

Antidiabetic Activity of *Sida linifolia* Juss. ex Cav. (Malvaceae) and *Uvaria chamae* P. Beauv. (Annonaceae) Fractions Using *ex Vivo* Methods

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Abstract

Background: Diabetes is a disease characterized by chronic hyperglycemia with acute and chronic complications. We have previously reported that the hydroalcoholic extract of *Uvaria chamae* leaves and of the whole plant of *Sida linifolia* exerted antihyperglycemic activity *in vitro* on glucose uptake using yeast cells and *ex vivo* on glucose absorption by Wistar rats' muscle and intestine. **Objectives:** The present study aimed to evaluate the antidiabetic and antioxidant activities of *Uvaria chamae* and *Sida linifolia* extracts fractions. **Methods:** For fractionation, solvents of increasing polarity (n-hexane, chloroform, ethyl acetate, butanol, and distilled water) were used. Also, an evaluation of the *in vitro* antioxidant activity (DPPH, FRAP) and the *ex vivo* effect on glucose absorption by Wistar rat muscle and jejunum was performed. **Results:** Aqueous fractions of *Uvaria chamae* (0.2 mg/mL) and *Sida linifolia* (0.1 mg/mL) exhibited the strongest antihyperglycemic activities ($P < 0.05$) in rat muscle and jejunum glucose absorption tests. Phytochemistry analysis revealed the presence of phenols, flavonoids, condensed tannins, sterols, triterpenes, and reducing sugars in the fractions. Phenol, flavonoid, and tannin contents were highest in the butanol and aqueous fractions, which held the highest *in vitro* antioxidant activities. **Conclusion:** Based on the results found in *ex vivo* antidiabetic experiments, the fractions of *Uvaria chamae* and *Sida linifolia* can be used in further investigations as such as antidiabetic properties assessments using *in vivo* methods.

Keywords

Plants, Fractionation, Glucose Transportation, Antioxidant, Rats

1. Introduction

Diabetes mellitus (DM) is a public health problem that currently affects over 537 million people globally; the number is estimated to rise to approximately 643 million by 2030 [1]. This disease has emerged as a significant issue in contemporary society owing to the grave long-term health ramifications linked to it [2]. Diabetes is accompanied by a loss of quality of life and the appearance of risk factors related to mortality. Diabetes outbreak seems to be due to disturbance of carbohydrate, fat, and protein metabolism [3]. Today, synthetic medicines are available and are effective in the treatment of a wide range of diseases; however, some people still prefer herbal medicines as they are viewed as being less harmful to the human body [4]. Plants have been used as therapies since ancient times. Roots, seeds, bark, leaves, and flowers have all been used for remedial purposes. Currently, estimates suggest that over 800 plant species possess significant hypoglycemic characteristics; however, many of them remain to be scientifically evaluated [5]. Various experimental and clinical investigations have been conducted based on the medicinal herbs, among which a significant decrease in patients' blood glucose has been observed [6]. Medicinal plants often contain micronutrients, amino acids, protein, mucilage, essential oils, triterpenoids, saponins, alkaloids, flavonoids, phenolic acids, tannins, and coumarins, which play an effective role in the prevention and treatment of various diseases, including Diabetes mellitus [7]. *Sida linifolia* and *Uvaria chamae* are two of these plants traditionally used in the treatment of diabetes in Togo [8]. We previously reported on antioxidant, anti-inflammatory, and antidiabetic activities of *Uvaria chamae* leaf and *Sida linifolia* whole plant extracts [9]. In the antidiabetic activities assessment using *ex vivo* models, the total extracts significantly increased isolated rat muscle glucose uptake and lowered glucose absorption by rat jejunum. Based on these observations, the current study investigated the two plant extract fractions, analyzing their chemical characteristics and evaluating their antihyperglycemic and antioxidant activities.

2. Material

2.1. Plant Material

Uvaria chamae leaves and *Sida linifolia* whole plant were collected respectively in Togo respectively in Agoè Apepito (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 by the Laboratory of Botany and Plant Ecology of the Faculty of Sciences in the 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 into powder at the Department of Pharmaceutical Sciences of the Faculty of Health Sciences in the University of Lomé (Togo).

2.2. Ethical Consideration

Experiments were conducted following the institutional guidelines and ethics of

the Laboratory of Physiology/Pharmacology of the University of Lomé, Togo (ref: 003/2022/CB-FDS-UL).

2.3. Animals

Wistar rats of both sexes weighing 80 to 150 g were pooled and used for *ex 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*.

2.4. Chemicals

DPPH, TPTZ, quercetin, metformin, glucose, gallic acid, and ascorbic acid were purchased from Sigma Aldrich (USA). Ethanol and methanol were purchased from VWR (France).

3. Methods

3.1. Extraction and Fractionation

The extracts were obtained using cold maceration in alcohol at 10% (m/v) with hydro-ethanolic solutions (80% for *Sida linifolia* and 50% for *Uvaria chamae*). The mixtures were subjected to mechanical stirring for 72 hours. The macerate was filtered and evaporated under reduced pressure at 45°C. The crude extracts obtained were stored in the refrigerator at 4°C until use.

Two methods were used to fractionate our extracts:

For *Uvaria chamae*, the extracts were dissolved at 10%, m/v in distilled water and shaken with n-hexane, chloroform, ethyl acetate, butanol, (1:1) for 24 hours and then allowed to stand for 48 hours before separating the phases in a separating funnel. The fractions were evaporated under reduced pressure at 45°C. The fractions were respectively labelled U5HE, U5CH, U5AE, U5BU, and U5ED for the hexane, chloroform, ethyl acetate, butanol, and aqueous fractions (**Figure 1(a)**).

For *Sida linifolia*, the extracts were fractionated in solvents of increasing polarity. The extracts were mixed with the solvents (10%, m/v) under mechanical stirring for 24 hours, then filtered before being redissolved in the solvent of the next polarity. The solvents were used in this order: n-hexane, chloroform, ethyl acetate, butanol, and distilled water. The resulting fractions were evaporated under reduced pressure at 45°C. The fractions were respectively labelled S8HE, S8CH, S8AE, S8BU, S8ED for the hexane, chloroform, ethyl acetate, butanol, and aqueous fractions (**Figure 1(b)**).

The yield was calculated using the following formula:

$$\text{Yield (\%)} = (\text{mass of fraction obtained}) / (\text{mass of extract used}) \times 100.$$

3.2. Phytochemical Screening

The chemical screening consisted of the search for large chemical groups such as alkaloids, tannins, sterols, triterpenes, phenols, flavonoids, saponins, and reducing

sugars through the described methods [10].

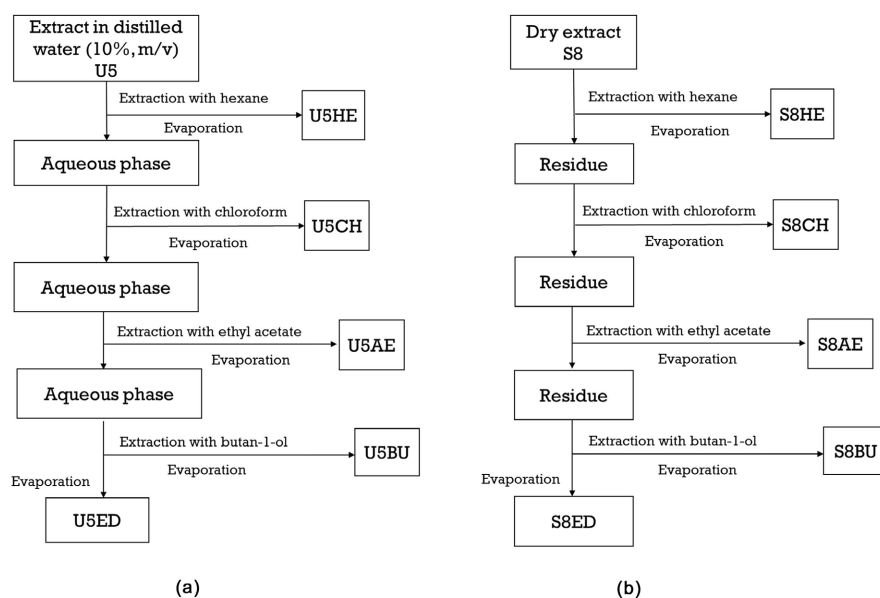


Figure 1. Fractionation of *Uvaria chamae* (a) and *Sida linifolia* (b).

3.3. Determination of Total Phenol Content

To 0.1 ml of aqueous solution of the fraction was added 2 ml of a sodium carbonate solution (2%). After five minutes, 100 μL of Folin-Ciocalteu reagent (1 N) was mixed with it, kept for 30 minutes at room temperature, and the absorbance was read against a blank using a spectrophotometer at 750 nm. A calibration curve is made simultaneously under similar conditions with gallic acid as a positive control (10 - 100 $\mu\text{g}/\text{ml}$). The results were expressed as milligram gallic acid equivalent per gram of dry fraction (mg GAE/g) [11].

3.4. Determination of Flavonoid Contents

To 2 mL of methanol, AlCl_3 (2%), 2 mL of the fraction was 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 $\mu\text{g}/\text{ml}$ was prepared under the same conditions as the fraction. The calibration curve was plotted using the different concentrations of quercetin, and the results were expressed as milligram quercetin equivalent per gram of dry fraction (mg QE/g) [12].

3.5. Determination of Condensed Tannin Contents

A volume of 50 μL of the sample was added to 1500 μL of 4% vanillin methanolic solution and mixed vigorously. Then, a volume of 750 μL of concentrated HCl was added. The resulting mixture was left in the dark at room temperature for 20 minutes. The absorbance was measured at 550 nm. Ranging concentrations of catechin (0 - 1000 $\mu\text{g}/\text{ml}$) as standard were used to draw the calibration curve, and

the results were expressed in mg Catechin Equivalent per gram of dry fraction (mg CE/g) [13].

3.6. *Ex Vivo* Methods

3.6.1. Effect of Glucose Uptake by Rat Muscle

The animals were sacrificed after 12 hours fasting, and the psoas muscle (0.5 g) was harvested. The muscles were then rinsed in cold Krebs' Ringer bicarbonate buffer and placed in tubes containing Krebs' Ringer bicarbonate buffer with 11.1 mM glucose and incubated for 60 minutes at 37°C. After incubation, the muscles were taken out. The glucose content of the incubated medium was measured by the GOD-POD method. The uptake of glucose was calculated in mg/g tissue. Glucose uptake per gram of tissue was calculated as the difference between the initial and final glucose content in the incubated medium. The effect of fractions at the doses of 0.1 and 0.2 mg/ml was measured. A 2 mg/ml metformin solution was used as a positive control [9].

3.6.2. Effect on Glucose Uptake by the Jejunum

The reduction of glucose concentration in an incubation solution containing 5 cm of freshly isolated rat jejunum (sacrificed after 12 hours of fasting) and different fractions' concentrations was measured using Chukwuma *et al.* (2018) method [14]. Briefly, a 5 cm jejunal segment from the isolated rat gut intestinal gut was first inverted to expose the villi and then incubated in 8 ml of Krebs' buffer containing 11.1 mM glucose and fractions (0.1 and 0.2 mg/ml). Glucose with Krebs' buffer was used as a control. Glucose concentrations were measured in all incubation solutions before and after the 120 min incubation period at 37°C by using a 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 = $(G_1 - G_2)/\text{length of jejunum}$ used in cm, G1 and G2 are glucose concentrations before and after the incubation, respectively.

3.7. Evaluation of Antioxidant Activity

3.7.1. DPPH Test

The anti-radical activity of the different fractions was evaluated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a relatively stable free radical. One hundred microliters (100 µl) of the fraction 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) [15] [16].

The percentage of inhibition of radical activity was calculated according to the formula:

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

The IC₅₀ was generated by GraphPad Prism 8.

3.7.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The iron III reducing capability of the extract was assessed using the Eloh *et al.* (2024) method with some modifications. The FRAP test involves assessing the extract capacity to release an electron, thereby converting Fe³⁺ into Fe²⁺. This conversion can be quantified by measuring the resulting formation of Fe²⁺ ions. The FRAP solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of Fe³⁺-TPTZ (10 mM) in HCl (40 mM), and 2.5 ml of FeCl₃·6H₂O (20 mM/l). To 900 μL of the FRAP solution, 30 μL of the fraction (1 mg/ml) and 90 μL of distilled water were incorporated. The optical density was measured at 593 nm when the intense blue color became apparent. Calibration was conducted using an iron sulfate solution (FeSO₄). Each concentration (250 - 1000 μg/ml) was tested three times, and the results were expressed in EC₅₀, which is the median effective concentration. The standard used was the ascorbic acid [17].

3.7.3. Statistical Analysis

Statistical analysis was performed using GraphPad Prism 8 (USA). Results were reported as mean ± standard error of the mean or standard deviation. The replicates (n = 3) were samples from different animals. Data were subjected to one-way analysis of variance (ANOVA). These analyses were followed by the Tukey post-test, which performs multiple comparisons. Differences were considered significant if p < 0.05.

4. Results

4.1. Yields

The following yields were obtained after the plants' extraction (Figure 2).

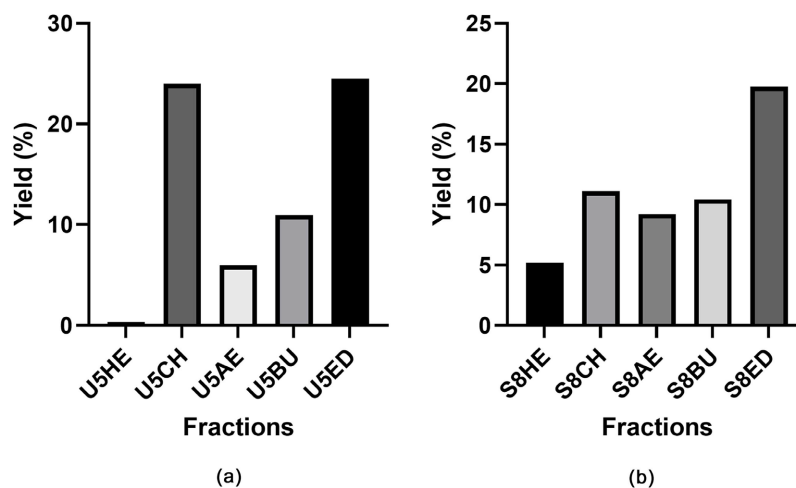


Figure 2. Yields of the fractions of the extracts; (a): *Uvaria chamae*; (b): *Sida linifolia*.

4.2. Phytochemical Analysis

Phytochemical analysis revealed the presence of several groups in the fraction (Table 1), and the determination of total phenols and flavonoids contents showed that the highest levels of phenols and flavonoids were found in S8ED for *Sida linifolia* and U5BU for *Uvaria chamae* (Table 2).

Table 1. Phytochemical screening of *Sida linifolia* and *Uvaria chamae* fractions.

Groups	U5HE	U5CH	U5AE	U5BU	U5ED	S8HE	S8CH	S8AE	S8BU	S8ED
Alkaloids	-	+	-	+	-	-	-	-	+	-
Condensed tannins	-	-	-	+	+	-	-	-	+	+
Sterols	+	+	+	+	+	+	+	+	+	+
Triterpenes	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-
Phenols	-	-	-	+	+	-	-	-	+	+
Flavonoids	-	-	-	+	+	-	-	-	+	+
Reducing sugars	+	+	+	+	+	+	+	+	+	+

+: presence; -: absence.

Table 2. *Uvaria chamae* and *Sida linifolia* total phenolic and flavonoid contents.

Fractions	Total phenolic contents (mg GAE/g)	Flavonoid contents (mg QE/g)	Condensed tannin contents (mgCE/g)
S8BU	128.74 ± 6.12	41.03 ± 2.31	41.08 ± 21.82
S8ED	169.30 ± 7.05	44.57 ± 1.48	160.58 ± 33.62
U5BU	273.86 ± 9.31	118.45 ± 0.67	110.70 ± 25.39
U5ED	88.74 ± 1.59	17.12 ± 0.97	40.05 ± 17.17

Values are expressed as means ± standard errors; GAE: gallic acid equivalent; QE: quercetin equivalent; CE: catechin equivalent.

4.3. Effects of Fractions on Glucose Uptake in Isolated Rat Muscle

The effects of *Uvaria chamae* and *Sida linifolia* extract fractions on glucose uptake in isolated rat muscle are presented in Figure 3. Results showed that U5ED (0.2 mg/ml), U5AE (0.1 and 0.2 mg/ml) increased significantly glucose absorption by muscles ($p < 0.001$, 0.01, and 0.5, respectively, compared to control (Figure 3(a)) for *Uvaria chamae*. For *Sida linifolia*, glucose uptake was significantly higher for the groups that received S8ED ($p < 0.01$) compared to the control (Figure 3(b)).

4.4. Effects of Fractions on Intestinal Glucose Absorption

The results showed that all doses of U5ED, U5BU for *Uvaria chamae* decreased significantly ($P < 0.01$, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively) the absorption of glucose by the rat jejunum (Figure 4) compared to the control group (Figure 4(a)). For *Sida linifolia*, almost all fractions decreased the glucose absorption by

the jejunum compared to the control group (Figure 4(b)).

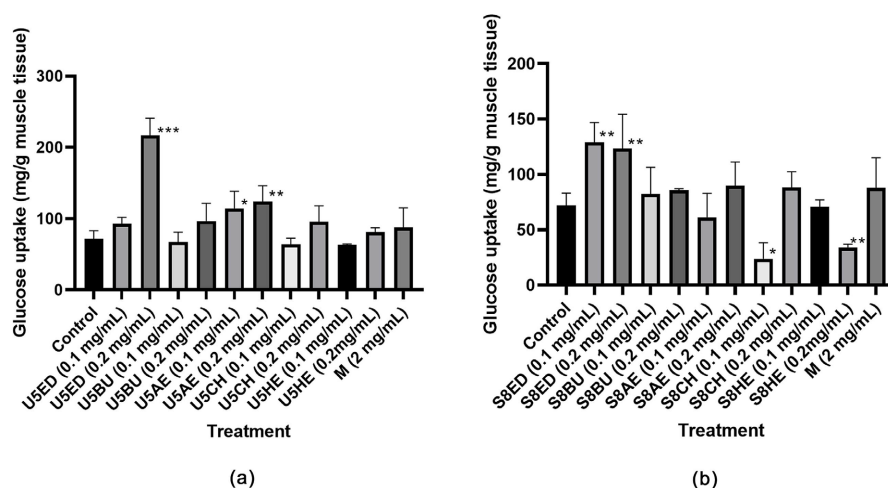


Figure 3. Effects of fractions on glucose uptake in isolated rat muscle (a): *Uvaria chamae*; (b): *Sida linifolia*; M: metformin; (Values are expressed as means \pm standard error of the mean (n = 3); *P < 0.05, ** P < 0.01, ***P < 0.001 vs control).

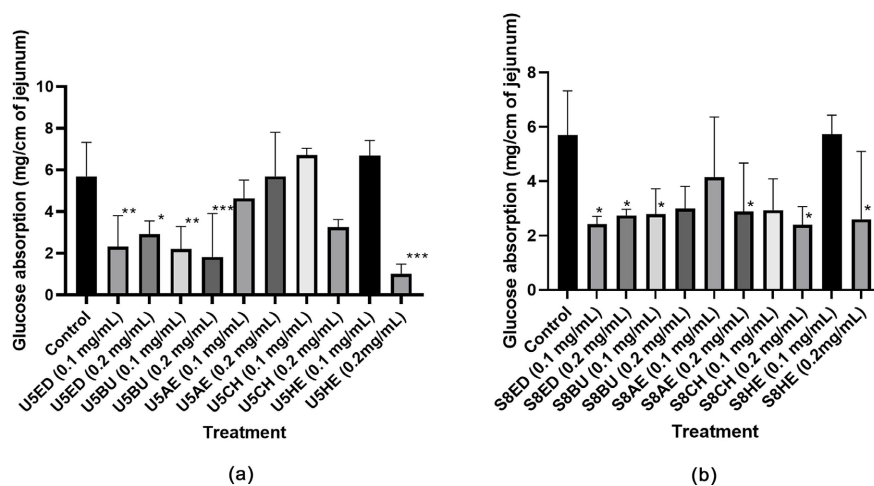


Figure 4. Effects of fractions on intestinal glucose absorption, (a): *Uvaria chamae*; (b): *Sida linifolia*; (values are expressed as means \pm standard error of the mean (n = 3); *P < 0.05, **P < 0.01, ***P < 0.001 vs control).

4.5. Antioxidant Assays

The scavenging capacity of the fractions was assessed and presented in the following table (Table 3).

Table 3. Antioxidant activity of *Sida linifolia* and *Uvaria chamae* fractions.

Fractions	DPPH	FRAP
	IC50 ($\mu\text{g/mL}$)	EC50 ($\mu\text{g/mL}$)
U5HE	2241.84	344.83
U5CH	1397.14	331.43

Continued

U5AE	246.74	306.45
U5BU	231.01	133.33
U5ED	691.86	511.63
S8HE	1158.41	1092.68
S8CH	902.01	311.54
S8AE	543.21	180.00
S8BU	533.81	55.56
S8ED	822.92	113.33
Quercetin	52.70	-
Ascorbic acid	-	40.91

Values are expressed as means; GAE: gallic acid equivalent; QE: quercetin equivalent; CE: catechin equivalent.

5. Discussion

In this study *Uvaria chamae* leaves and of the whole plant of *Sida linifolia* total extracts were fractionated with increasing polarity solvents leading to obtaining different yields [18]. Fractionation involves the transfer of several compounds from one phase to another through partially miscible liquid phases that interact with each other during their intimate contact. Fractions obtained often have different properties and can help in the isolation of bioactive natural compounds for drug development [19]. Preparation of the fractions in the present study is then the first step of *Uvaria chamae* leaves and of the whole plant of *Sida linifolia* total extracts purification. Indeed, the phytochemical showed that the phenolic compounds were isolated in the most polar solvents (butanol and water). We then performed *ex vivo* methods to assess the antidiabetic properties of the fractions. Diabetes mellitus (DM) is a complex chronic systemic disease associated with metabolic disorders, including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia; high blood glucose causes acute and chronic complications. Some acute complications are mainly associated with coma, hypoglycemia, and ketoacidosis, while chronic complications are most destructive to the body organs [20]. The treatment aims to control the hyperglycemia, which can be achieved by improving glucose uptake by muscle (biguanides) or by decreasing absorption of glucose by the small intestine (biguanides and α -glucosidases) [21]. The *ex vivo* test with the muscle and the rat jejunum allows to evaluation such activity [22]. The reference used was the metformin for it is a biguanide mostly used in diabetes treatment [21]. The doses were chosen following the use of these doses in previous studies [23]. Results showed that the fractions assessed were able to significantly increase the glucose uptake by the muscle, especially U5ED (0.2 mg/ml) for *Uvaria chamae* and S8ED (0.1 and 0.2 mg/ml) for *Sida linifolia*. Those fractions, U5ED and S8ED, also significantly decreased the absorption of the rat jejunum ($P <$

0.05). Similar results were found for other extracts fractions of natural plants as well [24] [25]. Oxidative stress has also been discovered to also play important roles in the development of diabetes mellitus. It is believed that oxidative stress is involved in the development of vascular complications in diabetes mellitus. Oxidative stress is usually caused by free radicals in the body. Free radicals are short-lived chemical entities containing one or more unpaired electrons. They exert damage by passing the unpaired electrons to the cell, resulting in oxidation of the cell's components and molecules [26]. The result of the antioxidant tests showed that the fractions had free radical scavenging activities, and U5ED for *Uvaria chamae* and S8ED for *Sida linifolia* demonstrated the highest activities along with the butanol fractions. The phytochemical screening showed the presence of phenolic compounds in the fractions. The antidiabetic activities of plants have been linked to several chemical groups such as alkaloids, phenolic compounds, flavonoids, terpenes, tannins, anthocyanins, and polysaccharides [27]. Polyphenolic compounds, especially flavonoids, are natural polyphenolic molecules of plant origin known for their antidiabetic, antioxidant, anti-inflammatory, and anticarcinogenic properties. Dietary intake of flavonoids might prove to be important for alternative diabetes treatments or the reduction of the risk of the disease. Attempts have been made to determine their potential in preventing β -cell apoptosis, promoting β -cell proliferation and insulin secretion, and enhancing insulin activity and insulin-stimulated glucose uptake [28]. They are also known for having a protective action on cellular antioxidant defense against oxidative damage induced in diabetes by stimulation of the endogenous antioxidant system [29]. The present data show an association between the presence of the phenolic content and the antidiabetic activity demonstrated by the extract fractions of the two plants.

6. Conclusion

Data collected on the fractions of *Uvaria chamae* leaves and *Sida linifolia* whole plant total extracts show that some of their fractions studied contain biomolecules for the treatment of diabetes. Nevertheless, *ex vivo* tissue assays support screening for antihyperglycemic potential but do not establish *in vivo* efficacy, bioavailability, or mechanism of action. Further investigation may help in the comprehension of the mechanisms of action of these molecules (*in vivo* assays) and the identification of active compounds for drug development and for alternative therapies.

Author Contributions

S.C.J.S. worked on the design and performed the experimental work; Y.T.K. worked on the conception of the *ex vivo* methods; M.C.A. performed the antioxidant assays; E.B. and M.A. worked on the interpretation of data; D.A., A.D. and B.B. worked on the conception.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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