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Original Article

Phytochemistry, Antihyperglycemic, Antioxidant and Anti-Inflammatory Properties of *Uvaria Chamae* and *Sida Linifolia* Extracts: Potential Implication in Diabetic Disease

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ABSTRACT

Introduction: *Uvaria chamae* and *Sida linifolia* are plants traditionally used in Togo in diabetes treatment, an affection that often leads to several complications. This study aimed to evaluate the antihyperglycemic, anti-inflammatory, antioxidant activity and toxicity of these two plants extracts. **Methods**: A phytochemical analysis was carried out on extracts obtained either by decoction or maceration in ethanol of *Uvaria chamae* leaves and *Sida linifolia* whole plant. Evaluation of the antihyperglycemic activity consisted in glucose absorption test using yeast and rats' muscle and jejunum. DPPH test, total antioxidant capacity assay, hemolysis and egg albumin denaturation inhibition assays and evaluation of extracts acute toxicity were performed. **Results**: Hydroalcoholic extract of *Uvaria chamae* showed the strongest antihyperglycemic activity ($p<0.05$); the highest phenolic contents (147.93 ± 1.01 mg/g), the best total antioxidant capacity (153.33 ± 4.07), the lowest IC_{50} (µg/mL) for DPPH test (296.96 ± 91.69), a capacity of hemolysis (825.99 ± 29.24) and egg albumin denaturation (738.10 ± 92.26) inhibition assays. In the same way, hydroalcoholic extract of *Sida linifolia*, showed the strongest antihyperglycemic activity ($p<0.05$), the highest phenolic contents (71.60 ± 2.16 mg/g), the best total antioxidant capacity (146.98 ± 2.81), lowest IC_{50} (µg/mL) for DPPH test (788.28 ± 112.54), the hemolysis (882.03 ± 20.86) and egg albumin denaturation (1966.18 ± 35.94) inhibition assays. None of the extracts showed acute toxicity in rats. **Conclusion**: the hydroalcoholic leaves extract of *Uvaria chamae* and of the whole plant of *Sida linifolia* could be candidates in the treatment of diabetes and its complications.

Keywords: Decoction, Hyperglycemia, Neuroprotection, Phytochemistry.

INTRODUCTION

Diabetes mellitus has become a common and widespread disease that affects a large number of people in both developed and developing countries [1]. It is a metabolic disease characterized by chronic hyperglycemia resulting from insulin deficiency, insulin resistance or both [2]. Morbidity and mortality are not directly caused by diabetes but relatively by long-term complications in diabetic patients. Prolonged and uncontrolled hyperglycemia is a risk that is associated with diabetic angiopathy, retinopathy, nephropathy and neuropathy [3]. Neuropathic damage is responsible for a loss of protective sensitivity of the lower limbs, neuropathic pain [4,5]. Thus, glycemic control strategy is necessary for the prevention of diabetes morbidity and mortality [4]. Furthermore, inflammation and oxidative stress are implicated into the physiopathology of diabetes mellitus. Thus, the suppression of oxidative stress/inflammation should be therapeutic targets for insulin resistance [6]. Current treatment of the disease is pharmacological molecules such as insulin, biguanides, dipeptidylpeptidase-4 inhibitors, sodium-glucose co-transporter 2 inhibitors, sulfonylureas, thiazolidinediones [7]. However, long term treatment of these medications has led to huge demand for efficacious, decreased side effects and affordable agents for the treatment of diabetic condition [8]. Many plants have been reported to have antidiabetic activities, but few studies have proven the safety and the efficiency of those as antidiabetic agents, even though plant compounds with both antihyperglycemic, anti-inflammatory and antioxidative properties would be useful drugs for the control of glycemia and evolution of diabetes and its related complications [9,10]. *Uvaria chamae* and *Sida linifolia* are plants that are known in Togo for the treatment of diabetes and its complications, but also in inflammatory diseases. The aim of the study is to perform a phytochemical analysis (qualitative and quantitative) of the plant extracts and to assess the antihyperglycemic, anti-inflammatory, antioxidant activity and the toxicity of these two plant extracts.

MATERIAL AND METHODS

Ethical consideration

Experiments were conducted in accord with the institutional guidelines and ethics of Laboratory of Physiology/Pharmacology of University of Lomé, Togo (ref: 003/2022/ CB-FDS-UL).

Chemicals

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), metformin and ascorbic acid were purchased from Sigma Aldrich (USA).

Plant material

The plant material used were *Uvaria chamae* leaves and *Sida linifolia* whole plant. They were collected

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in Togo respectively in Agoè Apessito (6°17'42.8" N; 1°09'49.0" E), on March 13, 2022 and in Dalavé (6°22'43.7 "N; 1°10'35.2" E), on august 22, 2022. The plants were identified in the Laboratory of Botany and Plant Ecology of the Faculty of Sciences, University of Lomé (Togo), and a sample was kept in the herbarium of the said Laboratory under numbers TOGO15906 and TOGO15907. The harvested plant materials were dried and ground to powder at the Department of Pharmaceutical Sciences of the Faculty of Health Sciences in University of Lomé (Togo).

Animals

Wistar rats of both sexes weighing 80 to 200 g were used for *ex vivo* and in vivo tests. The study animals were obtained from the Department of Pharmaceutical Sciences. They were all maintained in hygienic environmental conditions with a light/dark period of 12/12 h) in standard cages and fed with food and water ad libitum.

Extraction

The extracts were obtained using two extraction techniques: maceration in alcohol and decoction. For maceration, the plant powders were mixed with hydro-ethanolic solutions as indicated in table 1. The mixtures were subjected to mechanical stirring for 72 hours. The macerates were filtered and evaporated under reduced pressure at 45°C. For the decoctions, the powders were mixed with distilled water and boiled for 15 min. After cooling, the decoctions were filtered and evaporated under reduced pressure at 45°C. The crude extracts obtained (Table 1) were stored in the refrigerator at 4°C until use. The yield was calculated using the formula below:

Yield (%) = (mass of extract obtained)/ (mass of plant powder used) ×100

Phytochemical screening

The chemical screening consisted of the search for large chemical groups such as alkaloids, tannins, sterols, triterpenes, phenols, flavonoids, saponins, anthocyanins, reducing sugars and mucilage through described methods [11].

Determination of total phenols contents

A 0.1 mL of aqueous solution of 1 mg/mL extract were mixed with 2 ml of a newly prepared 2% sodium carbonate solution and the whole vortexed. After five minutes, 100 µL of Folin-Ciocalteu reagent (1 N) were added to the mixture, kept for 30 minutes at room temperature and the absorbance was read against a blank using a spectrophotometer at 750nm. A calibration curve was made simultaneously under similar conditions considering gallic acid as a positive control. The results were expressed as milligram of gallic acid equivalent per gram of dry extract (mg GAE/g) [12].

Determination of flavonoids contents

To 2 mL of $AlCl_3$ at 2% (m/v) in pure methanol, 2 mL of extract (1 mg/mL) also in methanol were added. The mixture was incubated for 10 minutes away from light and the absorbance was measured at 415 nm against a blank using a UV-Visible spectrophotometer. The standard range of 10 to 100 µg/mL was prepared under the same conditions as the extract. The calibration curve was plotted using the different concentrations of quercetin and the results were expressed as milligram of quercetin equivalent per gram of dry extract (mg QE/g) [13].

Table 1: *Uvaria chamae* and *Sida linifolia* extracts preparation.

	Techniques	Solvents	Extracts
Uvaria chamae	Maceration	Ethanol-water mix (80:20, v/v)	U8
		Ethanol-water mix (50:50, v/v)	U5
	Decoction	Distilled water (DW)	UD
Sida linifolia	Maceration	Ethanol-water mix (80:20, v/v)	S8
		Ethanol-water mix (50:50, v/v)	S5
	Decoction	Distilled water (DW)	SD

Evaluation of antioxidant activity

DPPH test

The anti-radical activity of the different extracts was evaluated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a relatively stable free radical, according to the method described by Kagnou et al. (2020) with some modifications [13]. One hundred microliters (100 µL) of extract were added to 2 mL DPPH (0.004% prepared in methanol). The mixture was homogenized and the absorbance reading was taken with a spectrophotometer at 517 nm after 30 minutes of incubation at room temperature in the dark. Three tests were carried out for each sample. The standard was quercetin (100 to 1000 mg/mL). The percentage of inhibition of radical activity was calculated according to the formula:

Inhibition (%) = (Absorbance (control)-absorbance (sample))/ (absorbance (control)) ×100

The IC_{50} was generated by GraphPad Prism 8.

Total antioxidant capacity

The test was carried out according to the method described by Nwankwo et al. (2023). The mixture of 300 µL of each extract and 3,000 µL of phosphomolybdate reagent was incubated at 95°C for one and a half hours. After centrifugation at 3000 rpm for 5 minutes, the absorbance of the cold supernatant solution was measured at 765 nm by a UV-Visible spectrophotometer. The standard was ascorbic acid (25 to 200 µg/mL). Its absorbance was measured under the same conditions as the samples and for each concentration. The test was repeated three times. The calibration curve was plotted using the different concentrations of ascorbic acid and the results are expressed in milligram of ascorbic acid equivalent per gram of dry extract (mg AAE/g) [14].

Acute toxicity

Acute oral toxicity was assessed in accordance with OECD Guideline N° 423 for Chemicals Testing (December 17, 2001). Rats were fasted, but allowed to drink water for 3 hours before extracts' administration. A first step consisted of administering extracts at 5000 mg/kg to 3 rats. The control group, consisting of 3 rats also received distilled water at 0.5 mL/100g body weight. The animals are observed individually 30 minutes after administration of the products, then every hour for the first 4 hours. Observations focused on ptosis, piloerection and general behavior of the animals (convulsion, salivation, diarrhea, spontaneous locomotor activity, reaction to external stimulus). Batch mortality was assessed during the first 24 hours after product administration. The body weight of the rats was measured on D1, D3, D7 and D14 after administration of the products [15].

Evaluation of antihyperglycemic activity

For the evaluation of the antihyperglycemic activity of the extracts, *in vitro* (effect on glucose uptake by yeast cells and glucose adsorption capacity assay) and *ex vivo* (effect on glucose absorption by muscle and intestine assay) methods were performed.

Effect on glucose absorption by muscle

The capacity of the different concentrations (0.5 and 1 mg/L) of extracts on muscle glucose uptake was measured in harvested rat psoas muscle according to the procedure described by Oyebode et al. [16]. The muscle was collected, briefly cleaned with Kreb's buffer and diced into small chunks of the same weight (500 mg). Each chunk was then incubated in 8 ml of Kreb's buffer, pre-mixed with 11.1 mM glucose (control) and varying concentration of the extract. A 2000 mg/mL metformin solution was used as a positive control. The incubation time lasted for

an hour at 37 °C. Before and after the incubation period, 1 mL sample was collected from each incubation tube and the glucose concentration was measured by using commercial assay kit. The uptake of glucose by the muscle was extrapolated as the quantity of glucose (mg) used up by each gram of muscle tissue using the following formula:

Muscle glucose = (G1 – G2) / weight of muscle tissue (g)

Where G1 and G2 are glucose concentrations before and after the incubation respectively.

Effect on glucose uptake by the intestine

The time-dependent reduction of glucose concentration in an incubation solution containing 5 cm of freshly isolated rat jejunum and different extracts' concentrations (0.5 and 1 mg/mL) was measured using Chukwuma et al. (2018) method [17]. Briefly, a 5 cm jejunal segment from the isolated rat intestinal gut was first inverted to expose the villi and then incubated in 8 mL of Kreb'sbuffer (118 mM NaCl, 5 mM KCl, 1.328 mM $CaCl_2{\cdot}2H_2O$, 1.2 mM KH_2PO_4, 1.2mM $MgSO_4$ and 25mM $NaHCO_3$) containing 11.1 mM glucose and extracts (0.5 and 1 mg/mL). Glucose with Kreb's buffer was used as a control. Glucose concentrations were measured in all incubation solutions before and after the 2 h incubation period at 37°C by using commercial assay kit. The intestinal glucose absorption was calculated as the amount (mg) of glucose absorbed per cm of rat jejunum using the following formula:

Intestinal glucose absorption per cm of jejunum = G1−G2 / length of jejunum used in cm Where, G1 and G2 are glucose concentrations (mg/dL) before and after the incubation, respectively.

Effect of extracts on glucose uptake by yeast cells

Commercial baker's yeast was washed in distilled water with repeated centrifugation (3000 x g, 5 min) until a clear supernatant was obtained. Furthermore, a 10% (v/v) suspension was prepared with it. Different concentrations of the six extracts (0.5–2.5 mg) were added to 1 ml of glucose solution (5–25 mM), the mixture was incubated for 10 min at 37 °C. The reaction was initiated by adding 100 µL of yeast suspension, vortexed and incubated at 37°C. After 60 min, the tubes were centrifuged (2500 x g, 5 min) and glucose was estimated in the supernatant. The percentage of activity (increase in glucose uptake by yeast cells) was calculated using the following formula [18]:

% activity= (control absorbance - sample absorbance)/ (control absorbance) ×100

Determination of the glucose adsorption capacity of the extracts

The extracts (250 mg) were added separately to 250 ml of glucose solution of increasing concentrations (5, 10, 15and 20 mM). The reaction mixture was shaken and incubated in a shaking incubator at 37 °C for 6 h, centrifuged at 4000 x g for 20 min. The glucose content in the supernatant solution was determined by the glucose oxidase-peroxidase method. The absorbance was read at 520 nm. The glucose adsorption capacity was determined according to the following formula [19]:

Glucose Bound= (Glucose 1 - Glucose 6)/ (Weight of extracts) ×Volume of solution, where

Glucose 1: Concentration of the original glucose solution.

Glucose 6: Glucose concentration after 6 h.

Evaluation of anti-inflammatory activity

Red blood cell membrane stabilization test

The anti-inflammatory activity of the extracts was evaluated by a red blood cell membrane stabilization test. The blood used was retro-orbital blood collected from Wistar rats. One millimeter of each extract (62.5 µg/mL to 500 µg/mL) was added to 1 mL of red blood cell suspension and incubated at 56°C for 30 min. The reacted mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was measured at 560 nm. Aspirin was used as standard. Saline was used as a control for the experiment and the percentage inhibition of hemolysis was calculated using the following formula:

Inhibition (%) = (control absorbance - sample absorbance)/ (control absorbance) ×100

IC_{50} was generated by GraphPad Prism 8 [20].

Egg albumin denaturation inhibition assay

The reaction mixture consisted of 0.2 ml of Isa Brown strain egg albumin, 2.8 ml of phosphate-buffered saline (pH 6.4), and 2 ml of various concentrations (62.5 to 500 µg /mL) of extracts or standard medications (aspirin). All samples were set aside at 37°C for 25 minutes followed by heating for 5 minutes at 70°C. The cooled solutions were centrifuged at 3000 rpm for 10 minutes. Then, the absorbance of the supernatant solutions was measured at 660 nm. Aspirin was used as standard. The buffer solution was used as a control for the experiment and the percentage inhibition of protein denaturation was calculated using the following formula:

Inhibition (%) = (control absorbance - sample absorbance)/ (control absorbance) ×100

IC_{50} was generated by GraphPad Prism 8 [21].

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8 (USA) and Microsoft Office Excel 2013 (USA). Data were subjected to one-way analysis of variance (one-way ANOVA). Results were reported as mean ± standard error of the mean or standard deviation. Differences were considered significant if $p < 0.05$.

RESULTS

Yields

The following yields were obtained after the plants' extraction (table 2):

Phytochemical analysis

Phytochemical analysis shows the presence of several groups in the extract (table 3) and the determination of total phenols and flavonoids contents showed that the highest levels of phenols and flavonoids were found in U5 an S8 (table 4).

Acute toxicity

The acute toxicity test shows that none of the extracts at 5000 mg/kg modifies the general behavior of the rats and does not cause the death of the rats within 24 hours following administration of the extract. The weight of the animals did not vary over the entire trial period either (table 5).

Bioactivity assays

Effects of extracts on glucose uptake in isolated rat muscle without insulin

The effects of *Uvaria chamae* and *Sida linifolia* extracts on glucose uptake in isolated rat muscle without insulin are presented in figure 1. Glucose uptake at 1 mg/mL concentration of U5 was significantly higher ($p < 0.05$) than the control, when the other extracts at the concentrations of 0.5 and 1 mg/mL did not show any significant difference compared to the control (figure 1A). For *Sida linifolia*, glucose uptake at 0.5 and

Table 2: *Uvaria chamae* and *Sida linifolia* extraction's yields.

Extracts	Yields (%)
U8	13.23
U5	17.86
UD	17.03
S8	5.03
S5	7.04
SD	13.10

Table 3: Phytochemical composition of *Uvaria chamae* and *Sida linifolia's* extracts.

Groups	U8	U5	UD	S8	S5	SD
Alkaloids	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Tannins	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	+	-	-	-	-
Steroids	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+
Reduced sugars	+	+	+	+	+	+
Mucilage	-	-	-	-	-	+

+: presence; -: absence

Table 4: *Uvaria chamae* and *Sida linifolia* total phenolic and flavonoids contents.

Extracts	Total phenolic contents (mg GAE/g)	Flavonoids contents (mg QE/g)
U8	127.07 ± 2.78	48.13 ± 1.26
U5	147.93 ± 1.01	68.29 ± 3.80
UD	110.07 ± 2.71	25.59 ± 0.02
S8	71.60 ± 2.16	22.08 ± 1.06
S5	58.43 ± 0.24	6.57 ± 0.81
SD	35.10 ± 3.80	2.43 ± 1.01

Values are expressed as means ± standard errors (n=3); GAE: gallic acid equivalent; QE: quercetin equivalent

Table 5: Effect of *Uvaria chamae* and *Sida linifolia* extracts on the weight evolution of rats.

Extracts	Rats' body weight (g)			
	D1	D3	D7	D14
Control	103.33 ± 5.70	109.33 ± 2.33	109.33 ± 4.7	119 ± 1
U8	120.67 ± 13.25	119.33 ± 11.46	119.33 ± 12.55	128 ± 13.08
U5	98 ± 11.72	102.67 ± 12.02	104 ± 11.15	112.33 ± 10.84
UD	103.67 ± 10.27	106 ± 10.02	108.33 ± 9.87	110.67 ± 6.69
S8	116.33 ± 9.87	113.67 ± 8.67	114 ± 10.26	120.67 ± 11.05
S5	112 ± 8.54	117.33 ± 6.23	122.33 ± 5.78	126.67 ± 5.84
SD	112 ± 9.07	115.33 ± 9.39	119.33 ± 8.69	124 ± 6.51

Values are expressed as means ± standard error of the mean (n=3)

1 mg/mL concentrations of S8 were significantly higher ($p < 0.001$) than the control, when the other extracts at the concentrations of 0.5 and 1 mg/mL did not show any significant difference compared to the control (figure 1B).

Effects of extracts on glucose uptake in isolated rat muscle with insulin

The effects of *Uvaria chamae* and *Sida linifolia* extracts on glucose uptake in isolated rat muscle with insulin are presented in figure 2. The extracts at the concentrations of 0.5 and 1 mg/mL did not show any significant difference compared to the control as for *Uvaria chamae* (figure 2A) or *Sida linifolia* (figure 2B) extracts.

Effects of extracts on intestinal glucose absorption

The results of the effects of the different concentrations of the ethanol extract on glucose absorption in rat jejunum are presented in figure 3. All extracts excepted from U8 significantly influenced the glucose jejunum absorption. The extracts at the concentrations of 0.5 ($p<0.01$) and 1 mg/mL ($p<0.001$) showed lower glucose absorption compared to the control whether for *Uvaria chamae* (figure 3A) or *Sida linifolia* (figure 3B) extracts.

Glucose adsorption capacity of Uvaria chamae and Sida linifolia extracts

In vitro glucose adsorption capacity of the extracts has been shown in figure 4. The data indicated that the extracts possessed a glucose adsorption capacity at all tested concentrations. The highest adsorption capacity was 37.56 ± 4.03 % for U5 and 19.99 ±0.43 % for S5.

Effect of extracts on glucose uptake by yeast cells

The percentage increase in glucose uptake by the yeast cell at different glucose concentrations (25mM, 10mM and 5mM) is shown respectively in figures 5, 6 and 7. U8 exhibited significantly higher activity than other plant extracts at all glucose concentrations showing the maximum increase in 5mM glucose concentration (16,14 ± 1,18 %) at 1 µg/mL of plant extract (figure 5).

Antioxidant activity

The table 6 describes the results of antioxidant activity of *Uvaria chamae* an *Sida linifolia* extracts (0.1 to 1mg/mL). Among the *Uvaria chamae* extracts, U5 showed the lowest IC_{50} and S8 for the *Sida linifolia* extracts; they both remained higher than quercetin, the standard for the DPPH test (table 6). For total antioxidant capacity, the same extracts showed the higher activity even though their activity were less than ascorbic acid the standard for total antioxidant capacity (TAC) test (table 6).

Table 6: Antioxidant capacity of *Uvaria chamae* and *Sida linifolia* extracts (0.1 to 1 mg/mL): DPPH and total antioxidant capacity test.

Extracts	DPPH	TAC
	IC_{50} (µg/mL)	AAE (mg/g)
U8	525.45 ± 184.86	195.88 ± 7.80
U5	296.96 ± 91.69	153.33 ± 4.07
UD	657.20 ± 186.01	72.23 ± 0.88
S8	788.28 ± 112.54	146.98 ± 2.81
S5	942.68 ± 157.19	81.62 ± 5.52
SD	1160.20 ± 465.55	30.79 ± 1.33

Values are expressed as means ± standard errors (n=3); AAE: ascorbic acid equivalent; TAC: total antioxidant capacity.

Table 7: Anti-inflammatory activity of *Uvaria chamae* and *Sida linifolia* extracts (62.5 to 500 µg/mL): test for inhibition of hemolysis and inhibition of egg albumin denaturation.

Samples	IC_{50} (µg/mL)	
	Haemolysis	Egg albumin denaturation
U8	1270.40 ± 459.26	1471.73 ± 388.10
U5	825.99 ± 29.24	738.10 ± 92.26
UD	612.66 ± 21.43	630.03 ± 30.10
S8	882.03 ± 20.86	1966.18 ± 35.94
S5	1498.30 ± 424.35	2171.03 ± 8.83
SD	1715.21 ±150.17	616.35 ± 188.59
Aspirin	161.66 ± 2.61	391.75 ± 9.58

Values are expressed as means ± standard errors (n=3)

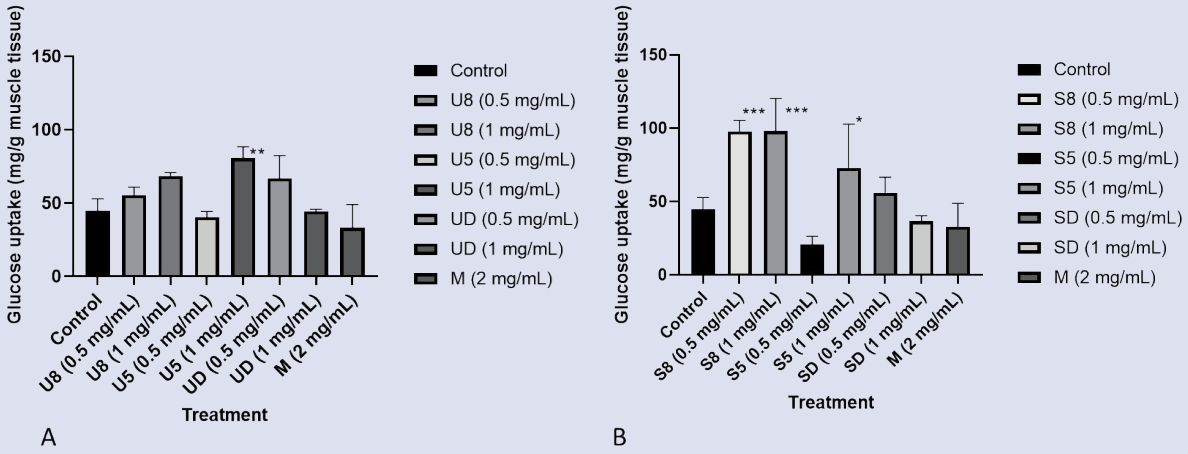

Figure 1: Effects of extracts on glucose uptake in isolated rat muscle without insulin
A: Uvaria chamae's extracts effects; B: Sida linifolia's extracts effects. Values are expressed as means ± standard error of the mean (n=3). *p<0.05, ***p<0.001 vs control.

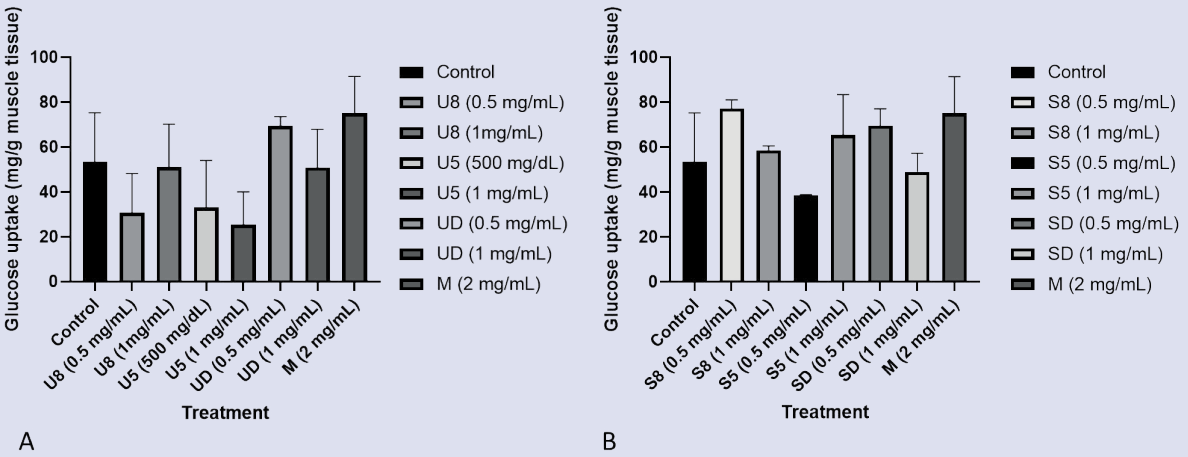

Figure 2: Effects of extracts on glucose uptake in isolated rat muscle with insulin
A: Uvaria chamae's extracts effects; B: Sida linifolia's extracts effects. Values are expressed as means ± standard error of the mean (n=3).

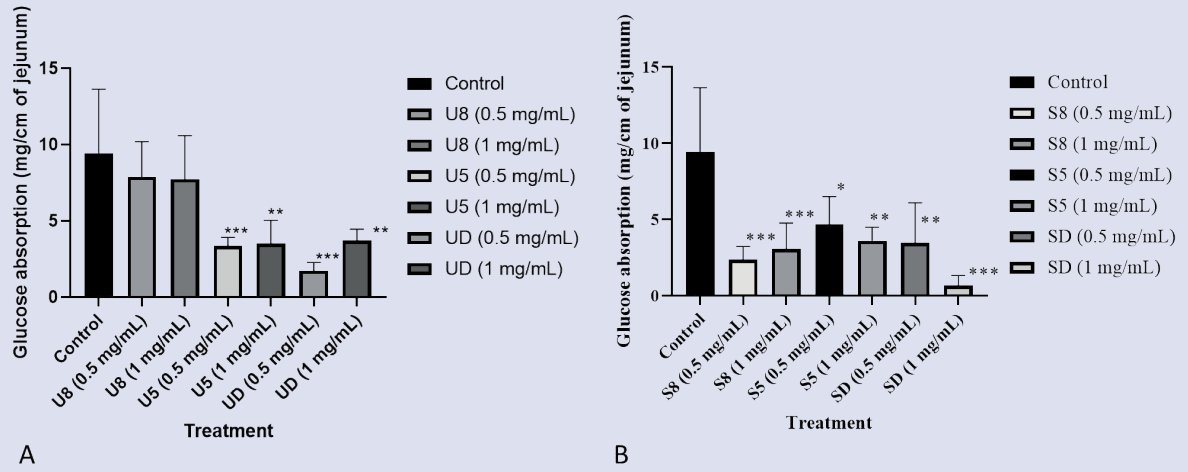

Figure 3: Effects of extracts on intestinal glucose absorption
A: Uvaria chamae's extracts effects; B: Sida linifolia's extracts effects. Values are expressed as means ± standard error of the mean (n=3). *p<0.05, **p<0.01, ***p<0.001 vs control.

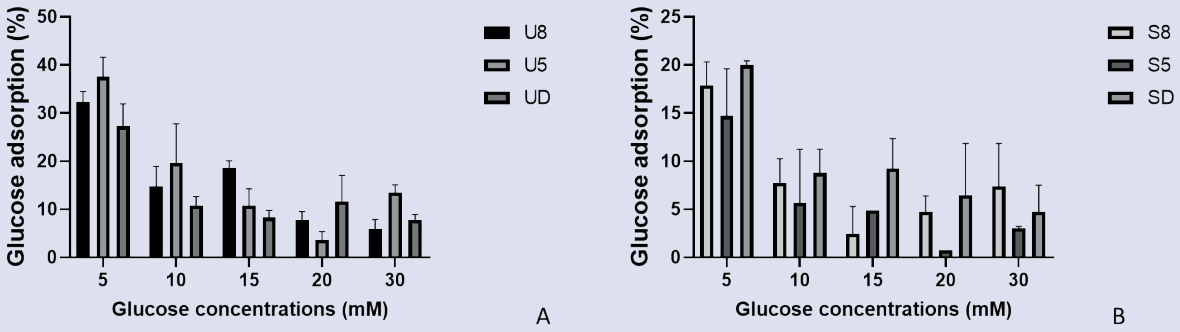

Figure 4: Glucose adsorption capacity of *Uvaria chamae* (A) and *Sida linifolia* (B) extracts (1mg/mL). Values are expressed as means ± standard error of the mean (n=3).

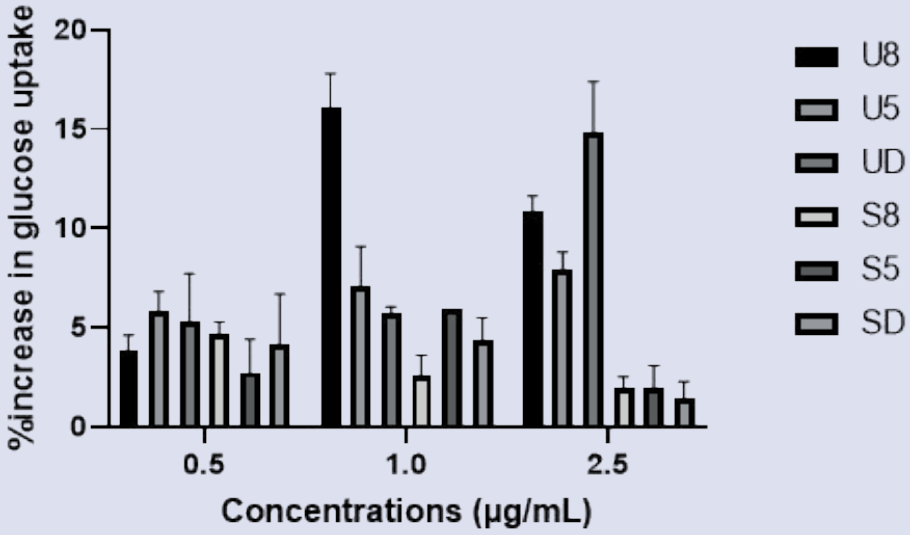

Figure 5: The percentage increase in glucose uptake by yeast cells due to the effect of *Uvaria chamae* and *Sida linifolia* extracts at 5mM glucose concentration. Values are expressed as mean ± standard error of the mean (n = 3)

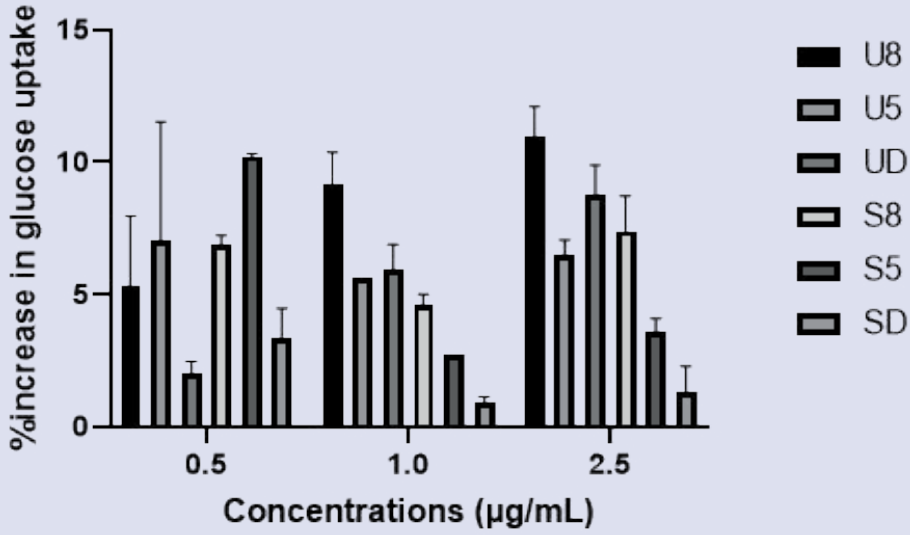

Figure 6: The percentage increase in glucose uptake by yeast cells due to the effect of *Uvaria chamae* and *Sida linifolia* extracts at 15mM glucose concentration. Values are expressed as mean ± standard error of the mean (n = 3)

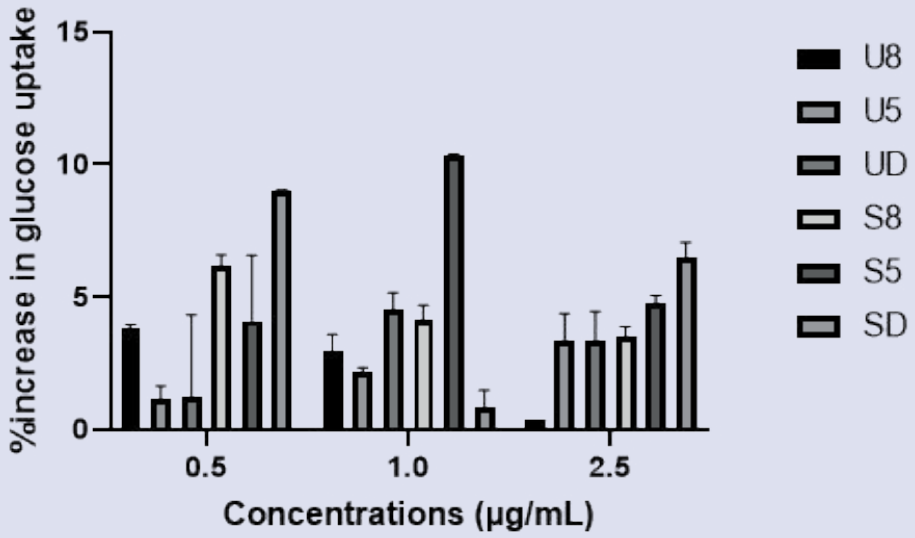

Figure 7: The percentage increase in glucose uptake by yeast cells due to the effect of *Uvaria chamae* and *Sida linifolia* extracts at 25mM glucose concentration. Values are expressed as mean ± standard error of the mean (n = 3)

Anti-inflammatory activity

The data of antioxidant activity of *Uvaria chamae* and *Sida linifolia* extracts (62.5 to 500 µg/mL) were depicted in table V7. Among the *Uvaria chamae* extracts, UD showed the lowest IC_{50} and S8 for the *Sida linifolia* extracts; they both remained higher than aspirin, the standard for the hemolysis inhibition test (table 7). For the egg albumin denaturation inhibition test, UD and SD showed the lowest IC_{50} therefore the higher activity even though their activity were less than aspirin the standard (table 7).

DISCUSSION

Diabetes is a disease characterized by high levels of blood glucose [22]; its intracellular concentration may be increased. The glycolysis pathway is then saturated, and other metabolic pathways usually not active are involved (polyol pathway, advanced glycation end product pathway, hexosamine pathway and protein kinase C pathway) responsible for the morbity and complications [23,24]. Managing the disorder and it's complications can then be done by lowering those levels through increasing muscle absorption of the blood glucose or by impeding the glucose absorption by the intestine for examples [25,26]. The extracts of *Uvaria chamae* and *Sida linifolia* have shown to improve the absorption of glucose by the muscle, glucose uptake at 1 mg/mL concentration of U5 was significantly higher ($p < 0.05$) than the control and the other extracts of the same plant, glucose uptake at 0.5 and 1 mg/mL concentrations of S8 were significantly higher ($p < 0.001$) than the control and the other extracts of *Sida linifolia*. The mechanisms of this activity may be explained by the facilitated transportation of the glucose through cell membranes. The effects of the extracts in the yeast glucose absorption increase showed that the extracts had increased the glucose absorption and U8 exhibited significantly higher activity than other plant extracts at all glucose concentrations showing the maximum increase in 5mM glucose concentration (16.14 ± 1.18 %) at 1 µg/mL of plant extract. The studies on the transport of non-metabolizable sugars, metabolizable glycosides have suggested that sugar transport across the yeast cell membrane is mediated by stereospecific membrane carriers and takes place by facilitated diffusion process[27]. On a general basis, the uptake of glucose by skeletal muscles is due to the accumulation of functional glucose transporting molecules in the cell membrane. The glucose transporting molecules are regulated by leptocytes and/or myocytes in response to high secretion of insulin in blood, resulting in hypoglycemic effect [19]. The extracts may then be increasing muscle glucose uptake by facilitating it's transportation through cells [28]. In the meantime, the effect on intestine glucose absorption was assessed. All extracts excepted from U8 significantly decreased the glucose jejunum absorption. The extracts at the concentrations of 0.5 ($p<0.01$) and 1 mg/mL ($p<0.001$) showed lower glucose absorption compared to the control. The glucose adsorption capacity assay also has been conducted and showed that all extracts possessed a glucose adsorption capacity at all tested concentrations. The highest adsorption capacity was U5's (37.56 ± 4.03 %) and S5's (19.99 ±0,43 %), reducing available quantity of glucose for the intestine to absorb in its lumen, therefore, reducing the postprandial glucose level [19] This result was also found by Motto et al who evaluated the adsorption capacity of plants as *Anogeissus leiocarpus* [29]. Similar studies have reported the anti-diabetic in vivo activities of *Uvaria chamae* and *Sida linifolia*, which showed to reduce blood glucose level in normal and diabetic rats [30,31]. Oxidative stress and inflammation are known to majorly be partaking in the pathogenesis of diabetic complications and a symbiotic relationship also appears to exist between oxidative stress and inflammation [32]. The antioxidant capacity of U chamae and *Sida linifolia* has been assessed through DPPH and CAT. It resulted that all extracts showed antioxidant activities, U5 and S8 showed the highest activity. The extracts also showed anti-inflammatory activity. The two plants has shown to have in other studies, antioxidative, anti-inflammatory and anti-nociceptive properties [14,30]. Those pharmacological capacities as antidiabetic, or antioxidant and anti-inflammatory ones could be due to plants components such as phenols, flavonoids, reduced sugars, steroids and triterpenoids. In fact, phenols, flavonoids reduced sugars, steroids and triterpenoids are known to have antidiabetic, antioxidant and anti-inflammatory properties [14,19,33] and the highest quantities of phenols and flavonoids were found in U5 and S8, which may explain their highest activities.

CONCLUSION

The present study assessed the *in vitro* and *ex vivo* antidiabetic properties of *Uvaria chamae* leaf and *Sida linifolia* whole plant extracts as well as their *in vitro* antioxidant and anti-inflammatory activities. The results showed that those extracts, especially the hydroalcoholic leaves extract of *Uvaria chamae* (U5) and of the whole plant of *Sida linifolia* (S8) can be considered for further studies in diabetic disease and its complications.

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ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; DW: Distilled water; EA: egg albumin; mg AAE/g: milligram of ascorbic acid equivalent per gram of dry extract; mg GAE/g: milligram of gallic acid equivalent per gram of dry extract; mg QE/g: milligram of quercetin equivalent per gram of dry extract.

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