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Article

Antidiabetic activity of combined extracts of *Hibiscus sabdariffa* Linn. and *Stevia rebaudiana* Bert. on streptozotocin-induced diabetes Wistar rats

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Abstract

The increasing prevalence of diabetes mellitus and the limitation in its conventional therapies underscores the need for alternative treatments. *Hibiscus sabdariffa* Linn. and *Stevia rebaudiana* Bert. have demonstrated individual antidiabetic activities attributed to their secondary metabolites, including flavonoid and phenolic compounds. This study employed an experimental in vivo design using rats induced with STZ. Thirty rats were divided into five groups (n=6): normal control, negative control, positive control (Glibenclamide), and two treatment groups receiving the combined aqueous extracts of H.sabdariffa and S.rebaudiana (RSAE) at 500 and 1000 mg/kgBW. Diabetes was induced using STZ (50 mg/kgBW administered i.p). Blood glucose levels were measured fasting and 2 hr postprandial at days 0, 3, and 14 after administration of RSAE. Data were analyzed using One-Way ANOVA. RSAE exhibited dose - dependent hypoglycaemic activity in STZ - induced diabetic rats, significantly reducing ($p < .005$) fasting blood glucose by up to 50.7 % and two - hour post - prandial glucose by 44.97% at 1000 mg/kgBW.

Keywords: *Blood glucose level, extract, herbal, medicinal plants, post-prandial glucose*

Graphical Abstract

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Introduction

Type 2 Diabetes mellitus (T2DM) is the fastest-growing metabolic disorder worldwide. Approximately 589 million adults worldwide are living with diabetes and its prevalence is projected to rise to 853 million by 2050 [1]. Current pharmacological management of T2DM typically begins with metformin which frequently combined with sulfonylureas such as glibenclamide when metformin alone no longer maintains glycaemic targets. Glibenclamide remains one of the most widely prescribed second-line agents because it is inexpensive, has a rapid onset, and can lower HbA1c by 1–2 % [2,3]. Although these agents generate favourable outcomes for many patients, their long-term use is frequently limited by adverse effects and increasing therapeutic resistance [4]. For instance, chronic glibenclamide therapy is associated with progressive β-cell exhaustion, weight gain, and an increased risk of cardiovascular events, while secondary failure rates can exceed 40% within five years [5,6]. These limitations, together with the rising prevalence of drug-induced hypoglycaemia and the economic burden of lifelong pharmacotherapy, leading to growing interest in plant-derived antihyperglycaemic agents that are affordable and well-tolerated [7,8].

Hibiscus sabdariffa Linn., widely consumed as a herbal tea, has garnered considerable attention for its ability to lower both fasting and post-prandial blood glucose in a variety of animal models and even preliminary human trials [8–16]. These pharmacological effects are attributed to its abundant polyphenols content, particularly anthocyanins, quercetin and protocatechuic acid. Anthocyanins (e.g., cyanidin-3-glucoside), quercetin and protocatechuic acid in *H. sabdariffa* are reported to scavenge reactive oxygen species, up-regulate GLUT-4, inhibit α-amylase and α-glucosidase and preserve pancreatic β-cell integrity [17–22]. Despite these clear benefits, *H. sabdariffa* has sour taste which resulting palatability challenge. Therefore, it is necessary to pair *H. sabdariffa* with a natural, non-caloric sweetener that can both mask its acidity and contribute its own antidiabetic properties.

Leaves of *Stevia rebaudiana* Bert. are known for its diterpene glycoside stevioside (4–13 % w/w) and rebaudioside A (2–4 %), which are 200–300 times sweeter than sucrose [23,24]. It can mask the intrinsic sourness of *H. sabdariffa* infusions, offering an organoleptic advantage for patient compliance. Beyond its sweetness, steviol glycosides stimulate glucose-dependent insulin secretion via TRPM5-mediated Ca^{2+} influx, up-regulate GLUT-4 and down-regulate hepatic PEPCK, producing fasting- and post-prandial glucose reductions in STZ- and diet-induced rodent models and improving weight maintenance in small human trials [25,26]. *S. rebaudiana* leaves also contains polyphenols (chlorogenic, ferulic, caffeic and gallic acids), flavonoids (rutin, kaempferol-3-rutinoside) and essential nutrients that collectively endow strong antioxidant capacity (ORAC) lipid peroxidation and advanced glycation end-product formation [23,27]. Furthermore, the review by Peteliuka et al. (2021) summarizes the antidiabetic mechanism of action of *S. rebaudiana* including insulinotropic signalling and carbohydrate-digestive enzyme blockade. Stevioside, rebaudioside A and steviol potentiate TRPM5-dependent Ca^{2+} oscillations in β-cells, driving glucose-regulated insulin release without stimulating hypoglycaemia [24].

Our preliminary work demonstrated that a 3:1 combination of *H. sabdariffa* and *S. rebaudiana* freeze-dried powder (1000 mg/kg BW) demonstrated a 51 % fall in fasting blood glucose in alloxan-induced

hyperglycaemic rats [28]. These findings, together with our previous literature review of phytochemistry and pharmacology of *H. sabdariffa* and *S. rebaudiana***,** suggest complementary, multi-target actions including synergistic enzymatic blockade**,** delaying carbohydrate absorption, β-cell protection & insulinotropism. Antioxidant properties of *H. sabdariffa* mitigate STZ-induced oxidative stress while steviol glycosides from *S. rebaudiana* directly enhance insulin release [21,29].

A broad spectrum of diabetogenic protocols has been reported, including single diabetogenic agent induction such as alloxan and streptozotocin (STZ), combination of STZ and nicotinamide, high fat diet combining with low-dose STZ, partial pancreatectomy, and various genetic manipulations [30]. However, chemically induced models created with sub-diabetogenic doses of alloxan or streptozotocin (STZ) remains the most widely use models because it generates hyperglycaemia rapidly, inexpensive and can be reproduced in most standard rodent facilities [31,32]. Among the chemical options, STZ has emerged as the preferred diabetogenic agent. Unlike alloxan, whose narrow therapeutic window, pronounced nephro- and hepatotoxicity and erratic glycaemic response compromise reproducibility, STZ enters β-cells via GLUT-2 transporters and produces a predictable alkylation-mediated DNA lesion that precipitates oxidative stress, progressive insulin secretory failure and the mixed insulin-deficient/insulin-resistant state characteristic of late-stage T2DM [33,34].
Building on this preliminary evidence and pathophysiological relevance of the STZ-induced diabetes animal model, the present study aims to characterise hypoglycaemic potential of *H. sabdariffa* dan *S. rebaudiana* combination. In this study we compared the effects of two oral doses (500 and 1000 mg/kgBW) on fasting and post-prandial glycaemia in STZ-induced diabetic Wistar rats over a 14-day period, using glibenclamide as an active reference.

Materials and Methods

Ethical approval

The study protocol was reviewed and approved by the Health Research Ethics Commission (Komisi Etik Penelitian Kesehatan), Faculty of Medicine, Universitas Padjadjaran (Ethical Approval No. 525/UN6.KEP/EC/2024), and was conducted in accordance with the ARRIVE 2.0 guidelines and the Guide for the Care and Use of Laboratory Animals.

Plant Material and Extraction

Two thousand grams of a commercially available 3:1 blend of *H. sabdariffa* calyces and S*. rebaudiana* leaves (Rosvia™, 3Sadulur Herbal, West Java, Indonesia) were infused in 5.000 mL of water heated to 73–74 °C with continuous stirring for 5 min, then vacuum-filtered through Whatman No. 1 paper. The filtrate was then transferred into sealed vessels and lyophilized at the Laboratory of Agricultural Industrial Technology, Universitas Padjadjaran (Indonesia). Freeze-drying yielded a dry aqueous extract (RSAE) with recovery 34.7%. Freeze-dried powder was stored in -20°C for further analysis.

Animals and Experimental Design

Thirty male Wistar rats (3–4 months old, 120–200 g) were obtained from the Animal Breeding Facility, Institut Teknologi Bandung and housed in groups of 10 per standard cage on corn-cob bedding. The animal room was maintained at 22 ± 2 °C with 50–60 % relative humidity and a 12 hr light and 12 hr dark cycle. Rats had *ad libitum* access to standard laboratory chow and filtered water. Group allocation was determined using

Federer's formula for sample size is (t-1) (n-) ≥ 15 where *t* is the number of groups (5) and *n* the number of animals per group. To ensure adequate power and allow for potential attrition, we selected *n* = 6 animals per group (total *N* = 30). After a 7-day acclimatization, rats were randomized into five groups (n = 6 each) including Normal control (CN), Negative control (C-) given STZ and 0.5 % of Na-CMC, Positive control (C+) given STZ and glibenclamide 5 mg/kgBW, Test group I (Test I) given STZ and combination of *H. sabdariffa* and *S. rebaudiana* (RSAE) 500 mg/kgBW, and Test group II (Test II) given STZ and RSAE 1000 mg/kgBW. Diabetes was induced by a single intraperitoneal injection of STZ 50 mg/kgBW. Blood glucose level was check after 72 hr of STZ administration. Rats with fasting blood glucose > 126 mg/dL were included in the study and subjected to further treatments. Extracts, glibenclamide, and Na-CMC were administered orally once daily for 14 days.

Blood glucose measurements

Fasting blood glucose (FBG) and 2 hours post-prandial glucose (PPG) were measured on days 0, 3, and 17 using Easy-Touch glucometer (GlucoDr™). The hypoglycemic effect of each treatment was expressed as the percentage change in blood glucose between Day 3 (D1'after STZ induction) and Day 17 (D14' after treament), calculated using equation 1.

To calculate the percentage reduction in blood glucose relative to the untreated diabetic control at Day 14' was calculated using equation 2. Same formula is applied for the 2 hr post-prandial glucose (PPG).

$$\Delta \text{ Day } 1' - \text{Day } 14' \ (\%) = \frac{\text{FBG Day 3} - \text{FBG Day 14}}{\text{FBG Day 3}} \ x \ 100\% \quad (1)$$

$$\frac{\text{FBG Negative control group} - \text{FBG Treated Group}}{\text{FBG Negative control group}} \ x \ 100\% \quad (2)$$

Total Phenolic Content (TPC)

The TPC of each freeze-dried extract was measured by the Folin–Ciocalteu method following the Farmakope Herbal Indonesia (FHI) monograph. Briefly, 0.5 mL of extract solution (1 mg/mL in distilled water) was mixed with 2.5 mL of ten-fold diluted Folin–Ciocalteu reagent and allowed to react for 5 min at room temperature. Then, 2.0 mL of 7.5 % (w/v) sodium carbonate solution was added, the mixture was vortexed, and incubated in the dark for 60 min at 25 °C. Absorbance was measured at 760 nm against a reagent blank. Gallic acid standards (0–200 µg/mL) were prepared in the same manner to generate a calibration curve. TPC was calculated as mg gallic acid equivalents (GAE) per gram of freeze-dried aqueous extract (mg GAE/g) and reported as the mean ± SD of three independent determinations.

Total Flavonoid Content (TFC)

TFC was determined by the aluminum chloride ($AlCl_3$) colorimetric method as described in the Farmakope Herbal Indonesia (FHI) monograph. Aliquots of 0.5 mL extract solution (1 mg/mL) were transferred to 10 mL volumetric flasks. To each flask were added 0.1 mL of 10 % (w/v) $AlCl_3$ solution and 0.1 mL of 1 M potassium acetate; the volume was adjusted to 10 mL with distilled water. After gentle mixing, the reaction mixtures were incubated at room

temperature for 30 min. Absorbance was recorded at 415 nm versus a blank containing all reagents except extract. Quercetin standards (0–100 µg/mL) were treated identically to construct the calibration curve. TFC was expressed as mg quercetin equivalents (QE) per gram of aqueous extract (mg QE/g), reported as the mean ± SD of three independent determinations.

Statistical Analysis

Data are presented as mean ± SD. Normality (Shapiro–Wilk) and homogeneity (Levene) were verified, one-way ANOVA followed by Newman–Keuls (homogeneous) or Games–Howell (heterogeneous) post-hoc tests was applied with α = 0.05 using IBM SPSS ver. 30.

Result and Discussion

The primary objectives of the management of diabetes are to reduce the incidence and burden of complications and to improve quality of life. Historically, these objectives were pursued through control of hyperglycaemia [35]. In the present study, fasting blood glucose (FBG) and post-prandial blood glucose (PPG) measurements were employed to capture distinct yet complementary aspects of glycaemic control. FBG levels primarily reflect the balance between hepatic glucose production and peripheral insulin sensitivity during periods without nutrient intake. Therefore, an elevated FBG is indicative of heightened gluconeogenesis and impaired insulin action, hallmark features of early type 2 diabetes pathogenesis. In contrast, PPG profiles reveal the capacity of pancreatic β-cells to mount a rapid secretory response to carbohydrate ingestion and the effectiveness of peripheral tissues in disposing of post-absorptive glucose surges [36–39]. By assessing both parameters, we have been able to delineate the multifaceted antihyperglycaemic activity of the combination of *H. sabdariffa* and *S. rebaudiana*. These dual effects on fasting and post-prandial glycaemia not only underscore the therapeutic breadth of the *H.sabdariffa* and *S. rebaudiana* but also align with existing literature that has reported both insulin-sensitizing and digestive enzyme–modulatory properties for their constituent phytochemicals [20,26,40–42].

Fasting Blood Glucose (FBG)

To evaluate the antihyperglycaemic efficacy of the *H. sabdariffa* and *S. rebaudiana* combination (RSAE), fasting blood glucose (FBG) was measured at baseline (Day 0), 72 hr post–STZ induction (Day 1') and after 14 days of treatment (Day 14'). All data are presented as mean ± SD (n = 6 per group). Statistical assumptions at Day 14' (Shapiro–Wilk, $p > 0.05$; Levene's test, $p > 0.05$) permitted one-way ANOVA followed by Newman–Keuls post-hoc comparisons (α = 0.05).

At baseline, all groups were normoglycaemic (63–85 mg/dL). By Day 3, STZ-treated groups C(–), C(+), Test 1, Test 2 exhibited a marked rise in FBG versus the normal control C(N), confirming successful diabetes induction (Table 1). After 14 days of treatment, glibenclamide C(+) reduced FBG by 38.3% and achieved a 54.5% decrease relative to C(–). The 500 mg/kg extract (Test 1) elicited a 33.3% intra-treatment drop and a 39.1% reduction versus C(–), while the 1000 mg/kg dose (Test 2) produced a 28.2% intra-treatment drop and the greatest relative improvement, 57.4% lower than C(–) (Table 1).

Table 1. Fasting blood glucose level measurement

Group	Day 0	Day 3 (D1') (mg/dL)	Day 17 (D14') (mg/dL)	Δ D1' – D14' (%)*	Δ vs C (-) (%)
C(N)	71.83 ± 9.68	73.17 ± 5,98	86.17 ± 7.05	–17.8	n/a
C(–)	72.50 ± 5.99	370.33 ± 46.86	409.17 ± 48.72	–10.4	Reference
Glibenclamide 5 mg/kgBW	84.83 ± 14.05	301.50 ± 60.30	186.00 ± 36.09	38.3	54.5
Test 1 500 mg/kgBW	79.83 ± 9.11	374.33 ± 47.00	249.50 ± 39.16	33.3	39.1
Test 2 1000 mg/kgBW	63.67 ± 7.42	242.50 ± 11.65	174.00 ± 35.59	28.2	57.4

*Negative value shows an increase

Figure 1 shown the significance result based on statistical analysis. Untreated group C (-) maintained markedly elevated fasting blood glucose levels at Day 14' (409.2 ± 48.7 mg/dL). Administration of glibenclamide 5 mg/kgBW (C+) produced antihyperglycaemic effect, lowering FBG to 186.0 ± 36.1 mg/dL ($p < 0.001$ vs. C(–). The RSAE combination extract at 500 mg/kg (Test 1) reduced FBG to 249.5 ± 39.2 mg/dL ($p < .001$), while the 1000 mg/kg dose (Test 2) achieved the greatest decrease, reaching 174.0 ± 35.6 mg/dL ($p < .001$). These results confirm a dose-dependent antihyperglycaemic activity of the combined extract.

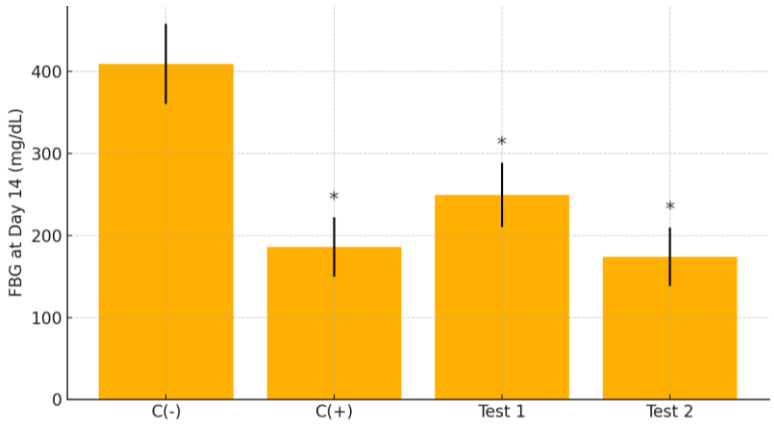

Figure 1. Fasting Blood Glucose at Day 14' (Mean ± SD). Error bars represent ±SD. Asterisks denote values significantly different from the negative control (C(–), $p < 0.001$).

Post-prandial Blood Glucose (PPG)

Two-hour post-prandial glucose (PPG) levels were measured at baseline (Day 0), 72 hr post–STZ (Day 1') and after 14 days of treatment (Day 14'). Data is presented as mean ± SD (n = 6); statistical assumptions at Day 14' (Shapiro–Wilk: W = .92–.98, $p > .05$; Levene's test: $p = .003$) dictated one-way ANOVA followed by Games–Howell post-hoc analysis ($\alpha = .05$). At Day 0, all groups exhibited comparable PPG values (79.7–

100.5 mg/dL). By Day 3 (D1'), STZ-treated group (C(–), C(+), Test 1, and Test 2) displayed pronounced hyperglycaemia (401.7–425.7 mg/dL) relative to the normal control (86.8 mg/dL), confirming successful hyperglycemic induction. After 14 days of treatment (Day 14'), glibenclamide achieved a 35.9 % reduction from the Day 1', the 500 mg/kg RSAE combination (Test 1) decreased PPG by 33.4 %, while the 1000 mg/kg dose (Test 2) produced the greatest effect, a 41.4 % drop compare to Day 1' and 46.2% reduction vs C(-) (Table 2).

Figure 2 then further ilustrated the signficance of blood glucose reduction of the treated group at Day 14' compare to the negative control (C-) group. Glibenclamide (significantly reduced PPG to 226.3 ± 30.2 mg/dL ($p < 0.001$ vs. C(–)), corresponding to a 50.0 % reduction. The RSAE combination at 500 mg/kg (Test 1) lowered PPG to 283.5 ± 26.4 mg/dL (37.4 % reduction, $p < 0.001$), while the 1000 mg/kg dose (Test 2) achieved a PPG of 243.5 ± 28.3 mg/dL—46.2 % lower than C (–) ($p < 0.001$).

Table 2. Post-prandial glucose level measurement

Group	Day 0	Day 3 (D1') (mg/dL)	Day 17 (D14') (mg/dL)	Δ D1' – 14' (%)*	Δ vs C (-) (%)
C(N)	79.67 ± 12.94	86.83 ± 10.80	103.00 ± 6.63	-18.6 %	n/a
C(–)	87.67 ± 9.05	401.67 ± 38.40	452.83 ± 51.04	-12.7 %	Reference
Glibenclamide 5 mg/kgBW	100.50 ± 10.84	353.33 ± 17.13	226.33 ± 30.22	35.9 %	50.0 %
Test 1 500 mg/kgBW	82.67 ± 5.61	425.67 ± 45.45	283.50 ± 26.40	33.4 %	37.4 %
Test 2 1000 mg/kgBW	86.83 ± 7.03	415.83 ± 21.57	243.50 ± 28.26	41.4 %	46.2 %

*Negative value shows an increase

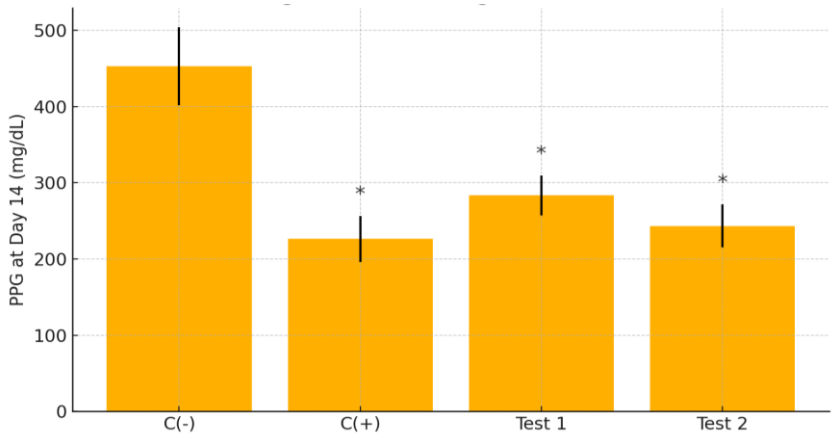

Figure 2. Two-Hour Post-Prandial Blood Glucose at Day 14' (Mean ± SD). Error bars represent ±SD. Asterisks denote values significantly different from the negative control (C(–), $p < 0.001$).

Epidemiological data indicated there have associated increased postprandial glucose (PPG) with a higher risk of macrovascular events, and long-term high fasting blood glucose (FBG) with microvascular complications [43–45]. Therefore, a drug that can simultaneously reduce basal hepatic glucose output and suppress postprandial glucose spikes would be highly desirable. Our findings thus support the potential of this herbal combination as a holistic glycemic modulator, providing comparable efficacy to conventional sulfonylurea therapy in streptozotocin-induced diabetic rats while reducing the inherent risk of hypoglycemia. In general, the measurement of both FBG and PPG has offered a comprehensive evaluation of the antidiabetic mechanisms at work and highlights the potential of *H. sabdariffa* sepals and *S. rebaudiana* leaves extract as an adjunct or alternative to conventional hypoglycaemic medications.

After 14 days of treatment, there was a decrease in fasting blood glucose (FBG) levels, which implies that the bioactive substances in this extract, particularly the flavonoids and polyphenols, may reduce basal hyperglycemia. This could be accomplished by improving insulin receptor signaling and blocking the hepatic gluconeogenic enzymes [46,47]. Simultaneously, the significant suppression in postprandial glucose spikes (PPG) suggests a different mode of action that involves the inhibition of the small intestine's α-amylase and α-glucosidase enzymes. The carbohydrate hydrolysis and glucose absorption are slowed as a result [48,49]

Total phenolic content (TPC)

The total phenolic content was determined using gallic acid as the reference standard. The gallic acid standard curve exhibited linearity with the linear regression equation $A=0.0030C-0.0115$ with $R^2 = 0.9993$ (Figure 3). Table 3 presents the mean absorbance at 760 nm and the corresponding total phenolic content (TPC) expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g).

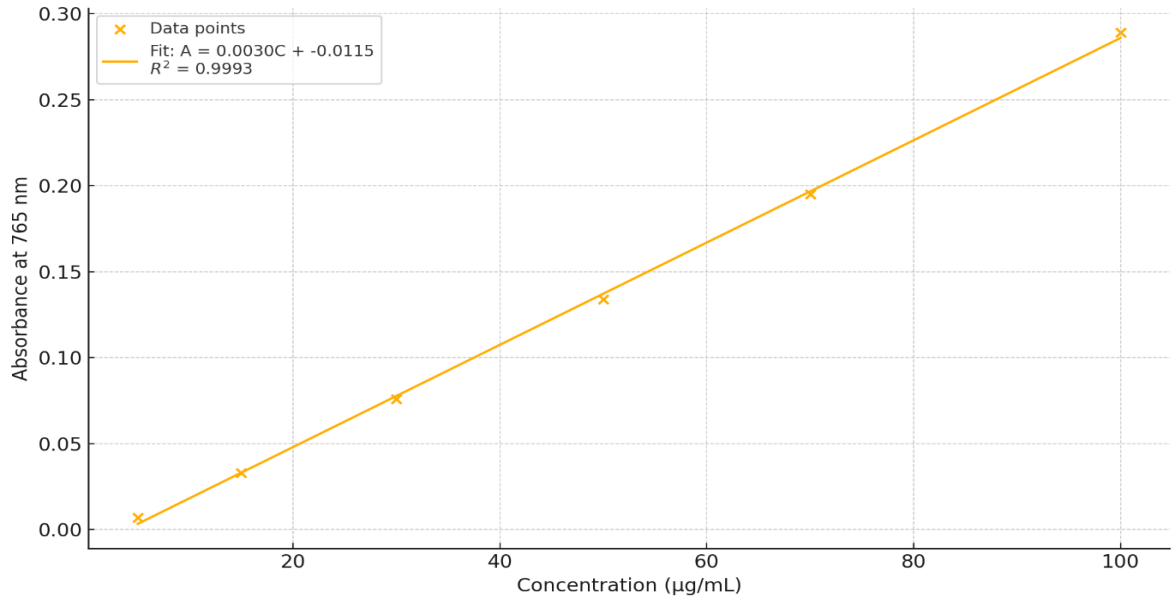

Figure 3. Gallic-acid standard curve

Table 3. Total Phenolic Content of *H. sabdariffa*, *S. rebaudiana*, and Their Combination

Samples	Absorbance (mean ± SD)	TPC (mgGAE/g)	RSD (%)
H. sabdariffa extract	0.174 ± 0.001	102.75 ± 0.06	0.57
S. rebaudiana extract	0.215 ± 0.001	188.33 ± 0.05	0.24
RSAE combination (3:1)	0.203 ± 0.001	118.85 ± 0.09	0.49

Total flavonoid content (TFC)

The total flavonoid content was determined using quercetin as the reference standard. The quercetin standard curve exhibited linearity with the linear regression equation A=0.0075C−0.0705 with R^2 = 0.9985 (Figure 4). Table 4 summarizes the mean absorbance at 415 nm and the corresponding total flavonoid content (TFC), expressed as mg quercetin equivalents per gram of dry extract (mg QE/g).

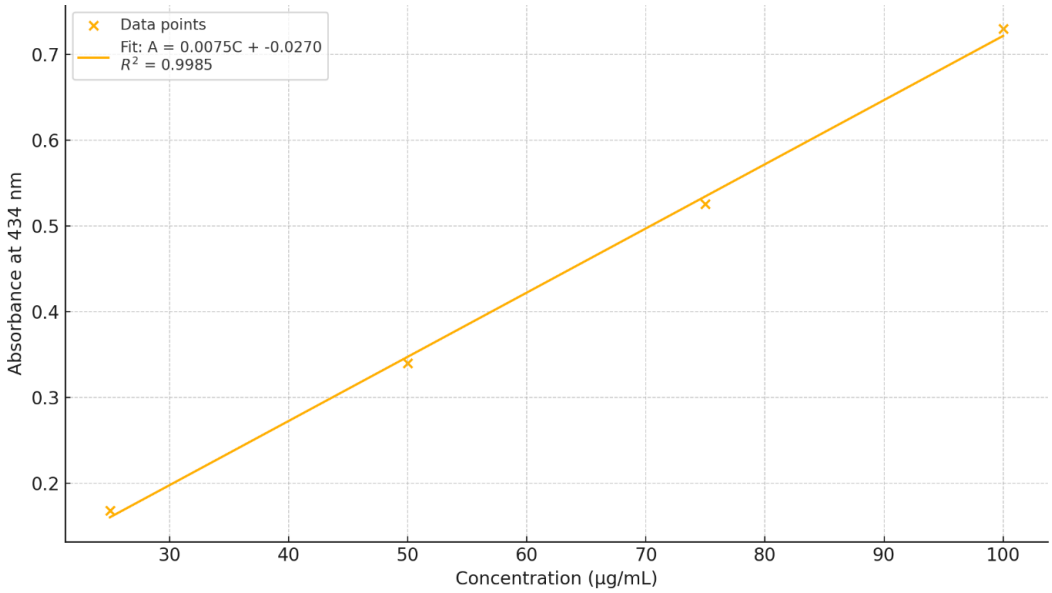

Figure 4. Quercetin standard curve

The *H. sabdariffa* sepals and *S. rebaudiana* leaves combination's rich complement of their phenolic and flavonoid constituents, the quantitative distribution in our extracts refflect the glycaemic improvements observed in vivo [47]. The test results showed the stevia leaves extract had a significantly higher phenolic content at 188.33 mg gallic-acid-equivalent (GAE) per g along with 6.99 mg quercetin-equivalent (QE) per g of total flavonoid content, whereas the rosella extract alone had 102.75 mgGAE/g of total phenolics and 3.10 mgQE/g of total flavonoids. The combination of 3:1 *H.sabdariffa* sepals and *S.rebaudiana* leaves extract yielded intermediate values (118.85 mgGAE/g of total phenolics and 4.79 mgQE/g of flavonoids), reflecting the additive contributions of both plants.

H. sabdariffa phenolic groups is dominated by anthocyanins—mostly delphinidin-3-sabdariffoside and cyanidin-3-sabdariffoside—and protocatechuic acid,

which together represent over 60 % of its total phenolics [50,51]. These compounds demonstrated potent in vitro inhibition of α-amylase and α-glucosidase (IC_{50} = 50–120 µg mL^{-1}) and have been shown to delay post-prandial glycaemic peaks in human bread-feeding trials [51,52]. Moreover, rosella anthocyanins promote GLUT-4 translocation and upregulate PPAR-γ expression in 3T3-L1 adipocytes, which facilitates greater peripheral glucose uptake [53]. By reducing reactive oxygen species and NF-κB signaling, protocatechuic acid further shields β-cells, promoting endogenous insulin secretion and increased cell viability [54].

Hepatic AMPK has been shown to be activated by the phenolic content of *S. rebaudiana*, which is high in caffeic, ferulic, and chlorogenic acids[55]. This activation increases GLUT-2 phosphorylation while suppressing important gluconeogenic enzymes like PEPCK. Rat models fed a high-fat diet and STZ have shown these effects [55–57]. Rutin and kaempferol-3-rutinoside are part of the flavonoid subfraction of *S. rebaudiana*, which inhibits intestinal starch hydrolysis by acting as a competitive α-glucosidase inhibitor. Steviol glycoside (stevioside) potentiates glucose-stimulated insulin secretion via TRPM5-mediated calcium influx in beta cells [57]. Recent work by Ajiboye et al. (2025) has extended the antidiabetic profile of Hibiscus sabdariffa beyond systemic glycaemic control to the male reproductive system as complication of T2DM. Flavonoid-enriched *H. sabdariffa* extract at 150 and 300 mg/kgBW markedly improved testicular redox status including decrease in malondialdehyde, increase in GSH, SOD, CAT, GPx and GST activities, accompanied by restoration of steroidogenesis and by normalisation of cholesterol turnover through up-regulation of 3β- and 17β-hydroxysteroid dehydrogenases [58]. Collectively, these data show that H. sabdariffa flavonoids not only counter systemic hyperglycaemia but also protect highly oxidative-sensitive tissues such as the testes by integrating antioxidant, anti-inflammatory, metabolic and gene-regulatory mechanisms.

Limitation

Despite its promising findings, this study has several important limitations. First, the use of a single, chemically induced model of diabetes (STZ-induced Wistar rats) captures primarily the insulin-deficient and oxidative-stress aspects of type 2 diabetes but does not fully replicate the multifactorial pathogenesis seen in humans, such as diet-induced insulin resistance or the chronic inflammatory milieu. Second, our 14-day treatment period, while sufficient to demonstrate acute antihyperglycaemic effects, does not address long-term efficacy, safety, or the risk of tolerance development. Third, although we quantified total phenolic and flavonoid content and correlated these broad classes with glycaemic improvements, we did not isolate or bio-assay individual compounds thus, the precise bioactive constituents remain undefined. Fourth, we focused exclusively on blood glucose measurements and did not assess insulin levels, pancreatic histopathology, or markers of β-cell mass and function, which would more directly confirm protective or regenerative effects on pancreatic islets.

Conclusion

In conclusion, the combined aqueous extracts of *H. sabdariffa* and *S. rebaudiana* exhibited dose-dependent antihyperglycaemic activity in STZ-induced diabetic Wistar rats, significantly reducing fasting blood glucose and two-hour post-prandial glucose over 14 days administration. These effects compare favorably with glibenclamide and are underpinned by the high phenolic and flavonoid contents of the extracts, which

mechanistic evidence suggests act through complementary pathways.

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Author Contributions

Conceptualization, AT and RMF; Methodology, AT and YS; Formal Analysis, DA, AT, and RMF; Investigation, AT and DA; Writing – Original Draft Preparation, AT and RMF; Writing – Review & Editing, RMF, YS, and MM; Funding Acquisition, RMF. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

References

[1]. International Diabetes Federation *IDF Diabetes Atlas, 11th Edn.*; Brussels, Belgium, 2025;

[2]. Feingold K Oral and Injectable (Non-Insulin) Pharmacological Agents for the Treatment of Type 2 Diabetes. In *Endotext*; Feingold KR, Ahmed SF, Anawalt B, Eds.; 2024.

[3]. Richardson,C.R., Borgeson, J.R., Van Harrison R Management of Type 2 Diabetes Mellitus. In *Ann Arbor (MI): Michigan Medicine*; University of Michigan, 2021.

[4]. Sahin, I.; Bakiner, O.; Demir, T.; Sari, R.; Atmaca, A. Current Position of Gliclazide and Sulfonylureas in the Contemporary Treatment Paradigm for Type 2 Diabetes: A Scoping Review. *Diabetes Therapy* 2024, *15*, 1687–1716, https://doi.org/10.1007/s13300-024-01612-8.

[5]. Padhi, S., Nayak, A.K., Behera, A. Type II Diabetes Mellitus: A Review on Recent Drug Based Therapeutics. *Biomedicine & Pharmacotherapy*, 2020, *131*, 110708, https://doi.org/10.1016/j.biopha.2020.110708.

[6]. Ling, Z., Wang, Q., Stangé, G., In't Veld, P., Pipeleers, D. Glibenclamide Treatment Recruits Beta-Cell Subpopulation into Elevated and Sustained Basal Insulin Synthetic Activity. *Diabetes*, 2006, *55*, 78–85.

[7]. Jacob, B.; Narendhirakannan, R.T. Role of Medicinal Plants in the Management of Diabetes Mellitus: A Review. *3 Biotech* 2018, *9*, 4, https://doi.org/10.1007/s13205-018-1528-0.

[8]. Yedjou, C.G.; Grigsby, J.; Mbemi, A.; Nelson, D.; Mildort, B.; Latinwo, L.; Tchounwou, P.B. The Management of Diabetes Mellitus Using Medicinal Plants and Vitamins. *International journal of molecular sciences,* 2023, 24(1).https://doi.org/10.3390/ijms241090 85.

[9]. Amos, A.; Khiatah, B. Mechanisms of Action of Nutritionally Rich Hibiscus Sabdariffa's Therapeutic Uses in Major Common Chronic Diseases: A Literature Review. *Journal of the American Nutrition Association*, 2022, *41*, 116–124, https://doi.org/10.1080/07315724.2020.1848662.

[10]. Bule, M.; Albelbeisi, A.H.; Nikfar, S.; Amini, M.; Abdollahi, M. The Antidiabetic and Antilipidemic Effects of Hibiscus Sabdariffa: A Systematic Review and Meta-Analysis of Randomized Clinical Trials. *Food Research International*, 2020, *130*, https://doi.org/10.1016/j.foodres.2020.108980.

[11]. Mozaffari-Khosravi, H.; Jalali-Khanabadi, B.-A.; Afkhami-Ardekani, M.; Fatehi, F.; Noori-Shadkam, M. The Effects of Sour Tea (Hibiscus Sabdariffa) on Hypertension in Patients with Type II Diabetes. *J Hum Hypertens* 2009, *23*, 48–54, https://doi.org/10.1038/jhh.2008.100.

[12]. 12. Salem, M.A.; Zayed, A.; Beshay, M.E.; Abdel Mesih, M.M.; Ben Khayal, R.F.; George, F.A.; Ezzat, S.M. Hibiscus Sabdariffa L.: Phytoconstituents, Nutritive, and Pharmacological Applications. *Advances in Traditional Medicine* 2022, *22*, 497–507, https://doi.org/10.1007/s13596-020-00542-7.

[13]. Adeyemi, D.O.; Adewole, O.S. Hibiscus Sabdariffa Reduces Renal Damage in Experimental Streptozotocin-Induced Diabetes. *Journal of Morphological Sciences*, 2020, *37*, 2–12, https://doi.org/10.51929/jms.37.2.2020.

[14]. Haro, G. Antidiabetic Effect of Roselle Calyces Extract (*Hibiscus sabdariffa* L.) in Streptozotocin Induced Mice. *International Journal of PharmTech Research,* 2014, *6(5)*, 1703–1711.

[15]. Salleh, N.H.; Zulkipli, I.N.; Mohd Yasin, H.; Ja'Afar, F.; Ahmad, N.; Wan Ahmad, W.A.N.; Ahmad, S.R. Systematic Review of Medicinal Plants Used for Treatment of Diabetes in Human Clinical Trials: An ASEAN Perspective. *Evidence-based Complementary and Alternative Medicine* 2021, https://doi.org/10.1155/2021/5570939

[16]. Susilawati, Y.; Febriyanti, R.M.; Febrina, E.; Chaerunisaa, A.; Sumiwi, S.A. A Comprehensive in Vivo Study of the Antihypertensive Properties and Toxicity of Roselle (*Hibiscus sabdariffa* L.). *Pharmacia* 12AD, *70*, 1521–1530. https://doi.org/10.3897/pharmacia.70.e109119.

[17]. Amin, A.R.; Kassab, R.B.; Abdel Moneim, A.E.; Amin, H.K. Comparison Among Garlic, Berberine, Resveratrol, Hibiscus Sabdariffa, Genus Zizyphus, Hesperidin, Red Beetroot, Catha Edulis, Portulaca Oleracea, and Mulberry Leaves in the Treatment of Hypertension and Type 2 DM: A Comprehensive Review. *Nat Prod Commun* 2020, *15*, https://doi.org/10.1177/1934578X20921623.

[18]. Mojica, L.; Rui, L.; Gonzalez De Mejia, E. *Hibiscus sabdariffa* L. Phytochemical Composition and Nutraceutical Properties. *ACS Symposium Series* 2012, *1109*, 289–305, doi:10.1021/bk-2012-1109.ch017.

[19]. Abdullah, B.; Khairunnisa, S.D.; Muhammad, M.I.; Atwinda, R.S. Isolation of Anthocyanin from Indonesian Purple Roselle (*Hibiscus sabdariffa* L.) Calyces. In Proceedings of the AIP Conference Proceedings; 2020; 2197.

[20]. Alegbe, E.O.; Teralı, K.; Olofinsan, K.A.; Surgun, S.; Ogbaga, C.C.; Ajiboye, T.O. Antidiabetic Activity-Guided Isolation of Gallic and Protocatechuic Acids from Hibiscus Sabdariffa Calyxes. *J Food Biochem*, 2019, *43*, https://doi.org/10.1111/jfbc.12927.

[21]. Anjabtsawa, D.; Febriyanti, R.M.; Diantini, A. Exploring the Phytochemical Properties and Therapeutic Potential of Hibiscus Sabdariffa Linn. and Stevia Rebaudiana Bert. *Indonesian Journal of Biological Pharmacy*, 2025, 4(2), 91-102. https://doi.org/10.24198/ijbp.v4i2.54035.g24888

[22]. Ajiboye, B.O.; Famusiwa, C.D.; Ajaesowuonu, J.F.; Chibueze, O.P.; Adejumo, A.A.; Onikanni, S.A.; Ajeigbe, K.O.; Ajuwon, O.R.; Oyinloye, B.E. Flavonoid-Rich Extracts of Hibiscus

Sabdariffa Leaf Attenuate Cardiomyopathy in Streptozotocin-Induced Rats. *Comp Clin Path* 2024, *33*, 927–941, https://doi.org/10.1007/s00580-024-03615-0.

[23]. Khilar, S.; Singh, A.P.; Biagi, M.; Sharma, A. An Insight into Attributes of *Stevia rebaudiana* Bertoni: Recent Advances in Extraction Techniques, Phytochemistry, Food Applications and Health Benefits. *Journal of Agriculture and Food Research,* 2022, *10*, 100458, https://doi.org/10.1016/j.jafr.2022.100458.

[24]. Peteliuk, V.; Rybchuk, L.; Bayliak, M.; Storey, K.B.; Lushchak, O. Natural Sweetener Stevia Rebaudiana: Functionalities, Health Benefits and Potential Risks. *EXCLI Journal, 20, 1412–1430. https://doi.org/10.17179/excli2021-4211*

[25]. Kamal, N.H.; Essmat, A.; El Askary, H.I.; El Hefnawy, H.M.; Wahab, S.M.A.; Meselhy, M.R. Chemical Profile and Beneficial Effect of Standardized Extract of Stevia Rebaudiana Bertoni Leaves on Metabolic Syndrome in High Fat Diet Streptozotocin-Induced Diabetic Rats. *Applied Biological Chemistry,* 2022, 65(5), https://doi.org/10.1186/s13765-022-00724-8.

[26]. Patel, S.; Navale, A. The Natural Sweetener Stevia: An Updated Review on Its Phytochemistry, Health Benefits, and Anti-Diabetic Study. *Current diabetes reviews,* 20(2), https://doi.org/10.2174/1573399819666230501210803

[27]. Shahu, R.; Jobby, R.; Patil, S.; Bhori, M.; Tungare, K.; Jha, P. Phytochemical Content and Antioxidant Activity of Different Varieties of *Stevia rebaudiana*. *Horticulture, Environment, and Biotechnology*, 2022, *63*, 935–948, https://doi.org/10.1007/s13580-022-00451-5.

[28]. Febriyanti, R.M.; Putri, R.N.; Diantini, A.; Levita, J. Hypoglycaemic Properties of The Combination of Roselle Calyx (*Hibiscus sabdariffa* Linn.) and Stevia Leaves (*Stevia rebaudiana* B.) in Alloxan-Induced White Rats. *Indonesian Journal of Pharmaceutical Science and Technology* 2025, *12*, 90–97, https://doi.org/10.24198/ijpst.v12s2.59646.

[29]. Anjabtsawa, D.; Febriyanti, R.M.; Diantini, A. A Review of Antidiabetic Potential of Indonesian Medicinal Plants on Streptozotocin (STZ)-Induced Rats. *Indonesian Journal of Biological Pharmacy*, 2024, 4(1), 24–37. https://doi.org/10.24198/ijbp.v4i1.54037

[30]. Radenković, M.; Stojanović, M.; Prostran, M. Experimental Diabetes Induced by Alloxan and Streptozotocin: The Current State of the Art. *J Pharmacol Toxicol Methods* 2016, *78*, 13–31, https://doi.org/10.1016/j.vascn.2015.11.004.

[31]. Singh, R.; Gholipourmalekabadi, M.; Shafikhani, S.H. Animal Models for Type 1 and Type 2 Diabetes: Advantages and Limitations. *Front Endocrinol (Lausanne)* 2024, *15*, 1359685, https://doi.org/10.3389/fendo.2024.1359685.

[32]. Pandey, S.; Chmelir, T.; Chottova Dvorakova, M. Animal Models in Diabetic Research—History, Presence, and Future Perspectives. 11(10), 2852. https://doi.org/10.3390/biomedicines11102852

[33]. Ghasemi, A.; Jeddi, S. Streptozotocin as a Tool for Induction of Rat Models of Diabetes: A Practical Guide. *EXCLI J* 2023, *22*, 274–294,

https://doi.org/10.17179/excli2022-5720.

[34]. Deeds, M.C; Anderson, J.M., Armstrong, A.S., Gastineau, D.A., Hiddinga, H.J. Jahangir, A., Eberhardt, N.L., Kudva, Y.C., Single Dose Streptozotocin-Induced Diabetes: Considerations for Study Design in Islet Transplantation Models. *Laboratory animals,* 2011, 45(3), 131–140. https://doi.org/10.1258/la.2010.010090

[35]. Galindo, R.J.; Trujillo, J.M.; Low Wang, C.C.; McCoy, R.G. Advances in the Management of Type 2 Diabetes in Adults. *BMJ Medicine* 2023, *2*, e000372, https://doi.org/10.1136/bmjmed-2022-000372.

[36]. Rizza, R.A. Pathogenesis of Fasting and Postprandial Hyperglycemia in Type 2 Diabetes: Implications for Therapy. *Diabetes* 2010, *59*, 2697–2707, https://doi.org/10.2337/db10-1032.

[37]. Lu, X.; Xie, Q.; Pan, X.; Zhang, R.; Zhang, X.; Peng, G.; Zhang, Y.; Shen, S.; Tong, N. Type 2 Diabetes Mellitus in Adults: Pathogenesis, Prevention and Therapy. *Signal Transduct Target Ther* 2024, *9*, 262, https://doi.org/10.1038/s41392-024-01951-9.

[38]. Su, Q.; Liu, J.; Li, P.; Qian, L.; Yang, W. Relative Contribution of Fasting and Postprandial Blood Glucose in Overall Glycemic Control: Post Hoc Analysis of a Phase IV Randomized Trial. *Diabetes Ther* 2018, *9*, 987–999, https://doi.org/10.1007/s13300-018-0403-7.

[39]. Ketema, E.B.; Kibret, K.T. Correlation of Fasting and Postprandial Plasma Glucose with HbA1c in Assessing Glycemic Control; Systematic Review and Meta-Analysis. *Arch Public Health* 2015, *73*, 43, https://doi.org/10.1186/s13690-015-0088-6.

[40]. Ifie, I., Marshall, L.J., Ho, P., Williamson, G. *Hibiscus sabdariffa* (Roselle) Extracts and Wine: Phytochemical Profile, Physicochemical Properties, and Carbohydrase Inhibition. *Journal of agricultural and food chemistry,* 2016, *64*, 4921–4931, https://doi.org/10.1021/acs.jafc.6b01246.

[41]. Jamrozik, D.; Borymska, W.; Kaczmarczyk-Żebrowska, I. Hibiscus Sabdariffa in Diabetes Prevention and Treatment—Does It Work? An Evidence-Based Review. *Foods* 2022, *11*, https://doi.org/10.3390/foods11142134.

[42]. Joseph, D.; George, J.; Mathews, M.M.; Mathew, F.; Varghese, B.; Sunny, B. A Comprehensive Exploration on Therapeutic Options of Stevia Rebaudiana with Emphasize on Anti-Diabetic Attribute. *Research Journal of Pharmacy and Technology,* 2019, *12*, 4981–4988, https://doi.org/10.5958/0974-360X.2019.00863.1.

[43]. Tong, Y.; Liu, F.; Huang, K.; Li, J.; Yang, X.; Chen, J.; Liu, X.; Cao, J.; Chen, S.; Yu, L.; et al. Changes in Fasting Blood Glucose Status and Incidence of Cardiovascular Disease: The China-PAR project. Journal of diabetes, 15(2), 110–120. https://doi.org/10.1111/1753-0407.13350

[44]. Thiab, S.; Akhal, T.; Akeblersane, M.; Sheth, H.; Atkin, S.L.; Butler, A.E. Microvascular Complications in Prediabetes: A Systematic Review & Meta-Analysis. *Diabetes Res Clin Pract* 2025, *225*, 112261, https://doi.org/10.1016/j.diabres.2025.112261.

[45]. Hershon, K.S.; Hirsch, B.R.; Odugbesan, O. Importance of Postprandial Glucose in Relation to A1C and Cardiovascular

Disease. *Clinical Diabetes* 2019, *37*, 250–259, https://doi.org/10.2337/cd18-0040.

[46]. Liu, M.; Liu, C.; Zhaxi, P.; Kou, X.; Liu, Y.; Xue, Z. Research Progress on Hypoglycemic Effects and Molecular Mechanisms of Flavonoids: A Review. *Antioxidants* 2025, *14*, 378, https://doi.org/10.3390/antiox1404037.

[47]. Praparatana, R.; Maliyam, P.; Barrows, L.R.; Puttarak, P. Flavonoids and Phenols, the Potential Anti-Diabetic Compounds from Bauhinia Strychnifolia Craib. Stem. *Molecules* 2022, *27*, 2393, https://doi.org/10.3390/molecules27082393.

[48]. Dimitriadis, G.D.; Maratou, E.; Kountouri, A.; Board, M.; Lambadiari, V. Regulation of Postabsorptive and Postprandial Glucose Metabolism by Insulin-Dependent and Insulin-Independent Mechanisms: An Integrative Approach. *Nutrients* 2021, *13*, 159, https://doi.org/10.3390/nu13010159.

[49]. Kazeem, M.I.; Adamson, J.O.; Ogunwande, I.A. Modes of Inhibition of *α* -Amylase and *α* -Glucosidase by Aqueous Extract of *Morinda Lucida* Benth Leaf. *BioMed Research International,* 2013, 1–6, https://doi.org/10.1155/2013/527570.

[50]. 50. DU, C.T.; FRANCIS, F.J. Anthocyanins of Roselle (*Hibiscus sabdariffa*, L.). *Journal Food Sciences,* 1973, *38*, 810–812, https://doi.org/10.1111/j.1365-2621.1973.tb02081.x.

[51]. 51. Lazalde-Cruz, R.; Miranda-Romero, L.A.; Tirado-González, D.N.; Carrillo-Díaz, M.I.; Medina-Cuéllar, S.E.; Mendoza-Martínez, G.D.; Lara-Bueno, A.; Tirado-Estrada, G.; Salem, A.Z.M. Potential Effects of Delphinidin-3-O-Sambubioside and Cyanidin-3-O-Sambubioside of Hibiscus Sabdariffa L. on Ruminant Meat and Milk Quality. *Animals* 2021, *11*, 2827, https://doi.org/10.3390/ani11102827.

[52]. Ademiluyi, A.O.; Oboh, G. Aqueous Extracts of Roselle (*Hibiscus Sabdariffa* Linn.) Varieties Inhibit *α* -Amylase and *α* -Glucosidase Activities *In Vitro*. *J Med Food* 2013, *16*, 88–93, https://doi.org/10.1089/jmf.2012.0004

[53]. Feng, S.-Y.; Wu, S.-J.; Chang, Y.-C.; Ng, L.-T.; Chang, S.-J. Stimulation of GLUT4 Glucose Uptake by Anthocyanin-Rich Extract from Black Rice (*Oryza sativa* L.) via PI3K/Akt and AMPK/P38 MAPK Signaling in C2C12 Cells. *Metabolites* 2022, *12*, 856, https://doi.org/10.3390/metabo12090856.

[54]. Zhang, S.; Gai, Z.; Gui, T.; Chen, J.; Chen, Q.; Li, Y. Antioxidant Effects of Protocatechuic Acid and Protocatechuic Aldehyde: Old Wine in a New Bottle. *Evidence-Based Complementary and Alternative Medicine* 2021, *2021*, 1–19, https://doi.org/10.1155/2021/6139308

[55]. Simlat, M.; Ptak, A.; Wójtowicz, T.; Szewczyk, A. The Content of Phenolic Compounds in *Stevia rebaudiana* (Bertoni) Plants Derived from Melatonin and NaCl Treated Seeds. *Plants* 2023, *12*, 780, https://doi.org/10.3390/plants12040780.

[56]. Carrera-Lanestosa, A.; Coral-Martínez, T.; Ruíz-Ciau, D.; Moguel-Ordoñez, Y.; Segura-Campos, M.R. Phenolic Compounds and Major Steviol Glucosides by HPLC-DAD-RP and *Invitro* Evaluation of the Biological Activity of Aqueous and Ethanolic Extracts of Leaves and Stems: *S. rebaudiana* Bertoni (Creole Variety INIFAP C01). *Int J Food Prop* 2020, *23*,

199–212, https://doi.org/10.1080/10942912.2020.1716789.

[57]. Philippaert, K.; Pironet, A.; Mesuere, M.; Sones, W.; Vermeiren, L.; Kerselaers, S.; Pinto, S.; Segal, A.; Antoine, N.; Gysemans, C.; et al. Steviol Glycosides Enhance Pancreatic Beta-Cell Function and Taste Sensation by Potentiation of TRPM5 Channel Activity. *Nat Commun* 2017, *8*, 14733, https://doi.org/10.1038/ncomms14733.

[58]. Ajiboye, B.O.; Famusiwa, C.D.; Falode, J.A.; Okwukwe Akojuru, D.; Owolabi, B.T.; Adejumo, A.A.; Oyinloye, B.E.; Ajuwon, O.R.; Ogedengbe, O.O.; Ojo, O.A. Protective Effects of Flavonoid-Rich Extracts from *Hibiscus sabdariffa* Leaves on Streptozotocin-Induced Testicular Damage in Rats. *Phytomedicine Plus*, 2025, *5*, 100808, https://doi.org/10.1016/j.phyplu.2025.100808