistent trends across the treatment groups, indicating that the extract possesses potential antidiabetic activity. The number of animals used in this study was also determined in accordance with the ethical clearance, which required the use of the minimum number of animals necessary to obtain meaningful results. For future studies, this input will be an important consideration in determining the number of animals to be included.

Review:

10. The treatment duration was four weeks. Do the authors have any data on whether the improvements in islet morphology and metabolic parameters are sustained after treatment cessation? Could this extract potentially reverse established diabetic complications?

Answer:

We thank the reviewer for this insightful question. In the present study, the treatment duration was four weeks, consistent with many previous antidiabetic studies that generally employ this timeframe to evaluate efficacy (Based on these references below, the duration of treatment varied from 14 to 45 days, and all of the experiments were terminated with animal sacrifice).

After treatment cessation, no further observations were made on metabolic parameters or islet morphology, as the animals were sacrificed at the end of week 4 for biochemical assessments, including blood glucose, total cholesterol, and triglyceride levels. In addition, pancreatic and skeletal muscle tissues (gastrocnemius) were collected, which precluded longer-term follow-up in the same animals. Therefore, we do not have data on whether the observed improvements in islet morphology and metabolic parameters persisted. Most previous antidiabetic studies have likewise used a four-week observation period with terminal sacrifice of the experimental animals.

There are some references that we considered to create an animal model:

- Elsadek, M. F., & Ahmed, B. M. (2022). Effect of sakuranin on carbohydrate-metabolizing enzyme activity modifications in streptozotocin-nicotinamide-induced diabetic wistar rats. *Saudi Journal of Biological Sciences*, 29(3), 1402-1406.
- Iheagwam, F. N., Iheagwam, O. T., Onuoha, M. K., Ogunlana, O. O., & Chinedu, S. N. (2022). Terminalia catappa aqueous leaf extract reverses insulin resistance, improves glucose transport and activates PI3K/AKT signalling in high fat/streptozotocin-induced diabetic rats. *Scientific reports*, 12(1), 10711.
- Motto, A. E., Lawson-Evi, P., & Eklug-Gadegbeku, K. (2022). Antidiabetic and antioxidant potential of total extract and supernatant fraction of the roots of Anogeissus leiocarpus in HFD-fed and Streptozotocin-induced diabetic rats. *Biomedicine & Pharmacotherapy*, 154, 113578.

Review:

11. The introduction notes that *S. vulcani* has been more extensively studied. Given the similarity between *S. bracteosa* and *S. vulcani*, are there plans for a comparative study to evaluate which species has superior antidiabetic activity or a more favorable safety profile?

Answer:

Thank you for your valuable insight. We believe it would be feasible to conduct a comparative study between two species (*Saurauia vulcanii* and *Saurauia bracteosa*) to determine which one demonstrates stronger antidiabetic activity and greater safety for long-term use. The results of such a study could serve as a basis for deciding which plant should be further developed as a standardized herbal drug or phytopharmaceutical. This valuable suggestion will guide our future research and may provide important information to society—particularly in North Sumatra, where these plants have been traditionally used—as well as to the academic community more broadly.

Review:

12. The manuscript is generally clear and understandable, with a logical structure and sound scientific content. However, the English writing contains several grammatical errors, awkward phrasings, inconsistent terminology, and stylistic issues that affect readability and professionalism. These issues are common in non-native English writing and can be improved with careful editing.

Answer:

Thank you for the valuable corrections regarding the grammar and structure of this article. We have checked the grammar and structure of this article thoroughly and revised it accordingly.

Examples and Evaluation

1. Subject-Verb Agreement and Article Usage

Example from Text (Abstract):

"*S. bracteosa* extract doses of 200 and 400 reduced hemoglobin A1c (HbA1c) levels..."

Improved Version:

"Treatment with *S. bracteosa* extract at doses of 200 and 400 mg/kg significantly reduced hemoglobin A1c (HbA1c) levels..."

Comment: Adding "mg/kg" clarifies the unit, and "treatment with" improves flow. The verb agrees with the plural subject.

Answer:

The corrections have been made and highlighted in yellow.

Revision Page: 1 lines: 19-22

Treatment with *S. bracteosa* extract at doses of 200 and 400 mg/kg significantly reduced hemoglobin A1c (HbA1c) levels, increased the immunoreactive score of glucose transporter 4 (GLUT4) expression, and diameters of islets of Langerhans.

Revision Page 6 lines: 213-218

Figure 3 presents the significant differences in serum HbA1c levels between the negative and normal control groups. Treatment with *S. bracteosa* extract at doses of 200 and 400 mg/kg, along with 45 mg/kg of metformin, led to a significant decline in serum HbA1c levels compared with the untreated diabetic group ($p < 0.05$). The greatest reduction was observed in the group receiving a 400 mg/kg dose of *S. bracteosa* extract.

2. Awkward or Unnatural Phrasing

Example from Text (Background):

"*Saurauia bracteosa* an endemic plant in North Sumatra, Indonesia, has been traditionally used..."

Corrected:

"*Saurauia bracteosa* is an endemic plant in North Sumatra, Indonesia, and has been traditionally used..."

Answer:

The corrections have been made and highlighted in yellow.

Revision Page: 2 lines: 42-50

Saurauia bracteosa is an endemic plant in North Sumatra, Indonesia, and has been traditionally used as an antidiabetic remedy by the Batak Toba and Karo people and may have the potential to manage diabetes (2,3).

Example from Text (Methods):

"The Federer's formula, used to determine the minimum number of rats: $(T-1)(N-1) \geq 15$..."

Improved:

"The minimum number of rats was determined using Federer's formula: $(T-1)(N-1) \geq 15$, where T is the number of groups and N is the number of animals per group."

Answer:

The corrections have been made and highlighted in yellow.

Revision Page: 3 lines: 92-94

The minimum number of rats was determined using Federer's formula: $(T-1)(N-1) \geq 15$, where T is the number of groups and N is the number of animals per group.

3. Inconsistent or Incorrect Terminology

Example from Text (Results):

"The in vivo test results showed that S. bracteosa extract regulated the blood glucose levels in T2DM animal model..."

Improved:

"The in vivo results showed that S. bracteosa extract significantly lowered blood glucose levels in a T2DM rat model."

Answer:

The corrections have been made and highlighted in yellow.

Revision Page 1, lines 17-19

The *in vivo* results showed that *S. bracteosa* extract significantly lowered blood glucose levels in the T2DM rat model (100, 200, and 400 mg/kg).

4. Redundancy and Wordiness

Example from Text (Discussion):

"The marked decrease in blood glucose and HbA1c levels following extract administration suggests enhanced long-term glycemic control."

Issue: "marked decrease" and "following extract administration" are acceptable but could be tightened.

More Concise:

"The significant reduction in blood glucose and HbA1c levels indicates improved long-term glycemic control."

Answer:

These sentences were removed as the discussion section underwent substantial revisions to incorporate the reviewer's suggestions.

5. Incorrect Preposition or Word Choice

Example from Text (Methods):

"Pancreatic and gastrocnemius muscle tissues were excised from the rats."

Better:

"Pancreatic and gastrocnemius muscle tissues were harvested for histological and immunohistochemical analysis."

Answer:

The corrections have been made and highlighted in yellow font.

Revision Page: 4, lines: 121-122

Pancreatic and gastrocnemius muscle tissues were harvested for histological and immunohistochemical analysis.

6. Incomplete Sentences

Example from Text (Results):

"In the group receiving a 400 mg/kg dose of S. bracteosa extract."

Correction:

"The greatest reduction in HbA1c was observed in the group receiving a 400 mg/kg dose of S. bracteosa extract."

Answer:

The corrections have been made and highlighted in yellow.

Revision Page: 6, lines: 217-218

The greatest reduction was observed in the group receiving a 400 mg/kg dose of *S. bracteosa* extract.

7. Inconsistent Verb Tense

Example from Text (Discussion):

"Diabetic animal models exhibit characteristic histopathological changes... The preservation of pancreatic islet morphology and increased islet diameter suggest that the extract may have a protective effect..."

Comment: Mixed present ("exhibit", "suggest") and modal ("may have") is acceptable, but consistency in tense is important. Since the discussion interprets current results, present tense is appropriate.

Answer:

The Discussion section has been thoroughly revised based on the reviewers' suggestions.

Revision: Page: 8-10 lines: 271-348

A type 2 diabetes mellitus (T2DM) model was induced using a high-fat and high-fructose diet, which promotes obesity, elevates circulating free fatty acids, glycerol, and leptin, and reduces adiponectin, collectively impairing insulin signalling (17). To further induce partial β -cell dysfunction without destruction, a single low dose of streptozotocin (25 mg/kg) was administered (18). This combined protocol is widely recognised as a reliable and reproducible approach for developing T2DM, as it mimics both insulin resistance and β -cell impairment. The treated animals subsequently exhibited persistent hyperglycemia, confirming successful model establishment.

Administration of *S. bracteosa* leaf extract for four weeks significantly reduced blood glucose and HbA1c levels. LC–MS analysis identified multiple secondary metabolites with antidiabetic potential. Rutin improves insulin sensitivity by inhibiting insulin receptor substrate-1 (IRS-1) phosphorylation and reducing suppressor of cytokine signalling 3 (SOCS3) activity. Furthermore, p-coumaric acid, apigenin, rutin, prunin, and oleanolic acid activate the phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway and enhance GLUT4 expression (19-23). Ursolic acid also ameliorates insulin resistance by activating Akt–GLUT4 signaling and exerting anti-inflammatory effects (24). Collectively, these compounds may act synergistically to improve insulin sensitivity and increase cellular glucose uptake. This is supported by immunohistochemical findings showing increased GLUT4 expression in the gastrocnemius muscle of extract-treated groups, confirming that enhanced insulin signalling contributes to the glucose-lowering effects.

The dose–response relationship in blood glucose reduction observed in weeks 3 and 4 indicated that further dose escalation no longer enhanced the therapeutic effect. This phenomenon may occur for several reasons. In pharmacodynamics, the ceiling effect (E_{max}) refers to the

maximal response a drug can elicit. Once this plateau is reached, additional dose increases will not confer further benefit but may instead elevate the risk of adverse effects. This principle underlies the dosing limitations commonly observed with many antidiabetic agents (25).

Rutin, oleanolic acid, juglalin, and ursolic acid have been reported to enhance endogenous antioxidant defences, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby reducing oxidative stress and suppressing inflammatory responses through inhibition of the NF- κ B signalling pathway and downregulation of proinflammatory cytokines such as TNF- α and IL-1 β (26-28). These protective effects are essential in preventing streptozotocin-induced pancreatic β -cell apoptosis. In addition, flavonoids have been shown to activate pancreatic and duodenal homeobox 1 (PDX-1), a key regulator of β -cell function and proliferation (29). Therefore, the secondary metabolites present in this extract may contribute not only to β -cell protection but also to β -cell regeneration. These findings are consistent with the present study, which demonstrated reduced β -cell injury and evidence of improved β -cell restoration following extract administration.

With respect to lipid regulation, flavonoids such as quercetin have been investigated as potential inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) (30). Additionally, quercetin promotes cholesterol-to-bile acid conversion and cholesterol efflux. Meanwhile, triterpenoids such as ursolic acid have been identified to primarily target hydroxy-3-methylglutaryl coenzyme A synthase 1 (HMGCS1), a key upstream enzyme of HMG-CoA reductase in lipid metabolism (31). By acting on sequential steps of the mevalonate pathway, these compounds may exert synergistic effects in suppressing cholesterol biosynthesis. Additionally, quercetin is linked to the Akt signalling pathway. It regulates the expression of sterol regulatory element-binding proteins (SREBPs), which in turn control the expression of HMG-CoA reductase and the LDL receptor, both of which are crucial for maintaining cholesterol homeostasis (32). On the other hand, quercetin ameliorates hypertriglyceridemia by suppressing lipogenic proteins acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and sterol regulatory element-binding protein-1c (SREBP-1c) while upregulating β -oxidation proteins such as peroxisome proliferator-activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1A), thereby enhancing fatty acid catabolism and reducing triglyceride accumulation (33). According to available evidence, fewer chemical constituents from *S. bracteosa* extract are known to reduce triglyceride levels compared to total cholesterol, suggesting that triglyceride modulation may only become significant at higher concentrations of the extract.

This study has several limitations. First, LC-HRMS profiling was conducted qualitatively without quantification, so the exact levels of the identified metabolites remain undetermined. Second, immunohistochemistry showed increased GLUT4 expression but did not confirm membrane translocation; more specific approaches (e.g., membrane fractionation or confocal imaging) are needed. Finally, safety assessments were not performed in this study. Future work should include quantitative phytochemical analysis, molecular pathway validation (e.g., Western blot), and comprehensive safety evaluation.

8. Awkward Passive Voice

Example from Text (Methods):

"The tissue was incubated with GLUT4 monoclonal antibody for 30 min."

Comment: Passive voice is standard in methods sections, but clarity matters.

□ This is acceptable in scientific writing, though adding the agent can help:

"Sections were incubated with a GLUT4 monoclonal antibody (Bioworld, China) for 30 min at room temperature."

Answer: The corrections have been made and highlighted in yellow.

Revision Page: 4 lines: 152-153

Sections were incubated with a GLUT4 monoclonal antibody (Bioworld, China) for 30 min at room temperature.

9. Punctuation and Spacing Issues

Example from Text (Figure Legend):

"ns: not significant; * $p < 0.05$; ** $p < 0.01$ "

Correction:

"ns: not significant; * $p < 0.05$; ** $p < 0.01$ "

Answer: The corrections have been made and highlighted in yellow.

Revision Page: 13 lines: 485-487

Figure 3. Hemoglobin A1c levels after 4 weeks of treatment. For each group, the data are presented as mean $\pm$ standard error of the mean of four replicates. ns: not significant; * $p < 0.05$; ** $p < 0.01$

10. Overuse of Repetition

Example from Text (Conclusion):

"*Saurauia bracteosa* leaf extract demonstrated antidiabetic effects... by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, and preserving islet morphology. The extract also improved lipid profiles..."

Improved:

"*Saurauia bracteosa* leaf extract demonstrated antidiabetic effects by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, preserving islet morphology, and improving lipid profiles."

Answer: The corrections have been made and highlighted in yellow.

Revision Page: 10, lines: 351-357

Saurauia bracteosa leaf extract demonstrated antidiabetic effects by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, preserving islet morphology, and improving lipid profiles (total cholesterol and triglycerides). LC-HR MS analysis identified 22 organic compounds that are likely responsible for these effects. Collectively, these findings highlight the potential of *Saurauia bracteosa* as a plant-based therapeutic candidate for managing type 2 diabetes and its associated metabolic complications.

Reviewer 6:

Dear Editor

Have a nice day.

The author investigated Phytochemical Characterization and Antidiabetic Evaluation of Saurauia bracteosa Leaf Extract in a Type 2 Diabetes Mellitus Model.

I would suggest reconsidering the manuscript for publication only after the following major revision.

1. Provide an LC-HRMS chromatogram.

Answer: We have added the LC-HRMS chromatogram in Figure 1.

Page: 13 Lines: 477-478

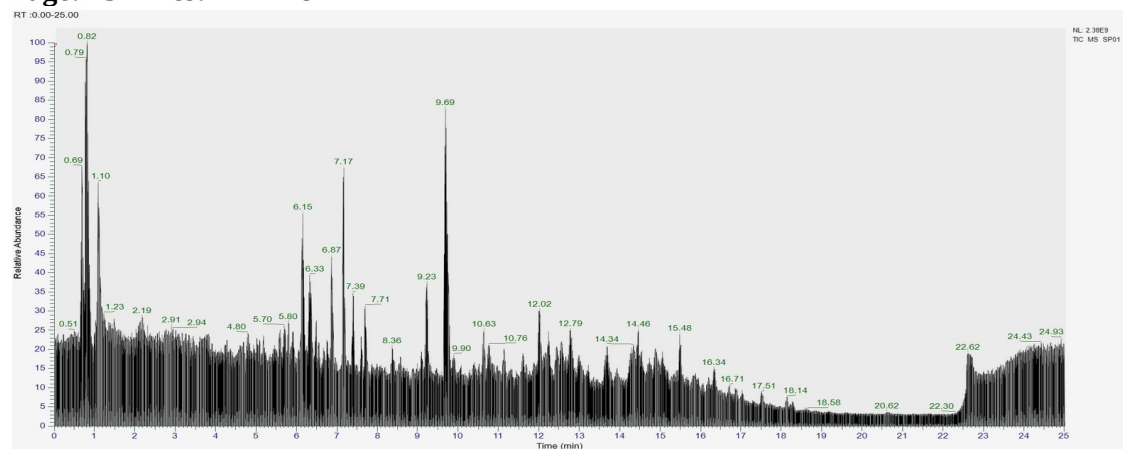


Figure 1. Liquid chromatography–high resolution mass spectrometry (LC-HRMS) chromatogram of *Saurauia bracteosa* leaf extract

2. It is necessary to rewrite the conclusion section more completely.

Answer:

We have rewrite conclusion more completely

Revision Page: 10 Line: 351-357

Saurauia bracteosa leaf extract demonstrated antidiabetic effects by reducing blood glucose and HbA1c levels, enhancing GLUT4 expression, preserving islet morphology, and improving lipid profiles (total cholesterol and triglycerides). LC-HR MS analysis identified 22 organic compounds that are likely responsible for these effects. Collectively, these findings highlight the potential of *Saurauia bracteosa* as a plant-based therapeutic candidate for managing type 2 diabetes and its associated metabolic complications.

3. In the section on cellular mechanisms, more up-to-date and accurate references should be provided.

Answer:

Additional up-to-date and relevant references have been incorporated into the Discussion section to better explain the cellular mechanisms. We have also linked the cellular mechanisms to GLUT4 expression to align with our in vivo findings.

Revision Page: 8-10, lines: 271-348

A type 2 diabetes mellitus (T2DM) model was induced using a high-fat and high-fructose diet, which promotes obesity, elevates circulating free fatty acids, glycerol, and leptin, and reduces adiponectin, collectively impairing insulin signalling (17). To further induce partial β -cell dysfunction without destruction, a single low dose of streptozotocin (25 mg/kg) was administered (18). This combined protocol is widely recognised as a reliable and reproducible approach for developing T2DM, as it mimics both insulin resistance and β -cell impairment. The

treated animals subsequently exhibited persistent hyperglycemia, confirming successful model establishment.

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The dose–response relationship in blood glucose reduction observed in weeks 3 and 4 indicated that further dose escalation no longer enhanced the therapeutic effect. This phenomenon may occur for several reasons. In pharmacodynamics, the ceiling effect (E_{max}) refers to the maximal response a drug can elicit. Once this plateau is reached, additional dose increases will not confer further benefit but may instead elevate the risk of adverse effects. This principle underlies the dosing limitations commonly observed with many antidiabetic agents (25).

Rutin, oleanolic acid, juglalin, and ursolic acid have been reported to enhance endogenous antioxidant defences, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby reducing oxidative stress and suppressing inflammatory responses through inhibition of the NF- κ B signalling pathway and downregulation of proinflammatory cytokines such as TNF- α and IL-1 β (26-28). These protective effects are essential in preventing streptozotocin-induced pancreatic β -cell apoptosis. In addition, flavonoids have been shown to activate pancreatic and duodenal homeobox 1 (PDX-1), a key regulator of β -cell function and proliferation (29). Therefore, the secondary metabolites present in this extract may contribute not only to β -cell protection but also to β -cell regeneration. These findings are consistent with the present study, which demonstrated reduced β -cell injury and evidence of improved β -cell restoration following extract administration.

With respect to lipid regulation, flavonoids such as quercetin have been investigated as potential inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) (30). Additionally, quercetin promotes cholesterol-to-bile acid conversion and cholesterol efflux. Meanwhile, triterpenoids such as ursolic acid have been identified to primarily target hydroxy-3-methylglutaryl coenzyme A synthase 1 (HMGCS1), a key upstream enzyme of HMG-CoA reductase in lipid metabolism (31). By acting on sequential steps of the mevalonate pathway, these compounds may exert synergistic effects in suppressing cholesterol biosynthesis. Additionally, quercetin is linked to the Akt signalling pathway. It regulates the expression of sterol regulatory element-binding proteins (SREBPs), which in turn control the expression of HMG-CoA reductase and the LDL receptor, both of which are crucial for maintaining cholesterol homeostasis (32). On the other hand, quercetin ameliorates hypertriglyceridemia by suppressing lipogenic proteins acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and sterol regulatory element-binding protein-1c (SREBP-1c) while upregulating β -oxidation proteins such as peroxisome proliferator-activated receptor alpha (PPAR α) and carnitine palmitoyltransferase 1a (CPT1A), thereby enhancing fatty acid

catabolism and reducing triglyceride accumulation (33). According to available evidence, fewer chemical constituents from *S. bracteosa* extract are known to reduce triglyceride levels compared to total cholesterol, suggesting that triglyceride modulation may only become significant at higher concentrations of the extract.

This study has several limitations. First, LC-HRMS profiling was conducted qualitatively without quantification, so the exact levels of the identified metabolites remain undetermined. Second, immunohistochemistry showed increased GLUT4 expression but did not confirm membrane translocation; more specific approaches (e.g., membrane fractionation or confocal imaging) are needed. Finally, safety assessments were not performed in this study. Future work should include quantitative phytochemical analysis, molecular pathway validation (e.g., Western blot), and comprehensive safety evaluation.

4. What is the effective role of Juglalin and Quercitrin?

Answer:

Thank you for your valuable question. To date, there are no direct studies reporting the antidiabetic activity of juglalin. However, juglalin has been shown to exert anti-inflammatory, antioxidant, and lipid-lowering effects in experimental models (Li et al., 2021). Since chronic inflammation and dyslipidemia are closely linked to the development and progression of type 2 diabetes, these pleiotropic effects may indirectly contribute to glycemic control. In this context, juglalin's ability to ameliorate dyslipidemia is particularly relevant, as dyslipidemia represents a common metabolic complication of diabetes mellitus. Consistently, in our study *S. bracteosa* leaf extract improved lipid parameters, notably by reducing total cholesterol and triglyceride levels, which may be partly attributable to the presence of juglalin and related phytoconstituents.

In contrast, quercitrin, another flavonoid glycoside identified in *S. bracteosa*, has demonstrated more direct antidiabetic activity. In STZ-induced diabetic rats, quercitrin (30 mg/kg) significantly lowered fasting glucose, increased insulin levels, and restored antioxidant defenses while protecting pancreatic, hepatic, and renal tissues (Babujanathanam et al., 2011). Similarly, quercitrin was identified among active flavonoids that improved glycemia and lipid profiles in alloxan-induced diabetic mice. These effects were further supported by strong α -amylase and α -glucosidase inhibition (Van et al., 2022; Xu et al., 2024).

Taken together, these findings suggest that juglalin may indirectly support glycemic control via lipid modulation and anti-inflammatory effects, while quercitrin exerts direct hypoglycemic and antioxidant actions, alongside enzyme inhibition and lipid regulation. The presence of both compounds in *S. bracteosa* may therefore contribute synergistically to its observed antidiabetic and metabolic benefits.

Reference:

Li Q, Ge C, Tan J, Sun Y, Kuang Q, Dai X, Zhong S, Yi C, Hu LF, Lou DS, Xu M. Juglanin protects against high fat diet-induced renal injury by suppressing inflammation and dyslipidemia via regulating NF- κ B/HDAC3 signaling. *Int Immunopharmacol*. 2021 Jun;95:107340. doi: 10.1016/j.intimp.2020.107340. Epub 2021 Mar 2. PMID: 33667999.

Babujanathanam, R., Kavitha, P., Mahadeva Rao, U.S. *et al.* Quercitrin a bioflavonoid improves the antioxidant status in streptozotocin: induced diabetic rat tissues. *Mol Cell Biochem* **358**, 121–129 (2011). <https://doi.org/10.1007/s11010-011-0927-x>

Van, L. V., Pham, E. C., Nguyen, C. V., Duong, N. T. N., Le Thi, T. V., & Truong, T. N. (2022). In vitro and in vivo antidiabetic activity, isolation of flavonoids, and in silico molecular docking of stem extract of *Merremia tridentata* (L.). *Biomed. Pharmacother*, 146, 112611.

Xu, X., Ding, L., Fu, Y., Wang, Y., & Cai, S. (2024). Polyphenol-rich extract of Chinese sumac (*Rhus chinensis* Mill.) fruits and its main component decrease in vitro starch digestibility of bread: Exploring the potential mechanisms from the perspective of molecular interactions. *LWT*, 195, 115856.

5. How do you explain the effect of the solvent (ethanol)?

Answer:

Thank you for the question. Ethanol was used as the solvent for extraction. After maceration, the solvent was removed completely by rotary evaporation under reduced pressure (≤ 40 °C). For dosing, the dried extract was reconstituted in 0.5% carboxymethylcellulose (CMC) in water, and the diabetic control group received the same CMC vehicle. Thus, no ethanol was present in the dosing vehicle, and the pharmacodynamic effects cannot be attributed to ethanol.

Revision Page: 2 Line: 70-73

Crude extract was concentrated by rotary evaporation (40 °C). The dried extract was reconstituted in 0.5% carboxymethylcellulose sodium (CMC-Na) solution for oral administration, with no ethanol remaining.

6. For Figures 3 and 4, numerical data (with tables) should also be provided.

Answer:

I have added numerical data to tables for Figures 3 and Figure 4.

Figure 3 → **Table 2.** Effect of *Saurauia bracteosa* extract on the diameter of Langerhans islets after four weeks of treatment

Revision Page: 7 lines: 232-233

Table 2. Effect of *Saurauia bracteosa* extract on the diameter of Langerhans islets after four weeks of treatment.

Groups	Average (mm) $\pm$ SEM
Negative control	14.34 $\pm$ 3.83 ^{ab}
Extract 100 mg/kg	23.61 $\pm$ 3.35 ^{ab}
Extract 200 mg/kg	26.37 $\pm$ 4.62 ^{ab}
Extract 400 mg/kg	34.75 $\pm$ 5.41 ^c
Metformin 45 mg/kg	32.15 $\pm$ 3.12 ^c
Normal control	32.21 $\pm$ 3.63 ^c

Legend: Data represent the mean of 10 islets of Langerhans. ^a $p < 0.05$: significantly different from the normal control group; ^b $p < 0.05$: significantly different from the positive control group; ^c $p < 0.05$: significantly different from the negative control group.

Revision Page: 8 lines: 251-252

Figure 4 → **Table 3.** Immunohistochemical scoring of GLUT4 expression in skeletal muscle (cell distribution, staining intensity, and IRS total score)

Table 3. Immunohistochemical scoring of GLUT4 expression in skeletal muscle (cell distribution, staining intensity, and IRS total score)

Experimental group	Cell distribution score $\pm$ SD	Staining intensity score $\pm$ SD	Total IRS (Distribution $\times$ Intensity) $\pm$ SD
Negative control	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
Extract 100 mg/kg	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
Extract 200 mg/kg	1.0 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00
Extract 400 mg/kg	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00
Metformin 45 mg/kg	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00
Normal control	1.00 $\pm$ 0.00	2.00 $\pm$ 0.00	2.00 $\pm$ 0.00

Legend: Data were obtained from 3 slides. SD = standard deviation. Cell distribution score of immunoreactive cells, 0: no immunoreactive cells; 1: <10% immunoreactive cells; 2: 10-50% immunoreactive cells; 3: 51-80% immunoreactive cells; 4: >80% immunoreactive cells. Staining intensity score, 0: no staining; 1: weak (pale) staining intensity; 2: moderate (bright) staining intensity; 3: strong (dark) staining intensity. Immunoreactive score (IRS) = cell distribution score $\times$ staining intensity score, 0: no protein expression; 1-3: low protein expression; 4-8: moderate protein expression; 9-12: high protein expression.

OPEN PEER REVIEW

Revision (1)

Esmail Mohammadian : Reviewer

30 Nov 2025

Dear Editor

Have a nice day.

The addressed issues and concerns have been effectively resolved. Consequently, the reviewer concurs to proceed with the publication of this paper in its current state.

REFEREE : Reviewer

6 Dec 2025

Dear author

Most of the corrections of manuscript requested by the referees have been addressed and included in the text of the article.

Good luck

REFEREE : Associate Editor

6 Dec 2025

The revision adequately addresses most concerns. Please provide: (1) explanation for the zero variability in GLUT4 IHC scores, (2) explicit discussion of statistical power limitations due to $n=4$, and (3) stronger emphasis on the critical need for safety assessment. After these minor clarifications, the manuscript is suitable for publication.

REFEREE : EIC

6 Dec 2025

I am pleased to inform you that your revised manuscript has been evaluated by the reviewers and the editorial team, and we have decided to accept it for publication pending minor clarifications.

The reviewers concurred that you have made substantial improvements and have adequately addressed the majority of concerns raised in the previous review round. The methodological justifications, expanded discussion, and language revisions have significantly strengthened the scientific quality and readability of your manuscript. Final Minor Revisions Required:

Before we proceed with final acceptance, please address the following three minor points in a brief response letter (no need to modify the manuscript unless specified):
 GLUT4 Immunohistochemistry Data Variability: Table 3 reports zero standard deviations (e.g., 1.00 ± 0.00) across multiple groups. Please provide a brief explanation for this lack of variability—was the scoring system binary, or does this reflect the precision of the measurement method?

Sample Size Statistical Power: While you appropriately acknowledged $n=4$ as a

limitation, please add one sentence to the Discussion explicitly stating that this small sample size may have limited statistical power to detect dose-dependent differences between treatment groups.

Safety Assessment Priority: Please strengthen the final sentence of your limitations paragraph to emphasize that comprehensive toxicity evaluation is a critical prerequisite before any therapeutic development, rather than a general future direction.

Once you submit your brief response addressing these points, we will proceed directly to publication without further review.

Next Steps:

Please submit your response letter within 7 days via the online submission system.

Upon receipt, we will issue a formal acceptance letter and forward your manuscript to our production team for copyediting and typesetting.

You will receive page proofs for final approval within approximately 2-3 weeks.

Thank you for your diligent efforts in revising the manuscript. We appreciate your contribution to the scientific literature on natural products and diabetes research.

Congratulations on your successful revision.

Sincerely,

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Reply to Editor in Chief, Associate Editor, and Reviewer

Reviewer 1:

Dear author

Most of the corrections of manuscript requested by the referees have been addressed and included in the text of the article.

Good luck

Answer: Thank you for your kind assessment and for acknowledging the revisions. We sincerely appreciate your valuable comments and guidance, which have helped improve the quality of our manuscript.

Associate Editor's Comments:

Associate Editor 0:

The revision adequately addresses most concerns. Please provide: (1) explanation for the zero variability in GLUT4 IHC scores, (2) explicit discussion of statistical power limitations due to $n=4$, and (3) stronger emphasis on the critical need for safety assessment. After these minor clarifications, the manuscript is suitable for publication.

Answer: We have added an explanation for the lack of variability in GLUT4 IHC scores, as well as the limitations of statistical power and the critical need for safety assessment, to the results and discussion section, particularly in the limitations section of the study.

Revision: Page: 10, lines: 345-349

The lack of variability in GLUT4 scores in the immunohistochemistry analysis stems from consistently strong staining across samples, which may partly result from the small number of animals and thus diminishes the ability to detect subtle biological differences.

Page: 10, line: 349-353

The small sample size ($n=4$) may also have limited the statistical power to detect dose-dependent differences between treatment groups. However, the sample size was determined using the Federer formula and aligned with ethical considerations for animal use, and the observed outcomes consistently support the antidiabetic potential of the extract.

Page: 10, line: 353-359

Additionally, the absence of toxicity testing prevents firm conclusions about the extract's safety profile. Therefore, future studies should include larger animal groups to improve statistical robustness, incorporate quantitative phytochemical analysis and molecular pathway validation (e.g., Western blot), and prioritize comprehensive toxicity evaluation as a critical prerequisite before the extract can be considered for therapeutic development.

Editor in Chief

GLUT4 Immunohistochemistry Data Variability: Table 3 reports zero standard deviations (e.g., 1.00 ± 0.00) across multiple groups. Please provide a brief explanation for this lack of variability—was the scoring system binary, or does this reflect the precision of the measurement method?

Answer: The absence of variability ($SD = 0$) in the GLUT4 IHC scores is attributable to the use of a semi-quantitative, categorical Immunoreactive Score (IRS) system, in which each sample is assigned a discrete value based on defined ranges of cell distribution and staining intensity. In

the present study, GLUT4 staining patterns appeared highly uniform across all slides, resulting in identical IRS values for all animals within the same group. Therefore, the zero variability reflects the consistency of the staining outcomes rather than an analytical error. Future studies with a larger number of animals and digital image-based quantitative methods may provide greater resolution for detecting inter-individual differences.

Sample Size Statistical Power: While you appropriately acknowledged $n=4$ as a limitation, please add one sentence to the Discussion explicitly stating that this small sample size may have limited statistical power to detect dose-dependent differences between treatment groups.

Answer: We thank the reviewer for this valuable suggestion. As recommended, we have added a sentence to the Discussion acknowledging that the small sample size ($n=4$) may have limited the statistical power to detect dose-dependent differences. The revised sentence is now included in the last paragraph of the Discussion.

Revision Page: 10, lines: 349-353

The small sample size ($n=4$) may also have limited the statistical power to detect dose-dependent differences between treatment groups. However, the sample size was determined using the Federer formula and aligned with ethical considerations for animal use, and the observed outcomes consistently support the antidiabetic potential of the extract.

Safety Assessment Priority: Please strengthen the final sentence of your limitations paragraph to emphasize that comprehensive toxicity evaluation is a critical prerequisite before any therapeutic development, rather than a general future direction.

Answer: Thank you for this valuable suggestion. We have revised the final sentence of the limitations paragraph to clearly emphasize that a comprehensive toxicity evaluation is a critical prerequisite before any therapeutic development of the extract, rather than a general recommendation for future work.

Revision Page: 10, lines: 353-359

Additionally, the absence of toxicity testing prevents firm conclusions about the extract's safety profile. Therefore, future studies should include larger animal groups to improve statistical robustness, incorporate quantitative phytochemical analysis and molecular pathway validation (e.g., Western blot), and prioritize comprehensive toxicity evaluation as a critical prerequisite before the extract can be considered for therapeutic development.

OPEN PEER REVIEW

Revision (2)

REFEREE : EIC

13 Dec 2025

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.

Your work underwent a rigorous peer-review process, and the reviewers and editorial board were impressed by the originality, scientific rigor, and relevance of your findings. We believe your contribution will be of significant interest to our readership and will advance the field of natural pharmaceutical products.

Before publication, our production team will contact you regarding final formatting, proofs, and any necessary administrative steps. Please ensure that all authors have approved the final version of the manuscript and that any required ethical or copyright documentation is in order.

On behalf of the editorial board, I congratulate you and your co-authors on this excellent work and thank you for choosing to publish in JJNPP.