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Evaluation of the Hypoglycemic Potential of the Ethanolic Bulb Extract of Allium Chinense G. Don (Sibujing) on High-Glucose Induced Hyperglycemic Swiss Albino Mice

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Abstract: This study investigated the hypoglycemic potential of the ethanolic bulb extract of *Allium chinense* G. Don, commonly known as Sibujing, a spice used in Maranao cuisine. Given the rising global prevalence of hyperglycemia and diabetes mellitus, with 14,416 deaths due to diabetes mellitus in the Philippines alone in 2023, effective treatments are crucial. Swiss albino mice were induced with hyperglycemia via a high-glucose diet to evaluate the extract's potential in managing elevated glucose levels. Phytochemical screening of the crude extract revealed the presence of flavonoids, alkaloids, phenols, saponins, steroids, and terpenoids, with the absence of tannins. Quantitative analysis showed a total phenolic content (TPC) of 13.2±1.0 mg gallic acid per gram extract and a total flavonoid content (TFC) of 9.8±0.1 mcg quercetin per gram extract. Acute oral toxicity testing indicated an LD50 >2000 mg/kg body weight, with no observed deaths or toxic symptoms. However, statistical analysis using One-Way ANOVA and post-hoc tests demonstrated that administration of the crude extract at varying doses (50, 300, 2000 mg/kg) did not result in a significant reduction in blood glucose levels compared to both the negative control (normal saline solution) and the positive control (metformin, 50 mg/kg). All pairwise comparisons yielded non-significant results (p>0.05), suggesting that under the conditions of this study, the ethanolic bulb extract of *A. chinense* did not exhibit significant hypoglycemic activity comparable to metformin.

Keywords: *Hyperglycemia, High Glucose, Crude Extract, Allium Chinense G. Don, Swiss Albino Mice.*

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I. INTRODUCTION

Diabetes Mellitus (DM), a chronic disease characterized by elevated blood glucose levels (hyperglycemia), is a significant global public health concern. Metabolic disorders leading to insufficient insulin production or utilization affect millions worldwide. The World Health Organization (WHO) reported 642 million people living with DM globally, with 1.6 million deaths annually. In the Philippines, diabetes mellitus was the fourth leading cause of death in 2023, accounting for 6.2% (14,416) of total deaths (Philippine Statistics Authority, 2023). This escalating prevalence underscores the urgent need for effective and accessible treatments.

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Traditional medicine, particularly the use of medicinal plants, has gained increasing interest in diabetes management due to the high cost and potential side effects of conventional medications (Zanzabil, 2023; Rondilla et al., 2021). Over 80% of the world's population relies on traditional medicine for primary healthcare (WHO, 2023). Allium chinense G. Don, locally known as Sibujing, is a widely available plant species in the Philippines traditionally used as a condiment in Mindanao (Vera Cruz et al., 2023). While other Allium species like A. sativum (garlic) and A. cepa (onion) have established antidiabetic properties due to their sulfur-containing compounds (Nakamura et al., 2021; Choi et al., 2022; Pandey et al., 2022), the medicinal properties of A. chinense G. Don remain insufficiently studied, particularly its potential in managing Type 2 Diabetes Mellitus (T2DM).

Sulfur compounds are essential for plant health and human disease prevention, imparting antioxidant, anti-inflammatory, and antimicrobial properties (Hill et al., 2022). The Allium family is known for thiosulfinates, which contribute to maintaining glucose tolerance and beta-cell viability by reducing oxidative damage and improving insulin secretion (Sabiu et al., 2019). This study aimed to investigate the potential hypoglycemic activity of the ethanolic bulb extract of A. chinense G. Don in high-glucose induced hyperglycemic Swiss albino mice. Specifically, the research sought to determine the extract's effect on blood glucose levels, compare its efficacy to a standard antidiabetic drug (metformin), and identify the secondary metabolites present. The findings could contribute to the existing knowledge of A. chinense G. Don and its potential as a natural remedy for T2DM.

II. MATERIALS AND METHODS

➢ *Plant Material and Extraction*

Bulbs of Allium chinense G. Don were collected from Wato-Balindong, Lanao del Sur, Philippines. The plant material underwent authentication and phytochemical analysis at the Biological Science Laboratory of Mindanao State University-Iligan Institute of Technology (MSU-IIT). The bulbs were cleaned, air-dried, and pulverized into a fine powder using a pulverizer and sieved through No. 40 and No. 60 sieves to ensure uniform particle size (Tinoy et al., 2024; Veltkamp, 2024). Two hundred grams of the powdered bulb were macerated in 1 liter of 90% ethanol (1:10 ratio) for one week with intermittent agitation at 150 rpm using an orbital shaker (Kumar et al., 2023; Salem et al., 2016). Ethanol was chosen as the solvent due to its polarity, miscibility with water, and safety profile compared to methanol (Abubakar et al., 2020; Pohanka, 2016). After maceration, the crude extract was concentrated via water bath evaporation (Tinoy et al., 2023).

The percentage yield was calculated using the formula: %w/w yield=(weight of crude extract in g/weight of dried bulbs in g)×100.

➢ *Phytochemical Analysis*

Qualitative phytochemical screening was performed on the ethanolic bulb extract of A. chinense G. Don to detect the presence of flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids, based on procedures adapted from Tinoy et al. (2024).

- Flavonoids (Hydrochloric Acid Reduction Test): Defatted extract dissolved in 80% alcohol, treated with 12M HCl, and heated. Red color indicates positive.
- Alkaloids (Wagner's Test): Extract treated with 2M HCl and NaCl, filtered, then reacted with Wagner's reagent. Brown precipitate indicates positive.
- Saponins (Froth Test Method): Extract shaken vigorously with water. Stable froth (2 cm for 30 minutes) indicates positive.
- Tannins (Ferric Chloride Test): Extract boiled with water, filtered, and treated with 1% FeCl3. Blue or black precipitate indicates positive.
- Steroids (Salkowski Test): Defatted extract treated with FeCl3, then concentrated H2SO4 layered at the bottom. Brown, blue, or green rings indicate positive.
- Phenols (Sodium Hydroxide Method): Extract dissolved in water, treated with 20% NaOH. Blue color indicates positive.
- Terpenoids (Liebermann-Burchard Test): Extract dissolved in chloroform, treated with acetic anhydride and concentrated H2SO4. Dark green color indicates positive.
- Quantitative analysis for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was also conducted.
- Total Phenolic Content (Folin-Ciocalteu Method): Extract reacted with Folin-Ciocalteu reagent and sodium hydroxide. Absorbance measured at 760 nm or 765 nm using a spectrophotometer. Results expressed as mg gallic acid equivalent (GAE) per gram of extract.
- Total Flavonoid Content (Aluminum Chloride Colorimetric Assay): Extract reacted with aluminum chloride and sodium acetate. Absorbance measured at 415 nm using a UV-vis spectrophotometer. Results expressed as mcg quercetin equivalent (QE) per gram of extract.

➢ *Animal Housing and Ethical Considerations*

Adult Swiss albino mice were obtained from the AMCC animal house and acclimatized for 7 days in the AMCC Pharmacy Laboratory at 25±2∘C and 70% humidity. They were fed standard rodent pellets and water ad libitum. The room maintained 10-20 air changes per hour with a 12-hour light/dark cycle. Cages with wood shavings were cleaned thrice weekly with sodium hypochlorite (OECD, 2001). All animal procedures adhered to ethical guidelines, and animals were humanely euthanized via cervical dislocation at the study's end (IACUC Guidelines, 2014).

➢ *Acute Oral Toxicity Test*

The acute toxicity test followed OECD Guidelines 423. Twelve healthy Swiss albino mice were used (3 mice per step). The crude extract was administered via oral gavage at starting doses of 5 mg/kg body weight, followed by 50 mg/kg, 300 mg/kg, and 2000 mg/kg if no toxic symptoms were observed. Animals were observed for 30 minutes, 4 hours, 24 hours, and daily for 14 days for any toxic signs, reactions, or delayed effects. Internal organs were inspected post-dissection for lesions or deformities. The LD50 was estimated based on these observations.

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➢ *Induction of Diabetes and Treatment Administration*

After acclimatization, mice were induced with hyperglycemia by oral gavage of 20 g/kg of 50% D-glucose for 2 weeks (Zhan et al., 2019). Blood glucose levels were monitored weekly via tail pricking using a OneTouch Select glucometer and test strips (OECD, 2001). Once 25 mice reached a blood glucose level of 240 mg/dL (Melior Discovery, 2020), they were randomly divided into 5 groups (n=5 per group) for treatment over an 8-week period:

- Group 1 (Low Dose): 50 mg/kg A. chinense extract
- Group 2 (Mid-Dose): 300 mg/kg A. chinense extract
- Group 3 (High Dose): 2000 mg/kg A. chinense extract
- Group 4 (Positive Control): 50 mg/kg metformin (standard dose, Sahu et al., 2024)
- Group 5 (Negative Control): 100 mL/kg normal saline solution

All test substances were administered via oral gavage (Zake et al., 2021). Blood glucose levels were monitored at 15-minute intervals for the first 1 hour and 45 minutes post-administration.

➢ *Statistical Analysis*

Data was analyzed using One-Way Analysis of Variance (ANOVA) to compare baseline blood glucose levels among groups and to assess the overall effect of treatment. Repeated measures ANOVA were performed to evaluate changes in blood glucose levels over time and the interaction between time and treatment. Post-hoc comparisons were conducted to identify specific group differences when a significant overall effect was observed (Ostertagova & Ostertag, 2013; Libretexts, 2024). Statistical significance was set at $p<0.05$.

III. RESULTS AND DISCUSSIONS

➢ *Preparation of Plant Extract*

The crude ethanolic bulb extract of Allium chinense G. Don was dark-brown, semi-solid in mass, and had a pungent odor.

Fig 1 Crude Ethanolic Extract of *A. Chinense* G. Don

The average percentage yield based on the three replicants of 20 grams of dried powdered bulbs of A. chinense G. Don soaked in 200 ml of ethanol is 66.67%. Table 1 shows the individual yield obtained from each replicant.

Table 1 The percentage yield of the crude ethanolic bulb extract of *A. chinense* G. Don

Replicant	Weight of dried leaves (g)	Weight of crude extract (g)	Percentage yield (%)
1	20	3.3	66
2	20	3.3	66
3	20	3.4	68
Mean Value			66.67

Both replicants 1 and 2 yielded 66% while the third replicant yielded 68% in every 20 grams of the crude extract hence, the mean value is 66.67%.

➢ *Phytochemical Analysis*

The phytochemical screening for secondary metabolites of the ethanolic bulb extract of *A. chinense* G. Don followed the procedures and tests found in chapter 3 to determine the presence of phytochemical compounds such as flavonoids, alkaloids, saponins, tannins, and steroids (Tinoy et al. 2024). A quantitative analysis of the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of the crude extract was also performed to quantify the number of flavonoids and phenols present in the extract which are some of the phytochemicals responsible for the hypoglycemic activity of the *Allium* family (Nakamura et al.) (Hill et al.).

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Table 2 shows the results for the tests conducted.

Table 2 Results for Phytochemical Tests for Secondary Metabolites of *A. chinense* G. Don

TESTS	VISIBLE RESULTS	IMPLICATIONS
Flavonoids	Hydrochloric Acid Reduction: Development of red color indicating a positive result for flavonoids.	+++
Alkaloids	Wagner's Test: Formation of a brown precipitate indicating a positive result for alkaloids.	+++
Phenols	Sodium Hydroxide Test: Formation of blue color indicating the presence of phenols.	+++
Saponins	Froth Test Method: Formation of 2 cm tall froth for 30 minutes indicating a positive result for saponins.	+++
Tannins	Ferric Chloride Test: No development of blue precipitate indicating a negative result for tannins.	-
Steroids	Salkowski Test: Formation of brown rings at the boundary region of the extract and sulfuric acid indicating a positive result for steroids.	++
Terpenoids	Liebermann-Burchard Test: Formation of dark green precipitate at the top of the solution indicating a positive result of terpenoids.	++

The phytochemical analysis of the ethanolic crude extract of *A. chinense* G. Don as shown in Table 2 shows a positive result for alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, and a negative result for tannins.

Additionally, a quantitative analysis of the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) was performed to determine the concentrations present in the ethanolic bulb extract that may contribute to its hypoglycemic activity (Sabiu et al. 2019) (Nakamura et al. 2021) (Chakraborty et al. 2022) (Hill and Foroozand et al. 2023). Table 3 shows the results for the quantitative analysis of *A. chinense* G. Don.

Table 3 Quantitative analysis of the phenols and flavonoids of *A. chinense* G. Don

Quantitative Test	Visible Results
Total Phenolics	13.2±1.0 mg gallic acid per gram extract
Total Flavonoids	9.8±0.1 mcg Quercetin per gram extract

The TFC and TPC of the crude extract of *A. chinense* G. Don showed that the TFC is 9.8±0.1 mcg Quercetin per gram extract and the TPC is 13.2±1.0 mg gallic acid per gram extract indicating that there is a sufficient amount that can potentially cause a hypoglycemic activity.

➢ *Acute Toxicity Tests*

No deaths were recorded after 24 hours of administration of the various doses (3, 50, 300, 2000 ml/kg body weight) of the ethanolic bulb extract of *A. chinense* G. Don. The low dose was 50 mg/kg for the low dose, 300 mg/kg for the mid dose, and 2000 mg/kg for the high dose. The intestines, spleen, kidney, and liver were also in good condition. There were no lesions, perforations, or any deformities of any kind nor any changes to the texture of the internal organs of the mice. Therefore, the LD50 is estimated at LD50 >2000 mg/kg body weight in mice.

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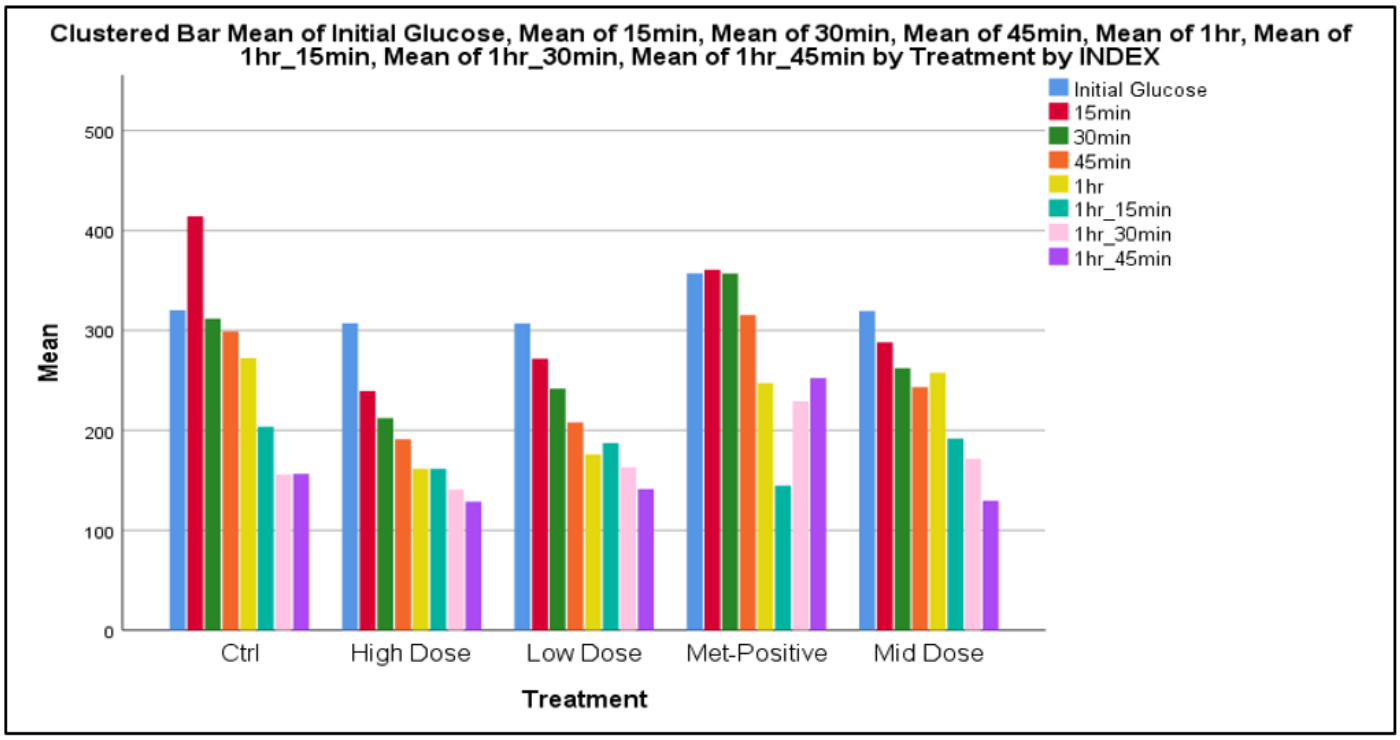
➢ *Statistical Analysis*

The first part of the data was analyzed using the One-Way Analysis of Variance (ANOVA) method because it involves comparing the antidiabetic effects of the ethanolic bulb extract of *A. chinense* G. Don to the positive and negative control in Swiss albino mice to determine any significant differences between the two variables.

A one-way analysis of variance (ANOVA) was performed to assess baseline differences in blood glucose levels among high-glucose induced diabetic Swiss albino mice assigned to control, low dose, mid dose, high dose, and metformin-positive groups. The analysis indicated no significant differences at baseline, $F(4,20)=0.335, p=0.848$. A repeated measures ANOVA revealed a significant main effect of time, $F(7,140)=18.404, p<0.001$, suggesting that blood glucose levels significantly changed over the course of the study.

However, the interaction between time and treatment was not significant, $F(28,140)=0.970, p=0.515$, indicating that the patterns of change over time were similar across groups. The between-subjects effect of treatment was also not statistically significant, $F(4,20)=2.03, p=0.128$, suggesting that administration of the ethanolic bulb extract of *Allium chinense* G. Don at different doses did not result in significant differences in blood glucose levels compared to the control and metformin-positive groups.



- *Comparison of Crude Extract and Positive Control*

A repeated measures ANOVA was conducted to compare the hypoglycemic activity of the ethanolic bulb extract of *Allium chinense* G. Don at varying doses with that of the standard antidiabetic agent, metformin, in diabetic Swiss albino mice. The between-subjects effect of treatment was not statistically significant, $F(4,20)=2.03, p=0.128$, indicating no significant differences in overall blood glucose levels among the groups. Although post hoc pairwise comparisons were performed for transparency, all comparisons yielded non-significant results ($p>0.05$), further confirming the absence of meaningful differences between treatment groups, including between the extract doses and metformin.

These findings suggest that the ethanolic bulb extract did not exhibit hypoglycemic activity comparable to that of metformin under the conditions of this study. Below is a bar graph showing the relationship between the blood glucose levels of the mice and various doses over time.

IV. DISCUSSION

The present study aimed to evaluate the hypoglycemic potential of the ethanolic bulb extract of *Allium chinense* G. Don in high-glucose induced hyperglycemic Swiss albino mice. While the phytochemical analysis confirmed the presence of several bioactive compounds, including flavonoids, alkaloids, phenols, saponins, steroids, and terpenoids, which are often associated with antidiabetic properties in other plant species (Tran et al., 2020; Nakamura et al., 2021; Chakraborty et al., 2022; Hill et al., 2023), the experimental results did not demonstrate a significant hypoglycemic effect.

The quantitative analysis showed a TPC of 13.2±1.0 mg GAE/g extract and a TFC of 9.8±0.1 mcg QE/g extract. These values indicate the presence of phenolic and flavonoid compounds, which are known for their antioxidant and potential glucose-lowering activities. For instance, *A. cepa* (onion) has been shown to reduce blood glucose levels due to compounds like quercetin and s-methylcysteine sulfoxide,

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which promote glucose uptake and enhance insulin sensitivity (Chakraborty et al., 2022; Ngan Tran et al., 2020).

However, despite the presence of these phytochemicals in *A. chinense* G. Don, their concentration or specific composition might not be sufficient, or their mechanism of action might differ from those in other *Allium* species to elicit a significant hypoglycemic effect under the study conditions.

The acute toxicity test indicated that the ethanolic bulb extract of *A. chinense* G. Don is safe up to a dose of 2000 mg/kg body weight, with no observed mortality or adverse effects. This suggests a wide therapeutic window if any efficacy were to be found.

However, the core finding of the study, as revealed by the repeated measures ANOVA, is the lack of a statistically significant difference in blood glucose reduction across the various doses of *A. chinense* extract, the negative control, and the metformin-positive control. While blood glucose levels did change significantly over time in all groups (likely due to the acute glucose challenge and subsequent physiological regulation), the extract did not demonstrate a distinct glucose-lowering pattern or magnitude comparable to metformin, nor did it significantly differ from the untreated hyperglycemic control. This suggests that the extract, at the tested doses and under the specific experimental conditions (high-glucose induction, Swiss albino mice model, and duration of observation), did not exert a notable antidiabetic effect.

This outcome contrasts with some related studies on other *Allium* species, such as *A. hookeri*, *A. sativum*, and *A. cepa*, which have shown promising antidiabetic effects in animal models (Choi et al., 2022; Jalal et al., 2007; Xie et al., 2023). The discrepancy could be attributed to several factors:

- Species-specific differences: While belonging to the same genus, the phytochemical profiles and biological activities of *A. chinense* may vary significantly from other *Allium* species.
- Extraction method: Although ethanol is a common solvent, different extraction methods or solvents might yield different concentrations or types of active compounds.
- Dosage and duration: The tested doses, while covering a wide range, might not have been optimal, or the 8-week treatment period might have been insufficient to observe a significant long-term effect or a more pronounced reduction in chronic hyperglycemia.
- Animal model: While Swiss albino mice are common, other diabetic mouse models (e.g., NOD, Akita, db/db, ob/ob mice) or different induction methods (e.g., alloxan or streptozotocin) might respond differently to the extract. The high-glucose induction method might have created a hyperglycemia model that was not sufficiently responsive to the extract's potential subtle effects.
- Metabolic complexity: T2DM involves complex pathophysiological mechanisms, including insulin resistance and β-cell dysfunction (Galicia-Garcia et al., 2020). The extract might not effectively target these specific pathways in the induced model.

The study's findings highlight the importance of rigorous scientific evaluation of traditional remedies. While *A. chinense* G. Don possesses various phytochemicals, its direct hypoglycemic efficacy, as tested in this study, was not statistically significant. This does not preclude other potential health benefits or different pharmacological activities.

V. CONCLUSION AND RECOMMENDATIONS

➢ *Summary of Findings*

The ethanolic bulb extract of *A. chinense* G. Don was successfully prepared, yielding an average of 66.67%. Phytochemical screening confirmed the presence of flavonoids, alkaloids, phenols, saponins, steroids, and terpenoids, but not tannins. Quantitative analysis revealed TPC of 13.2±1.0 mg GAE/g extract and TFC of 9.8±0.1 mcg QE/g extract. The acute toxicity test indicated an LD50 >2000 mg/kg body weight, confirming the extract's safety at the tested doses. However, statistical analysis using repeated measures ANOVA showed no significant differences in blood glucose levels among the various *A. chinense* extract doses, the negative control, and the metformin-positive control groups. The interaction between time and treatment was also not significant, indicating similar patterns of glucose change across all groups.

➢ *Conclusion*

Based on the current findings, the ethanolic bulb extract of *Allium chinense* G. Don did not exhibit a statistically significant hypoglycemic effect on high-glucose induced hyperglycemic Swiss albino mice under the conditions of this study. The presence of secondary metabolites such as flavonoids, saponins, alkaloids, phenols, terpenoids, and steroids, while confirmed, did not translate into a measurable reduction in blood glucose levels comparable to the standard antidiabetic drug metformin. Therefore, the null hypothesis, stating that the ethanolic bulb extract of *A. chinense* G. Don would have no significant effect on blood glucose levels compared to the control group treated with metformin, is accepted**.**

➢ *Recommendations*

Based on these findings, the researchers recommend the following for future studies:

- Alternative Diabetic Models: Utilize established diabetic mouse models such as Non-Obese Diabetic (NOD) mice, Akita mice, or db/db and ob/ob mice, which may more closely mimic human T2DM pathophysiology and offer different sensitivities to potential therapeutic agents.
- Chemical Induction Methods: Consider chemical induction of diabetes using alloxan or streptozotocin (STZ) via the intraperitoneal route, which can provide a more consistent and well-characterized diabetic state.
- Alternative Positive Controls: Explore therapeutic alternatives for positive controls, such as Linagliptin or Glibenclamide, which represent different classes of antidiabetic drugs and may provide a broader comparative context.
- Further Formulation Studies: Given the presence of saponins, investigate the formulation of a soap containing

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A. chinense G. Don extracts. This would require solubility testing in various solvents, compatibility testing with excipients, and evaluation of soap properties (appearance, color, pH, alkali content, fatty matter, lathering, cleaning efficiency, skin feel, shelf-life stability, dissolution).

- Toxicity and Irritation Testing for Formulations: Conduct comprehensive toxicity, skin irritation, and ocular irritation testing for any viable soap formulations.

Isolation and Characterization of Compounds: It is highly recommended to perform isolation testing to identify the specific active compounds, especially the phenols and flavonoids, responsible for any potential biological activity. Techniques such as partitioning (liquid-liquid extraction), column chromatography, and thin-layer chromatography can be employed to isolate and purify individual compounds for further testing. This would allow for a more targeted investigation of their pharmacological effects.

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