Asian Science Bulletin

OPEN ACCESS

Antihyperglycemic and Antioxidant Properties of *Sida linifolia* Juss. ex Cav. (Malvaceae) Hydroalcoholic Extract in ICR Mice

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ABSTRACT

Background and Objective: *Sida linifolia* Juss. ex Cav. (Malvaceae) is a medicinal plant used in Togo for the treatment of diabetes mellitus. The present study was designed to investigate the antihyperglycemic and antioxidant effects of the hydroalcoholic extract of *S. linifolia*. **Materials and Methods:** Antihyperglycemic activity was measured by oral glucose tolerance tests in glucose-loaded ICR mice and on their basal blood glucose. The effect of *S. linifolia* extract was evaluated *ex-vivo* on glucose absorption using inverted intestinal sacs and skeletal muscle isolated from rats. Antioxidant activities were assessed *in vitro* by the DPPH free radical scavenging method, iron-reducing power and total antioxidant capacity. Phytochemical groups were characterized by classical chemical staining using appropriate reagents and quantified by spectrophotometric method. **Results:** Hydroalcoholic extract of *S. linifolia* significantly reduced hyperglycemia in mice 30 min after glucose overload at doses of 100, 200 and 400 mg/kg compared to control mice. However, the reduction in hyperglycemia was greater in mice treated with the highest dose (400 mg/kg) of the extract for 180 min ($p<0.0001$). The effect of the extract on the basal glycemia of mice was also evaluated, but it did not show a significant decrease ($p>0.05$) compared to the normoglycemic control. The *ex-vivo* tests demonstrated that the extract inhibited glucose membrane transport through the intestine and increased peripheral glucose use. The antioxidant tests indicated a dose-dependent antioxidant activity of the extract. Phytochemical analysis revealed the presence of major phytochemical groups in the extract. **Conclusion:** *Sida linifolia* whole plant extract exhibited significant antioxidant and antihyperglycemic properties probably attributed to its phenolic compounds. The use of this plant extract in the production of herbal medicines can help treat diabetes and its complications.

KEYWORDS

Sida linifolia, oral glucose tolerance *in vivo*, glucose absorption *ex vivo*, antioxidant *in vitro*, diabetes, skeletal muscle

ISSN: 1813-0070 (Print)
https://doi.org/10.3923/asb.2025.08.19

Received: 25 May, 2024
Accepted: 17 Dec. 2024
Published: 31 Mar. 2025

INTRODUCTION

Diabetes is a group of metabolic disorders that cause chronic hyperglycemia due to a defect in insulin secretion, insulin action or both[1]. The development of diabetes is influenced by both genetic predisposition and environmental factors. The condition is identified by a clinically diagnosed high blood glucose level, determined by fasting blood glucose levels ≥126 mg/dL (7 mmol/L) or ≥200 mg/dL (11.1 mmol/L) after an oral glucose tolerance test[2]. Diabetes mellitus is linked to chronic hyperglycemia and reduced antioxidant potential, resulting in oxidative stress. This stress is partly responsible for diabetic complications, including retinopathy, nephropathy, neuropathy and cardiovascular diseases[3,4].

The prevalence of diabetes has been estimated at 10.5% in 2021 and could reach 12.2% in 2045 according to the International Diabetes Federation[5]. Type 2 diabetes represents 90% of all cases of diabetes and is characterized by insulin resistance followed by progressive insulinopenia. Conventional medical treatment aims to normalize blood sugar levels. Although new therapeutic molecules have been developed, regular administration of insulin and oral antidiabetic drugs can cause several side effects[6]. Herbal medicine has been used for many years by different cultures around the world to treat diabetes[7]. Due to their low cost and increased tolerances, medicinal plants are a viable alternative to the limited therapeutic options currently available for the treatment of diabetes.

Plants play a significant role in treating diabetes, as evidenced by their use in Togolese traditional medicine. *Sida linifolia* Juss ex. Cav, an herbaceous plant from the Malvaceae family, is one such plant used to treat diabetes. According to an ethnopharmacological survey by Kpodar *et al.*[8], the whole plant of *Sida linifolia* Juss ex. Cav is used as a decoction in traditional medicine to treat diabetes mellitus. *Sida linifolia* is recognized for its antimicrobial, nephroprotective, anti-inflammatory and anti-nociceptive properties, as well as its potential in treating diabetes mellitus. However, there is insufficient scientific data to support its effectiveness. This study aims to evaluate the antihyperglycemic effect and antioxidant potential of the hydroethanolic extract of *Sida linifolia* Juss. ex. Cav.

MATERIALS AND METHODS

Study area: The study took place from January to March, 2023 in the Laboratory of the Physiopathology, bioactive substances and safety research unit of the Department of Animal Physiology at the Faculty of Science of the University of Lomé, Togo.

Animals: Sprague Dawley rats (250±50 g) and Institute of Cancer Research (ICR) mice (25±5 g) were obtained from the Department of Animal Physiology, University of Lomé. Mice were used for oral glucose tolerance tests, while rats were used for glucose absorption tests. The animals were acclimated for at least one week before the start of the experiment and were raised under standard laboratory conditions, with a temperature of 25±2°C, relative humidity of 40-45% and a 12 hrs light/dark cycle. They were provided with a standard diet and had *ad libitum* access to food and water. Principles of laboratory animal care as described in institutional guidelines and ethics of the Laboratory of the Physiopathology, bioactive substances and safety of the University of Lome-Togo (ref: 001/2012/CB-FDS-UL) were followed.

Collection and identification of plant material: Plant material selected for this study was based on an ethnobotanical survey[8]. It consisted of whole plants of *Sida linifolia* Juss. ex Cav. (Malvaceae) harvested in the Attitogon locality (prefecture of Bas-Mono, Togo). The botanical authentication was conducted at the herbarium of the Laboratory of Botany and Plant Ecology of the Faculty of Science (University of Lomé) and a voucher specimen was deposited under the number 15897 TOGO. The plants were washed with water, cut into small pieces and dried at the Animal Physiology Laboratory at 25±2°C away from light. They were then powdered with a mill before extraction.

Extraction: The whole plant of *Sida linifolia* (500 g) was ground into a powder and macerated in a mixture of distilled water and ethanol (5:5) for 72 hrs with intermittent manual agitation. The resulting mixture was filtered twice using hydrophilic cotton and Whatman filter paper (Ø150 mm). The filtrate was then evaporated at 45°C under vacuum using a rotary evaporator (Rotavapor Buchi R120). The resulting dry extract was stored in a refrigerator at 4°C until use[9].

***In vivo* and *ex-vivo* antihyperglycemic activity**

Effect of *S. linifolia* extract on hyperglycemic mice: Oral glucose tolerance test (OGTT): The antihyperglycemic activity of the extract was assessed using the glucose tolerance test method, following the procedure described by Motto *et al*.[9]. Five groups of mice were randomly assigned (n = 5). After 9 hrs of fasting, hyperglycemia was induced by oral administration of D-glucose (4 g/kg b.wt.) at a rate of 5 mL/kg. Blood glucose levels were measured before the administration of all solutions. The control group received only distilled water and the treated groups received extracts of *Sida linifolia* at doses of 100, 200 and 400 mg/kg. The reference group received metformin at a dose of 100 mg/kg via oral administration thirty minutes before glucose loading. Blood glucose levels were measured using a glucometer (Accu check, Germany) from the tail vein blood at 0 min before and 30, 60, 120 and 180 min after glucose loading[9].

Effect of *S. linifolia* extract on normoglycemic mice: Three groups of five fasted mice were used in this study. Group 1 received 5 mL/kg b.wt., of distilled water (normoglycemic control group). Group 2 received a dose of 400 mg/kg of hydroalcoholic extract of *Sida linifolia* and Group 3 received a dose of 100 mg/kg of metformin (reference group). Blood glucose levels were measured as before[10].

Intestinal absorption of *S. linifolia* extract in rat everted jejunal sacs: Glucose absorption from the intestine was determined using the method described by Hamilton and Butt[11]. Ten Sprague Dawley rats were fasted for 24 hrs with access to water. After sacrificing the animals, their isolated jejunum was everted using a rod and cut into 8 cm length segments. The fragments were rinsed with 0.9% NaCl at 37°C and then placed in an oxygenated Krebs-Henseleit Bicarbonate Buffer (KHB, pH = 7.4) for further analysis. The culture medium consists of Krebs-Henseleit Bicarbonate buffer (mM/L): $NaHCO_3$ 25, NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.2 and Na_2EDTA 9.7 mg/L. The intestine fragments were filled with 1 mL of KHB solution (serous fluid). They were weighed and then placed in Erlenmeyer flasks containing 10 mL of KHB as a mucous liquid. Incubation was carried out at 37°C under oxygenation and continuous stirring[12].

To examine the effect of *S. linifolia* extract on glucose absorption, glucose was added to the mucosal compartment fluid at different concentrations (40, 60, 80 and 100 mM/L) immediately before the start of the experiment. The same compartment was then treated with *S. linifolia* extract (40 and 80 mg/mL). After 60 min of incubation, the fragments were removed, blotted and emptied and the glucose levels in the solutions from the fragments were measured. Glucose concentration was measured in both serous and mucous fluids using the GOD-PAP kit resulting from the Trinder method. The absorption of glucose from the intestine leads to a decrease in glucose in the mucous fluid. This leads to an increase in glucose in the serous fluid and accounts for the released glucose. However, the retention of glucose in the tissues during its passage causes an imbalance between the glucose lost in the mucosal fluid and the glucose gained into the serosal fluid. Both forms of glucose (absorbed and released) were expressed as mM/g fresh tissue/hrs[12].

Effect of the *S. linifolia* extract on uptake of glucose in rat isolated skeletal muscle: The method used by Povi *et al*.[13] was adopted. Male rats were euthanized by cervical dislocation after 24 hrs of fasting. The femoral muscles were then removed and cut into small 250 mg pieces. These pieces were pre-incubated in the presence of 95% O_2 and 5% CO_2 and 10 mL of Kreb's Ringer Bicarbonate buffer (KRB) at 37°C for

10 min. During handling, the KRB solution was replaced by KRB-G (KRB containing 11.1 mM glucose). Twelve batches (in triplets) were then formed by adding extract or metformin and insulin or no insulin, as follows:

- **Group 1:** Muscle+KRB-G, represent the control group
- **Group 2:** Muscle+KRB-G+insulin (100 mIU/L)
- **Group (3, 4) and (5, 6):** Muscle+KRB-G+*S. linifolia* at 125 mg/mL with or without insulin (100 mIU/L), respectively
- **Group (7, 8) and (9, 10):** Muscle+KRB-G+*S. linifolia* at 250 mg/mL with and without insulin (100 mIU/L), respectively
- **Lot 11:** Muscle+KRB-G+metformin at 20 mg/mL
- **Lot 12:** Muscle+KRB-G+insulin (100 mIU/L)+metformin at 20 mg/mL

Aliquots (10 µL) of the incubation solution were collected for 3 hrs at 60, 120 and 180 min. The glucose concentration was measured using an enzymatic colorimetric test, GOD-POD liquid (Cypress diagnostic, Belgium). The following batches were tested: It is assumed that the glucose lost in the incubation solution represents the glucose absorbed by the muscle.

$$\text{Glucose absorbed} = \frac{\text{Initial glucose} - \text{Final glucose}}{0.25}$$ [13]

While, 0.25 represents the fresh weight of the muscle. The absorbed glucose is expressed in mmole/g fresh muscle.

***In vitro* antioxidant activity of *S. linifolia* extract**

Free radical scavenging (DPPH) test: DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity of *Sida linifolia* hydroalcoholic extract was estimated using the method described by Kpemissi *et al.*[14]. Briefly, 0.25 mL of methanol extract solution (31.25, 62.5, 125, 250, 500 and 1000 µg/mL) was added to 1.5 mL of DPPH solution (100 µmol/L). Ascorbic acid was used as the standard. Ascorbic acid at different concentrations (3.125, 6.25, 12.5, 25, 50 and 100 µg/mL) was used as a positive control. The absorbance was measured in triplicate at 517 nm. Then, the scavenging activity of DPPH* was calculated as follows:

$$\text{DPPH}^* \text{ scavenging} = \frac{A_0 - A_1}{A_0} \times 100$$ [14]

Where:

A_0 = Absorbance of the control

A_1 = Absorbance of the test with the extract

The 50% inhibition concentration (IC_{50}) was determined from the equation of the inhibition percentage of DPPH.

Ferric ion-reducing antioxidant power (FRAP): The concentration of the extract was varied (50, 100, 200, 400 and 800 µg/mL) and mixed with 2.5 mL of 0.2 M PBS buffer solution (pH = 6.6) and 2.5 mL of 1% potassium ferrocyanide solution using the slightly modified method of Oyaizu[15]. The resulting mixture was incubated in a water bath at 50°C for 20 min. After cooling, 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. After centrifuging at 3000 rpm for 10 min, 2.5 mL of the resulting supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1% solution of Ferric Chloride ($FeCl_3$). The absorbance of the reaction mixture was measured at 700 nm.

Total antioxidant capacity (phosphomolybdate assay): Total antioxidant capacity of the extract was determined using the phosphomolybdate method[16]. This test is based on the reduction of Mo (+6) ion to Mo (+5) by the extract, resulting in the formation of a green Phosphate-Mo complex (+5) at acidic pH. To perform the test, 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added to 0.3 mL of the extract solution (1 mg/mL) prepared in methanol. Ascorbic acid was used as the standard. The absorbance of the reaction mixture was measured at 695 nm after incubation at 95°C for 90 min and then compared to the blank.

Phytochemical study

Phytochemical screening of *S. linifolia* extract: Preliminary phytochemical screening was carried out to determine the presence of active phytoconstituents in the extract using the colorimetric and precipitation methods described by Harborne[17].

Estimation of total phenolic compounds and tannin contents: The total phenolic compounds and tannin content of *S. linifolia* extract were determined by the colorimetric Folin-Ciocalteu method as described by Maksimović *et al.*[18]. Phenolic compounds were determined using the Folin-Ciocalteu reagent which is a mixture of phosphotungstic acid and phosphomolybdic acid. The latter is reduced during the oxidation of phenols in a mixture of blue oxides of tungsten and molybdenum. The wavelength was measured with a spectrophotometer at 735 nm. A blank was made in parallel.

A second dosage was carried out after fixation of the tannins with Polyvinylpyrrolidone (PVP). The difference between the two dosages made it possible to determine the total quantity of tannins. Gallic acid (GA) at different concentrations (0, 6.25, 12.25, 25 and 50 µg/mL) served as a standard. The absorbance of the reaction was measured at 735 nm using a US/VIS Spectrophotometer wavelength. The total phenolic and tannin content of the extract was expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract).

Estimation of total flavonoid content: Flavonoid content was estimated using the aluminum chloride colorimetric assay described by Povi *et al.*[19] with a few modifications. Flavonoids from a flavonoid-aluminum complex with aluminum chloride, which absorbs at 415 nm. About 1 mL of 2% aluminum chloride (20 mg in 100 mL distilled water) was added to 1 mL of the extract solution to be assayed. The mixture was mixed and incubated at the laboratory temperature (25±2°C) for 10 min. Absorbance was measured at 415 nm against the blank. For the blank, the extract was replaced by ethanol. Rutin (0, 5, 25, 50, 75, 100, 150 and 200 µg/mL) was used as a standard. Assays were carried out in triplicates. The amount of total flavonoids was expressed as rutin equivalents (mg RE/g extract).

Estimation of polysaccharides: The phenol-sulphuric acid method of Dubois *et al.*[20] has been used to determine the polysaccharides contained in plant extracts. Polysaccharides give a yellow-orange colour when treated with phenol and sulphuric acid. The extract solution was prepared at 1 mg/mL and 200 µL of it was added to 200 µL of the 5% aqueous phenol solution. The mixture was mixed using a vortex then 1 mL of concentrated sulfuric acid was added. The new mixture was mixed and heated to 100°C for 5 min in a water bath. The absorbance was measured at 480 nm after cooling on ice (for 30 min) protected from light. A blank was made in parallel. The standard was carried out with glucose: 0, 25, 50, 100, 150 and 200 µg/mL. The tests were carried out in triplicate. The polysaccharides were determined in glucose equivalents/g of extract.

Statistical analysis: Results were expressed as Mean±Standard Error of the Mean (SEM). Statistical analysis was performed by Two-way Analysis of Variance (ANOVA) followed by Dunnett's test to evaluate significant differences between groups. The level of significance was set at $p<0.05$ and statistical analysis was carried out using GraphPad Prism 6.01.

RESULTS

Effect of *S. linifolia* extract on hyperglycemic mice: Oral glucose tolerance test (OGTT): Figure 1(a) shows the variation in blood glucose levels. Thirty minutes after glucose overload, blood glucose levels increased significantly ($p<0.0001$), reaching a peak before gradually decreasing. Blood glucose levels in group control increased to 182.78% (from 82.75±1.88 to 234±7.735 mg/dL) at t30 before returning to a near-normal value around 180 min Fig. 1(a).

Administration of the extract significantly reduced hyperglycemia compared with the control group. The reduction in hyperglycemia at t30 was 15.39% for SL 100, 29.92% for SL 200 and 13.19 and 24.93%, respectively at t60 compared with controls. The 400 mg/kg b.wt., extract significantly reduced blood glucose levels over 120 min. This decrease was 32.62% ($p<0.0001$) at t30, 27.82% ($p<0.001$) at t60 and 31.87% ($p<0.01$) at t120. Metformin at a dose of 100 mg/kg, used as the reference drug, also significantly reduced blood glucose levels (Fig. 1a).

Area under the curve (AUC) (Fig. 1b) confirmed a significant reduction of blood glucose for both doses of extract (SL 200, SL 400) and metformin. Blood glucose levels decreased significantly more for extract 400 mg/kg ($p<0.01$) compared to controls.

Effect of *S. linifolia* extract on normoglycemic mice: Under our conditions, administration of SL extract at a dose of 400 mg/kg did not significantly (p 0.05) reduce basal blood glucose levels in normoglycemic mice compared with the control. Metformin 100 mg/kg, used as the reference drug did not also significantly reduce (Fig. 2a). The area under the curve showed no significant reduction in basal blood glucose compared with the control (Fig. 2b).

Effect of *S. linifolia* extract on intestinal absorption: In the presence of different concentrations of glucose (60, 80 and 100 mM), the *S. linifolia* extract at 80 mg/mL caused more significant loss (****$p<0.0001$) of glucose to the external environment than the 40 mg/mL extract (Table 1). The results show that there was a decrease in the amount of glucose released compared to the controls. The rate of glucose release into the serous medium increased with glucose concentration. Glucose release from the inverted intestine was reduced compared with controls. This reduction was more significant at 80 Mm (Table 1).

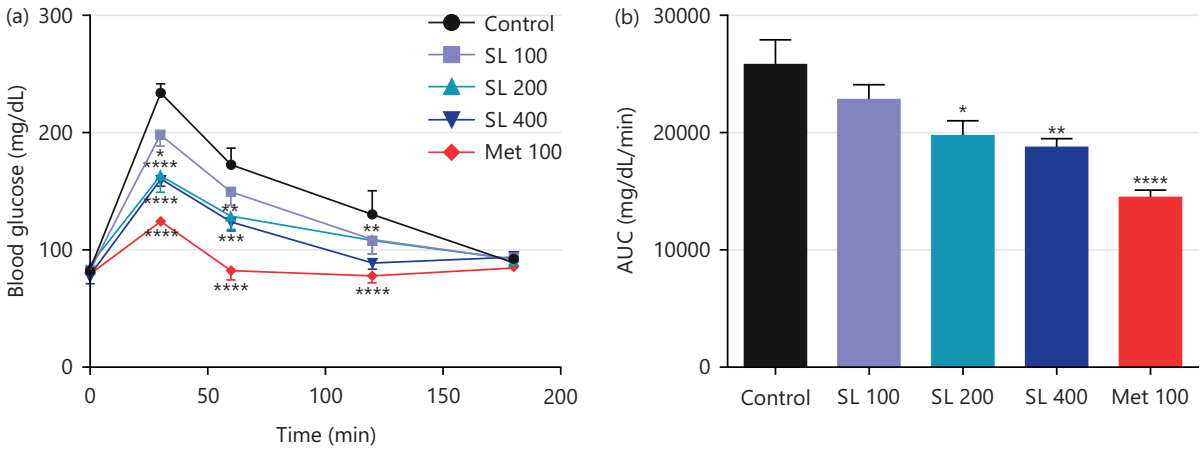

Fig. 1(a-b): Effects of *S. linifolia* extract on (a) Glucose tolerance and (b) Area under the curve

Control: Treated with distilled water, SL 100: Treated with extract at 100 mg/kg, SL 200: Treated with extract at 200 mg/kg, SL 400: Treated with extract at 400 mg/kg, Met 100: Treated with metformin at 100mg/kg, results were expressed as Mean±SEM, n = 5, *$p<0.05$, **$p<0.01$, ***$p<0.001$ and ****$p<0.0001$

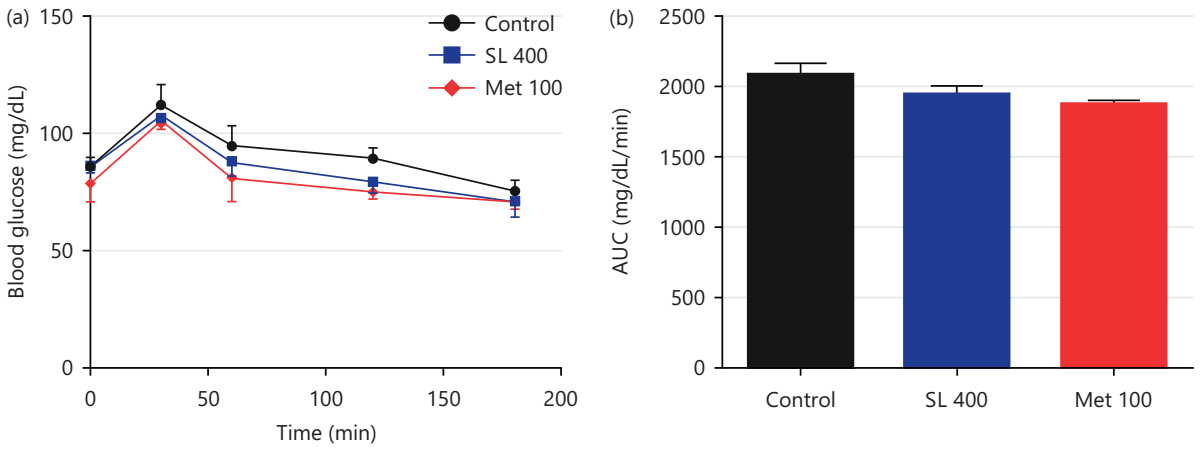

Fig. 2(a-b): Effect of *S. linifolia* extract on basal (a) Blood glucose levels of normoglycemic mice and (b) Area under the curve

Control: Treated with distilled water, SL 400: Treated with extract at 400 mg/kg, Met 100: Treated with metformin at 100 mg/kg, results were expressed as Mean±SEM and n = 5

Table 1: Effect of *S. linifolia* extract on the release of varying concentrations of glucose by everted gut sacs

	Released glucose (mM/g/ tissue wet weight)		
Glucose concentration (mM/L)	Control	SL (40 mg/mL)	SL (80 mg/mL)
40	35.945±0.034	31.989±0.673	32.255±0.479
60	53.829±0.099	50.750±0.411	50.863±0.519
80	71.816±0.026	69.127±1.164	65.876±0.485
100	91.268±0.085	87.583±0.436	86.290±0.748

Fragments of the everted gut sacs were incubated in Kerbs-Henseleit Buffer at 37°C and values are expressed as Mean±SEM of three experiments

Table 2: Effect of extract of *S. linifolia* on the absorption of glucose in the skeletal muscle

	Glucose absorbed by the muscle tissue (mM/g of fresh tissue)			
	30 min	60 min	120 min	180 min
Glucosed medium (GM)	1.989±0.285	3.443±0.225	4.087±0.201	11.213±0.300
GM+Insulin	3.241± 0.124*	4.055±0.177	7.610±0.241****	13.851±0.547****
GM+SL 1	2.625±0.146	5.727±0.434****	8.816±0.028****	10.923±0.351
GM+SL 1+Insulin	3.878±0.098	7.022±0.246*	8.866±0.183	14.912±0.463
GM+SL 2	5.084±0.370****	7.047±0.295****	9.355±0.571****	11.954±0.098
GM+SL 2+Insulin	6.833±0.053**	10.979±0.151****	12.049±0.386**	11.599±2.849
GM+Met	1.652±0.065	20.759±0.032	20.759±0.032	20.759±0.032
GM+Met+Insulin	21.475±0.129	24.242±0.885	24.242±0.885	24.242±0.885

Glucose absorbed: (Gli-Glf)/0.25 g, Gli: Initial glucose, Glf: Final glucose, 0.25 g: Weight of muscle tissue. The skeletal muscle tissue was incubated in a Kreb's-Ringer bicarbonate buffer solution containing 11.1 mM glucose at 37°C. GM: Glucosed medium, SL: Sida linifolia, SL 1: Extract at 125 mg/mL, SL 2: Extract at 250 mg/mL, Insulin: Insulin at 100 mIU/L and Met: Metformin at 20 mg/mL. Results are expressed as Mean±SEM, (N = 3), *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared to the controls

Effect of *S. linifolia* extract on glucose uptake in isolated rat skeletal muscle: The effect of the *S. linifolia* extracts at 125 and 250 mg/mL on glucose uptake in isolated rat muscle is presented in Table 2. After 120 min of incubation, glucose uptake was dose-dependently increased with or without insulin and was found to be (respectively 5.727±0.434 and 7.047±0.295 mM/g fresh tissue) with *S. linifolia* extract at 125 and 250 mg/mL compared to the control group (4.087±0.201 mM/g fresh tissue). Extract at 250 mg/mL showed better activity.

In vitro antioxidant activity of *S. linifolia* extract

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical scavenging activity: The free radical scavenging activity of DPPH of *S. linifolia* extract (IC_{50} = 218.658±0.507 µg/mL) increased in a dose-dependent manner and

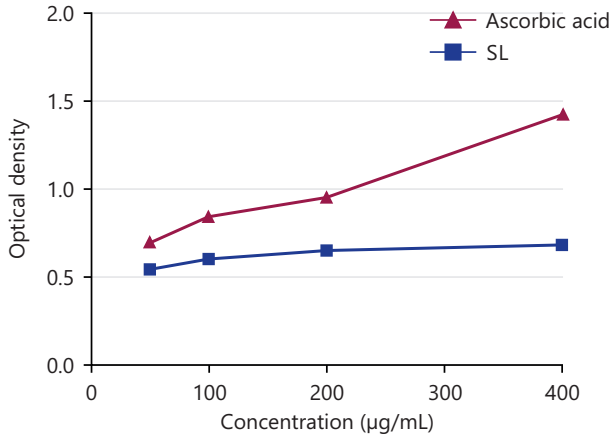

Fig. 3: Iron-reducing power of *S. linifolia* extract

Table 3: Quantification of total phenols, tannins, total flavonoids and polysaccharides of the *S. linifolia* extract

Phytochemical compounds	Content in *S. linifolia* extract	Reference molecule
Total phenol	18.607±0.346 (mg Eq GA/g)	Gallic acid
Tannins	10.031±0.074 (mg Eq GA/g)	Gallic acid
Total flavonoids	77.468±7.685 (mg Eq R/g)	Rutin
Polysaccharides	143.892±13.779 (mg Eq G/g)	Glucose

Total phenols and tannins are expressed in mg gallic acid equivalent/g extract. Flavonoids are expressed in mg Rutin Equivalent/g extract. Polysaccharides are expressed in mg Glucose EquiR and DRvalent/g extract and the results represent the Mean±SEM (N = 3)

was significantly ($p<0.0001$) elevated compared to that of ascorbic acid (IC_{50} = 26.894±0.065). However, a low IC_{50} reflects stronger DPPH scavenging activity.

Total antioxidant capacity: The antioxidant capacity value of the *S. linifolia* extract is 123.792±8.215 mg Eq AA/g (mg of ascorbic acid equivalent/g extract) in a dosed manner. It represents the capacity of all the antioxidant compounds present in the extract as ascorbic acid equivalents.

Ferric reducing antioxidant power assay: The ferric-reducing antioxidant power (FRAP) of the *S. linifolia* extract is presented in Fig. 3. The result showed the dose-dependent response for reducing the power of the extract of *S. linifolia*. However, this result showed a lower reducing power than ascorbic acid.

Phytochemical study

Identification of the major chemical groups in the hydroalcoholic extract of *S. linifolia*: Phytochemical screening indicated the presence of alkaloids, phenols, flavonoids, tannins, saponins and triterpenes in *S. linifolia* hydroalcoholic extract.

Estimation of total phenols, tannins, total flavonoids and polysaccharides contents: Total phenols, tannins, total flavonoids and polysaccharides contents of the *S. linifolia* extract were determined and the results were shown in Table 3.

DISCUSSION

The present study was designated to evaluate the antihyperglycemic and antioxidant properties of the hydroalcoholic extract of the whole plant of *S. linifolia*.

Hyperglycaemia is a key marker of diabetes. To assess the antihyperglycemic effect of the extract, fasting mice in a state of hyperglycemia induced by OGTT were used. The administration of glucose to mice resulted in a significant increase in blood glucose levels ($p<0.0001$), peaking at t = 30 min, followed by

a gradual decrease to near basal levels at t = 180 min. This increase can be explained by the fact that the absorbed glucose entered the general circulation before being utilized for the body's needs or stored under the direct action of insulin[9]. The cells responsible for glucose uptake, under the action of insulin and its storage in the form of glycogen, mainly in the liver, are the mechanisms responsible for the gradual decrease in blood glucose levels. This leads to the observed homeostasis around the 180th min[21].

The *S. linifolia* extract, when orally administered at doses of 200 and 400 mg/kg, resulted in a dose-dependent reduction in hyperglycaemia compared to the control. The reduction was more significant ($p<0.001$ at t = 30 min) in mice treated with the 400 mg/kg extract for 120 min. The group treated with the reference metformin also exhibited a significant reduction. The extract has been confirmed to reduce blood glucose levels, as evidenced by the total amount of glucose available in the blood per unit time determined by the area under the glucose tolerance curve. This proves that *S. linifolia* extract contains antihyperglycemic compounds, which are thought to be responsible for the pronounced reduction obtained at a dose of 400 mg/kg. According to Atchou *et al.*[10], the extract may have delayed or blocked the intestinal absorption of glucose or stored it primarily as glycogen.

The 400 mg/kg dose of the extract did not significantly reduce basal blood glucose in normoglycemic mice over 180 min. This suggests that the *S. linifolia* extract does not directly increase insulin secretion. The antihyperglycemic activity of *S. linifolia* extract is similar to that of metformin and could therefore prevent postprandial hyperglycemia in diabetics. Metformin belongs to the biguanide class of drugs. It reduces the absorption of carbohydrates in the gastrointestinal tract and inhibits hepatic gluconeogenesis by activating an intracellular pathway through AMP-Activated Protein Kinase (AMPK). Additionally, it increases the sensitivity of peripheral tissues to insulin and glucose absorption. Metformin does not stimulate insulin secretion[22].

Glucose uptake by cells is an important phenomenon in the maintenance of blood glucose levels. To investigate the impact of the extract on intestinal glucose absorption, we conducted the inverted intestine test. Glucose is transported into epithelial cells through the apical membrane via Sodium-Glucose Cotransporters (SGLT1) using secondary active transport coupled with Na^+. The intestinal glucose is absorbed across the basolateral membrane by facilitated diffusion (GLUT2) and then released into the blood[9]. The study results indicate a significant decrease in glucose released into the intestine compared to the control group. The *S. linifolia* extract at doses of 40 and 80 mg/mL reduces intestinal glucose absorption. This reduction is more pronounced at concentrations of 80 and 100 mMol/L. Furthermore, the amount of glucose absorbed by the intestine was found to be concentration-dependent. The extract is believed to regulate glucose homeostasis in rats by inhibiting the transporter proteins SGLT1 and GLUT2. This inhibition of glucose transporters by the extract, or its regulatory effect on glucose absorption, is attributed to its phytochemical compounds. Polyphenols are believed to be responsible for inhibiting intestinal glucose absorption[23]. Therefore, the extract from *S. linifolia* could be effective in reducing postprandial hyperglycemia.

Skeletal muscle is the major site of glucose disposal and insulin resistance in type 2 diabetes[24]. It is an insulin-sensitive tissue that plays a crucial role in regulating glucose transport and metabolism during physical exercise or in the postprandial period. Muscle glucose uptake induced by insulin and muscle contraction is facilitated by the translocation of the primary glucose transporter, GLUT4, to the cell surface of myocytes. The GLUT4 is translocated from intracellular compartments to the plasma membrane in response to insulin, where it enhances cellular glucose uptake[25]. The study demonstrated a significant increase in glucose uptake by skeletal muscle, both in the presence and absence of insulin. The amount of glucose uptake is higher in the presence of insulin. An increase in plasma insulin concentration leads to the translocation of GLUT4 via the phosphatidylinositol 3-kinase signalling pathway, resulting in the

uptake of blood glucose by skeletal muscle[13]. These observations demonstrate that the extract of *S. linifolia* enhances muscle sensitivity to insulin action via GLUT4. It is possible that the phenolic compounds in the extract are responsible for this activity. The hydroalcoholic extract of *S. linifolia* may increase muscle sensitivity to the action of insulin, similar to the effect of metformin.

Diabetes mellitus is linked to an increase in the production of reactive oxygen species and a decrease in antioxidant defences. Hyperglycemia induces oxidative stress which is characterized by an imbalance between the production of free radicals and their neutralization by endogenous antioxidants[26]. In people with diabetes, increased ROS production and lipid peroxidation are observed; two essential factors that contribute to the pathophysiology of diabetes[27]. Thus, the provision of exogenous antioxidants during the treatment of hyperglycemia can help prevent and reduce the risk of complications related to diabetes[28]. The extract of *S. linifolia* exhibited antioxidant activity against free radicals, indicating its potential therapeutic use against diabetes complications associated with oxidative stress. The reducing power of an extract is a significant indicator of its antioxidant capacity[29]. The results demonstrate a dose-dependent reducing power of the extract. The antioxidant activity of *S. linifolia* extract is believed to be a result of its high composition of bioactive compounds with known antioxidant properties. On the other side, Nwankwo *et al*.[30], reported excellent antioxidant effects with ethanolic leaf fractions of *S. linifolia*, which is thought to be due to the presence of polyphenols. These compounds interact directly with activated oxygen species to inhibit the formation of free radicals[31]. Polyphenols enhance the plasma's antioxidant capacity by accepting electrons from reactive oxygen species (ROS)[32].

The phytochemical composition of *S. linifolia* provides insight into the plant's pharmacological properties. The extract was found to contain total phenolic, flavonoids and tannins, which was consistent with the findings of Nwankwo *et al*.[30] who reported high levels of these compounds in the ethanolic leaf fraction. Phytotherapy is based on the therapeutic action of complex mixtures of different compounds that often act synergistically to exert their beneficial effects. The biologically active compounds present in the *S. linifolia* extract acted alone or in synergy to exert the observed antihyperglycemic activity.

The antioxidant activity of *S. linifolia* may contribute to its therapeutic effect in treating diabetic complications associated with oxidative stress. This activity helps protect against oxidative damage and insulin resistance.

CONCLUSION

It emerges from this study that the hydroalcoholic extract of the whole plant of *Sida linifolia* have a antihyperglycemic and antioxidant properties. The *ex-vivo* tests showed inhibition of glucose membrane transport through the intestine and increased peripheral glucose use, indicating an extra-pancreatic mechanism of action. Phytocompounds contained in the extract would be responsible for the activities observed. The observed activities are attributed to the phytocompounds present in the extract. Further research, including toxicity assessments and extract fractionation, is necessary to confirm their effectiveness in treating diabetes mellitus.

SIGNIFICANCE STATEMENT

Plants have several therapeutic properties including the treatment of diabetes. In diabetes, hyperglycemia causes metabolic syndrome and oxidative stress which leads to depletion of endogenous antioxidants and causes complications. Thus, antihyperglycemic prophylaxis and a simultaneous supply of exogenous antioxidants are essential. *Sida linifolia* Juss ex. Cav, this medicinal plant from traditional pharmacopeia was selected for this purpose to prove it's antihyperglycemic and antioxidant effectiveness through tests. Hydroalcoholic extract of *S. linifolia* reduced hyperglycaemia in mice after glucose overload, without reducing basal glycaemia. It also showed antioxidant activity and inhibited membrane glucose transport. Other, more advanced studies following induction of diabetes *in vivo* will validate the anti-diabetic properties of this plant and shed more light on its various mechanisms of action.

REFERENCES

1. Petersmann, A., D. Müller-Wieland, U.A. Müller, R. Landgraf and M. Nauck *et al*., 2019. Definition, classification and diagnosis of diabetes mellitus. Exp. Clin. Endocrinol. Diabetes, 127: S1-S7.
2. ADA, 2020. 2. Classification and diagnosis of diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care, 43: S14-S31.
3. Saraswat, N., N. Sachan and P. Chandra, 2020. Anti-diabetic, diabetic neuropathy protective action and mechanism of action involving oxidative pathway of chlorogenic acid isolated from *Selinum vaginatum* roots in rats. Heliyon, Vol. 6. 10.1016/j.heliyon.2020.e05137.
4. Pitocco, D., M. Tesauro, R. Alessandro, G. Ghirlanda and C. Cardillo, 2013. Oxidative stress in diabetes: Implications for vascular and other complications. Int. J. Mol. Sci., 14: 21525-21550.
5. Sun, H., P. Saeedi, S. Karuranga, M. Pinkepank and K. Ogurtsova *et al*., 2022. IDF diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res. Clin. Pract., Vol. 183. 10.1016/j.diabres.2021.109119.
6. Nissen, S.E. and K. Wolski, 2007. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N. Engl. J. Med., 356: 2457-2471.
7. Bakoma, B., B. Berke, K. Eklu-Gadegbeku, A. Diallo and A. Agbonon *et al*., 2012. Total phenolic content, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia of *Bridelia ferruginea* extracts. Res. J. Phytochem., 6: 120-126.
8. Kpodar, M.S., P. Lawson-Evi, B. Bakoma, K. Eklu-Gadegbeku, A. Agbonon, K. Aklikokou and M. Gbeassor, 2015. Ethnopharmacological survey of plants used in the treatment of diabetes mellitus in South of Togo (Maritime Region). J. Herb. Med., 5: 147-152.
9. Motto, E.A., P. Lawson-Evi, Y. Kantati, K. Eklu-Gadegbeku, K. Aklikokou and M. Gbeassor, 2020. Antihyperglycemic activity of total extract and fractions of *Anogeissus leiocarpus*. J. Drug Delivery Ther., 10: 107-113.
10. Atchou, K., P. Lawson-Evi, K. Metowogo, B. Bakoma, K. Eklu-Gadegbeku, K. AkliKokou and M. Gbeassor, 2020. Antihyperglycaemic and antioxidant activities of *Crataeva adansonii* DC. ssp. *adansonii* leaves extract on ICR mice. J. Drug Delivery Ther., 10: 30-38.
11. Hamilton, K.L. and A.G. Butt, 2013. Glucose transport into everted sacs of the small intestine of mice. Adv. Physiol. Educ., 37: 415-426.
12. Therasa, S.V., T. Thirumalai, N. Tamilselvan and E. David, 2014. *In-vivo* and *ex-vivo* inhibition of intestinal glucose uptake: A scope for antihyperglycemia. J. Acute Dis., 3: 36-40.
13. Povi, L.E., A.E. Motto, K. Atchou, K. Tona, K. Eklu-Gadeg and K. Aklikokou, 2021. Insulinomimetic activity assessment of antidiabetic plants used in togolese pharmacopoeia, *in ovo* and *ex vivo* study. J. Biol. Sci., 21: 1-9.
14. Kpemissi, M., K. Eklu-Gadegbeku, V.P. Veerapur, A.V. Potârniche and K. Adi *et al*., 2019. Antioxidant and nephroprotection activities of *Combretum micranthum*: A phytochemical, *in-vitro* and *ex-vivo* studies. Heliyon, Vol. 5. 10.1016/j.heliyon.2019.e01365.
15. Oyaizu, M., 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. Diet., 44: 307-315.
16. Prieto, P., M. Pineda and M. Aguilar, 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal. Biochem., 269: 337-341.
17. Harborne, J.B., 1973. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 2nd Edn., Chapman & Hall, London , United Kingdom, ISBN: 9780412105401, Pages: 278.
18. Maksimović, Z., Đ. Malenčić and N. Kovačević, 2005. Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresour. Technol., 96: 873-877.
19. Povi, L.E., B. Batomayena, T.A. Hode, E.G. Kwashie, A. Kodjo and G. Messanvi, 2015. Phytochemical screening, antioxidant and hypoglycemic activity of *Coccoloba uvifera* leaves and *Waltheria indica* roots extracts. Int. J. Pharm. Pharm. Sci., 7: 279-283.
20. DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

21. Mingueneau, M., A. Chaix, N. Scotti, J. Chaix, A. Reynders, C. Hammond and J. Thimonier, 2015. Hands-on experiments on glycemia regulation and type 1 diabetes. Adv. Physiol. Educ., 39: 232-239.
22. Kim, Y.D., K.G. Park, Y.S. Lee, Y.Y. Park and D.K. Kim *et al*., 2008. Metformin inhibits hepatic gluconeogenesis through AMP-activated protein kinase-dependent regulation of the orphan nuclear receptor SHP. Diabetes, 57: 306-314.
23. Loureiro, G. and F. Martel, 2019. The effect of dietary polyphenols on intestinal absorption of glucose and fructose: Relation with obesity and type 2 diabetes. Food Rev. Int., 35: 390-406.
24. Zhang, Y., T. Hu, H. Zhou, Y. Zhang, G. Jin and Y. Yang, 2016. Antidiabetic effect of polysaccharides from *Pleurotus ostreatus* in streptozotocin-induced diabetic rats. Int. J. Biol. Macromol., 83: 126-132.
25. Khan, A. and J. Pessin, 2002. Insulin regulation of glucose uptake: A complex interplay of intracellular signalling pathways. Diabetologia, 45: 1475-1483.
26. Ang, L.Z.P., R. Hashim, S.F. Sulaiman, A.Y. Coulibaly, O. Sulaiman, F. Kawamura and K.M. Salleh, 2015. *In vitro* antioxidant and antidiabetic activites of *Gluta torquata*. Ind. Crops Prod., 76: 755-760.
27. Bhatti, J.S., A. Sehrawat, J. Mishra, I.S. Sidhu and U. Navik *et al*., 2022. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radical Biol. Med., 184: 114-134.
28. Sarikaphuti, A., T. Nararatwanchai, T. Hashiguchi, T. Ito and S. Thaworanunta *et al*., 2013. Preventive effects of *Morus alba* L. anthocyanins on diabetes in Zucker diabetic fatty rats. Exp. Ther. Med., 6: 689-695.
29. Li, H., X. Wang, Y. Li, P. Li and H. Wang, 2009. Polyphenolic compounds and antioxidant properties of selected China wines. Food Chem., 112: 454-460.
30. Nwankwo, N.E., E.C. Ezeako, F.N. Nworah, A.L. Ogara and S.A. Oka *et al*., 2023. Bioactive compounds, anti-inflammatory, anti-nociceptive and antioxidant potentials of ethanolic leaf fraction of *Sida linifolia* L. (Malvaceae). Arab. J. Chem., Vol. 16. 10.1016/j.arabjc.2022.104398.
31. Gong, X., X. Li, Y. Xia, J. Xu, Q. Li, C. Zhang and M. Li, 2020. Effects of phytochemicals from plant-based functional foods on hyperlipidemia and their underpinning mechanisms. Trends Food Sci. Technol., 103: 304-320.
32. Gothai, S., P. Ganesan, S.Y. Park, S. Fakurazi, D.K. Choi and P. Arulselvan, 2016. Natural phyto-bioactive compounds for the treatment of type 2 diabetes: Inflammation as a target. Nutrients, Vol. 8. 10.3390/nu8080461.